Testosterone undecanoate associated polycythaemia in males with late-onset hypogonadism: Private practice Emalahleni

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PREFACE

This mini-dissertation is presented in an article format, where the results are discussed in Chapter 3.

The mini-dissertation is divided into four chapters:

- Chapter 1 provides a brief overview of the study and all ethical considerations needed to successfully complete the study

- Chapter 2 comprises a detailed literature review that addresses relevant topics (hypothalamic-pituitary-gonadal axis pathophysiology, epidemiology of late-onset hypogonadism forms, diagnosis, treatment and treatment-associated side effects). This section is then concluded with a chapter summary (section 2.8)

- Chapter 3 offers answers to the empirical investigation (results of this study in article format) as set out in Chapter 1, (section 1.3.4) of this mini-dissertation. The article, written according to the journal requirements, is submitted for peer review and possible publishing in the Journal of Endocrinology, Metabolism, and Diabetes of South Africa (JEMDSA - 2018 – 0016)

- Chapter 4 includes the conclusions, study limitations, strengths and recommendations for further studies.

At the end of this mini-dissertation, the references and annexures that hide prescribers' personal information follow (Chapter 4).

The article co-authors are the supervisor and co-supervisor. They both read and approved the mini-dissertation (which includes the manuscript). Acknowledgements follow in the next section.

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ABSTRACT

Title: Testosterone undecanoate associated polycythaemia in males with late-onset hypogonadism: Private practice Emalahleni

Late-onset hypogonadism (LOH) is a clinical and biochemical syndrome which affect one of the biochemical processes in the human body, and then present with a set of associated symptoms, e.g. age-related testosterone deficiency. This age-related testosterone deficiency, as seen in LOH is then associated with a cluster of symptoms which mostly include loss of libido, erectile dysfunction, fatigue, depression and loss of body hair. Testosterone is the only evidence-based treatment for LOH. Benefits of treatment include: favourable effects on cognitive function, sexual parameters, body composition and quality of life with demonstrated decreased mortality rates. Testosterone replacement therapy (TRT) is known to induce changes in certain blood parameters that stimulate haematopoiesis, which might well result in polycythaemia, also known as erythrocytosis (an excessive increase in the number of red blood cells).

The blood parameter changes observed pre- and post-treatment are expressed as haematocrit (Hct) percentages. Supra-physiological Hct values can be expected in the LOH patient during the first few weeks to months following treatment initiation and should reach a plateau within the first 12 months of therapy. Therefore, emphasis should be placed on frequent patient monitoring during these times that assesses signs and symptoms associated with polycythaemia to prevent testosterone-induced complications. Even though thrombosis, strokes and cardiovascular events are known complications of polycythaemia, factors such as diet, disease state and socioeconomic factors may also influence the haematopoietic process. Sufficient high-powered large cohort studies are still needed to fully explain the implication of Hct changes in the LOH patient.

The primary aim of this study was to investigate the effect of TRT on total-testosterone (TT) levels and Hct, with the focus on polycythaemia that occurred in LOH treatment-naïve patients. The study took place in a private urology practice in Emalahleni, formerly known as Witbank. This was a retrospective, observational, descriptive study. Data collected were TT levels (n = 49) and Hct (n = 50) values at the point of diagnosis (day zero) and at three months' post-treatment initiation. The risk for polycythaemia was determined by the probability of polycythaemia in the study population. The change between the two-time points was determined by the dependent t-test. Cohen's d-value was then used to evaluate the practical significance of the results (with d ≥ 0.8 defined as a large effect with practical significance).

The prevalence of polycythaemia was 34% (n = 50). The mean change in Hct over the study period was 3.49% (standard deviation [SD (4.46%)]). The mean increase in TT levels over the

study period was 4.21 nmol/L (SD 6.47). The rise in Hct was statistically significant, p-value < 0.001. The practical effect size was 0.73, suggestive of a practically significant impact. The increase of TT was statistically significant (p-value < 0.001). The practically significant effect was 0.68, suggestive of a larger effect size. A negative correlation between Hct and TT was noted after the study period. The prevalence of polycythaemia is higher for the South African population than for their international counter parts, and the practical implication of the statistical findings is not yet fully explained. Monitoring for changes in Hct values, especially during the treatment initiation phase, and then annually, is therefore suggested.

Keywords: Late-onset hypogonadism (LOH), haematocrit (Hct), testosterone replacement therapy (TRT), Depot-testosterone undecanoate

OPSOMMING

Titel: Testosteroon undekanoaat-geassosieerde polisitemie in mans met laat-aanvang-hipogonadisme: privaat praktyk Emalahleni

Laat-aanvang-hipogonadisme (LAH) is 'n kliniese en biochemiese sindroom, wat een van die biochemiese prosesse in die liggaam affekteer en dan presenteer met geassosieerde simptome soos ouderdom-verwante testosteroon gebrek. Hierdie ouderdom-verwante testosteroon gebrek, soos waargeneem in LAH word dan geassosieer met n groep simptome wat meestal verlaagde libido, erektiele disfunksie, moegheid, depressie en 'n verlies aan liggaamshare insluit. Testosteroonterapie is die enigste getoetste metode wat bewys is om 'n positiewe effek te hê op LAH. Voordele van behandeling sluit onder andere in 'n verbetering van kognitiewe funksies, seksuele funksies, voordelige liggaamsmassa-verhouding en 'n algehele verbetering in kwaliteit van lewe en lewensverwagting. Testosteroonvervangingsterapie (TVT) is bekend daarvoor om bloedsel-vorming te stimuleer, wat mag lei tot polisitemie oftewel eritrositose (die vermeerdering van die aantal rooi bloedselle (RBSe) wat waargeneem word wanneer 'n bloedmonster getoets word).

Die bloed-parameterverandering wat waargeneem word voor en na behandeling word uitgedruk as die hematokrit (Hkt)-persentasie. Bo-verwagte hoë Hkt-waardes kan verwag word gedurende die eerste paar weke tot maande na behandeling geïnisieer word in die LAH pasiënt, waarna 'n plato bereik word gedurende die eerste 12 maande van terapie. Daarvolgens moet klem geplaas word op gereelde pasiënt-monitering gedurende die terapie-inisiëringsfase om te evalueer vir tekens en simptome wat geassosieer word met polisitemie om testosteroon-geïnisieerde komplikasies te minimaliseer. Selfs al is trombose, beroertes en kardiovaskulêre siekte-toestande bekende komplikasies van polisitemie, het faktore soos dieet, ander siektetoestande en sosio-ekonomiese toestande ook 'n invloed op die bloedselvormingsproses. Voldoende studies is nog nodig om die effek van Hkt-verandering in die LAH-pasiënt te verduidelik.

Die primêre doel van die studie was om die effek te evalueer wat TVT op totale testosteroon (TT)-vlakke en Hkt het, met 'n fokus op polisitemie wat ontstaan in die LAH-pasiënt waarin behandeling die eerste keer geïnisieer is. Die studie het plaasgevind in 'n private praktyk in Emalahleni, wat voorheen bekend gestaan het as Witbank. Die studie was 'n retrospektiewe, waarnemende, beskrywende en alomvattende studie. Die data wat versamel is, het TT-vlakke (n = 49) en Hkt-waardes (n = 50) ingesluit van wanneer die pasiënt gediagnoseer is (dag nul) en dan weer TT-vlakke en Hkt-waardes soos geneem aan die begin van maand 3 na behandeling geïnisieer is. Die risiko vir polisitemie is bepaal deur die moontlikheid vir polisitemie van die studiepopulasie.

Die verandering tussen die twee tydsintervalle (maand 0 en maand 3) is bepaal deur middel van die afhanklike t-toets. Cohen se d-waarde is gebruik om die prakties betekenisvolle veranderinge van die resultate te verduidelik (met 'n d-waarde ≥ 0.8 wat 'n aanduiding is van 'n groot effek met prakties betekenisvolle verandering).

Die voorkoms van polisitemie was 34%. Die gemiddelde Hkt-verandering gedurende die studietydperk was 3.49% (standaard afwykings [SD (4.46%)]). Die gemiddelde TT-vlakke het met 4.21 nmol/L (SD 6.47) gedurende die studietydperk verhoog. Die verhoogte Hkt-waardes wat tydens die studietydperk genoteer is, was statisties beduidend; p-waarde < 0.001. Die prakties betekenisvolle verandering was 0.73, wat 'n aanduiding is van 'n groot prakties betekenisvolle verandering. Die verhoging wat opgemerk is in die TT-vlakke gedurende die studietydperk was statisties betekenisvol (p-waarde < 0.001). Die prakties betekenisvolle verandering was 0.68, wat 'n aanduiding is van 'n groot prakties betekenisvolle verandering. 'n Negatiewe korrelasie tussen Hkt en TT is genoteer na die studie tydperk. Die voorkoms van polisitemie is hoër vir die Suid-Afrikaanse populasies as vir internasionale populasies en die praktiese implikasie van hierdie statistiese bevindings moet nog ten volle verduidelik word. Monitering van Hkt-waardes, veral gedurende die behandelingsinisiëringstydperk en dan jaarliks, word dus aanbeveel.

Trefwoorde: Laataanvang-hipogonadisme (LAH), hematokrit (Hkt), testosteroon-vervangingsterapie (TVT), Depo-testosteroon undekanoaat

LIST OF DEFINITIONS

Agenesis	The failure or non-development of an organ body part or tissue (Oxford Concise Medical Dictionary, 2007:15).
Autosomal	Any chromosome that is not a sex chromosome and present in pairs (Berkow & Fletcher, 1992:2286).
Androgens	Molecules responsible for the development of the male internal and external genitals (Kaufman & Vermeulen, 2005:834).
Aneuploidy	The condition where the chromosome number of a specific cell is not the same as the norm (Re & Birkhoff, 2015:10).
Anosmia	Complete loss of smell (Zitzmann *et al.*, 2014:36).
Erythrocyte	A red blood cell with the main function being a transporter of haemoglobin (oxygen-carrying protein). An increase in the number of erythrocytes is known as erythrocytosis (Oxford Concise Medical Dictionary, 2007:251) as appose to anaemia when the erythrocyte count is low (Oxford Concise Medical Dictionary, 2007:29).
Erythropoiesis	The process of red blood cell development, which normally originates from the bone marrow (Oxford Concise Medical Dictionary, 2007:251).
Genital ambiguities	Synonym to cases where the external examination of the genitalia does not appear to be clearly male or female (Mayoclinic, 2015).
Gonads	The male or female reproductive organ (Oxford Concise Medical Dictionary, 2007:306) that produces mature sex cells of both male (spermatozoa and female (ovum) (Oxford Concise Medical Dictionary, 2007:520).

Gonadotropins	The collective name for the hormones that is produced and secreted from the pituitary gland in response to stimulation from gonadotropin releasing hormone (GnRH) originated in the hypothalamus. The two main gonadotropins are luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Boehm *et al.*, 2015:548).
Haematocrit	The percentage of erythrocytes present in a blood sample (MedicineNet, 2014).
Haemoglobin	The main oxygen-carrying protein that is present within the red blood cell (Oxford Concise Medical Dictionary, 2007:316).
Hypogonadism	A clinical condition that develops from the failure of the gonads from both men and women to produce physiological levels of androgens (Zarotsky *et al.*, 2014:1), also known as testosterone deficiency (TD) in men (Lunenveld *et al.*, 2015:1) and menopause in women (Wiereman *et al.*, 2014:3492).
Hyposmia	Partial loss of smell (Zitzmann *et al.*, 2014:36).
Hypothalamic-pituitary gonadal axis	A physiological system that consists of the hypothalamus, pituitary gland and the gonads. This system regulates the release and inhibition of hormones via a positive and negative feedback system. The Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus that up regulate the pituitary gland to synthesise and secrete luteinizing hormone and follicle stimulating hormone that in return activate the gonads to produce testosterone via the Leydig cells and initiate spermatogenesis from the Sertoli cells. The hypothalamus decreases the secretion of GnRH via a negative feedback system associated with an increase in testosterone levels. For the purpose of this study the hypothalamic-pituitary gonadal axis denotes the signalling system between the hypothalamus, pituitary gland and the gonads that regulate the normal physiological function of the testes (Jones *et al.*, 2015:102; Matthew *et al.*, 2015:1).

Karyotype	Denote the number and the structure of specific chromosome sets of an individual or species (Oxford Concise Medical Dictionary, 2007:388).
Late-onset hypogonadism (LOH)	Hypogonadism in males that developed after puberty, therefore males diagnosed with late-onset hypogonadism have fully developed secondary sex characteristics (Dohle *et al.*, 2014:4).
Phenotype	Denote the physical characteristics of an individual because of the interactions between genes (Bardsley *et al.*, 2013:1085; Bonomi *et al.*, 2017:123-125).
Plasma	The non-living liquid portion of the blood in which cells are suspended (MedicineNet, 2013).
Polycythaemia	An increase in haematocrit in the blood. This can be due to a decrease in the plasma volume of the blood or an abnormal increase in the quantity of the red blood cells (Berkow & Fletcher, 1992:1188-1189). For the purpose of this study polycythaemia will be defined as a haematocrit equal to, or exceeding 50% (Flora *et al.*, 2010:386).
Prostate specific antigen (PSA)	The enzyme produced by the glandular epithelium of the prostate and is secreted in larger than normal quantities in the blood during certain conditions e.g. inflammation or prostate enlargement. The PSA is also used as a cancer marker but appear not to be cancer specific and no clear cut-off level is defined yet (Oxford Concise Medical Dictionary, 2007:588).
Secondary sexual characteristics	The change of the external genitalia including the appearance of pubic-, axillary- and facial hair, increase of volume of the larynx and/or deepening of voice with increased lean body mass (Oxford Concise Medical Dictionary, 2007:646).

Testosterone   The main male androgen that is secreted by the testes due to stimulation from the hypothalamic-pituitary gonadal axis system. Testosterone is also responsible for the secondary sex characteristics of males e.g. deepening of voice and secondary hair growth (Kaufman & Vermeulen, 2005:834).

Total testosterone   The testosterone value that consists of free-testosterone (FT), testosterone bound to albumin and the portion of testosterone that is bound to sex hormone binding globulin (SHBG) (Stanford & Jones, 2006:26).

LIST OF ABBREVIATIONS

AR   Androgen receptor

ARs   Androgen receptors

AIS   Androgen insensitivity syndrome

AMS   Aging male scorecard

ADAM   Androgen deficiency in the aging male

BACH   Boston Area Community Health

BAT   Bioavailable testosterone

BMI   Body mass index

BPH   Benign prostatic hyperplasia

CAIS   Complete androgen insensitivity syndrome

CHH   Congenital hypogonadotropic hypogonadism

DM2   Diabetes mellitus type 2

ED   Erectile dysfunction

FDA   Food and Drug Administration

FSH   Follicle-stimulating hormone

FT   Free testosterone

GnRH   Gonadotropin releasing hormone

Hb   Haemoglobin

Hct   Haematocrit

HIV   Human immunodeficiency virus

Hkt   Hematokrit

HPG axis	Hypothalamic-pituitary gonadal axis
IIEF	International index of erectile function scorecard
IIEF-5	International index of erectile function-5 scorecard
I-PSS	International Prostate Symptom Scorecard
KS	Klinefelter's syndrome
LH	Luteinizing hormone
LAH	Laat aanvang-hypogonadisme
LOH	Late-onset hypogonadism
LUTS	Lower urinary tract symptoms
MAIS	Mild androgen insensitivity syndrome
MMAS	Massachusetts Male Aging Study
NWU	North-West University, Potchefstroom Campus
PADAM	Partial androgen deficiency in the aging male
PAIS	Partial androgen insensitivity syndrome
PSA	Prostate specific antigen
RBSe	Rooi bloedselle
SAPC	South African Pharmacy Council
SHBG	Sex hormone binding globulin
TT	Total testosterone / Totale testosteroon
TESE	Testicular sperm extraction
TRT	Testosterone replacement therapy
TVT	Testosteroonvervangingsterapie

VPSS   Visual prostate symptom scorecard

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CHAPTER 1: INTRODUCTION

1.1   Introduction and background to the study

The disruption of mental health is well documented in the elderly, and has now also been linked to androgen deficiency as a potential contributory factor (Liu *et al.*, 2016:1). Hypogonadism is the universal term used to describe the failure of the gonads, resulting in the production of reduced amounts of physiologically active androgens (Basaria, 2014:1250). This directly affects several processes, from mental status to development and growth in the human body (Dwyer *et al.*, 2015:R17; Stanworth & Jones, 2008:25). Testosterone is the dominant and most abundant form of androgen (Bassil *et al.*, 2009:427-429; Bornman & Reif, 2007:62).

Male hypogonadism, also known as testosterone deficiency, is a mixed disorder of the hypothalamus, exacerbated pituitary gland and the testes, resulting in an androgen deficiency syndrome that can present with adverse effects on the functioning of multiple organs (Nieschlag & Behre, 2010:169). These effects may include, for example, reduced fertility, sexual dysfunction, decreased muscle development and bone mineralisation, metabolic syndrome, and disturbances of fat metabolism (Basaria & Dobs, 1999:132; Dohle *et al.*, 2014:6; Kaufman & Vermeulen, 2005:843; Kumar *et al.*, 2010:297). The above-mentioned clinical consequences of this condition are related to the age of onset and severity thereof (Dohle *et al.*, 2014:13-15).

Late-onset hypogonadism (LOH), according to the authors of the guidelines on male hypogonadism, Dohle *et al.* (2014:4), is a form of hypogonadism that arises in a male who had normal development through puberty and therefore developed accordingly in sexual characteristics. It is common to relate LOH to hypogonadism (Stanworth & Jones, 2008:25). Late-onset hypogonadism in males has a rather slow onset, unlike menopause in women where the occurrence is rather rapid and well defined (Kumar *et al.*, 2010:299).

The diagnosis of LOH in men is made by investigation of the clinical and biochemical symptoms in conjunction with the demonstration of a decreased testosterone level. Androgens play a key role in the maintenance of associated and diverse secondary sex characteristics in men, e.g. deepening of voice pitch, maintaining of muscle mass, strength and facial hair (Kaufman & Vermeulen, 2005:834). The most prevalent symptoms experienced by males with LOH include *inter alia,* decreased sexual thoughts, weakened morning erections, erectile dysfunction (ED), hot flushes, visceral obesity and small testes (Dohle *et al.*, 2014:13).

According to Lackner *et al.* (2011:1311), men are classified as being middle aged between the ages of 45 and 60 years; 6% of this cohort will present with low androgen levels, with an even

higher prevalence in older men (Dohle *et al.*, 2014:6; Hall *et al.*, 2008:3870; Kumar *et al.*, 2010:299). According to Campbell and Stein (2014:440), only five South African articles have reported on male sexual dysfunction since 1970. Nevertheless, a study presented by Bornman and Reif (2007:62) reported that healthy South African men aged 20 to 49 years have sub-physiological total testosterone (TT) levels (below 12.5-30.5 nmol/L) compared to their international counterparts. Consequently, the assumption can be made that South African men will qualify sooner for testosterone replacement therapy (TRT) compared to the international cohort.

Decreased TT levels have also been associated with several chronic diseases and it has been demonstrated that patients may benefit from TRT, e.g. enhanced libido and sexual performance, muscle mass increase, favourable bone density measurements, better mood and cognitive function, and quality of life (Bassil *et al.*, 2009:427; Dohle *et al.*, 2014:18). Although symptomatology of age-related testosterone depletion has been elucidated, literature is inconclusive as to whether TRT effectively addresses all aspects of the clinical syndrome (Heidari *et al.*, 2015:435; Lackner *et al.*, 2011:1310; Saad *et al.*, 2011:675; Yamaguchi *et al.*, 2010:52). Recent studies have demonstrated that TRT may be associated with the worsening of hypertension, congestive cardiac failure and weight gain; with serious and sometimes life-threatening side effects, including enhanced prostate cancer growth and hepatotoxicity associated with oral testosterone use (Aleksova & Allan, 2015:36; Basaria & Dobs, 1999:133-138; Dohle *et al.*, 2014:22-23; Golden 2003:553-555; Haider *et al.*, 2010:349; Hamilton, 2003:854; Jick & Hagberg, 2012:260).

Numerous treatment options are available, including formulations containing different testosterone derivatives administered in various dosage forms such as capsules, intramuscular injections, depot-intramuscular injections, buccal testosterone or transdermal patches. Nevertheless, according to the Monthly Index of Medical Specialities (Snyman, 2016:359), only the depot-intramuscular injection is specifically indicated for LOH in South Africa. The Standard Treatment Guidelines and Essential Medicines List (2015:8.4) states that testosterone cypionate is used to treat hypogonadism in the primary healthcare setting without the mention of LOH. Each of these dosage forms presents its own unique challenges, varying from affordability to availability, as well as physiological, biochemical and clinical side effect profiles (Bhasin & Bremner, 1997:3-8; Kumar *et al.*, 2010:300).

Irrespective of the age of the patient diagnosed with LOH, the primary aim of TRT is to improve general wellbeing, sexual function, muscle strength and bone mineralisation (Basaria, 1999:136; Coviello *et al.*, 2008:914; Dohle *et al.*, 2014:17). The aforementioned has been evaluated by Dohle *et al.* (2014:25) and Saad *et al.* (2011:675), where changes in general wellbeing are noted within three to four weeks, increase in libido is experienced within three weeks, muscle strength was observed between three and four months, effects on bone observed after six months and erythropoiesis evident at three months – plateauing between month nine and 12. This exogenous administration of TRT will supplant the already low to low normal known physiological testosterone levels to within the normal physiological range. The hypothalamic-pituitary gonadal axis mechanism of testosterone after treatment withdrawal as explained in section 2.1 and 2.2 may take up to 12 months to regain normal physiological function.

The administration of TRT does not only affect the haematocrit (Hct) levels of treated patients, but also relevant biomarkers such as testosterone levels (Kang & Li, 2015:1). An increase in Hct is the most prevalent side effect of TRT, known as the trade hallmark of polycythaemia. The Hct, defined as the percentage of red blood cells present in a blood sample, numerically expressed as a value equal to or higher than 50%, is classified as a contraindication for TRT (Dohle *et al.*, 2014:18; Flora *et al.*, 2010:386). Polycythaemia associated with TRT is known to result in complications, for example blood clots (that may cause stroke, myocardial infarction or pulmonary embolism), as well as spleen enlargement, which may compromise its role in erythrocyte removal and the immune system (Golden, 2003:553). It is therefore crucial that all males, especially the patients treated with TRT for LOH, be monitored (Basaria & Dobs, 1999:136; Coviello *et al.*, 2008:914; Jick, 2012:267). To understand the impact of TRT on Hct, leading to polycythaemia, it is important to understand the effect of TRT on erythropoiesis (Golden, 2003:553).

Prescriptions for TRT have increased tremendously in recent years, generally due to increased public awareness campaigns (Samoszuk, 2016:12). Besides being the number one reason for men seeking medical advice, sexual dysfunction is the number one clinical symptom accompanying low testosterone levels (Corona *et al.*, 2012:251). Old age is associated with lower androgen levels, accompanied with decreased physical- and emotional symptoms that impair quality of life. Treatment using depot-testosterone undecanoate will make the symptoms bearable; sadly, side effects are inevitable. Therefore, controlling medicine-induced side effects is important (Kumar *et al.*, 2010:297).

1.2   Problem statement

Patients receiving TRT are at risk of developing polycythaemia, among other physiological effects, such as a change in the measured TT levels. Even though the usage of TRT has increased over the years (Samoszuk, 2016:12), it does not reflect LOH prevalence as other conditions also rely on treatment with TRT, e.g. ED (Marais, 2016:11). The cohort of LOH patients is left rather vulnerable when considering the few published studies related to male sexual wellbeing in South Africa over the past four decades (Campbell & Stein, 2014:440) and the limited number of patients receiving treatment after diagnosis (Carruthers, 2009:21).

This study addressed these issues by investigating depot-testosterone undecanoate associated polycythaemia in males with LOH, in a retrospective cohort, while simultaneously evaluating the variance of previously recorded TT levels before and after treatment.

1.3   Research aims and objectives

The following section contains specific set goals (aims) that were reached by following a set plan (objectives) in a structured manner.

1.3.1   Research aims

The aim of the present study was to investigate the effect of TRT on TT levels and Hct, with the focus on polycythaemia, in men diagnosed with LOH in a private urology practice located in Emalahleni for the period of 1 July 2013 to 1 March 2017.

1.3.2   Specific research objectives

To accomplish the stated aim of this study, specific literature- and empirical objectives were met as set out under the following two subheadings:

1.3.2.1   Literature objectives

The literature objectives were to:

- Define LOH in men and review the literature for prevalence, demographic data, pathogenesis, clinical presentation (both sexual and non-sexual), diagnosis, treatment and monitoring

- Describe the treatment options currently available for LOH patients, including the benefits and associated risks

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- Investigate possible complications and/or side effects due to initiation of TRT and its monitoring and management.

1.3.2.2 Empirical research objectives

The empirical objectives of the study were met by:

- Retrospectively observing the effect of TRT, if any, on the Hct- and TT values of patients diagnosed with LOH

- Determining the prevalence of polycythaemia in patients with an increased Hct currently diagnosed with LOH and treated with depot-testosterone undecanoate

- Determining, if possible, whether the percentage variance in TT level per patient can be used to predict the change in the Hct levels measured.

1.3.3   Literature review

The literature review involved an intense topic-related study, which helped the researcher to motivate and explain the empirical study.

The researcher made use of databases such as Google Scholar™, EBSCOHost®, Science Direct® and Scopus®).

Scientific information was gathered and filtered for more productive and focused results, and this was done by using keywords and phrases, Boolean- and proximity operators, and parenthesises for example:

- 'Late-onset hypogonadism (LOH)', 'Aging male', 'Androgen deficiency'

- 'Testosterone treatment', 'complication*'

- 'Polycythemia', 'Polycythaemia', 'Thrombocytosis' and 'Haematocrit'.

1.3.4   Empirical investigation

The empirical study was a retrospective, longitudinal, observational study. The Hct- and TT laboratory results of males on TRT and diagnosed with LOH were evaluated retrospectively on

month zero (the time when treatment was initiated by the treating specialist) and then again at three months of treatment as per previously published recommendation (Lunenfeld *et al.*, 2015:8).

1.4 Research methodology

1.4.1 Study design

The proposed study design was a quantitative, observational, descriptive, retrospective, cohort study that is a subcategory of the non-experimental design (Brink, 2010:10-290; Conaglen *et al.*, 2014:574; Lackner *et al.*, 2011:1310).

The study design can be subdivided into the following aspects:

- Non-experimental study design: A study where the aim of the researcher is not to influence or to control the independent variable that has an effect on the dependant variable, but rather to describe the effect that the independent variable has on the dependant variable (Brink, 2010:102)

- Quantitative: Variables are measurable (Aldous *et al.*, 2013:25)

- Observational: The researcher will measure the implicated variables, but will not intervene (Aldous *et al.*, 2013:25)

- Descriptive: The occurrence in a population and applicable risk factors are described (Brink, 2010:102-103)

- Cohort study: Where a group of people who share the same specified characteristics that the researcher would like to study, are monitored over a specific time period (Lackner *et al.*, 2011:1310)

- Retrospective: When data generated in the past is investigated in the present (Conaglen *et al.*, 2014:574; Lackner *et al.*, 2011:1310).

The researcher used data for the research questions, aims and objectives by retrospectively observing the change of variables over the three-month period. Furthermore, the proposed study design competently addresses the current aims and objectives and therefore it was seen as the most appropriate for the study.

1.4.2   Study setting

The study made use of data from patients who attended one of the two consulting urologists at a private practice located in Mpumalanga. The practice is located adjacent to a private hospital and apart from their daily consultations and operations, run a male clinic as part of their community involvement. The practice serves people throughout Mpumalanga, ranging from Emalahleni (formerly known as Witbank) to Groblersdal and Nelspruit. It is however not a mutely exclusive Mpumalanga urology practice and patients from any province are welcome at the practice. This multi-focal study setting is a great advantage for the study, due to the large population area that it serves.

1.4.3   Sampling

No sampling was performed since all patients who met the inclusion criteria were used in this specific study.

1.4.4   Target & study population

The target population for this particular study was all male patients living with LOH in South Africa.

The study population consisted of all male patients diagnosed with LOH at the specific private practice from 1 July 2013 when the practice was opened, until 1 March 2017, to give newly diagnosed LOH patients the opportunity to complete the second dose of TRT. Permission had to be obtained from the Health Research Ethics Committee (NWU-00082-17-S1) before data collection could commence at the beginning of June 2017 and patients had to meet the inclusion criteria as stated in section 1.4.4.1. This study period was chosen to optimise the quality of data that was captured, since no South African LOH database has been developed to date. The longest possible time has been allocated to optimise statistical analysis of the collected data.

1.4.4.1   Inclusion criteria

The inclusion criteria for the proposed study were:

- All treatment naïve male patients diagnosed with LOH at the specific private urological practice according to the European Guidelines for LOH

- Patients treated with depot-testosterone undecanoate for LOH within the participating urology practice.

1.4.4.2 Exclusion criteria

The exclusion criteria for the proposed study were:

- Patients who are unable to start treatment or have to stop due to prostate cancer or being diagnosed with prostate cancer during the course of treatment (Dohle *et al.*, 2014:18)

- Prostate-specific antigen (PSA) > 4 ng/ml at time of diagnosis (Huhtaniemi, 2014:197)

- Male breast cancer (Bornman & Reif, 2007:62)

- Severe sleep apnoea (Bassil *et al.*, 2009:440)

- Haematocrit exceeding 50% (of blood sample) at time of diagnosis, as this is seen as a relative contraindication for treatment initiation (Bhasin *et al.*, 2010:2536; Dohle *et al.*, 2014:18; Lunenfeld *et al.*, 2015:7) notwithstanding the fact that patients would also be seen as polycythaemia patients (Flora *et al.*, 2010:386)

- Patients receiving TRT, while not being diagnosed with LOH by the participating urology practice, as TRT is not exclusively for the treatment of LOH.

1.5 Data source

The data source, developed by the male clinic data manager, was derived from a more comprehensive version used by the participating practice. The data source containing de-identified data for this study was in the form of an Excel® spreadsheet (Annexure A), equipped with the following data fields:

- Number of patients

- Total testosterone values measured in nmol/L

- Haematocrit percentage at the time of diagnosis (month zero) and three months later.

Annexure A contains an additional column, which was used by the researcher to identify patients with or without polycythaemia.

1.5.1 Validity and reliability of data source

Assessment of a data source not only ensures that results are captured and collected, but also ensures that the quality of the data is gathered in a way that promotes evidence-based medicine (Heale & Twycross, 2015:66). Validity is the ability of the data source to define the aim of the study accurately through data collection. Reliability ensures that the data collected are precise (Ehrlich & Joubert, 2014:123; Heale & Twycross, 2015:66). A data source is evaluated not only for the way in which data collection takes place, but also for the validity and reliability of the source, which should be balanced to promote constructive research without compromising validity or reliability (Aldous *et al.*, 2013:43).

This specific data source was designed with validity and reliability in mind without compromising patient anonymity and confidentiality. Consequently, any information linking a patient to his personal profile was de-identified by the male clinical data manager by means of deleting the cells linking the patient's personal information to this study. The validity of the data source (Annexure A) matches criteria as set out in Brink *et al.* (2006:159), where only data needed to conduct a study are captured. Patient reliability is free from bias as results are obtained from a laboratory and could not be influenced by the participant's state of mind as they are biological measurements. Furthermore, transferring relevant data electronically to the data source used in this study confirmed reliability, limiting manual random finger errors that might arise from capturing data from hard copies.

The data collection source complied with guidelines as set out in Brink *et al.* (2006:159-165) relating to validity and reliability of data, making the data source relevant for this specific study.

1.6 Data collection process

The researcher was not actively involved in the data collection process. Total testosterone levels and Hct values needed to complete the study were provided by the clinical data manager currently employed at the urology practice. The data needed were TT levels and Hct values as recorded by the clinical data manager at the time of diagnosis (month zero) and three months later. These values were then given to the researcher upon granted ethical approval from the Health Research Ethics Committee of the North-West University (NWU), Potchefstroom Campus (NWU-00082-17-S1).

A goodwill permission (Annexure B) to use relevant patient data was obtained from the urology practice, because informed consent by patients is not compulsory, as noted by the National Health

Act 61 of 2003 chapter-2 number 16. Data that finally met the criteria to achieve the aim of the current study were transferred electronically to the developed data source (Annexure A) by the clinical data-capturer, currently employed by the participating urology practice and double-checked by the practice manager who is also a full-time employee of the stated urology practice. The aforementioned processes de-identified the data, which made it functional for the researcher and statistician (Ms M Cockeran) to work with.

Patient confidentiality and anonymity were ensured by de-identifying the collected data. The developed data source is password protected, with only the urologists and the clinical data manager knowing the password. Relevant data were sent to the researcher electronically via the clinical data manager. The researcher then evaluated data for detection of polycythaemia (where an Hct is defined as erythrocytes conquering ≥ 50% of a blood sample as evaluated at three months' post-treatment). A positive or negative result was documented in the space provided under the heading *polycythaemia*, of (Annexure A). At the end of the data collection period, the researcher then electronically sent the completed data source to the statistician currently employed by the NWU, who then processed the data for further interpretation by the researcher. Results were stored on the researcher's personal computer, which is password- and virus protected. The results will remain on the researcher's computer until completion of the study. It will then be deleted by the researcher, under supervision of the clinical data manager currently employed by the urology practice.

1.6.1 Recruitment of participants

The researcher did not perform active recruitment. Diagnosed LOH patients, as set out in section 1.4.4.1 (inclusion criteria), were identified retrospectively by the clinical data manager.

1.7 Statistical analysis

The Statistical Analysis System®, SAS 9.3® (SAS Institute Inc., 2009) was used to analyse the data in consultation with Ms M Cockeran, currently employed by the NWU.

Categorical variables were reported as frequencies and percentages. Continuous variables reported as mean ± SD (normally distributed data) or median (25th, 75th) percentiles (skewed data). The distribution of variables was evaluated by means of histograms and Q-Q plots. Variables were logarithmically transformed to improve the normality thereof. Possible outlying values were identified by using box-and-whiskers plots, with z-score values larger than the absolute value of three. The dependent t-test was used to compare the change between the two-

time points. Cohen's d-value was used to determine the practical significance of the results (with d ≥ 0.8 defined as a large effect with practical significance).

Table 1.1:   Statistical analysis

Objective	Variables	Descriptive statistics	Inferential statistics	Practical significance
Determine the effect of TRT on the Hct- and TT values of patients diagnosed with LOH.	Hct at baseline and follow-up. TT at baseline and follow-up.	Mean ± SD 95% CI Median (25th percentile, 75th percentile)	Dependent t-test	Cohen's d
Determine the prevalence of polycythaemia in patients with an increased Hct value.	Number of patients with polycythaemia.	Frequency (%)	-	-
Determine whether the percentage variance in TT level per patient can be used to predict the change in the Hct levels measured.	Hct at baseline and follow-up. TT at baseline.	Mean ± SD 95% CI Median (25th percentile, 75th percentile)	ANCOVA	

1.8   Ethical considerations

The ethical considerations for this study are laid out in the following section.

1.8.1   Permission and informed consent

Informed consent per patient did not take place, as the National Health Act no. 61 of 2003, chapter-2, number 16 states that a healthcare worker may use data obtained from patient records for research purposes if no effort is made by the researcher to identify the patient. A goodwill permission letter (Annexure B) was obtained from the participating urology practice giving permission to use the relevant data collected by the practice in order to successfully complete the undertaken research study. Furthermore, a letter granting permission to use the participating practice name, including the names of the individual specialists' names if needed during the writing process of the mini-dissertation or article, was provided and can be viewed in (Annexure C).

1.8.2 Anonymity

Anonymity is part of human rights for participating in research, as set out in Brink *et al.* (2006:31) to ensure that participants are treated with the necessary respect and dignity.

Participant anonymity was assured by using the de-identified data source (Annexure A), designed by the clinical data manager.

1.8.3 Confidentiality

Patient confidentiality was sustained at all times (during the collection- and analysis process). The data source was password protected, populated with de-identified patient data. Only the participating specialists and clinical data-capturer knew the password. The researcher did not have access to the original identifiable data. Data were already de-identified at the time of analysis, thereby posing no risk to the patients. Analysed data were only available to the statistician, the supervisor, as well as the co-supervisors and the researcher.

1.8.4 Justification of research study

To reach the stated aim of this study, only patient data that met the inclusion criteria as set out in section 1.4.4.1 were included. By doing so, patient bias was excluded, giving every patient an equal chance to form part of the study.

1.8.5 Benefit-risk ratio analysis

In this specific study, the benefits outweighed the risks. Additionally, this study poses no direct or indirect risks related to the physical or emotional wellbeing of patients, as the study was done in retrospect.

1.8.6 Anticipated benefits

The following subdivision will elaborate on the benefits that the study holds for the participants.

1.8.6.1 Direct benefits

There was no direct benefit for any patient involved in this study due to the nature of the study design.

1.8.6.2 Indirect benefits

This study benefitted the LOH community at large indirectly by means of describing the most frequent side effect experienced by LOH patients, namely polycythaemia. This was achieved by means of recording Hct values due to administered TRT that took place in the participating urologist practice. According to Jick (2012:260), the safety of different TRT dosing formulations has not yet been fully explained. The Food and Drug Administration (FDA) only requires adequate pharmacokinetic studies for newly registered testosterone products (Kloner *et al.*, 2016:547), creating a lack of confidence pertaining to the efficacy of testosterone products. This study might also assist in predicting the increase in Hct because of TRT. It is known that the safety profile of testosterone therapy may vary post-marketing, *inter alia,* commercial motivations of sponsors, "framework of clinical registration trials" and between centre variances in clinical practice (Middleton *et al.*, 2015:512).

1.8.7 Anticipated risks and precautions

Anticipated risks to the participants and researcher, including precautions taken to minimise those risks, are explained in the following section.

1.8.7.1 Anticipated risks to the participants and precautions taken

This was a medium-risk study, where any discomfort that may have been experienced by the patients was not due to the study, but then again would have been part of the diagnostic process of the specialist. Anticipated risks, e.g. anonymity and confidentiality, were limited by the fact that the personal information linking a patient to the study was not included in the data source used by the researcher.

1.8.7.2 Anticipated risks to the researcher and precautions taken

The researcher had no contact with the participating subjects or any of their family members, ensuring low professional risk. Data values could have been captured incorrectly (on the original data source) by the clinical data-capturer, and for that reason, values were double-checked by the practice manager. The process of capturing laboratory values is part of the normal daily routine for the clinical data-capturer (no new procedure), ensuring minimal opportunity for incorrect values to be captured.

1.8.8   Reimbursement of study participants

There was no incentive or reimbursement to study participants, since there was no participant-researcher contact.

1.8.9   Data management

The de-identified data, as per the data collection tool, was sent to the statistician electronically, with no hard copies supplied to anyone. The statistician saved it electronically with an encrypted password. Data collected together with the analysed results were supplied to the administrative person in charge of records of the research entity, Medicine Usage in South Africa, where it will be kept for a period of seven years as per legislation. The outcomes of the processed data were electronically supplied to the researcher and study leaders who analysed the results. The researcher will keep the supplied results on his personal computer, equipped with an antivirus program and protected with a ten-digit encrypted password until the study is finalised; the researcher will then delete all data under supervision of the research assistant of Medicine Usage in South Africa.

1.8.10 Dissemination of research results

The results did form part of a mini-dissertation at the NWU. Results were made available to the public through publication in an appropriate peer-reviewed scientific journal. Findings were also discussed with the participating medical practice upon finalisation of results. The mini-dissertation or parts thereof may also be presented at a conference.

1.8.11 Role of members in the research team

The research team consisted of a registered family practitioner, two academic senior lecturers with doctorate degrees, all registered as pharmacists, one statistician from the NWU and the researcher (Mr HL Bester).

The researcher is a registered pharmacist with the South African Pharmacy Council (SAPC) and is permanently employed at a private hospital pharmacy as a senior pharmacist. He has been working there for the past six years. His responsibility is the planning of daily duties of co-workers as well as standing in for the pharmacy manager when she is not available. The researcher also plays a key role in the antimicrobial stewardship bundle compliance of his hospital. The researcher was responsible for planning all matters relating to this study, e.g. keeping to time lines as set out by the NWU, sending data to the statistician after the data collection period, writing of the literature study and interpreting the analysed results.

Dr Jesslee du Plessis was the study supervisor, and is employed at the NWU as a senior lecturer in Clinical Pharmacy and as a researcher in the research entity Medicine Usage in South Africa. She is also registered with the Health Professions Council of South Africa as a general practitioner. She has published numerous articles and reviews academic journals.

Dr Marlene Julyan was a co-supervisor and is employed at the NWU, with a special interest in the academic field of ancient medicine, clinical pharmacy and primary healthcare. She has also published numerous articles.

Ms Marike Cockeran was the statistician on the team. She is employed by the NWU, with the sole responsibility (for this study) to process the de-identified data into statistical data. She was awarded the best MSc student prize and formed part of a team that published four articles in 2015.

All ideas and information were presented via the co-supervisors, to the supervisor in one consolidated report. The researcher was responsible for collecting the relevant study information and doing the writing of the mini-dissertation and manuscript. The supervisor leads and oversees the study to ensure the prompt completion of tasks.

1.8.12 Conflict of interest

There was no conflict of interest to be declared.

1.9 Study limitations

Study limitations identified after consultation with the treating urologists regarding the study were documented as follows:

- This was a retrospective study and no present evidence was available that the treating specialists assessed the relevant patients' haematopoietic supplementation history, and therefore there was no way of knowing what the patient's haematological status was regarding supplementation when the study data were recorded

- Patients were not stratified according to their disease state, e.g. diabetic-, obese patients, patients living with human immunodeficiency virus (HIV), hypercholesteraemic-, anaemia-patients or age.

- According to Lunenfeld et al. (2015:3), the ideal time for TT blood sampling is between 07:00 am and 11:00 am, because of circadian blood level variance. This study did not note the time when a sample was taken by the laboratory.

1.10 Chapter summary

This chapter demonstrated the methods that were used to materialise the objectives as stated in section 1.3.2 of this mini-dissertation. Ethical approval to conduct the study was granted by the NWU. A literature review on the hypothalamic-pituitary gonadal axis (HPG axis) and TRT in the LOH patient with associated benefits and risk related to therapy is provided in Chapter 2.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction and background to the study

Late-onset hypogonadism, a physical- and biochemical syndrome, is regarded as a disturbance relating to the function of the HPG axis (see section 2.2), which regulates positive and negative feedback pathways of endogenous hormones involved in the endocrine system (Jones *et al.*, 2015:102; Matthew *et al.*, 2015:1). Hormonal levels are dependent on the optimal functioning of this endocrine system, where any changes in the functionality of this endocrine system might lead to pathological conditions, e.g. LOH, which manifests clinically as reduced morning erections, vigour and hot flushes (Dohle *et al.*, 2014:13). These signs and symptoms are not pathognomonic (specific signs, symptoms or conditions that are characteristic of a disease state) to LOH, because primary- and secondary hypogonadism patients also share some of the signs and symptoms that LOH patients experience (Huhtaniemi, 2015:389). This is not strange, as TT levels are also reduced in these patients. Primary- and secondary hypogonadism can be idiopathic or acquired and will be discussed in more detail in section 2.5, as primary- and secondary hypogonadism manifests in many ways.

Testosterone replacement therapy remains the mainstay of treatment for all forms of hypogonadism (Khera *et al.*, 2016:908-909). Despite the black box warning issued by the FDA in March 2015 to intensify awareness relating to increased risk for stroke and heart attack, in South Africa, only two injectable formulas are available with only one, depot-testosterone undecanoate, specifically indicated for hypogonadism (Snyman, 2016:359). Parental TRT-induced polycythaemia with the focus on LOH patients is the side effect most frequently encountered in treated patients (Bian, 2010:20), and will form the basis of the current study.

2.2 A brief overview of the hypothalamic-pituitary-gonadal axis pathophysiology

The HPG axis is an important neuroendocrine system that regulates body functions, metabolic rates and reproduction. It consists of the hypothalamus, situated in the forebrain. The pea size pituitary gland just under the hypothalamus in the bone of the skull, and the gonadal glands in the case of a male are located in the scrotum (Oxford Concise Medical Dictionary, 2007:558). The pituitary gland can be divided into the anterior (front) and posterior (back) sides. The anterior half of the pituitary is responsible for secreting adrenocorticotropic hormones, growth hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone and prolactin. The posterior side secretes oxytocin and vasopressin (Brenner & Stevens, 2010:351).

Due to the intense complexity of the neuroendocrine HPG axis, only mechanisms of actions needed to underpin the current study will be explained.

The hypothalamus releases the gonadotropin releasing hormone (GnRH) in a pulsating fashion every 60 to 90 minutes. Gonadotropin-releasing hormones interact with the GnRH receptors located on the anterior pituitary that initiate the release of LH and FSH (Boehm *et al.*, 2015:548; Dwyer *et al.*, 2015:R15). Luteinizing hormones stimulate Leydig cells (located in the testes) to release testosterone, while FSH stimulates spermatogenesis in the Sertoli cells (located in the testes) (Basaria, 2014:1250; Boehm *et al.*, 2015:549).

Testosterone is predominantly produced by the testes, and the adrenal glands play a pivotal role in the male reproductive system and sexual function (Hines *et al.*, 2016:69; Kaufman & Vermeulen, 2005:834). Furthermore, testosterone is the precursor of oestrogen, which is derived from testosterone via enzymatic conversion with aromatase (Brenner & Stevens, 2010:375-377). Fertility, libido, muscle strength and erythropoiesis are maintained by testosterone (Kumar *et al.*, 2010:299), whereas testosterone-derived oestrogen maintains skeletal integrity, and modulates lipid metabolism and cardiovascular physiology. Testosterone is the most abundant male androgen in a group that also includes dihydrotestosterone, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulphonate (Kaufman & Vermeulen, 2005:834). Testosterone is more physiologically active when compared to its derivatives, because androgen receptors (ARs) do not readily interact with androstenedione, dehydroepiandrosterone or dehydroepiandrosterone sulphonate, and it is believed that the androgenic effects of these steroid hormones are elicited primarily due to its conversion to testosterone in the target tissue (Kaufman & Vermeulen, 2005:834-835). Circulating testosterone in the plasma is highly bound to sex hormone-binding globulin (SHBG). Only the free fraction of the hormone, as with any biologically-active moiety, is physiologically active (Kaufman & Vermeulen, 2005:834). Briefly, testosterone and the concentration of SHBG are positively correlated in that increasing concentrations of SHBG will, in the absence of pathology, result in compensating increases of testosterone. As a result, the free testosterone (FT) is maintained within the physiological window. In the case of underlying gonadal failure, an increase in the SHBG concentration will result in reductions in FT. Factors that may contribute to an increase in the SHBG concentration include hyperthyroidism (Hackett *et al.*, 2008:1850), HIV (Millar *et al.*, 2016:E322) and the use of anticonvulsants (Millar *et al.*, 2016:E322), whereas reductions in the SHBG concentration are associated with obesity, diabetes and excessive glucocorticoid administration (Millar *et al.*, 2016:E322).

The production of testosterone in the testes is the result of HPG axis stimulation. Any condition or stimulus that interferes with the HPG axis hormonal pathway induces a deregulation in the

testosterone levels, causing clinical and physical relevant pathology. Late-onset hypogonadism, as well as primary- and secondary hypogonadism are examples of HPG axis failure or deregulation.

2.3 Epidemiology of late-onset hypogonadism

Late-onset hypogonadism is known by different clinical terms that include testosterone deficiency syndrome, andropause, androgen deficiency in the aging male (ADAM), partial androgen deficiency in the aging male (PADAM) and hypogonadism (Jones *et al.*, 2015:101-102). To confirm LOH, two morning testosterone values below the suggested value of 12.1 nmol/L for TT and 243 pmol/L for FT should be obtained at least on two separate occasions in association with related signs and symptoms (Dohle *et al.*, 2014:13). Miller *et al.* (2016:E322) recommend the use of free- or bioavailable testosterone (BAT), whereas Taniguchi and Matsuda (2017:376) used TT levels, irrespective of the different forms of testosterone (free-, bioavailable-, or TT levels) used as a diagnostic marker in association with signs and symptoms for LOH. The poor correlation between symptoms reported by patients and age-specific testosterone levels complicates the determination of the exact prevalence of LOH (Basaria, 2014:1253; Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:3; Millar *et al.*, 2016:E323; Surampudi *et al.*, 2011:2).

There is no set standard to determine LOH prevalence. Some epidemiology studies use different age groups in association with signs and symptoms, whereas others combine signs, symptoms and age with low levels of TT (Surampudi *et al.*, 2012:3). It is therefore challenging to group epidemiology studies. For instance, the Boston Area Community Health (BACH) survey used testosterone levels in combination with symptoms of androgen deficiency. An androgen-related deficiency of 37.7% was observed in men over 50 years of age, where 15% of treated patients presented with low TT, 9.9% presented with low FT, and 8.4% presented with low TT and FT. This left 15% of the patients with low levels of TT or FT without presenting with symptoms (Carruthers, 2009:22). In another study conducted by Heineman (2005:34-38) in European men, the prevalence was 17.7% lower than in the BACH study. The Massachusetts Male Aging Study (MMAS) also used testosterone levels in combination with specific signs and symptoms and published a prevalence of 5.6% for the age group of 30 to 79 years of age. The low prevalence might be attributed to the extensive age range. The 'Hypogonadism in Males' study reported a hypogonadism prevalence of 38.7% in men over 45 years of age (Mulligan *et al.*, 2006:762), and was confirmed by Lunenfeld *et al.* (2015:2).

Within the South African context, a study performed by Bornman and Reif (2007:62) reported mean TT levels for white males between the ages of 20 and 29 years (14.6 nmol/L), 30 and 39 years (13.9 nmol/L), 40 and 49 years (11.4 nmol/L) and for black males between the ages of 30 and 39 years (16.9 nmol/L). Importantly, the study confirmed that both ethnic groups had TT levels within the lower half of the average international TT range of 12.5 to 30.5 nmol/L, indicating that healthy South African males have TT levels lower than their international counter parts.

As a result, from the previous mentioned studies, it is clear, that studies measuring crude prevalence (specific testosterone levels accompanied with associated signs and symptoms) of males suffering from LOH are needed in South Africa to ensure effective diagnoses and treatment for patients living with LOH in South Africa. The latter is supported by an article that reported an androgen crude prevalence of 6% for men of the United States of America in relation to a 38.7% prevalence when diagnosis for androgen deficiency was made for LOH based on evaluating the bio-chemical levels only, without including symptoms (Bazaria, 2014:1253). Prevalence studies on LOH have been contradicting, although the age-related decline in testosterone levels remains well documented (Bazaria & Dobs, 1999:131; Hassan & Barkin, 2016:20; Huhtaniemi, 2014:192).

A step-wise approach for diagnosing and differentiating between the diagnoses of the different forms of hypogonadism will follow in the next section.

2.4 Diagnostic criteria of hypogonadism forms

In the following section, a broad overview is provided on how to accurately apply diagnostic criteria to differentiate between classical forms of hypogonadism.

2.4.1 History taking, physical examination and biochemical investigations

A full case history and physical examination with adequate time for patients to express concerns form the cornerstone to accurately diagnose hypogonadism. The diagnosis for all types of hypogonadism is made by evaluating the patient history, biochemical markers, signs and symptoms (sexual and non-sexual). Deoxyribonucleic acid testing is conducted in cases where AIS hypogonadism is suspected. This form of hypogonadism is described under special investigations of hypogonadism in section 2.5.1.1 (Aversa & Morģentaler, 2015:641; Basaria, 2014:1253; Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:2-3).

2.4.1.1 History taking

History taking (Dohle *et al.*, 2014:13) mostly includes details on:

- The time of onset

- The extent of signs and symptoms present

- Questions related to pharmacological molecules that might influence testosterone levels.

The following can be suspected as pharmacological (Dohle *et al.*, 2014:13) differential causes for testosterone variations:

- Drug abuse

- The use of corticosteroids

- Alcohol abuse

- Opiate containing products

- The previous use of testosterone-related medicine.

History taking points towards the aetiology (see section 2.5) of hypogonadism. The following sexual signs and symptoms are the most prevalent in hypogonadism patients:

- Low libido (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:2)

- Sex-related erectile dysfunction (Aversa & Morǵentaler, 2015:641; Lunenfeld *et al.*, 2015:2)

- Reduced morning erection (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13 Lunenfeld *et al.*, 2015:2)

- Difficulty in achieving an orgasm (Aversa & Morǵentaler, 2015:641).

Frequent non-sexual symptoms of hypogonadism include:

- Fatigue (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:2)

- Depressed mood (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:2)

- Poor concentration (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13)

- Decreased body hair (Dohle *et al.*, 2014:13)

- Small testes (Dohle *et al.*, 2014:13)

- Gynaecomastia (Dohle *et al.*, 2014:13)

- Visceral obesity (Aversa & Morǵentaler, 2015:641; Dohle *et al.,* 2014:13)

- Hot flushes (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13).

The patient history can be used to differentiate between LOH and other forms of hypogonadism (Lunenfeld *et al.*, 2015:1) and can be used to include the need for possible genetic testing (see section 2.4.1.4) (Zitzmann *et al.*, 2014:82). A large waist and obesity are the strongest non-sexual indicators of LOH.

2.4.1.2 Physical examination

Physical indicators and examinations of suspected hypogonadism patients are:

- Decreased muscle mass (Aversa & Morǵentaler, 2015:642; Lunenfeld *et al.*, 2015:2)

- Obesity (Aversa & Morǵentaler, 2015:641; Dohle *et al.*, 2014:13; Lunenfeld *et al.,* 2015:2)

- Reduced bone density (Dohle *et al.,* 2014:13; Lunenfeld *et al.*, 2015:2)

- Anaemia (Aversa & Morǵentaler, 2015:642)

- Deviation from the normal male diamond shape pubic hair distribution is indicative of the stage of puberty (Dohle *et al.*, 2014:13; Zitzmann *et al.*, 2014:82)

- Abnormal body fat distribution (Dohle *et al.*, 2014:13; Lunenfeld *et al.*, 2015:2; Zitzmann *et al.*, 2014:83)

- Visual disturbances (Zitzmann *et al.*, 2014:82)

- Reduced penis size (Zitzmann *et al.,* 2014:83)

- Altered testes shape and consistency (Zitzmann *et al.*, 2014:83).

The penis size and consistency of the testes (normally 4 cm long and 12 ml in volume) are also important physical indicators that should form part of the examination (Zitzmann *et al.*, 2014:83). Soft and smaller testes are related to lower levels of testosterone. Heart rate and blood pressure measurements are also useful indicators that can monitor cardiovascular risk (Aversa & Morǵentaler, 2015:642). Modern technique sonographs of the scrotum and prostate may also form part of the physical examination. Sonographs can be used to measure the volume of the testes and visualise varicocele, previous inflammatory conditions of the epididymis and even tumour-related growths (Zitzmann *et al.*, 2014:88). Visual disturbances indicate a pituitary involvement and prolactin levels should be taken to either exclude or confirm prolactinoma (Zitzmann *et al.*, 2014:82). The presence of anosmia can be used to differentiate between Kallmann syndrome and congenital hypogonadotropic hypogonadism (CHH) (Trabado *et al.*, 2014:80).

Low testosterone levels are a known factor responsible for hypogonadism-associated signs and symptoms. Therefore, a biochemical hormone analysis (see section 2.4.1.3) should be performed to validate the suspicion of abnormal testosterone levels (Basaria, 2014:1254).

2.4.1.3   Biochemical hormone analysis

A biochemical analysis is the best screening test for hypogonadism and should be performed in patients suspected of having hypogonadism during the physical examination and history taking process (see section 2.4.1.2 and 2.4.1.1). Biochemical evaluation of TT is the accepted international analyte (Aversa & Morǵentaler, 2015:642-643). Delay the use of morning testosterone levels if a patient is recovering from an illness as the sub-acute phase might influence the testosterone levels. Blood testosterone levels should be drawn between 07:00 a.m. and 11:00 a.m., or within three hours of awakening due to diurnal variations (Lunenfeld *et al.*, 2015:3; Morales *et al.*, 2015:1373).

The average normal TT level proposed as physiological normal is 12.5 to 30.5 nmol/L (Bornman & Reif, 2007:62). Suspected hypogonadism patients present with TT and/or FT levels below the normal suggested value of 12.1 nmol/L for TT and 243 pmol/l for FT (Dohle *et al.*, 2014:13). In the case of inconclusive first sampling, a second sample should be taken together with LH and FSH samples to asses for HPG axis functionality (Aversa & Morǵentaler, 2015:643). Elevated LH

and FSH levels in relation to a very low TT level and a normal prolactin level are normally suggestive of primary hypogonadism. A low to normal TT with low to normal LH and FSH level with an elevated prolactin level are suggestive of secondary hypogonadism and a low TT level in combination with a low or normal LH and FSH is normally indicative of LOH (Aversa & Morǵentaler, 2015:644; Khera *et al.*, 2016:915-918). Refer to Table 2.1 for a visual representation of some of the most frequent encountered hypogonadism forms in relation to hormonal findings during the biochemical hormone analysis. All biochemical markers listed in Table 2.1 is available in South Africa and is part of the holistic approach, when hypogonadism is suspected in a patient.

Table 2.1:   Hypogonadism forms in relation to hormone levels

Types of hypogonadism forms	TT	LH	FSH	Prolactin
Primary Hypogonadism	↓↓	↑	↑	N
Secondary Hypogonadism	↓/N	↓/N	↓/N	↑
Late-onset Hypogonadism	↓	↓/N	↓/N	

Abbreviations: ↓↓, Very low levels; ↑, elevated levels; N, Normal hormonal levels; ↓/N, Low to normal levels; TT, Total testosterone; LH, Luteinizing hormone; FSH, Follicle stimulating hormone

Food intake does not influence testosterone levels. Sex hormone-binding globulin should also be measured, and possible factors associated with SHBG solved (Aversa & Morǵentaler, 2015:642). Sex hormone-binding globulin is a liver-derived protein that acts as a hormone transporter. Only the unbound hormone fraction is physiologically active (AACC, 2018). Sex hormone-binding globulin levels increase with age and should form part of the biochemical investigation when LOH is suspected (Zirken & Tenover, 2012:1111-1118).

The following conditions are all associated with a decrease in SHBG:

- Obesity (Aversa & Morǵentaler, 2015:642; Khera *et al.*, 2016:915)

- Diabetes mellitus type 2 (DM2) (Aversa & Morǵentaler, 2015:642)

- Hypothyroidism (Aversa & Morǵentaler, 2015:642)

- Acromegaly (Aversa & Morǵentaler, 2015:642)

- Nephrotic syndrome (Aversa & Morǵentaler, 2015:642).

Factors associated with an increased level of SHBG are (Khera *et al.*, 2016:915):

- Hyperthyroidism

- Oestrogens

- Human immunodeficiency virus

- Hepatitis C

- Alcoholic cirrhosis.

2.4.1.4   Specialised investigations

Chromosomal investigations are recommended for patients where the diagnosis related to a specific hypogonadism aetiology remains inconclusive, even after a thorough examination as set out in section 2.4.

Gene testing may lead to the discovery of new forms of hypogonadism in cases where clinical hypogonadism is diagnosed without certain elevation or deviation of endocrine functions in phenotypically normal males. Chromosomal abnormalities are not a frequent occurrence in the general public (Majzoub *et al.*, 2017:168) and are not performed routinely. Chromosomal investigations are normally performed when couples fail to conceive after at least 12 months of frequent unprotected intercourse (Majzoub *et al.*, 2017:168). Chromosomal investigations are also used in cases where signs, symptoms and physical appearance point towards hypogonadism, but are not conclusive. Such as in the case of the *de la Chapelle* (46, XX) syndrome where patients present with a short stature and infertility where the lack of length is due to the Y-chromosome specific growth factors (Majzoub *et al.*, 2017:168). Genetic findings of KS and XYY syndrome led to the discovery of the aberration of the number of chromosomes where an incomplete translocation of the Y-chromosome has been noted in the XX-male and male turner syndrome (Zitzmann *et al.*, 2014:37). For a full reference on chromosomal hypogonadism conditions, refer to section 2.5.

2.5 Aetiology and clinical presentation of primary-, secondary- and late-onset hypogonadism

Hypogonadism is the collective term used to describe the androgen deficiency experienced by males. This can be sub-categorised into four syndromes to narrow down the specific aetiology experienced by a male, namely primary-, secondary hypogonadism, LOH and androgen insensitivity/resistance (Dohle *et al.*, 2014:8).

2.5.1 Primary hypogonadism

Primary hypogonadism is classified as testicular failure, meaning even if exposed to supra-physiological levels of gonadotropin hormones (Basaria, 2014:1250-1253; Corona *et al.*, 2015:120), fertility cannot be restored, resulting in infertility. The only way of fathering a child is through donor sperm or adoption. Common causes of primary hypogonadism are:

- Androgen insensitivity syndrome

- Cryptorchidism

- Klinefelter's syndrome

- XX-disorder

- Noonan's Syndrome

- XYY-Syndrome.

2.5.1.1 Androgen insensitivity syndrome

The incidence of AIS is rare, with an estimated occurrence in newborns of 1 in 20 000 to 64 000 (Kar *et al.,* 2016:S358; Zitzmann *et al.*, 2014:67). These patients present with the 46, XY karyotype (Mongan *et al.*, 2015:572). The function of ARs depends on their geographical location in the body. Functions include male morphogenesis, production and maturation of germ cells and initiation of virilisation and development of secondary sexual and non-sexual characteristics (Gottlieb, 2005:43; Mongan *et al.*, 2015:570). Furthermore, androgen resistance occurs due to the inability of androgens to elucidate a full response on ARs due to undesirable AR mutations (Geethika *et al.,* 2016:1016; Gottlieb, 2005:43; Kar *et al.,* 2016:S358; Mongan *et al.*, 2015:569).

The severity of the syndrome depends on the degree of impairment associated with the AR, where infertility presents in almost all affected individuals (Gottlieb, 2005:42-43; Zitzmann *et al.*, 2014:66-69). To date, three sub-divisions have been identified for people affected by AIS (Geethika *et al.,* 2016:1016; Gottlieb, 2005:43; Mongan *et al.*, 2015:572; Zitzmann *et al.*, 2014:66-69).

- Complete androgen insensitivity syndrome: Patients appear phenotypically unambiguously female, although internal genitalia are lacking (no uterus and underdeveloped internal vagina) (Kar *et al.*, 2016:S361; Mongan *et al.*, 2015:570-572). This form of AIS occurs due to the complete loss of function of the AR (Geethika *et al.*, 2016:1016; Kar et al., 2016:S358; Mongan *et al.*, 2015:570)

- Partial androgen insensitivity syndrome: The external form of the genitalia may vary greatly (Geethika *et al.,* 2016:1016; Mongan *et al.*, 2015:572). This appears to be the rule and not the exception, with a normal but small appearance of the genitals, where some males present with hypospadias or a visible line or thickening of the scrotum (Mongan *et al.*, 2015:573). The testes frequently only partially descended. Patients sometimes present with gynecomastia, which becomes obvious after puberty. Physical appearances seem to be mainly male with a normal distribution of pubic and armpit hair (Geethika *et al.*, 2016:1017). The decision to choose male gender as a way of life is not always easy, as the patient will need surgical procedures on multiple occasions at best to correct hypospadias, including relocating the testes and penis enlargement procedures later in life (Mongan *et al.*, 2015:576)

- Mild androgen insensitivity syndrome: The MAIS presents phenotypically as a normal male (Mongan *et al.*, 2015:573), where impaired spermatogenesis results in infertility and is sometimes the only symptom experienced by the affected male (Zitzmann *et al.*, 2014:69). The external genitalia are undeniably male and the testes have descended (Geethika *et al.*, 2016:1016).

Gene testing, laboratory tests (GnRH, LH, FSH and testosterone levels), in combination with physical evaluations, clinical signs and symptoms help to confirm AIS (Geethika *et al.,* 2016:1016; Mongan *et al.*, 2015:573; Zitzmann *et al.*, 2014:69). However, these findings in combination with azoospermia or oligospermia cannot be used as pathognomonic diagnosis and AR gene bio-molecular techniques should be used to confirm a positive diagnosis when AIS is suspected (Zitzmann *et al.*, 2014:69). Laboratory assay results can be interpreted as follows:

- In the case of the CAIS patient, serum testosterone, LH, SHBG and oestradiol levels are within or higher than the reference range, where FSH appears to be within normal reference range limits (Kar *et al.,* 2016:S358; Mongan *et al.*, 2015:575)

- The laboratory panel test for PAIS will reveal increased levels of LH, testosterone and oestradiol, where FSH levels may be normal to increased

- Laboratory panel testing for the MAIS male phenotype will reveal increased levels of testosterone and LH.

The suspicion for AIS should be raised if a patient presents with gynecomastia and azoospermia in association with defective sexual characteristics (Zitzmann *et al.*, 2014:69).

Treatment is challenging and depends on the classification of the affected individual. Virtually all patients diagnosed with AIS will be infertile (Gottlieb, 2005:42; Kar *et al.,* 2016:S358; Zitzmann *et al.*, 2014:69), resulting in adoption or the use of donor sperm should a couple like to become parents. Cosmetic procedures form part of the treatment plan for aesthetic purposes (Zitzmann *et al.*, 2014:69), especially for patients who present with gynecomastia. A gonadectomy is recommended for CAIS patients following puberty due to the increased prevalence noted of germ cell tumours (Geethika *et al.,* 2016:1018; Kar *et al.,* 2016:S361; Mongan *et al.*, 2015:573). Androgen replacement therapy is administrated to increase libido, resolve unexplained fatigue and to prevent a loss in bone mineral density (Geethika *et al.,* 2016:1016; Mongan *et al.*, 2015:575-576).

2.5.1.2   Cryptorchidism

Cryptorchidism, also known as undescended testicle(s) syndrome (Diallo *et al.*, 2017:125; Zitzmann *et al.*, 2014:56), is associated with hypogonadism as a clinical consequence (Diallo *et al.*, 2017:125; Rey *et al.*, 2013:7). Once cryptorchidism is suspected, differentiation between pendular-, sliding testicles or anorchidism should be made swiftly. Postponement of an accurate diagnosis may lead to infertility if the testicles are not moved to their anatomical position (Zitzmann *et al.*, 2014:56). An increase in the risk for developing testicular tumours has been noted (Diallo *et al.*, 2017:128; Ludwikowski & González, 2013:5; Zitzmann *et al.*, 2014:56). Pendular testicles (where the testicles slide from the inguinal position to the scrotum once the cremasteric muscle relaxes) can be differentiated from sliding testicles (where the testicles are positioned on the outer ring of the inguinal area and can be pushed down to the scrotum, from where it will move back, spontaneously) (Hensel *et al.*, 2015:2; Zitzmann *et al.*, 2014:55). The absence of testicles is titled anorchidism (Zitzmann *et al.*, 2014:54). Cryptorchidism has a prevalence of 1 to 3% where a decrease in the incidence is noted among adults (Diallo *et al.*, 2017:127). Although research in

the field of urology is investigating the exact science of cryptorchidism, the cause remains unclear (Hensel *et al.*, 2015:1).

The human chorionic gonadotropin test is used to differentiate between cryptorchidism and anorchidism (Rey *et al.*, 2013:7; Zitzmann *et al.*, 2014:54). The test is performed by measuring testosterone levels before and 72 hours after the administration of 500 units of human chorionic gonadotropin. A positive test, indicative of bilateral anorchidism, is noted by minimal to no increase of the testosterone level from the base line value. A 1.5- to 2-fold increase in testosterone levels is observed in the case of cryptorchidism (Zitzmann *et al.*, 2014:54). Spontaneous decent of testes occurs mostly within the first three to six months of life. Spontaneous decent of testicles after six months is most unlikely (Hensel *et al.*, 2015:2). Unilateral cryptorchidism is more frequently diagnosed as opposed to bilateral cryptorchidism (Diallo *et al.*, 2017:127). A surgical investigation is needed upon the discovery of impalpable testes (Hensel *et al.*, 2015:2). The treatment should be guided in accordance with the surgical findings.

An inverse relationship exists between the age of an individual and the time of intervention to rectify cryptorchidism, as adulthood cryptorchidism interventions are associated with infertility (Diallo *et al.*, 2017:128; Ludwikowski & González, 2013:7). Surgical procedures remain the mainstay of treatment to reposition the undescended testes and should be performed within the first 12 to 18 months of life (Diallo *et al.*, 2017:128; Ludwikowski & González, 2013:5; Zitzmann *et al.*, 2014:56). In the case of anorchidism, cosmetic surgery is performed as prosthetic testicles are placed in the scrotum (Ludwikowski & González, 2013:7). Bilateral anorchidism should be treated with TRT when puberty is expected and should continue lifelong (Zitzmann *et al.*, 2014:55). If the testicles were removed as a result of a carcinoma, no androgen therapy should be used (Zitzmann *et al.*, 2014:55), as therapy is proven to counteract initial therapy. Monorchidism is not treated, as the remaining testis will completely fulfil the endocrine and exocrine functions (Zitzmann *et al.*, 2014:55).

2.5.1.3 Klinefelter's syndrome

Klinefelter syndrome is known to affect 152 to 223 cases per 100 000 males (Gies *et al.*, 2014:R67) and one in 660 newborn boys (Aksglaede & Juul, 2013:R67), making it the most frequent sex chromosome encountered form of primary hypogonadism. The classical numerical and visual form of chromosome karyotype is 47, XXY, affecting an estimate of 80 to 90% of patients, where 48, XXYY, 48, XXXY and the mosaic karyotype (47, XXY/46, XY) account for the remaining 10 to 20% of KS patients (Bonomi *et al.*, 2017:123; Gies *et al.*, 2014:R68).

Patients are rarely diagnosed with KS pre-puberty (< 9-14 years) (Bonomi *et al.*, 2017:128), where the majority of patients are diagnosed during adulthood as a result of hypogonadism, infertility and/or sexual dysfunction (Aksglaede & Juul, 2013:R67; Gies *et al.*, 2014:R68). Clinical signs and symptoms suggestive of KS pre-puberty include cryptorchidism (undescended testicles), behavioural problems and learning disabilities with excessively long legs, poor muscle build and gynecomastia with signs of androgen insufficiency and low testosterone levels linked to elevated levels of GnRH (Bonomi *et al.*, 2017:124; Gies *et al.*, 2014:R68; Groth *et al.*, 2013:23). Interestingly, patients with KS have normal testicular growth that parallel testosterone levels in the low normal to normal range until puberty, from where deterioration follows quickly (Gies *et al.*, 2014:R69). Spermatogenesis of the KS patient deteriorates progressively from early puberty to mid-puberty resulting in azoospermia and oligospermia. Histological findings indicate relatively normal seminiferous tubules, reduced germ cells and normal Sertoli/Leydig cells as the patient enters puberty. Extensive fibrosis and hyalinisation of the seminiferous tubules are the consequences of this syndrome, with a quick onset during mid-puberty (Bonomi *et al.*, 2017:124; Gies *et al.*, 2014:R71; Groth *et al.*, 2013:22).

To date, no resolution has been found for this chromosome disorder. Progress in the field of fertility and modern techniques has made it possible for some KS patients to father children (Groth *et al.*, 2013:22). The onset of treatment should be carefully planned as the initiation of testosterone for androgen-related signs and symptoms will decrease already low sperm counts to undetectable levels (Aksglaede & Juul, 2013:R70; Gies *et al.*, 2014:R71). Differentiation between the chromosomal abnormality and androgen deficiency should be clearly defined to enhance the benefit of TRT for each patient (Bonomi *et al.*, 2017:129). All forms of TRT are effective and dosage forms should be adapted to fulfil patient needs (Bonomi *et al.*, 2017:129; Groth *et al.*, 2013:26). Testicular sperm extraction (TESE) before treatment initiation is an option to preserve sperm, as the chances to conceive normally are very low (Aksglaede & Juul, 2013:R67). Testicular sperm extraction involves multiple samples to be taken from seminiferous tubules, where micro-TESE carefully identifies the epididymis seminiferous that contains sperm, giving the patient the best change to farther a child through intracytoplasmic sperm injection (Aksglaede & Juul, 2013:R70).

The treatment plan for KS patients is very unique, as treatment has to be given, changed, or stopped, depending on the age of the patient and whether or not the patient would like to father a child, taking into consideration the patient-specific aneuploidy and karyotyping of the syndrome.

2.5.1.4 XX-disorder

The non-autosomal syndrome 46, XX (Majzoub *et al.*, 2017:168; Vorona *et al.*, 2007:3458) is characterised by three distinct phenotypes. The first group displays a normal male phenotype, the second consists of males, with genital ambiguities, and the third group is characterised by the true hermaphrodite phenotype (Vorona *et al.*, 2007:3458). This rare syndrome is more often than not discovered when patients seek assistance for fertility complications (Chiang *et al.*, 2013:75; Majzoub *et al.*, 2017:168). The 46, XX has an estimated prevalence of 0.005% (Majzoub *et al.*, 2017:168; Vorona *et al.*, 2007:3458; Zitzmann *et al.*, 2014:60) and was first described in 1964 (Majzoub *et al.*, 2017:168). The aetiology, believed to originate from a translocation between the X- and Y-chromosomes in the spermatogenesis of the father, results in the inability to produce spermatozoa, causing infertility (Zitzmann *et al.*, 2014:60).

Clinical signs and symptoms of the affected patients include a short stature (due to the insufficient testosterone modulated growth spurt), poor hair growth with gynecomastia and female adipose distribution, and azoospermia with testicular atrophy is always detected (Chiang *et al.*, 2013:77; Majzoub *et al.*, 2017:169; Vorona *et al.*, 2007:3461-3462). These findings do not constitute a pathognomonic diagnosis (Majzoub *et al.*, 2017:169).

Treatment should be offered by means of a multidisciplinary team approach, owing to the various aspects of the condition (Majzoub *et al.*, 2017:171). Hypogonadism should be treated with TRT, where the treatment dose and route of administration should be guided by patient adherence (Majzoub *et al.*, 2017:171). Genetic and psychological counselling should be offered to the patient and partner to explain all aspects of the condition (Majzoub *et al.*, 2017:171). Due to the absolute absence of spermatogenic genes, the Y-chromosome germ cell proliferation is incomplete, resulting in the inability to produce spermatozoa (Zitzmann *et al.*, 2014:60). Artificial insemination by means of donor sperm or considering adoption is an option for couples who would like to start a family (Majzoub *et al.*, 2017:171).

2.5.1.5 Noonan's Syndrome

Noonan syndrome was first described by Jacqueline Noonan when she reported on specific phenotype findings observed in nine males. These male patients resemble the female Turner phenotype, combined with cryptorchidism (affecting 94% of boys) (Roberts *et al.*, 2013:333-337), with no discrimination between sex and race (Bhambhani & Muenke, 2014:2). The estimated prevalence of Noonan syndrome is one in 1 000 to 2 500 (Roberts *et al.*, 2013:333-337; Van der Burgt, 2007:1) with a 50% change of transmission to a sibling from the affected parent (Bhambhani & Muenke, 2014:3). Most cases of Noonan syndrome are due to new chromosome

mutations where familial cases appear to be inherent (Bhambhani & Muenke, 2014:3). The gene to blame for Noonan syndrome in 50% of cases is the protein tyrosine phosphatase, non-receptor type-11 (Chen *et al.*, 2014:11473; Van der Burgt, 2007:1).

The physical signs of Noonan syndrome are continuously clustered together and characterised by typical facial features, short stature, variable cognitive development, cardiac abnormalities and webbed neck (Bhambhani & Muenke, 2014:1; Chen *et al.*, 2014:11473; Roberts *et al.*, 2013:333; Van der Burgt, 2007:1). Physical signs, unlike the clinical symptoms of Noonan syndrome, depend on the age of the individual. The newborn will present with a large head compared to the rest of the face and wide-spaced eyes. The nose will be short, depressed and broad with full lips, a small chin and short neck. Excess skin will be visible at the back of the neck with swollen hands and feet (Bhambhani & Muenke, 2014:9). Thin fine hair with noticeable eyes, a bulging nose and "cupid bow appearance of the upper lip" are the trade hallmark of the Infant with Noonan syndrome (Bhambhani & Muenke, 2014:10). As the child grows older and reaches adolescence, the following features are noticeable and carry over to adulthood; the triangle-shaped head starts to develop with wrinkled skin and a wide forehead with a webbed neck, and a small chin is prominent. The sternum is depressed, and the nipples are widely spaced (Bhambhani & Muenke, 2014:11-12). Clinical symptoms include haematological, cardiovascular, genitourinary, skeletal, neurologic, gastrointestinal and dental abnormalities (Bhambhani & Muenke, 2014:13; Roberts *et al.*, 2013:333-335).

The diagnosis is challenging, as the physical appearance of the phenotype becomes less evident as the patients age (Van der Burgt, 2007:1); on the other hand, adding gene testing to the equation increases the accuracy of the diagnosis to 70% (Bhambhani & Muenke, 2014:1). The most recent diagnostic Noonan scoring system was developed in 1994, and entails the categorising of certain facial, cardiac, height, chest wall, family history or other *de novo* features, into a major or minor class according to the severity of the phenotype characteristic. The presence of two major class features or one major class feature and two minor features are diagnostic of Noonan syndrome; the presence of three minor criteria is also indicative of Noonan syndrome (Van der Burgt, 2007:2; Bhambhani & Muenke, 2014:14).

Even though the infertility of the Noonan male is not treatable (Zitzmann *et al.*, 2014:61), the rest of the affected systems are manageable and individualised treatment should be guided by a multidisciplinary team (Bhambhani & Muenke, 2014:15; Roberts *et al.*, 2013:339). Treating the cardiovascular complications is most important (Zitzmann *et al.*, 2014:61), as a third of patients have ongoing cardiac complications that require treatment (Roberts *et al.*, 2013:335). An article by Shaw *et al.* (2007:129) noted only three deaths due to cardiac complications.

Noonan syndrome is an incurable but manageable syndrome to date with a mortality rate of 9% for the age category just short of 61 years (Roberts *et al.*, 2013:335). Even though gene isolation holds great promise for the future (Van der Burgt, 2007:1), no treatment to date exists that will restore fertility for the Noonan syndrome patient, leaving them infertile.

2.5.1.6 XYY-Syndrome

Jacob's- or 47, XYY-syndrome is named after Jaqueline Jacobs (Re & Birkhoff, 2015:10). Following KS, 47, XYY is the second most common sex chromosome syndrome (Kim *et al.*, 2013:188). Published data record the incidence of Jacob's syndrome to be as high as one in 1 000 live births (El-Dahtory & Elsheikha, 2009:1; Re & Birkhoff, 2015:14; Zitzmann *et al.*, 2014:61). The true prevalence is still largely unknown as genetic testing is not routinely performed.

Prenatal diagnosis is made by chance, more often than not due to screening for other conditions such as Down syndrome; recommended for older expecting mothers together with genetic testing for families with a history of genetic abnormalities (Re & Birkhoff, 2015:10), whereas a postnatal diagnosis often occurs later in life due to decreased phenotypical differences compared to the 46, XY male (Kim *et al.*, 2013:190; Re & Birkhoff, 2015:10). The diagnosis is made by evaluating the signs and symptoms present and confirmed with genetic testing (Kim *et al.*, 2013:190). Historically, people were only genetically tested and karyotyped based upon clinical signs, symptoms and clinician experience (Bardsley *et al.*, 2013:1085), which led to some patients never being diagnosed. The most common clinical signs and symptoms of the syndrome appear to be an increased growth spurt during childhood resulting in a tall structure with variable degree of cognitive impairment and fertility inconsistencies (El-Dahtory & Elsheikha, 2009:1; Kim *et al.*, 2013:190; Re & Birkhoff, 2015:10-14).

Jacob's syndrome is inherited and usually takes place during the father's second meiotic division when sperm cells are formed (Re & Birkhoff, 2015:10; Zitzmann *et al.*, 2014:61), leading to the incorporation of an extra Y-chromosome in the sperm cell (El-Dahtory & Elsheikha, 2009:3; Kim *et al.*, 2013:188). Fertility inconsistencies can be explained in part as a result of mosaicism with the most common karyotype being (47, XYY/46, XY), resulting from the origin of the XYY non-disjunction (Re & Birkhoff, 2015:10). Some authors reported that the extra Y-chromosome disappears during later divisions, leading to the formation of this karyotype (47, XYY/46, XY) (El-Dahtory & Elsheikha, 2009:4). Contradicting data exist pertaining to the stigma of aggressive behaviour as no large randomised control studies exist currently. (Re & Birkhoff, 2015:10). Evidently, these people stand out due to taller stature that can create a sense of shyness that might force patients to live a confined life (Re & Birkhoff, 2015:9). The taller structure is probably

due to increased activity of the short stature homeo-box gene and other related Y-chromosomes (Re & Birkhoff, 2015:10).

Treatment should be tailored in accordance with patient needs. Most 47, XYY patients are sufficiently androgenised and fertile, but impaired spermatogenesis cannot be corrected. Androgen-related signs and symptoms, if need be, may be treated with TRT (Zitzmann *et al.*, 2014:61). Cognitive evaluation should be done, and treatment stratified accordingly, e.g. patients who present with seizures should be treated with the standard guidelines of anti-epileptic medication and the patient with impaired handwriting or speech should receive occupational assistance (Bardsley *et al.*, 2013:1092).

The fact remains that Jacob's syndrome is the second most reported sex chromosome aneuploidy reported following KS. The most prevalent signs and symptoms to date appear to be people of taller structure, with variable degrees of fertility ranges, where some of the affected patients have a decreased intellectual ability compared to control groups. This should not label patients as being infertile or incompetent.

2.5.2   Secondary hypogonadism

Secondary hypogonadism occurs as a result of HPG axis failure, resulting in hypogonadism (Kumar *et al.*, 2010:2; Zitzmann *et al.*, 2014:83). It is associated with several conditions, which consist of:

- Kallmann syndrome

- Congenital hypogonadotropic hypogonadism

- Pituitary disorders

- Hypopituitarism

- Hyperprolactinemia.

2.5.2.1   Kallmann Syndrome

Kallmann syndrome affects one in 8 000 newborns, with a higher incidence ratio in males compared to females (Dodé & Hardelin, 2009:139; Kaplan *et al.*, 2010:2796; Zitzmann *et al.*, 2014:36). Kallmann syndrome is caused by insufficient secretion of GnRH from the hypothalamus and incomplete migration of the GnRH neurons from the upper roof of the nose to the

hypothalamus (Forni & Wray, 2015:170; Kaplan *et al.*, 2010:2796; Zitzmann *et al.*, 2014:36-39). The insufficient secretion of GnRH results in reduced secretion of LH and FSH (from the pituitary) causing reduced sexual development in men, where the incomplete migration of the neurons causes anosmia or hyposmia (Dodé & Hardelin, 2009:140; Dweyer *et al.*, 2015:R17; Stieg *et al.,* 2017:2). A small percentage of people present with involuntary upper limb movement, abnormal eye movement, partial or complete agenesis of teeth or cleft lip and olfactory imperfections (Dodé & Hardelin, 2009:140; Kaplan *et al.*, 2010:2796). Genes that have been associated with Kallmann syndrome include KAL1 (responsible for the mutation on the X-chromosome), fibroblast growth factor receptor-1, fibroblast growth factor-8, as well as prokineticin receptor-2 and prokineticin-2 (Dodé & Hardelin, 2009:140; Kaplan *et al.*, 2010:2798; Zitzmann *et al.*, 2014:39). To date, more than 25 different genes have been associated with Kallmann syndrome (Boehm *et al.*, 2015:550). Most cases of Kallmann syndrome appear to happen by chance; then again, familial inheritance has been described (Dodé & Hardelin, 2009:142).

Early diagnosis is rarely made and features such as microphallus, cryptorchidism, hearing loss or agenesis should be cause for further investigation (Kaplan *et al.*, 2010:2798). Mostly, the diagnosis is made by eliminating other causes of hypogonadism at the time of puberty. A positive diagnosis is made based upon the presence of low serum gonadotropins (LH and FSH) and gonadal androgens in combination with anosmia or hyposmia (Dodé & Hardelin, 2009:140).

Treatment is aimed at restoring secondary sexual characteristics and inducing fertility. Fertility can be initiated by pulse therapy of GnRH to increase testicular growth and develop spermatogenesis where the secondary sexual characteristics are treated by means of administrating TRT (Dodé & Hardelin, 2009:144). The anosmia, if present, unfortunately cannot be corrected (Zitzmann *et al.*, 2014:41).

The transition from pre-puberty to puberty that progresses to adolescence can lead one on an emotional rollercoaster; notwithstanding the additional burden of a *de novo* or inherent syndrome. Kallmann syndrome is treatable and suspicion thereof should be dealt with swiftly to minimise emotional scarring.

2.5.2.2 Congenital hypogonadotropic hypogonadism

Congenital hypogonadotropic hypogonadism is associated with reduced secretion of GnRHfrom the hypothalamus (Boehm *et al.*, 2015:547; Laitinen *et al.*, 2012:534), which reduces pituitary function. The inability of the testes to produce androgens and sperm is the result of the incomplete secretion of LH and FSH from the under-stimulated pituitary gland (Trabado *et al.*, 2014:79; Zitzmann *et al.*, 2014:41). These individuals are therefore characterised as infertile in combination

with incomplete or absent puberty (Boehm *et al.*, 2015:547; Laitinen *et al.*, 2012:534), although fertility can be stimulated with treatment (Boehm *et al.*, 2015:556).

Congenital hypogonadotropic hypogonadism has a male dominance and is seldom diagnosed before adolescence, as CHH is difficult to differentiate from other causes of delayed puberty (Boehm *et al.*, 2015:547). The diagnosis for CHH is made based on excluding differential diagnoses such as pituitary or functional causes (Boehm *et al.*, 2015:551; Trabado *et al.*, 2014:80). Diagnostic criteria for CHH (Boehm *et al.*, 2015:553) follow:

- In-depth analysis of growth charts

- Genetic testing

- Signs such as external ear shape and number of teeth

- Evaluation of skin pigmentation.

Other clinical signs and symptoms include all the secondary sexual characteristics that depend on the GnRH pathway, as this hormonal pathway is lacking or may be underdeveloped leading to decreased concentrations of GnRH (Trabado *et al.*, 2014:80). There is a great overlap of clinical signs and symptoms between CHH and Kallmann syndrome, where both syndromes can present with developmental abnormalities (Boehm *et al.*, 2015:547) such as:

- Cleft lip or palate

- Skeletal abnormalities

- Ear abnormalities

- Dental and/or renal agenesis.

The differences are noted in the ability to smell by the CHH patient's contrary to the presence of anosmia or hyposmia in the Kallmann syndrome patients (Trabado *et al.*, 2014:80; Zitzmann *et al.*, 2014:36). The reason for this is due to the complete migration of the GnRH neurons in the CHH syndrome enabling them to smell, as opposed to the Kallmann syndrome lacking the connection between the hypothalamus and the olfactory lobe by the GnRH neurons (Boehm *et al.*, 2015:548; Trabado *et al.*, 2014:80; Zitzmann *et al.*, 2014:39).

Clinical signs and symptoms that warrant further investigation during neonatal and/or childhood phase are cryptorchidism and micro-penis, as these features depend on GnRH that is deficient in CHH patients. The timing and onset of puberty vary widely and are more often than not delayed or absent. The testicular volume of CHH patients rarely exceeds 4 ml during puberty (Boehm *et al.*, 2015:549). The CHH patient does not present with a growth spurt, but exhibits with linear steady growth, resulting in a short stature. The most common complaint during adolescents is the lack of secondary sexual development paired with lack of self-esteem and low libido. Mostly, in adulthood, CHH is diagnosed as patients seek help for infertility problems or even bone fractures (Boehm *et al.*, 2015:549; Laitinen *et al.*, 2012:534).

Progress in the field of research has led to the possible reversal of CHH (Boehm *et al.*, 2015:547; Laitinen *et al.*, 2012:534). Inhibitin B, insulin-like-3, Anti-Mullerian hormone (Trabado *et al.*, 2014:82-84) and kisspeptin were identified as diagnostic and treatment biomarkers (Boehm *et al.*, 2015:547). For some of the phenotypes, anosmia and renal agenesis patients of CHH, no treatment is available yet. Some patients can be offered treatment by means of surgery, e.g. the patients with cleft lip and/or palate and the ones with hearing loss (Boehm *et al.*, 2015:554). Early diagnosis is important to set treatment goals, which may be directed towards virilisation and/or fertility depending on when the patient is diagnosed (during childhood, adolescents or adulthood). This should be managed by a multidisciplinary healthcare team (Boehm *et al.*, 2015:554-558).

2.5.2.3   Pituitary disorders

Pituitary disorders occur due to inappropriate stimulation from the hypothalamus or a decreased response from the negative feedback system of the gonads (see section 2.2). The levels of GnRH, LH, FSH and testosterone can give great insight into the origin of some types of hypogonadism (Wong *et al.*, 2015:1562). Hypopituitarism is defined as a reduced amount of LH and FSH secretion from the pituitary (Stieg *et al.,* 2017:2), where hyperprolactinemia occurs due to the increased function of the pituitary, secreting prolactin that, in turn, suppresses the secretion of GnRH from the hypothalamus resulting in hypogonadism (Melmed *et al.*, 2011:274; Wong *et al.*, 2015:1563; Zitzmann *et al.*, 2014:46-51).

2.5.2.3.1 Hypopituitarism

Hypopituitarism is characterised as a reduced function of the pituitary gland (Stieg *et al.,* 2017:2). Some of the causes known to induce hypopituitarism (Stieg *et al.,* 2017:2-3) are:

- Masses in the region of the pituitary gland

- Trauma to the base of the scull or contusions

- Congenital defects or aneurysms.

Neurosurgical statistics have found that the most prevalent cause of hypopituitarism is pituitary adenomas, located in the anterior lobe of the pituitary (Zitzmann *et al.*, 2014:46-47), resulting in suppression of the synthesis and secretion of LH and FSH, causing hypogonadism (Zitzmann *et al.*, 2014:49). It is still unclear as to why pituitary adenomas develop.

The clinical signs and symptoms depend on the age and the sex of the patient (Zitzmann *et al.*, 2014:48). Reduced levels of LH and FSH are often the first indicators of hypopituitarism combined with symptoms of androgen deficiency (loss of libido, decreased sexual thoughts and infertility) (Zitzmann *et al.*, 2014:49). An endocrinologist should confirm hypopituitarism as the pituitary gland also forms part of the rest of the endocrine system (Wong *et al.*, 2015:1565).

The diagnosis for hypopituitarism is made by evaluating GnRH, LH, FSH and testosterone levels in combination with magnetic resonance imaging that will detect the mass (Zitzmann *et al.*, 2014:49). An insufficient rise of LH and FSH during exogenous administration of GnRH points towards a pituitary origin. The detection of a mass via magnetic resonance imaging will prove to be certain (Zitzmann *et al.*, 2014:49).

Therapy is aimed at treating the underlying cause responsible for the pituitary insufficiency (Zitzmann *et al.*, 2014:46). The recovery rate is respectable, depending on the causative condition (Zitzmann *et al.*, 2014:49). Therefore, temporary hypogonadism symptoms can be dealt with by means of hormone replacement therapy (Zitzmann *et al.*, 2014:49). In cases where only androgen substitution is needed, exogenous testosterone is the treatment of choice. Luteinizing hormone and FSH should form part of the treatment plan if the patient would like to father a child (Zitzmann *et al.*, 2014:49). The pituitary gland is not only secreting LH and FSH, and therefore, other hormones (cortisone, thyroxin and growth hormone) dependable on pituitary function should be substituted and should form part of the treatment plan for hypopituitarism (Wong *et al.*, 2015:1564; Zitzmann *et al.*, 2014:49).

2.5.2.3.2 Hyperprolactinemia

Prolactin is a hormone that is secreted by the pituitary gland with the primary function to stimulate breast milk production (Casanueva *et al.*, 2006:266; Melmed *et al.*, 2011:274; Wong *et al.*, 2015:1562). The normal physiological prolactin reference range for men and woman is 20 to 25 ng/ml (Casanueva *et al.*, 2006:267; Wong *et al.*, 2015:1566; Zitzmann *et al.*, 2014:51). Prolactin also exerts an inhibitory effect on the hypothalamus resulting in decreased amounts of

gonadotropins released from the pituitary gland (Casanueva *et al.*, 2006:266; Wong *et al.*, 2015:1562; Zitzmann *et al.*, 2014:51). The hypothalamus is responsible for secreting GnRH that, in turn, stimulates the pituitary to secrete gonadotropins, resulting in the stimulation of the gonads and other target receptors (see section 2.2). Overlapping signs of hyperprolactinaemia and hypopituitarism include reduced libido and/or sexual function with infertility (Casanueva *et al.*, 2006:266; Melmed *et al.*, 2011:276). Galactorrhoea is noted in hyperprolactinaemia, contrary to hypopituitarism (Melmed *et al.*, 2011:274; Zitzmann *et al.*, 2014:52-53).

There are many causes for hyperprolactinaemia (Casanueva *et al.*, 2006:265; Wong *et al.*, 2015:1562). As with hypopituitarism, hyperprolactinaemia also originates from one cell (monoclonal) (Zitzmann *et al.*, 2014:51). Some causes have been differentiated from other as a result of exponentiation of prolactin levels (Melmed *et al.*, 2011:275; Zitzmann *et al.*, 2014:51). Prolactin levels five to six times higher than normal appear to be because of medication (Casanueva *et al.*, 2006:266; Wong *et al.*, 2015:1564; Zitzmann *et al.*, 2014:52) such as:

- Imipramine

- Clomipramine

- Amitriptyline

- Domperidone

- Meprobamate

- Propranolol

- Metoclopramide

- Cimetidine.

An increase of less than twice the normal level leans towards the diagnosis of stress (physical and psychological) (Wong *et al.*, 2015:1563; Zitzmann *et al.*, 2014:52). Moderate increases of serum prolactin levels might be indicative of renal insufficiency, hypothyroidism or liver disease (Casanueva *et al.*, 2006:266; Melmed *et al.*, 2011:275; Wong *et al.*, 2015:1563). Finding consistently high levels of serum prolactin should be identified and by means of elimination of other known causes move towards eliminating differential diagnosis to find the offender cell (Casanueva *et al.*, 2006:267; Zitzmann *et al.*, 2014:52).

Treating the hyperprolactinemia patient depends on the correct diagnostic findings (Wong *et al.*, 2015:1562; Zitzmann *et al.*, 2014:52-53), where the aim of treatment is to restore gonadotropin concentrations by normalising the prolactin levels (Casanueva *et al.*, 2006:269). For patients who present with micro- and macro-adenomas, dopamine agonists, e.g. cabergoline, metergoline, bromocriptine and pergolide (Zitzmann *et al.*, 2014:53), are the drugs of choice (Casanueva *et al.*, 2006:269; Melmed *et al.*, 2011:283). The choice of treatment also depends on the ability of the patient to take frequent dosing or intermitted dosing as all treatment doses were found to be effective (Casanueva *et al.*, 2006:269).

Dopamine and prolactin are inversely related in the central nervous system, and consequently the administration of a dopamine agonist will decrease the prolactin levels leading to better treatment outcomes and shrinking of the adenoma (Casanueva *et al.*, 2006:265; Zitzmann *et al.*, 2014:53). Micro-adenoma is < 10 mm while macro-adenoma > 10 mm (Casanueva *et al.*, 2006:265; Zitzmann *et al.*, 2014:47). Treating a macro-adenoma acutely leads to rapidly reducing the size, resulting in better mortality and morbidity rates when preparing a patient for surgery. Treatment for a micro-adenoma depends on the response of the tumour. An estimate of 40% of pituitary adenomas is prolactinomas (Casanueva *et al.*, 2006:266; Melmed *et al.*, 2011:273; Wong *et al.*, 2015:1562). Normally, the best response is noted within a few months; tumours continue to shrink over a period of a few years (Casanueva *et al.*, 2006:270; Zitzmann *et al.*, 2014:52).

2.5.3 Late-onset hypogonadism

Late-onset hypogonadism is the most common form of hypogonadism (Corona *et al.*, 2012:251-252; Dohle *et al.*, 2014: 13; Khera *et al.*, 2016:916), more often than not overlooked as a result of the nonspecific symptoms clustered together, associated with a slow progression, including patients' choice to ignore symptoms (Davidiuk & Broderick, 2016:825; Khera *et al.*, 2016:916). A review by Millar *et al.* (2016:E321-E330) of available literature for a period of 48 years concludes that the prevalence of low testosterone levels of LOH patients varies considerably between studies. Furthermore, 29 studies reported on TT levels with a range of 6.9 to 15 nmol/L (200-433 ng/dl), nine studies used BAT to predict low testosterone with a range of 2.4 to 6.9 nmol/L (69.4-198.4 ng/dl) and four studies looked at using FT with a range of 0.16-0.24 nmol/L (4.6-7.0 ng/dl). Lastly, some studies used guideline reference values where a small number did not declare any rationale as to why they used certain cut-off values. A target testosterone level range of 400 to 700 ng/dl for patients receiving TT has been set by the endocrine society's clinical practice guidelines (Shoskes *et al.*, 2016:834).

Three trade hallmark sexual symptoms (Davidiuk & Broderick, 2016:825), mostly suggestive of LOH related to threshold testosterone values for the age groups of 40 to 79 years (Dohle *et al.*, 2014:13), are:

- Decreased frequency of sexual thoughts < 8 nmol/L

- Erectile dysfunction < 8.5 nmol/L

- Decreased frequency of morning erection < 11 nmol/L.

The circadian rhythm influences the testosterone levels, and therefore two morning levels between 08:00 and 10:00 (Hackett *et al.*, 2008:1849; Üçer & Gümüs, 2004:171) on different occasions, preferably one to three days apart, should be taken to confirm a low testosterone level. Aversa and Morģentaler (2015:643), Jones *et al.* (2015:103) and Morales *et al.* (2015:1373) suggest taking blood samples between 07:00 and 11:00. Testosterone levels should not be taken during a sub-acute illness or during the recovery phase, as this might influence the results leading to false low or elevated testosterone levels (Corona *et al.*, 2012:252; Khera *et al.*, 2016:915). It is further recommended to offer symptomatic men with TT levels under 8 nmol/L TRT and men with levels of 8 to 12 nmol/L a trial of TRT and men with TT levels above 12 nmol/L no TRT (Corona *et al.*, 2012:252; Üçer & Gümüs, 2014;171).

In Table 2.2 on the next page suggested cut off TT values are associated with a treatment plan.

Table 2.2: **Total testosterone levels of symptomatic men associated with the need for testosterone replacement therapy**

Total testosterone level	Testosterone replacement therapy
< 8 nmol/L	Yes
8-12 nmol/L	Trial of treatment recommended
> 12 nmol/L	No

Symptom scorecards are used to improve the diagnosis of LOH based upon the clinical symptoms and biochemical investigation (Zitzmann *et al.*, 2014:126). These scorecards should not be used on its own due to low specificity. To date, androgen-related symptom scorecards (Heinemann *et*

al., 2003:1; Martits *et al.*, 2014:286; Trinick *et al.*, 2011:10; Üçer & Gümüs, 2014:171) currently in use are:

- The aging male scorecard (AMS)

- The international prostate symptom scorecard (I-PSS)

- The visual prostate symptom scorecard (VPSS)

- The international index of erectile function scorecard (IIEF) that was shortened to an international index of erectile function 5-scorecard (IIEF-5).

A full description of the symptom scorecards is provided in section 2.5.3.1.1.

2.5.3.1 Late-onset hypogonadism diagnosis and clinical symptoms

The diagnosis for LOH is made by a combination of assessments that include patient history, biochemical values and physical appearances (Davidiuk & Broderick, 2016:825-826; Zitzmann *et al.*, 2014:126).

2.5.3.1.1 Patient history

Patient history has been found to be more consistent with the completion of questionnaires and supportive biochemical data before a diagnosis for LOH can be confirmed (Dohle *et al.*, 2014:13; Morley *et al.*, 2006:425; Üçer & Gümüs, 2014:171). Questions during history taking should be directed towards the patient's sexual- and professional life, in conjunction with a physical examination (Zitzmann *et al.*, 2014:126). The following assessment scorecards were designed to structure patient history in such a way to draw conclusions about therapy effectiveness and progress. A summary of the assessment scorecards to guide decision-making of results obtained will precede a full description of all:

- The **AMS** was developed to assess symptoms related to LOH that present in males, independent of other existing chronic conditions. The likelihood of a patient being diagnosed with LOH when this scorecard is used in combination with a low TT is much more reliable than when a diagnosis is made based on the TT level alone. The score should decrease during the treatment period. For a full description on the scorecard, refer to (Annexure D)

- The **I-PSS** was developed to assess symptoms related to the prostate. Incomplete bladder emptying, weak urinary stream and poor bladder control are the symptoms mostly experienced by these symptomatic patients. The I-PSS should decrease in the face of treatment efficacy. The I-PSS has set out to evaluate the effectiveness of TRT in the LOH patient. A full version of the I-PSS is available as (Annexure E)

- The **VPSS** was derived from the I-PSS questions. The VPSS consists of pictures that patients can relate to during bladder emptying. There is a strong association between the I-PSS and the VPSS and the assumption can be made that the VPSS can be used with confidence in people of lower education or the elderly. For the visual representation, refer to (Annexure F)

- The **IIEF-5** scorecard was developed to assess treatment efficacy on ED. Erectile dysfunction is one of the most prominent symptoms experienced in the LOH patient, where the increase in the total score will be indicative of an effective treatment plan. The IIEF-5 is attached as (Annexure G).

Full description:

Even though the **AMS** presents with low specificity, a considerably higher sensitivity makes the suspicion of LOH more reliable when this scorecard is used, as opposed to a TT level alone (Kaufman & Vermeulen, 2005:848-849; Morley *et al.*, 2006:424). The AMS was developed in Germany during 1999 to assess the symptoms of male menopause already known for women, including the effect on health-related quality of life (Heinemann *et al.*, 2003:2). The measured outcomes of the scale are to evaluate symptoms of aging (independent of already present chronic illness) in males under different circumstances, to assess the severity of symptoms experienced and to evaluate the change of symptoms, including the progress of symptoms experienced pre- and post-androgen treatment. The AMS originally started off with 200 questions that were narrowed down to a questionnaire consisting of 17 questions. For each one of the 17 questions, the patient has the option to choose a block that supplies options ranging from one to five (1 being no symptoms experienced, and five being extremely severe symptoms experienced). These measure patient perceptions pertaining to the question. At the end of the questionnaire, the blocks are totalled, and a value is obtained. This value is retained and compared with future values to draw conclusions relating to the effectiveness of therapy (Heinemann *et al.*, 2003:2). The scoring system is set out in the following manner (Trinick *et al.*, 2011:11):

- No symptoms: score 17 to 26

- Little symptoms: score 27 to 36

- Moderate symptoms: score 37 to 49

- Severe symptoms: score > 50 is indicative of severe symptoms.

During the course of androgen therapy, the score should decrease as lesser symptoms are experienced resulting in a better health-related quality of life experienced by the patient.

The AMS is a valuable tool as it is used and/or accepted worldwide with all translated versions done according to international standards (Heinemann *et al.*, 2003:1-5). Trinick *et al.* (2011:10-15) used the AMS in an online web survey and close to 11 000 male participants were evaluated. The mean age for the participants was 52 years, where 13% were below the age of 40 years. Twenty-seven percent were in their 40s, which is in line with the findings of Heinemann *et al.* (2003:2) for LOH symptoms experienced in men older than 40 years. Trinick *et al.* (2011:10-11) further concluded that the severity of symptoms peaked in relation to the mean age, suggesting that these males might benefit from TRT. Moore *et al.* (2004:80) used the AMS in a pre-post-12-week TRT treatment trial that included 1174 patients. During this period, an improvement in the AMS of 32%, on average, relative to the patient's reference point score was observed. The positive predictive value was 89%, negative predictive value 59%, with a sensitivity of 96%. Therefore, the assumption can be made that the AMS is an excellent self-administration scorecard independent of the physicians' examination. Even though the specificity was a mere 30%, specificity was also found to be consistently low by Kaufman and Vermeulen, (2005:848- 849) and Morley *et al.* (2006:424). Interestingly, the AMS was developed to measure symptom scores related to male menopause without identifying the hormone for the greatest variance in symptoms. Findings were made that the AMS model explained 51.6% of the sexual factor (Heinnemann *et al.*, 2003:2). In this regard (as discussed above) testosterone is the physiologically most active male masculinisation (Kumar *et al.*, 2010:297), consequently being the most prominent contributor to maintain sexual function in young men (Basar *et al.*, 2005:597). One could now delineate that it will also be the case in older men, translating to AMS values. However, the MMAS study by Basar *et al.* (2005:600) found no correlation between the AMS and TT, including beliefs that TT is not such a game changer as previously believed. Currently, best evidence suggests that 69.9% of males were positively identified by the AMS when biochemical markers relating to LOH were done. Furthermore, the study concluded that there was no statistical variance in the biochemical markers (TT, calculated FT and calculated BAT) used, including no correlation between age of participants and testosterone (Liu *et al.*, 2016:1). Liu *et al.* (2016:1)

conclude a positive association between the diagnosis of LOH and the AMS. No statistically significant association was found between TT and age. After the MMAS study from Basar *et al.* (2005:600), different testosterone and derivatives were explored, and it was found that BAT and FT have great efficacy pertaining to the patients with ED, one of the most prevalent symptoms of LOH (Basar *et al.*, 2005:600).

The **I-PSS** was developed by the American Urology Association, validated as a reliable and easy scorecard to evaluate symptoms relating to the prostate (Yap *et al.*, 2007:811-813; Öztürk *et al.*, 2011:228). This scorecard is a good indication of symptoms that are experienced during the LOH syndrome as prostate health also makes out a big part of lower urinary tract symptoms (LUTS) of men. The scorecard consists of seven questions:

- Ability to completely empty the bladder

- Urinary voiding

- Interruption during urinating

- Urinary urgency

- The force of urine steam

- The time and effort it takes to begin urinating

- Nocturia (bedwetting during the night).

Each question is rated from zero to five, where the number five is associated with the most severe symptom experienced by patients. Based on the total score, patients can be categorised as experiencing mild- (total zero - seven), moderate- (total eight -19) or severe (total 20-35) symptoms (Haltbakk *et al.*, 2005:1735; Yap *et al.*, 2007:812). South Africa is a third-world country with the majority of residents being unemployed without tertiary education. Heyns *et al.* (2014:357) conducted a study in Africa and concluded that the VPSS took less time to complete, especially in those with lower levels of education, making the VPSS an excellent self-assessment tool providing accurate information regarding LUTS.

Consequently, it is contextually appropriate to use the **VPSS** in men regardless of their education level to gather information about their prostate symptoms and quality of life (Heyns *et al.*, 2014:356-357; Serge *et al.*, 2014:223). The VPSS might benefit them more due to the correlation

between the VPSS and the I-PSS, where Öztürk *et al.* (2011:228) reported that older persons and people with primary and secondary levels of education are better off being assisted when completing the I-PSS. Patients, aged 60 years and older, including patients with primary and secondary school education, will complete the questionnaire more accurately with the assistance of a trained medical officer. The VPSS consists of pictograms measuring the force of the urinary stream, the frequency of urinating during the day and night, with a section measuring quality of life. An overall statistical correlation was found between the I-PSS and VPSS on the total score, the force of the urinary stream and the quality of life (Heyns *et al.*, 2014:353-357; Öztürk *et al.*, 2011:228; Serge *et al.*, 2014:220). Sexual symptoms most frequently reported is erectile dysfunction, decreased libido and difficulty in achieving an orgasm (Aversa & Morġentaler, 2015:641). Interestingly (Campbell & Stein, 2014:440) noted that erectile dysfunction was the sexual symptom most frequently reported by symptomatic LOH men.

Erectile dysfunction can be measured with the **IIEF-5** (Utomo *et al.*, 2015:1154), derived from the original 15 question IIEF following development in 1997 (Rosen *et al.*, 1997:828). This scorecard makes it possible for the physician to more accurately diagnose patients with and without ED (Rosen *et al.*, 1999:319-320), and even evaluate the progress of treatment through this self-assessment scorecard (Utomo *et al.*, 2015:1154). It is known that FT levels and ED mimic each other, making the IIEF-5 a good evaluator of ED (Basar *et al.*, 2005:597). The androgen deficiency of the AMS was developed in 2000 with the ability of detecting "men at risk for low androgen" and has a sensitivity of 88% (Bernie *et al.*, 2014:195; Mohamed *et al.*, 2010:20). This makes the AMS scorecard a great point of care test-kit for detecting onsite non-invasive low androgen, keeping the rather low specificity of 24 to 60% in mind (Bernie *et al.*, 2014:195; Mohamed *et al.*, 2010:20). When symptom scorecards are used as part of the panel of tests for confirming LOH, the specific tested testosterone level should be noted, as the different types of testosterone levels also correlate differently with symptom scorecards. Morley *et al.* (2005:424) demonstrated this with a study that included 148 males, where the sensitivity of the AMS was 83% with a specificity of 39% when the BAT was used, and a significantly lower correlation was found when these results were correlated with TT.

Apart from using symptom scorecards during the patient history investigation, non-sexual and sexual signs and symptoms are also investigated and these signs and symptoms also form an integral part of the patient history.

Sexual signs and symptoms are the most prevalent in hypogonadism patients and most frequently associated with LOH and low testosterone levels (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:13 Khera *et al.*, 2016:916; Üçer & Gümüs, 2014:171), which consist of:

- Low libido (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916; Üçer & Gümüs, 2014:171)

- Reduction in sex-related erection (Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916)

- Reduced morning erection (Dohle *et al.*, 2014:17)

- Loss of vigour (Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916).

Most prevalent, non-sexual symptoms associated with LOH but not pathognomonic are:

- Loss of muscle mass (Corona *et al.*, 2012:252; Üçer & Gümüs, 2014:171)

- Decreased cognitive function (Corona *et al.*, 2012:252)

- Depressive symptoms (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17; Üçer & Gümüs, 2014:171)

- Fatigue (Dohle *et al.*, 2014:17)

- Hot flushes (Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916)

- Loss of body hair especially in the region of the pubic area (Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916)

- Loss of bone density (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916; Üçer & Gümüs, 2014:171)

- Sarcopenia (Dohle *et al.*, 2014:17)

- Increased body mass index (BMI) (Khera *et al.*, 2016:916; Üçer & Gümüs, 2014:171)

- Decreased testicle size (Khera *et al.*, 2016:916).

With an ever increasing LOH incidence, it is of utmost importance to effectively diagnose and treat LOH, as this syndrome is associated with a great majority of negative, related co-morbidities that affect quality of life. The probability of effectively diagnosing LOH in patients is better when scorecards are combined with clinical signs, symptoms and biochemical analysis, as opposed to not using scorecards (Űçer & Gümüs, 2014:171).

2.5.3.2 Physical examination

A physical examination should be conducted and combined with patient history, signs and symptoms of the LOH patient (see section 2.5.3.1.1). The physical examination should include assessments on:

- Blood pressure

- Distribution of body hair (Khera *et al.*, 2016:916; Lunenfeld *et al.*, 2015:2)

- Examination of the testes and prostate (Khera *et al.*, 2016:916; Lunenfeld *et al.*, 2015:2)

- Bone density (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17; Khera *et al.*, 2016:916; Űçer & Gümüs, 2014:171)

- Patient mood (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17)

- Fatigue (Corona *et al.*, 2012:252; Dohle *et al.*, 2014:17)

- Presence of breast enlargement (Lunenfeld *et al.*, 2015:2)

- Determining BMI, weight and height – an increase of body weight is associated with LOH (Lunenfeld *et al.*, 2015:2).

The more symptoms detected, the greater the chance of a patient being diagnosed with LOH (Lunenfeld *et al.*, 2015:2) and the detection of sexual symptoms might be the most helpful (Khera *et al.*, 2016:916). The presence of symptoms alone does not constitute LOH and must therefore be combined with a low TT- or FT level (Lunenfeld *et al.*, 2015:2).

2.5.3.3 Biochemical hormone analysis

Biochemical analysis comprises of:

- Total testosterone levels

- Luteinizing hormone

- Prolactin levels

- Sex hormone binding globulin levels.

Testosterone deficiency symptoms are non-specific and vary in onset and intensity. Sexual symptoms such as loss of libido and ED are the most prevalent symptoms associated with LOH and low testosterone levels (Morales *et al.*, 2015:1372).

2.5.4 Late-onset hypogonadism co-morbidities and risk-factors

Co-morbidities are not only associated with disease but can occasionally be used as early indicators for other conditions (Rees & Kirby, 2014:10-13; Yassin *et al.*, 2015:163).

Late-onset hypogonadism risk factors that were found to be statistically significant are:

- Obesity (Hassin & Barkin, 2016:21; Zheng *et al.*, 2016:1)

- A decrease in bone density (Lunenfeld *et al.*, 2015:2)

- A decreased muscle mass (Lunenfeld *et al.*, 2015:2)

- Erectile dysfunction (Lunenfeld *et al.*, 2015:2)

- Diabetes mellitus 2 (Hassin & Barkin, 2016:21; Lunenfeld *et al.*, 2015:2).

Recent studies have controversial opinions concerning co-morbidities, risk factors and the effect of treatment on hypogonadism patients, as risk factors of hypogonadism more often than not co-exist with co-morbidities. Co-morbidities associated with LOH are:

- Diabetes mellitus 2 (Khera *et al.*, 2016:916; Lunenfeld *et al.*, 2015:2; Miner *et al.*, 2014:39)

- Metabolic syndrome (Khera *et al.*, 2016:916; Lunenfeld *et al.*, 2015:2)

- Chronic obstructive pulmonary disease (Khera *et al.*, 2016:916; Miner *et al.*, 2014:42)

- Osteoporosis (Khera *et al.*, 2016:916)

- Human immunodeficiency virus associated weight loss (Khera *et al.*, 2016:916; Miner *et al.*, 2014:42)

- Obesity (Miner *et al.*, 2014:42)

- Cardiovascular disease (Miner *et al.*, 2014:42).

Erectile dysfunction, one of the symptoms most accurately associated with hypogonadism, is now also linked to metabolic syndrome and cardiovascular disease. Most resent findings also include that ED is the second-best indicator of DM2, after age (Yassin *et al.,* 2015:165). Metabolic syndrome has an increased risk of LUTS and is strongly associated with hypogonadism (Corona *et al.*, 2015:120; Rees & Kirby, 2014:10-12).

Zheng *et al.* (2016:1) reported that DM2 patients are more likely to develop hypogonadism. Furthermore, 36.2% of DM2 patients have low TT values. A considerably higher prevalence was noted by a South African study that reported a 50% prevalence of low testosterone levels in a cross-sectional study involving 150 diabetic patients (Kemp & Rheeder, 2015:92). A descriptive study inclusive of 130 participants done by Yassin *et al.* (2015:163) also confirmed that DM2 patients are prone to hypogonadism, including an increase in risk factors for cardiovascular disease. Contrary to the study of Yassin *et al.* (2015:162-168), a multi-national large cohort longitudinal study inclusive of 750 diagnosed LOH patients done by Maggi *et al.* (2016:843) reported that deaths occurring while patients are receiving treatment for LOH are not related to the treatment, but rather to old age and their already existing cardiovascular condition. Rather than only reporting on hypogonadism and DM2, Yassin *et al.* (2015:163) also concluded a significant association between ED and the mean weight of participants, waist circumference, total cholesterol, fasting glucose, triglycerides and AMS. Obesity is defined by a BMI ≥ 30 kg/m^2

where these patients are three times as likely to develop LOH, compared to a person with a lower BMI (Corona *et al.*, 2015:121). The specific mechanism of action is not yet completely understood on how obesity induces hypogonadism. Possible solutions entail an increase in levels of inflammatory markers in obese people with increased levels of glucose due to a lack insulin secretion or receptor resistance. These over-productions in cytokines and higher than normal glucose levels induce lower than normal secretion of gonadotropin releasing hormones, followed by reduced levels of testosterone resulting in hypogonadism (Corona *et al.*, 2015:121-122). Corona *et al.* (2015:123) further hypothesised that hypogonadism-induced obesity is due to the lower than normal stimulation of ARs by testosterone resulting in a lower than normal metabolic cell rate, affecting energy production and lipolysis. The net effect of the lower than normal stimulation and decreased lipolysis result in higher than normal visceral fat percentages (Corona *et al.*, 2015:124).

Studies reporting on the prevalence of chronic obstructive pulmonary disease patients with LOH in middle-aged and elderly men are scanty; nevertheless, a rage of 22 to 69% was noted (Decaroli & Rochira, 2016:11). During the process of aging, frailty becomes a huge concern as this condition is not an acute circumvent episode, but progresses ruthlessly. Frailty not only influences the clinical picture of the patient, but also the physical routine, such as walking, and psychological wellbeing, such as memory and mental wellbeing (Decaroli & Rochira, 2016:12). As demonstrated by Lunenfeld *et al.* (2015:2), low levels of testosterone are inversely related to frailty. Chronic liver conditions, particularly cirrhosis and fatty liver syndrome, are related to an increased production in SHBG and oestrogen resulting in hypogonadism as the latter has an inhibitory effect on the release of LH from the pituitary gland (Decaroli & Rochira, 2016:11). Hypogonadism is highly prevalent in chronic kidney disease as a result of testicular damage (Decaroli & Rochira, 2016:12).

The parallel relationship between aging and low testosterone levels is well defined, as opposed to information relating to low testosterone levels during acute infectious diseases in the elderly (Decaroli & Rochira, 2016:12). Sepsis induces a decrease in testosterone, through a mechanism of inhibiting the release of LH from the pituitary gland. Other acute diseases also induce low testosterone levels, such as HIV, by means of the redistribution of visceral fat; the increase of SHBG and the intensification of the aging process are all contributing factors of low testosterone and orchitis (more associated with primary hypogonadism) (Decaroli & Rochira, 2016:13).

Associations between LOH and co-morbidities are quite challenging. In some instances, LOH will act as a catalyst for the co-morbidity and *vice versa*. It has also been noted that in some cases the assumptions had to be made that there is no clear evidence as to what the relationship between the co-morbidity and LOH is. It is therefore suggested to conduct studies related solely

to LOH and co-morbidities without correlating the different co-morbidities with each other to ensure crude prevalence and incidence between LOH and the possible co-morbidity.

2.5.5 Treatment options for late-onset hypogonadism

A wide variety of treatment formulations are currently on the marked for the treatment of LOH. All of them contain testosterone as no other alternative or molecule mimicking testosterone for the past 70 years was reported on (Shoskes *et al.*, 2016:834; Yassin & Haffejee, 2007:577). Currently, differences in treatment formulations are (Davidiuk & Broderick, 2016:826; Dohle *et al.*, 2014:21):

- The dosage forms

- Frequency of dosing

- Mechanism of delivering the drug into the systemic circulation

- Duration of action, which includes advantages and disadvantages of treatment formulations.

Parental formulations can be divided into long-acting depot-testosterone undecanoate and shorter acting testosterone injections. The long-acting depot-testosterone undecanoate injection presents with the most favourable pharmacokinetic profile (Yassin & Haffejee, 2007:577). Other treatment formulations are transdermal patches, topical gels, buccal tablets, implantable tablets and oral tablets (Shoskes *et al.*, 2016:834; Kumar *et al.*, 2010:301-302).

2.5.5.1 Injections

Conventionally, testosterone ester injection formulations are used for the treatment of conditions associated with a decrease of testosterone e.g. LOH (Yassin & Haffejee, 2007:577). An ester formulation of a product is simply the chemical reaction between the hydroxyl part of a molecule and the carbon part of another molecule. The esterification process makes the molecule more lipid soluble, which, in turn, enables the molecule to be released slower into the circulation (Chao & Page, 2016:111). Differences between testosterone esters are due to the variability in length of the side chain (made up by carbon, hydrogen and oxygen molecules) attached to the 17β-hydroxyl position of the testosterone molecule. This is responsible for the dosing interval variance (Yassin & Haffejee, 2007:580). Examples of testosterone esters are testosterone enanthate, testosterone cypionate, testosterone cyclohexanocarboxylate and testosterone propionate. Testosterone enanthate is dosed at 200 to 250 mg every two to three weeks (Yassin & Haffejee,

2007:579), where Folia (2007:4) reported on a testosterone enanthate ester suspended in a sesame oil and dosed it at 200 to 400 mg every two to four weeks or 100 to 200 mg every two weeks, the testosterone cypionate and testosterone cyclohexanocarboxylate dosing interval and actual dose closely resemble testosterone enanthate with a dose of 200 mg every two weeks. Seemingly, the pharmacokinetics of the latter three testosterone forms all closely resemble each other, including the super physiological levels of testosterone after dosing and insufficient testosterone levels prior to the next dose (Yassin & Haffejee, 2007:579). Testosterone esters were the mainstay of treatment with approximate doses of 300 mg every two weeks assisting hypogonal men to be eugonal (Yassin & Haffejee, 2007:579).

Long-acting depot-testosterone undecanoate ester consists of a long aliphatic side chain and was developed as a result of the World Health Organisation looking for a longer acting male contraception (Yassin & Haffejee, 2007:580). The first long-acting depot-testosterone undecanoate was developed in China, but due to the large dose-related volume, problems occurred at the injection site. Jenapharm/Schering reformulated the product to a smaller volume (4ml/1000mg), by suspending the testosterone ester in a refined castor oil (Yassin & Haffejee, 2007:580). Other suspension vehicles are cottonseed oil and sesame seed oil (Singh *et al.*, 2014:2596).

These oils keep the testosterone ester and fatty acid side chains intact longer after intramuscular administration, which contributes to the longer half-life, compared to the testosterone propionate, testosterone enanthate and testosterone cypionate formulations (Kumar & Kumar, 2010:301; Yassin & Haffejee, 2007:580). Interestingly, Haider *et al.* (2009:349) and Yassin and Saad (2007:181-182) both reported on an inclusive 122 hypogonal men-study over a period of 24 months (Haider *et al.*, 2009:350) and 12 months (Yassin & Saad, 2007:182), where testosterone undecanoate injections were given at zero and six weeks followed by a dose every 12 weeks. The study concluded that testosterone levels increased significantly with prostate safety retained. Furthermore, I-PSS followed a linear decrease over the treatment period with an increase in Hct and haemoglobin (Hb) concentration plateauing at month 15, not increasing above the upper normal limit (Haider *et al.*, 2009:352), demonstrating the effectiveness of the long-acting depot-testosterone undecanoate formulation. Conaglen *et al.* (2014:574-579) retrospectively evaluated 179 hypogonal men in the clinical setting for an estimate period of 24 months who received the standard dose and interval of long-acting depot-testosterone undecanoate (a 1 000 mg intramuscular at day one with a booster at week six followed by a dose every 10 to 14 weeks). The results concluded that this dosage form is safe, effective and well tolerated with only the minority of men experiencing atypical physiological responses that seemingly may well be resolved by a mere change in the dosing interval. Nevertheless, Layton *et al.* (2015:1187)

reported a greater risk of cardiovascular events and deaths in patients following injectable testosterone when compared to testosterone gel applications. Patches and gel preparations had a similar risk profile. On the other hand, Hadgraft and Lane (2015:46) reported no significant cardiovascular incidence between injectable and transdermal application; then again, the oral administration of testosterone was associated with an increase cardiovascular risk profile.

2.5.5.2 Oral, sublingual and buccal tablets

Oral tablets are extensively metabolised by the liver, contributing towards hepatocellular damage (Matthew *et al.*, 2015:2), as opposed to the sublingual and buccal dosage forms that are designed in such a way to avoid the hepatic first pass metabolism (Shoskes *et al.*, 2016:835; Yassin & Haffejee, 2007:577) giving them a quick onset of action with a more desirable dosage schedule compared to other formulations (Dohle *et al.*, 2014:20). Testosterone is quickly absorbed from the gastrointestinal track following oral administration. Traditionally, the use of the 17α- testosterone formulation has become obsolete as studies reported an increased tendency associated with this molecular structure of testosterone and liver carcinoma (Ebert *et al.*, 2005:140; Matthew *et al.*, 2015:2). Esterification of testosterone on the 17β position yields testosterone undecanoate (Shoskes *et al.*, 2016:835). The oral testosterone undecanoate is given up to six hourly compared to the daily sublingual and twice a day buccal dose (Dohle *et al.*, 2014:21).

The hepatic first pass effect is defined as the process where absorbed molecules from the gastrointestinal tract are transported to the systemic circulation via the portal vein. This process leads to deactivating of unwanted molecules by the liver leading to excretion. Molecules are quickly eliminated and excreted even after good gastrointestinal tract absorption. Molecules such as testosterone undecanoate are engineered to be more lipid soluble to increase the absorption by the lymphatic system (not part of the hepatic first pass effect) after gastrointestinal tract absorption, avoiding inactivation of the liver, to be transported in the unmetabolised form into the systemic circulation, where stimulation of receptors can take place after enzymatic breakdown of the molecule (Hu *et al.*, 2016:13701). The first pass metabolic effect plays an important role in drug metabolism, as certain drugs are activated by the hepatic first pass effect.

Oral testosterone undecanoate esterification on the 17β-hydroxyl position has resulted in the molecule to be highly lipid soluble and not dependable on the hepatic first pass effect for activation and distribution (Shoskes *et al.*, 2016:835; Yassin & Haffejee, 2007:578). On the down side, this dosage form is dependent on the simultaneously intake of a fatty meal to be able to follow lymphatic circulation (Dohle *et al.*, 2014:20). Oral testosterone undecanoate is the most widely

used and safest delivery system, rarely causing an increase in testosterone levels above the upper normal physiological level (Dohle *et al.*, 2014:20).

2.5.5.3 Transdermal patches, topical gels and sub-dermal pellets

When the transdermal route of drug administration is used, the skin will serve as a storage facility for the medicine, releasing the medicine molecules gradually into circulation. This mode of action also avoids the hepatic first pass metabolism (Hadgraft & Lane, 2015:44; Ullah *et al.*, 2014:105). Patients should avoid wetting the area of drug administration for at least three hours after application to ensure proper absorption. Skin irritation is commonly associated with this preparation form and may be treated with a self-prescription topical hydrocortisone cream (Ullah *et al.*, 2014:106).

Transdermal patches are normally applied daily. If a patch is removed prior to the next dose before noon, another one may be used; if the patch is removed after noon, the patient should wait and apply a new patch according to the next dosing schedule (Ullah *et al.*, 2014:106). Total testosterone levels can be measured following a two-week treatment plan, with blood being drawn early morning (Shoskes *et al.*, 2016:838; Ullah *et al.*, 2014:106) from where dose adjustments can be made. Hadgraft and Lane (2015:44) demonstrated that single-use patches removed after 24 hours increased baseline testosterone levels of 5.9 nmol/L to an average maximum level of 44.1 nmol/L after 5.7 hours of treatment following a decline to 17 nmol/L over the following 12 hours. An elimination half-life of 116 minutes follows the removal of the patch. A further four-week trail concluded that testosterone levels associated with this dosage form mimic normal diurnal variations, making this a satisfactory TRT preparation.

Testosterone gel applications are normally applied daily with a maximum testosterone concentration normally evident three hours post-dose (Ullah *et al.*, 2014:106), reaching steady state between 48 and 72 hours post-first dose (Hadgraft & Lane, 2015:45). A great variety of gels are currently registered in the United States of America; unfortunately, not dose interchangeable (Hadgraft & Lane, 2015:45; Ullah *et al.*, 2014:106). Testosterone dose adjustments can be made, where treatment monitoring depends on the time of testing and length of treatment duration of the specific product. For Androgel® 1% and 1.62%, dose titrations are done after two to four weeks of treatment, with the difference between available concentrations being the quantity of product applied to the skin. With the Androgel® 1.62%, a smaller quantity is needed for the same response following treatment with Androgel® 1%. Axiron® 2% gel is applied under each axilla (under arms) daily, like Foresta® 2%, Axiron® 2% and Testim® dose monitoring is done following a treatment period of about two weeks (Shoskes *et al.*, 2016:837-840; Ullah *et al.*, 2014:107). A long-term study done on the efficacy and safety of testosterone gel applications reported that testosterone

gels significantly improved symptoms associated with hypogonadism with no significant increased association with morbidity and mortality (Swerdloff & Wang, 2003:207). Comparable studies between testosterone gel formulations and transdermal patches revealed a more favourable pharmacokinetic profile of gels compared to the testosterone patch, even though both formulations reached normal testosterone levels within 24 hours. Finally, Hadgraft and Lane (2015:45) indicated that testosterone gels were superior to testosterone patches due to higher patient discontinuation rates of testosterone patches.

Sub-dermal implantable pellets are engineered to deliver testosterone in circulation at a constant rate, currently offering the longest duration of action among dosing formulations (Bassil *et al.*, 2009:431). Absorption occurs as a result of the erosion of the implantable pellet at a consistent rate. Patient age, diagnosis and occurrence of drug side effect will determine the dose required. Pellets are normally surgically placed in the hip area or other fatty area with dosing intervals of three to six months, subject to patient response and testosterone levels reached. Testosterone levels peak normally within one month (Shoskes *et al.*, 2016:837). Sub-dermal implantable pellets are not an inferior treatment option for hypogonadism compared to testosterone injections and testosterone gel applications, as concluded by Pastuszak *et al.* (2015:165). Seemingly, the greatest influence factor towards choice of treatment preparation is patient preference.

The route of administration is of paramount importance for the consumer. Transdermal TRT preparations have demonstrated not to be inferior to injectable counterparts. The end goal of TRT of the hypogonal male is eugodism (Shoskes *et al.*, 2016:834). With a target testosterone level range set between 400 and 700 ng/dl by the endocrine society clinical practice guidelines (Shoskes *et al.*, 2016:834), patient signs, symptoms and interpersonal variances should not be discredited (Ullah *et al.*, 2014:108). All exogenous formulations of testosterone are metabolised to oestradiol and dihydrotestosterone (Yassin & Haffejee, 2007:577), demonstrating good correlation with endogenous metabolites of testosterone. The transdermal patches, topical gels, oral- and sublingual tablets are all appropriate formulations with proven efficacy, but not regularly used due to the frequent dosing regimen. However, the depo-testosterone undecanoate injection formulation has the most favourable pharmacokinetic profile and is currently marketed as Nebido®️ in most countries, including South Africa.

2.6 Advantages and disadvantages of testosterone replacement therapy formulations

Testosterone replacement therapy has the ability to increase testosterone to physiologically normal levels (Jones *et al.*, 2015:105; Okada *et al.*, 2016:1). The following section will highlight the advantages (see section 2.6.1) and disadvantages (see section 2.6.2) of TRT dosing formulations.

2.6.1 Advantages of testosterone replacement therapy formulations

Some of the most prevalent TRT benefits are:

- Oral formulations follow an easily adaptable dosing regimen (Surampudi *et al.*, 2011:6)

- The testosterone undecanoate oral form has almost no undesirable effects on the liver (Dohle *et al.*, 2014:21)

- Transdermal dosing is associated with good absorption, near physiological circadian rhythms and less frequent skin irritation when compared to other forms of transdermal dosing formulations (Basaria, 2014:1256; Surampudi *et al.*, 2011:6)

- Testosterone implantable pellets are dosed less frequently than the rest of the TRT formulations (Matthew *et al.*, 2015:3)

- Testosterone implantable pellets release medicine at a constant rate with dose compliance; the best of all dosage forms (Bassil *et al.*, 2009:431; Bhasin *et al.*, 2010:2547)

- Conventional injectable testosterone, e.g. testosterone cypionate and testosterone enanthate, has a relatively short duration of action allowing for a shorter discontinuation time, if necessary, compared to the long-acting depot-testosterone undecanoate formulation (Dohle *et al.*, 2014:21)

- Long-acting depot-testosterone undecanoate injectable molecules have improved sexual desire and intercourse satisfaction when compared to the placebo (Hackett *et al.*, 2016:804)

- Parental depot-testosterone undecanoate has a better steady-state profile compared to the testosterone cypionate and testosterone enanthate dosing forms (Dohle *et al.*, 2014:21).

2.6.2   Disadvantages of testosterone replacement formulations

Less desirable side effects associated with TRT include:

- Oral TRT is associated with variable levels of testosterone above and below the mid-range (Dohle *et al.*, 2014:21)

- Headaches, less than optimal taste and gum pain are the side effects most frequently associated with the buccal dosage form (Surampudi *et al.*, 2011:6)

- Skin irritation after topical use (Dohle *et al.*, 2014:21) and drug transfer of externally applied formulations following direct contact with another person, including children, are the most undesirable effect experienced with topical testosterone formulations (Surampudi *et al.*, 2011:6)

- Implantable pellets are associated with infection and pain at the incision site (Aversa & Morǵentaler, 2015:645; Surampudi *et al.*, 2011:6), with extrusion noted in some cases (Basaria, 2014:1256)

- Super physiological spurts of testosterone spikes are associated with parental testosterone cypionate and testosterone enanthate shortly after each injection interval (Kumar & Kumar, 2010:301-302; Yassin & Haffejee, 2007:577)

- Long-acting depot-testosterone undecanoate injections are associated with pulmonary oil micro-embolism and polycythaemia that can be rectified by changing the dose interval with no post-injection haematoma observed even in patients taking blood thinning medication (Middleton *et al.*, 2015:511)

- Higher than normal post-dose testosterone might lead to symptoms such as acne and polycythaemia with higher than normal levels of oestradiol leading to gynecomastia in some patients (Yassin & Haffejee, 2007:579)

- Low testosterone levels pre-next dose can predispose as mood variations, ED and low energy levels (Yassin & Haffejee, 2007:579).

All dosing forms of testosterone will give a rise in serum testosterone levels; although a normal steady state might not be obtained by all testosterone formulations (Aversa & Morǵentaler, 2015:645).

2.7 Physiological benefits and risks associated with testosterone replacement therapy

Physiological benefits (see section 2.7.1) and risks (see section 2.7.2) will be discussed in the following section with special attention to TRT-induced polycythaemia (see section 2.7.2.1).

2.7.1 Physiological benefits of testosterone replacement therapy

Effective TRT has beneficial effects on bones, muscles, sexual functions, cognitive functions, erythropoiesis and an increase in overall wellbeing (Bain, 2010:16; Űçer & Gümüs, 2014:173; Zitzmann *et al.*, 2014:131). The symptoms of hypogonadism do not parallel physiological testosterone levels. Initial hypogonadism symptom improvement in relation to maximum physiological response was reported on by Saad *et al.* (2011:675), who reported the following:

- Sexual symptoms improve first within the first three weeks of treatment initiation

- Improvement in quality of life can be expected between weeks three and four

- Overall mental health improved between weeks three and six

- Positive effects on plasma lipids are noted after four weeks

- Prostate specific antigen gradual increase over the course of 12 months.

These are important findings as the patient treatment plan can be adjusted accordingly. Furthermore, screening for side effects of TRT can be done periodically according to the time of onset and the time to maximum effect. Note that some of the side effects of TRT are dose-dependent.

Most of these symptoms reach a maximum effect at month 12 (Saad *et al.*, 2011:675), although some studies were not able to conclude whether a plateau was reached even after 36 months of TRT (Saad *et al.*, 2011:675; Űçer & Gümüs, 2014:175). The former findings prove to be quite significant, taking into consideration planning for follow-up visits of patients. To regulate side effects, monitoring is done the third month of treatment and then annually thereafter (Űçer & Gümüs, 2014:177; Zitzmann *et al.*, 2014:137).

2.7.1.1   Effects on bone, muscle, sexual function and cognitive function

Androgens play an important role in the bone development of men throughout their life. The mortality rates of hip fractures are double for men compared to women (Űçer & Gümüs, 2014:174). Evidence exists that the conversion of testosterone by aromatase enzyme to 17β-oestradiol is more important in bone acquisition than testosterone itself (Bain, 2010:17; Zitzmann *et al.*, 2014:131; Saad *et al.*, 2011:677).

Men with lower testosterone values seem to benefit more from treatment than those with only marginally reduced testosterone levels (Bassil *et al.*, 2009:432; Zitzmann *et al.*, 2014:131). In the largest randomised placebo-controlled study conducted, a 4.2% increase in bone density was recorded (Zitzmann *et al.*, 2014:131). Bone density improvement at the femoral neck remains uncertain (Dohle *et al.*, 2014:18; Saad *et al.*, 2011:677-678).

Aging is associated with changes in body composition, e.g. muscle mass and fat redistribution (Bassil *et al.*, 2009:432; Űçer & Gümüs, 2014:175). Testosterone stimulates protein synthesis among other functions (Bain, 2010:18; Bassil *et al.*, 2009:432; Saad, 2011:677). Therefore, a decrease in muscle mass is expected from hypogonadism patients.

Treating hypogonadism with TRT has shown to decrease abdominal fat (Bain, 2010:18; Saad, 2011:678; Zitzmann *et al.*, 2014:133) and increase lean body mass (Basaria & Dobs, 1999:135). Hypogonadism patients are not the only ones who benefit from TRT. Administration of TRT to eugonal men also lead to increased protein synthesis, lean body mass and increased body strength (Basaria & Dobs, 1999:135). Evidence is predominantly in favour of the effects that TRT has on muscle mass, lean body mass and strength (Basaria & Dobs, 1999:135).

The number one clinical symptom experienced by hypogonal men is sexual symptoms (Aversa & Morǵentaler, 2015:641; Isidori *et al.*, 2015:106). Testosterone replacement therapy initiated in 23 hypogonal men resulted in a 61% improvement of sexual attitudes and performance (Basaria & Dobs, 1999:137). To date, evidence suggests that TRT treatment leads to better sexual outcomes in hypogonadal men compared to placebo studies (Basaria & Dobs, 1999:137). The latter was also found by Snyder *et al.* (2016:611), who reported on 790 hypogonal men in a double-blinded controlled trial. Authors have recommended treating patients timeously to prevent long-term risks of hypogonadism (Isidori *et al.*, 2015:106).

Treatment should be guided by patient symptoms and testosterone levels as indicated by Isidori *et al.* (2014:99-109) that conducted a literature review of the past 20 years. The Italian Society of Endocrinology recommends treatment for patients experiencing symptoms of low libido with a testosterone level < 8 nmol/L and suggests a trial of treatment for symptomatic patients with

testosterone levels between 8 and 12 nmol/L. Seventeen randomised controlled studies enrolled 1111 patients; improvement of symptoms was only found in those patients with marked reduced levels of testosterone < 8 nmol/L. In another study, including 1 431 participants, only patients with testosterone levels < 12 nmol/L reported improvement of symptoms (Isidori *et al.*, 2015:106).

Age has been associated with a decrease in androgen levels, combined with non-specific symptoms, including a decrease of cognitive awareness and depression (Basaria & Dobs, 1999:137; Űçer & Gümüs, 2014:175; Zitzmann *et al.*, 2014:135). Zitzmann *et al.* (2014:135) and (Basaria & Dobs, 1999:137; Morley, 2014:3) documented on the improvement of cognitive functions following TRT. As a result, cognitive function, as with other clinical signs and symptoms of hypogonadism, will show an improvement following TRT (Lackner *et al.*, 2011:1310).

A change in mood was noted after three to four weeks following TRT (Saad *et al.*, 2011:675). Bain (2010:17) and Űçer and Gümüs (2014:175) reported on the beneficial effects of TRT relating to mood, quality of life and depression. Emphasis should be placed on the fact that these beneficial effects were only noted in participants with a marked reduction in testosterone levels. Cherrier *et al.* (2015:421) found no improvement in measures of mood, cognitive function or quality of life in a randomised double-blinded placebo control study. The sample size was small with a mere 22 participants who met all the study criteria.

2.7.2 Physiological risks associated with testosterone replacement therapy

Consequences of under-treatment of LOH include cardiovascular diseases, metabolic syndrome, diabetes (Khera *et al.*, 2016:911-913; Carruthers, 2009:21), osteoporosis and Alzheimers (Carruthers, 2009:21). Lunenfeld *et al.* (2015:2) further added hot flushes, decreased libido, ED, osteoporosis and an increase in the worsening of co-morbidities of LOH, e.g. DM2 as a result of androgen deprivation therapy. It has been suggested to investigate males, *inter alia,* with the following conditions: low libido, diminished morning erections, depressed mood, fatigue, cognitive impairment, insulin resistance, metabolic syndrome, arterial hypertension and decreased muscle strength (Lunenfeld *et al.*, 2015:2), for LOH, as these burdens, due to low testosterone levels, are preventable. To treat the LOH patient is also associated with potential risks, e.g. polycythaemia, prostate cancer, LUTS and sleep apnoea.

2.7.2.1 Testosterone-associated polycythaemia

Synonyms for abnormal physiological levels of Hb and Hct at present are polycythaemia and/or erythrocytosis (Ohlander *et al.*, 2018:78). The exact mechanism of action related to testosterone-induced polycythaemia is not yet fully understood (Grech *et al.*, 2014:197). Haematocrit, oestrogen, hepcidin, erythropoietin and their influence on haematopoiesis will be discussed in the

form of a short synopsis in section 2.7.2.1.2 in relation to TRT, which induces polycythaemia. To fully understand the effects of testosterone-induced polycythaemia, one should first familiarise oneself with erythrocyte development under normal basal conditions, as explained in section 2.7.2.1.1 and related factors that impact on haematopoiesis.

2.7.2.1.1   Physiological erythrocyte development

It is known that different cell types all emerge from a pro-generator stem cell that under certain hormonal, humoral or activation from the environment initiate the growth, progression and meiosis from specific cell types via proliferation; ultimately changing to committed cells that further convert into erythrocytes, granulocytes, agranulocytes or platelets (Dzierzak & Philipsen, 2013:6-7; Kaushansky & Kipps, 2011:1069). Cell proliferation pathways related to that of testosterone-induced polycythaemia that commonly occur will be discussed after the aforementioned.

The journey of an erythrocyte starts as a haemocytoblast stem cell that becomes a committed pro-erythroblast. The early and late erythroblast phases follow the pro-erythroblast phase from where the normoblast stage starts (Marieb, 1998:631-633). The normoblast will eject its nucleus to become a reticulocyte, and will be released from the bone marrow to mature into an erythrocyte with a life span of roughly 120 days (Dzierzak & Philipsen, 2013:7; Hatlangadi *et al.*, 2011:6258). The sole function of erythrocytes is dedicated to that of oxygen distribution throughout the body. This function is fulfilled by the incorporation of the protein Hb during the early-late erythroblast stage that precedes the normoblasts erythrocyte phase (Ridley *et al.*, 1994:130). One of the most important elements that forms part of both the Hb and erythrocyte machinery is iron (Bachman *et al.*, 2010:4743; Kaushansky & Kipps, 2011:1067; Shahani *et al.*, 2009:705-706), where fluctuations of iron levels will manifest in the form of pathological illnesses such as anaemia or polycythaemia, which will have an effect on erythrocyte development time (Kaushansky & Kipps, 2011:1068; Marieb, 1998:639). As explained, such cell proliferation acts upon certain stimuli. The kidney-derived cytokine (erythropoietin) hypothesis is the one known to explain the erythrocyte initiation the best under normal physiological circumstances (Hatlangadi *et al.*, 2011:6258; Kaushansky & Kipps, 2011:1068). Kidney cells detect tissue hypoxia and secrete erythropoietin with the sole function to activate the bone marrow stem cell process to enhance erythrocyte production (Kaushansky & Kipps, 2011:1069; Ridley *et al.*, 1994:130). Erythropoietin is not able to increase the number of stem cells, but only serves as a catalyst (Ridley *et al.*, 1994:129). Furthermore, erythropoietin was found not only to initiate stem cell proliferation, but also to influence the Hb formation during the early-late and normoblast phases. This function of erythropoietin has led to the assumption that Hb and Hct levels may be independently increased to greater quantities in relation to that of erythrocytes (Shahani *et al.*, 2009:706). From here, the

idea is that erythropoietin will in a similar manner interact with testosterone to affect relevant systems independently.

2.7.2.1.2 Testosterone induced polycythaemia

Jones *et al.* (2015:106) and Ohlander *et al.* (2018:80) have reviewed the plausibility of a few proposed testosterone mechanisms of actions as reported by different authors over the years, leading to polycythaemia. Direct testosterone derived bone marrow over-stimulation, leading to polycythaemia, has been proposed by Shahani *et al.* (2009:706) and Velho *et al.* (2017:6), whereby testosterone enters the bone marrow via receptors to form a covalent bond with the nucleus of bone marrow. This testosterone nucleus complex causes bone marrow cells to increase where mRNA syntheses lead to multiplication of erythroid cell lines. These cell lines require stimulation from erythropoietin to further develop into erythrocytes, as explained earlier (Shahani *et al.*, 2009:706). The latter study was not able to demonstrate the erythropoietin involvement related to increased levels of observed Hb in relation to the initiated testosterone interventions. Studies by Velho *et al.* (2017:6), Delev *et al.* (2016:1489) and Ohlander *et al.* (2018:80) concluded that testosterone therapy most probably acts indirectly to stimulate erythropoietin as opposed to direct stimulation of the bone marrow derived erythropoiesis. Maggio *et al.* (2013:26), in addition to the indirect action of testosterone-derived erythropoietin hypothesis further demonstrated that the mean variance of testosterone-induced erythropoietin cannot be used to correlate the mean increase observed in Hct values. Said author then concluded that Hct variances are independent of erythropoietin, but then again, directly related to the mean change of serum testosterone levels. Therefore, the assumption can be made that testosterone-initiated therapy stimulates erythropoiesis via an indirect erythropoietin regulated mechanism of action rather than directly, as seen under normal physiological conditions.

An indirect mechanism of actions leading to polycythaemia includes hepcidin suppression by testosterone and the stimulation of oestrogen receptor-α after aromatase of testosterone to oestradiol (Ohlander *et al.*, 2018:80). Hepcidin is a liver-derived peptide, responsible for the destruction of the channels responsible for iron absorption (Delev *et al.*, 2016:1491). As mentioned earlier, iron forms part of the critical building blocks needed for erythrocyte formation and Hb formation. Bachman *et al.* (2010:4743) found that the dose of testosterone and the age of the patient were inversely related to the level of hepcidin suppression. Therefore, higher doses in older patients will lead to more profound hepcidin suppression than in younger patients, partly explaining the testosterone induced age-related differences between erythropoiesis of these two distinct groups following treatment. Findings published in 2013 specified that testosterone will activate erythropoietin leading to increased levels of both Hb and Hct in relation to reduced levels of ferritin (iron stores) and hepcidin (Bachman *et al.*, 2013:725). Finally, telomerase, a

testosterone-sensitive enzyme functionality, will increase once exposed to testosterone. The exposure leads to active cell proliferation initiating erythropoiesis, possibly causing polycythaemia, therefore, down-regulation of this enzyme by inhibiting oestrogen receptor-α but not β will select for increased erythrocyte proliferation (Ohlander *et al.*, 2018:80).

Polycythaemia, furthermore, is a dose-dependent side effect of TRT (Coviello *et al.*, 2008:4; Grech *et al.*, 2014:197). Both the dose and delivery system of the chosen testosterone product affect the magnitude of the Hct increase (Davidiuk & Broderick, 2016:830), where the injection formulation has been associated with having a higher risk for causing an elevated Hct (Coviello *et al.*, 2008:4; Jones *et al.*, 2015:103). Coviello *et al.* (2008:1) demonstrated a linear increase of Hb and Hct for younger and older men. The Hb and Hct are much more profound in older men than in their younger counter parts (Bachman *et al.*, 2013:726).

An increase in blood viscosity due to polycythaemia is a known link between cardiovascular events, deep vein thrombosis and cerebrovascular events, e.g. stroke (Coviello *et al.*, 2008:5; Osterberg *et al.*, 2014:3), where all of these conditions are associated with higher mortality rates (Jones *et al.*, 2015:107). Some studies documented that although an increase in blood viscosity is clearly a risk for cerebrovascular events, the relationship between Hct and deep vein thrombosis has yet to be proven (Davidiuk & Broderick, 2016:830; Jones *et al.*, 2015:107). This might be due to the lack of randomised control studies, where most studies to date were only conducted in retrospect (Jones *et al.*, 2015:107). On the other hand, Grech *et al.* (2014:190) documented no significant challenge between TRT, cerebrovascular events and deep vein thrombosis.

Jones *et al.* (2015:105) documented an Hct increase over the first five to six months of treatment, from where Hct will return to normal values after three to 12 months of treatment discontinuation (Davidiuk & Broderick, 2016:830; Jones *et al.*, 2015:105). The risk of developing polycythaemia is not linked to the duration of treatment, not even for long periods (Jones *et al.*, 2015:105). However, the highest incidence for polycythaemia was noted following treatment initiation. Jones *et al.* (2015:105) hypothesised that this might be due to the changes in hormonal homeostasis. If indeed a high Hct is detected during the treatment period, treatment discontinuation is recommended until Hct levels reduce to normal reference ranges, from where a reduced dose can be given (Grech *et al.*, 2014:197; Osterberg *et al.*, 2014:3). Furthermore Lunenfeld *et al.*, (2015:8) and Yassin & Haffejee (2007:585) recommends haematological assessment for increased levels of HCT before treatment initiation then again after three-four months and at month 12. Thereafter annual testing is recommended.

What is known is that testosterone has the ability to stimulate hematopoietic stem cells in such a way to hyper-proliferate, causing polycythaemia by means of indirect influence on erythropoietin concentrations. These changes are more profound in the older population from which the LOH male forms a part (Bachman *et al.*, 2013:725), leaving this population more vulnerable to Hct changes than the rest of the population. From a South African point of view this study found a higher polycythaemia prevalence [34% (n = 50)] in relation to similar studies conducted internationally. Haider *et al.* (2009:353) reported a polycythaemia prevalence of 5% as a pose to no polycythaemia cases documented by Conaglen *et al.*, (2014:579).

2.7.2.2 Testosterone replacement therapy and prostate cancer

Should TRT be used during prostate cancer and is it even beneficial? This has been a debate going on for some time, even though almost no large-scale randomised control studies exist to date (McBride *et al.*, 2016:52; Miner *et al.*, 2014:48). Such a study will require roughly 5 000 to 10 000 participants for a period of at least five to 10 years (Bain, 2010:19). Growth or exacerbation of prostate cancer due to TRT is one of the more serious risk factors (Miner *et al.*, 2014:48). Testosterone replacement therapy is contraindicated for untreated prostate cancer and relatively contraindicated in the presence of a high risk for prostate cancer or a PSA value > 4 ng/ml (Miner *et al.*, 2014:48). If indeed testosterone were to feed the cancer, one would expect a linear relationship between prostate cancer growth and testosterone levels. No such finding was documented, but rather an inverse relationship between testosterone and the development of prostate cancer was noted (Bain, 2010:19; Basaria & Dobs, 1999:133). Testosterone replacement therapy might even be used after successful treatment for prostate cancer to counteract all the co-morbidities and side effects associated with decreased levels of androgens (Bassil *et al.*, 2009:437).

We know that to date there is no evidence that supports the idea of testosterone causing *de novo* prostate cancer (Basaria & Dobs, 1999:133), nor little to no evidence exists that TRT enhances prostate cancer (Bain, 2010:19). Perhaps as a result of the hypothesis that the ARs of the prostate are only sensitive for androgens when the levels are very low, and the receptors are under-stimulated, once sufficiently stimulated, the tissues are no longer responsive (Bian 2010:19; McBride *et al.*, 2016:52; Miner *et al.*, 2014:48).

According to the guidelines endorsed by the European Association of Urology, TRT remains contraindicated for prostate cancer patients even after successful treatment of prostate cancer (Dohle *et al.*, 2014:23). In this light, taking into consideration the level of evidence with no golden rule to date, one should rather stay on the side of caution.

2.7.2.3 Effects of testosterone therapy on lower urinary tract symptoms

Lower urinary tract symptoms denote a cluster of symptoms related to urinary flow. The symptoms include nocturia, urinary incontinence, hesitancy and urgency. Benign prostatic hyperplasia (BPH) is the most common condition grouped with these symptoms and in its own right is characterised by obstructing urinary flow as a result of an enlarged prostate (Haltbakk *et al.*, 2005:1733). Lower urinary tract symptoms are measured by means of the I-PSS or the VPSS and their influence on quality of life (Haltbakk *et al.*, 2005:1733).

Zitzmann *et al.* (2014:129) found no correlation between TRT and LUTS, but patients with a high I-PSS were not included in these studies. McBride *et al.* (2016:53) reported no worsening of LUTS in TRT patients, including BPH patients. Bassil *et al.* (2009:437) published that many studies failed to show exacerbation of LUTS due to BPH while receiving TRT. In some cases, improvement of LUTS can be expected in the setting of TRT (Grech *et al.*, 2014:195; McBride *et al.*, 2016:53). The latter was demonstrated by a randomised control study of 46 male participants.

As good as the benefit of TRT on LUTS might sound, one must keep in mind that TRT will keep on stimulating prostate growth (Bassil *et al.*, 2009:436-437), leading to worsening of LUTS in the long run. Further studies that point out the benefits of TRT in LUTS patients consist of small sample sizes (Grech *et al.*, 2014:195-196). Therefore, studies of larger sample sizes over a period of at least 12 months (Saad *et al.*, 2011:675) are needed to fully elucidate the effect of TRT on LUTS.

2.7.2.4 Sleep apnoea and testosterone replacement therapy

Sleep apnoea has a prevalence of 25% among obese males (Hoyos *et al.*, 2012:599).

Testosterone replacement therapy is associated with sleep apnoea. During a recent study by Wittert (2014:263-264), patients received short-term doses of testosterone undecanoate on week six and 12 following a baseline test. Wittert (2014:263-264), Hoyos *et al.* (2012:603) and Grech *et al.* (2014:196) noted a worse oxygen desaturation index at week seven compared to week 18. This led to the conclusion that TRT can affect sleep apnoea more negatively during the treatment initiation phase than during later stages of treatment (Hoyos *et al.*, 2012:601; Wittert, 2014:263-264). Patients should therefore be monitored more closely, especially during the initiation phase.

A linear relationship has been demonstrated between TRT and sleep apnoea, but then again inconsistencies still exist between the extent that it will affect the patients, as no changes in physical, mental or increase in day time sleeping were noted in treated patients (Bain 2010:20). Sleep apnoea is associated with increased mortality rates and risk factors. Risk factors include

cardiovascular events, e.g. strokes, systemic hypertension, myocardial infarction and congestive heart failure (Jordan *et al.*, 2014:736).

As the search for the main offender causing or worsening sleep apnoea is still ongoing, between metabolic syndrome, obesity and TRT, current best evidence recommends against TRT for untreated diagnosed sleep apnoea patients (Dohle *et al.*, 2014:18).

2.8   Chapter summary

It is evident that TRT is an effective treatment option for LOH patients. Despite the effectiveness of TRT, side effects are unavoidable. Side effects, of which polycythaemia is the most prevalent, are associated with the parental TRT dosing formulation. Currently, no clear-cut rule exists as to when side effects will manifest. What is known is that most unwanted side effects manifest during the treatment initiation phase, e.g. the increase of Hct up to month six and the worsening of sleep apnoea at week seven compared to week 18. It is therefore wise to monitor for Hct levels at quarterly intervals and then annually thereafter. Testosterone replacement therapy will increase prostate growth, which might lead to the worsening of LUTS symptoms at any stage. Prostate screening (digital rectal examination and PSA) should be done before treatment initiation and then followed up with a PSA test at months three, six and 12 and then annually. Men with cardiovascular co-morbidities should be assessed by a cardiologist before TRT initiation, and then be closely monitored via a personal monitoring plan during treatment. Late-onset hypogonadism is growing in global prevalence, but remains under-treated and under-diagnosed. Further information should be given to patients and prescribers regarding the associated benefits and risks of TRT in the LOH patient.

CHAPTER 3:   ARTICLE MANUSCRIPT

3.1   Introduction

In the next section, the results of this study are presented in the form of an article. The manuscript draft can be found in section 3.2, followed by the author guidelines in section 3.2.1 and statements in section 3.2.2. The chapter summary is presented in section 3.3.

3.2 Manuscript

The following section presents the peer-reviewed, revised version (awaiting final approval), of the manuscript as submitted to the Journal of Endocrinology, Metabolism and Diabetes of South Africa (JEMDSA).

Journal of Endocrinology, Metabolism and Diabetes

Acute changes in haematocrit leading to polycythaemia in late-onset hypogonadism patients that receive testosterone replacement therapy – a South African study

--Manuscript Draft--

Full Title:	Acute changes in haematocrit leading to polycythaemia in late-onset hypogonadism patients that receive testosterone replacement therapy – a South African study
Manuscript Number:	JEMDSA - 2018 - 0016R1
Article Type:	Original Research
Keywords:	late-onset hypogonadism, haematocrit, polycythaemia, depot-testosterone undecanoate, testosterone replacement therapy
Manuscript Classifications:	Endocrinology; Medicine; Reproductive Endocrinology
Abstract:	Background: According to the literature, parenteral testosterone replacement therapy (TRT)-induced polycythaemia is associated with cardiovascular events. No or minimal data exist for the prevalence of TRT-induced polycythaemia in late-onset hypogonadism (LOH) patients from South Africa. Polycythaemia is the side-effect most frequently associated with parental TRT formulations. Design: This was a quantitative, observational, descriptive, retrospective study. Setting: The study setting was a private practice male clinic in Emalahleni. Subject: An all-inclusive sampling method was used. Outcome measures: The main outcome measure for polycythaemia was haematocrit (Hct). A Hct percentage of $\geq$ 50% at month 3 (post-treatment initiation) constituted a positive diagnosis for polycythaemia. For the rise in total testosterone (TT) and Hct, the variance was used as documented between pre- and post-treatment initiation. Results: The prevalence of polycythaemia was 34%. A statistically significant increase in both TT and Hct was observed. The Cohen's d effect size was 0.68 and 0.73, respectively, for TT and Hct. Conclusion: Depot-testosterone undecanoate parenteral formulation induces polycythaemia in LOH patients, where the rise in TT demonstrates the effectiveness of therapy.

Acute changes in haematocrit leading to polycythaemia in late-onset hypogonadism patients that receive testosterone replacement therapy – a South African study

Abstract

Background: According to the literature, parenteral testosterone replacement therapy (TRT)-induced polycythaemia is associated with cardiovascular events. No or minimal data exist for the prevalence of TRT-induced polycythaemia in late-onset hypogonadism (LOH) patients from South Africa. Polycythaemia is the side-effect most frequently associated with parental TRT formulations.

Design: This was a quantitative, observational, descriptive, retrospective study.

Setting: The study setting was a private practice male clinic in Emalahleni.

Subject: An all-inclusive sampling method was used.

Outcome measures: The main outcome measure for polycythaemia was haematocrit (Hct). A Hct percentage of $\geq 50\%$ at month 3 (post-treatment initiation) constituted a positive diagnosis for polycythaemia. For the rise in total testosterone (TT) and Hct, the variance was used as documented between pre- and post-treatment initiation.

Results: The prevalence of polycythaemia was 34%. A statistically significant increase in both TT and Hct was observed. The Cohen's d effect size was 0.68 and 0.73, respectively, for TT and Hct.

Conclusion: Depot-testosterone undecanoate parenteral formulation induces polycythaemia in LOH patients, where the rise in TT demonstrates the effectiveness of therapy.

Keywords: late-onset hypogonadism, haematocrit, polycythaemia, depot-testosterone undecanoate, testosterone replacement therapy

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Introduction

Hypogonadism is a syndrome related to androgen deficiency. This can be sub-classified into four separate syndromes to narrow down the specific aetiology: primary-, secondary hypogonadism, androgen insensitivity/resistance hypogonadism and LOH. The inability of the testes to produce androgens even if they are sufficiently stimulated is known as primary hypogonadism. A disruption of the neuroendocrine system result in secondary hypogonadism and androgen insensitivity/resistance hypogonadism is due to the inability of the androgens to elicit a full response on the androgen receptor. LOH is defined as the disruption of stimulus between the neuroendocrine system and the testes that under normal circumstances will lead to androgen production and secretion by the testes. LOH men had normal development through puberty and therefore developed accordingly in sexual characteristics.[1]

The aging population is expected to increase,[2] yet aging does not always parallel optimum health. Low levels of TT in association with sexual symptoms that comprise of low libido, erectile dysfunction and a poor morning erection is the clinical and biochemical symptoms that manifest the most in patients prior to a positive LOH diagnosis.[3,4] These symptoms are not only the first clinical symptoms to manifest, but according to Liu *et al.*, may be used in combination with low levels of testosterone to diagnose LOH.[5]

TRT is the cornerstone of treatment for LOH and other androgen deficiency syndromes.[6] Benefits of treatment include a rise in the TT level, where a TT level above 12 nmol/l is considered physiologically normal.[2] Additional clinically beneficial effects related to TRT include the increase of bone mineral density, favourable effects on muscle mass, sexual parameters, strength and mood.[2,7] Other secondary advantages associated with TRT is the improvement of the inverse relationship between low testosterone levels and metabolic syndrome, which includes clinical conditions such as central obesity, hypertension and unsatisfactory lipid profiles that are associated with a greater risk for cardiovascular disease and atherosclerosis.[2] Furthermore TRT significantly improve glycaemic control of the type 2 diabetes mellitus patient and it is recommended that all these male patients are also screened for hypogonadism.[4] In order to maximise patient adherence and co-operation, different formulations exist that should be prescribed in accordance with patient blood levels and ability to comply with dose frequency and route.[8]

Various types of TRTs are available, which include oral formulations, implantable pellets, parenteral formulations, buccal tablets, transdermal patches and topical gels.[9] Side-effects most frequently associated with TRT formulations are: less than optimal taste for oral tablets, person-to-person transfer due to close contact of individuals shortly after application of the topical gels, and gum pain is frequently reported with the use of buccal tablets.[8] Furthermore, transdermal patches cause skin rash in some patients, where extrusion of the implantable pellet has also been documented in a portion of treated patients.[10] Side-effects most frequently experienced related to the parenteral dosage formulations such as testosterone cypionate, testosterone propionate or testosterone enanthate are supra-physiological testosterone levels shortly after the dose, followed by sub-therapeutic levels prior to the next dose.[9,11] This phenomenon can partly be explained by molecular esterification. Esterification processes make molecules more lipid soluble, which, in turn, enables the molecule to be more readily released from circulation.[12] This might benefit the molecules' pharmacokinetic profile, as in the case of the depot-testosterone undecanoate formulation.[11] The pharmacokinetic benefits of the depot-testosterone undecanoate formulation include a more favourable spread of TT levels around the median physiological testosterone value, with a beneficial clinical implication of less frequent dosing than older generations of testosterone. However, an increase in the number of erythrocytes above the physiological normal levels, also known as erythrocytosis or polycythaemia, is the risk most frequently encountered with parenteral TRT,[13] even though the exact mechanism of action still needs to be explained.[14] Androgen replacement therapy induced polycythaemia originate via the bone marrow haematopoiesis pathway, as an alternative to the oxygen-erythropoietin cascade.[15]

Despite polycythaemia-induced cardiovascular complications, the depot-testosterone undecanoate parenteral formulation remains the only registered TRT in South Africa to treat LOH.[16,17] Subsequently, this study has set out to evaluate, in retrospect, the prevalence of polycythaemia among diagnosed LOH patients who have received depot-testosterone undecanoate TRT and the possible clinical significance thereof.

Method

Setting

The study took place in a private practice located in Mpumalanga, ranging from Emalahleni (formerly known as Witbank), Groblersdal and Nelspruit, South Africa. By undertaking a

study that covered all these towns, a large study population from different geographical areas was ensured. The study was approved by the Ethics Committee of the North-West University (NWU-00082-17-S1).

Subject selection

Inclusion criteria: Data were subject to the following criteria. Only data of confirmed LOH cases were included in the current study, where depot-testosterone undecanoate was initiated as normal standard practice according to the specialist prescription.

Exclusion criteria: Data of patients with signs and symptoms of conditions such as severe sleep apnoea, male breast cancer or a Hct $\geq$ 50% at the time of diagnoses, and prostate specific antigen > 4 ng/ml at the time of diagnoses were excluded from the study, as alternative treatment plans had to be applied by the treating practitioner.

Research procedure

This study investigated retrospective data of diagnosed LOH men who followed the European Association of Urology treatment regimen for depot-testosterone undecanoate for at least a three-month period. The observation was done in retrospect, where treatment was initiated on day 1, a booster at six weeks after the first injection, and then after 12 weeks.[9,1]

The data required to execute the study were obtained from the clinical data manager of the specified private practice. It included re-identified patient data of laboratory TT levels and Hct values as recorded by the clinical data capturer at the time of diagnoses (month 0), and three months post-treatment. A positive diagnosis for polycythaemia was made once a Hct percentage of $\geq$ 50% was obtained from the laboratory. The raw data was sent to Statistical Consultation Services of the North-West University for data analyses.

Data analysis

The Statistical Analysis System®, SAS 9.3® (SAS Institute Inc., 2009) was used to analyse the data. Categorical variables were reported as frequencies and percentages. Continuous variables were reported as mean ± SD (normally distributed data) or median (25th, 75th) percentiles (skewed data). Histograms and Q-Q plots were used to evaluate the distributions. Possible outlying values were identified by means of box-and-whiskers plots, with z-score values larger than the absolute value of three. The dependent t-test was used to compare the

change between the two time points. Cohen's d-value was used to determine the practical significance of the results (with $d \geq 0.8$ defined as a large effect with practical significance).

Results

The cohort of LOH patients was observed retrospectively for a three-month study period. Polycythaemia was observed in 34% of participants.

The mean Hct rose from 45.62% [standard deviation (SD) 4.79] to 49.11% (SD 3.98). The change in mean Hct over the study period was 3.49% (SD 4.46). The rise in Hct was statistically significant, p-value < 0.001. The effect size was 0.73, suggestive of a large impact.

At the beginning of the study, the participants' mean TT was 8.18 nmol/l (SD 3.71), and at the end of the three-month period, 12.39 nmol/l (SD 6.18). The mean increase of TT over the study period was 4.21 nmol/l (SD 6.47). The increase of TT was a statistically significant p-value < 0.001. Cohen's d-value of 0.68 suggests a large practically significant change over the two-time points. A negative association between Hct and TT was noted after the study period.

Discussion

Depot-testosterone undecanoate-induced polycythaemia occurred in 34% of study participants, with an overall study period recorded mean Hct of 49%. This mean Hct value was below the upper level stated as pathognomonic for polycythaemia (Hct $\geq$ 50%).[1] The rise in TT of the study participants (n=49) after initiated treatment proved to be practically significant, as TT levels increased to within the normal physiological TT level of 12 nmol/l.[2] The observed change in Hct and TT values was statistically and practically noteworthy, indicative of the importance to evaluate benefits and risks related to patient care.

Polycythaemia – the increase of erythrocytes leading to higher levels of blood viscosity – is a known risk when a patient receives TRT.[2,1] Furthermore, it is well documented that polycythaemia is a dose-dependent side-effect of TRT and that both dose and delivery system affect the magnitude of the Hct increase.[3,14,15,18] As observed in this study, a higher polycythaemia prevalence is noted in some study participants, as opposed to no cases of polycythaemia recorded in studies done by Conaglen *et al.* and Yassin and Haffejee.[9,11] A

possible reason for the difference noted in polycythaemia prevalence between studies is the studied population of hypogonadal men. Some authors reported Hct values that consisted of men who had previous encounters with TRT, where others used a mixed-patient population that comprised men with primary-, secondary-, and late-onset hypogonadism.[8,19] This study, however, only used treatment-naïve LOH patients. Furthermore, over- or under-reporting of polycythaemia might occur due to mean Hct values and standard deviations that mask individual results that are over the upper Hct limit. The individual masking effect was explained by Haider *et al.*, where the mean Hct of 122 LOH male patients at month 3 of initiated therapy revealed no cases of polycythaemia in relation to a 5% prevalence of polycythaemia after evaluating patients individually post-treatment (at month 3).[20] The latter might be resolved by grouping patients according to their own individualised Hct values.[20] Finally, a polycythaemia-induced Hct cut-off value still eludes prescribers and patients as discussions still exist as to what the peak cut-off Hct percentage should be. Haematocrit values ranging from 50 to 54% were chosen by most authors.[14,1] This study used an Hct percentage of $\geq 50\%$ as pathognomonic for polycythaemia, which is in line with the European Association of Urology guidelines.[1]

It is important to be aware of the possible mean increase in Hct, as a steep rise above the Hct threshold level may lead to adverse effects such as strokes, myocardial infarctions or deep vein thrombosis that might well lead to a pulmonary embolism.[20,21] Despite TRT-induced erythropoiesis leading to polycythaemia, randomised control studies still cannot demonstrate the direct relationship between TRT-induced erythrocytosis and cardiovascular events.[22] In a recent review published by Hackett, TRT patients presented with a 6% increase in risk for polycythaemia. However, mortality rates were lower in TRT cohort of patients than for treatment-deprived patients which demonstrate the beneficial effect of TRT for hypogonadism patients. There is a relationship between the mean change in Hct values of this study and other studies, which demonstrates the similarities of a mean change in Hct values between international patients and South African patients.[18] This is noteworthy for the South African population, as TRT-induced polycythaemia studies are not well defined in South Africa. Subsequently, Assi and Baz noted that any man presenting with a Hct > 52% can be diagnosed with polycythaemia[23] – further strengthening evidence that the South African male population might be comparable with international cohorts.

The statistically significant increase in testosterone experienced by the current cohort of subjects leads to TT levels close to or within normal physiological TT ranges; consistent with international guidelines.[1] The rise in TT levels depends on the baseline TT value.

Observed study limitations were the lack of hematopoietic supplementation history of patients and the fact that patients were not stratified according to possible underlying co-morbid conditions, e.g. diabetes, obesity, HIV and anaemia, as these are known conditions that have a variable effect on testosterone levels.

Conclusion

The prevalence of polycythaemia for the South African cohort of studied men was higher than that of their international counterparts. However, the mean increase of Hct was below the pathognomonic cut-off used to diagnose polycythaemia. The conclusion can therefore be made that polycythaemia is a statistically significant occurrence for some individuals, but when put into perspective related to the broader community, polycythaemia is an isolated occurrence that should be screened for during the first few weeks to months after treatment initiation. Therefore, prescribers should strongly recommend screening for polycythaemia as per published guidelines. The rise in TT levels was sufficient to conclude that less than optimal baseline TT levels will rise to within normal physiological TT levels. To conclude, despite a greater statistically significant risk for attaining TRT induced polycythaemia, further research needs to be conducted to determine the practical significance thereof.

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3.2.1 Author guidelines

The article manuscript was written according to the author guidelines of the *Journal of Endocrinology, Metabolism and Diabetes of South Africa*. The authors' guidelines can be accessed on the following website (www.jemdsa.co.za). The article manuscript was submitted May 2018 (manuscript number: JEMDSA – 2018 – 0016).

3.2.2 Statements

The supervisor (Dr Jesslee du Plessis), co-supervisor (Dr Marlene Julyan) and statistician (Ms Marike Cockeran) of the mini-dissertation were also evaluated and supervised the writing of this article manuscript. The mini-dissertation and article manuscript were prepared by Mr HL Bester. All statistical analyses were generated by Ms Marike Cockeran.

3.2.3 Other data

Objectives not discussed in the manuscript will be addressed in this section.

A negative association between Hct percentages, and mean TT levels was noted after the study period. The practical significant effect was 0.23 suggestive of a small effect size. The association between the mean variance of the TT levels and the Hct percentages was statistical insignificant, p-value > 0.05. This is not an isolated occurrence as neither Haider *et al.* (2009:353) nor Coviello *et al.* (2008:914) were able to use the mean TT levels as a forecaster to predict the change in Hct percentages. Mostly so as the mean standard deviation of the group, masks the excess TT levels' mean change of an individual patient in smaller cohorts. Larger cohort studies where patients are stratified according to risk factors and co-morbidities might offer a more precise explanation as to what the statistically significant association is between the mean TT levels and Hct percentages.

3.3 Chapter summary

In this chapter, results of this mini-dissertation were described and presented in an article format. In Chapter 4, conclusions and recommendations will follow.

CHAPTER 4:   CONCLUSIONS AND RECOMMENDATIONS

4.1   Introduction

Information regarding the prevalence of LOH for the South African population is limited, even more so any information regarding TRT-induced polycythaemia.

In the following section, conclusions are derived from the results, and linked to the aims and objectives as set out in section 1.3 of this mini-dissertation. The aim of the study was to investigate the effect of TRT on TT levels and Hct. The focus was on polycythaemia in men, diagnosed with LOH in a private urology practice located in Emalahleni for the period 1 July 2013 to 1 March 2017. The objectives (literature review section 4.2 and empirical investigation section 4.3) were stratified in such a way to answer the stated aim as set out in section 1.3.1 of this mini-dissertation. Finally, the chapter will conclude with recommendations and study limitations, which might well be addressed by future researchers. A reflection on the study will be found at the end of this chapter.

4.2   Literature review objectives

Conclusions drawn in the literature review were the result of an intensive topic review whereby the researcher made use of databases e.g. Google Scholar™, EBSCOHost®, Science Direct® and Scopus®. Filters to databases were applied to be more productive and focused. Phrases and keywords mostly applied to narrow the search were:

- 'Late-onset hypogonadism (LOH)', 'Aging male', 'Androgen deficiency'

- 'Testosterone treatment', 'complication*'

- 'Polycythaemia', 'Polycythemia', 'Thrombocytosis' and 'Haematocrit.

The literature objectives were successfully actioned and are presented in sections 4.2.1 to 4.2.3.

4.2.1 Define late-onset hypogonadism in men and review the literature for prevalence, demographic data, pathogenesis, clinical presentation (both sexual and non-sexual), diagnosis, treatment and monitoring

Late-onset hypogonadism is defined as a physical and biochemical syndrome related to the insufficient production of testosterone (Űçer & Gümüs, 2014:171). The cause may be idiopathic or acquired. Determining the crude prevalence among LOH patients is difficult to pinpoint, as factors such as reduced morning erections, vigour and hot flushes not only present in LOH, but also in other forms of hypogonadism (Dohle *et al.*, 2014:13). It was found that even if some landmark articles are academically and historically dated, they are still used as indicators for prevalence as they remain the only high powered sufficient populated studies (see section 2.3). The down side of these studies that were used to draw conclusions related to the LOH men is that the primary end-point of most (if not all) studies was not to determine the prevalence of hypogonadism.

This objective concluded that the exact prevalence determination of LOH remains difficult to determine despite ongoing research efforts. The pathogenesis, clinical presentation, diagnosis, treatment and monitoring thereof have been sufficiently explained. Late-onset hypogonadism should be considered in men that present with the following signs and symptoms, e.g. loss of libido and ED, obesity, depressed mood and hot flushes (Dohle *et al.*,2014:13) in conjunction with two measured morning low TT levels. The diagnosis of LOH should be made after evaluating signs, symptoms and a biochemical investigation (Aversa & Morģentaler, 2015:641). Morning TT levels within three hours of awakening or between 07h00 and 11h00 should be used due to the diurnal variation of TT levels (Morales *et al.*, 2015:1373). Furthermore, despite the availability of several treatment options, the depot-testosterone undecanoate parental TRT remains the only registered product to treat LOH in South Africa. Monitoring of treatment should be done according to the suspected side effect profile of each individual. The side effect mostly encountered with TRT is polycythaemia and the patient should be screened at months three, six and 12 after treatment initiation and then annually (Flora *et al.*, 2010:386).

4.2.2 Describe the treatment options currently available for late-onset hypogonadism patients, including the benefits and associated risks

A detailed analysis of the treatment options for LOH and associated benefits and risks was given respectively in sections 2.5.5 and 2.6. The following is merely a conclusion of TRT-associated benefits and risks.

Despite the vast majority of TRT available to treat LOH, only the depot-testosterone undecanoate parental TRT option is available in South Africa (Snyman, 2016:359). Other formulations available internationally are TRT gels, buccal tablets, implantable pellets and transdermal patches (Dohle *et al.*, 2014:21). Associated risks of TRT are highly dependable on the formulation, where person-to-person transfer is the side effect mostly encountered with the gels (Surampudi *et al.*, 2011:6). Implantable pellets are associated with extrusion (Basaria, 2014:1256), transdermal patches with allergic skin reactions and a variable pharmacokinetic testosterone level is observed with parental dosage forms (Dohle *et al.*, 2014:21). Benefits of formulations include rapid absorption and physiological testosterone levels for buccal tablets. A favourable steady-state testosterone level has been observed for transdermal patches where the depot-testosterone undecanoate parental formulation is the TRT associated with the least fluctuation of testosterone levels around the median.

Lastly, this study concluded that the greatest influencing factor when it comes to the choice of TRT is patient preference after a detailed discussion with the prescriber, with eugonadism as the main goal of treatment. Furthermore, benefits and risks should be explained to the patient in relation to the selected dosage formulation.

4.2.3 Investigate possible physiological complications and/or side effects due to initiation of testosterone replacement therapy, and its monitoring and management

Literature on the safety concerns of TRT showed that several safety concerns exist regarding TRT, even though efficiency has been well demonstrated by studies. These adverse effects include polycythaemia, prostate cancer, LUTS and sleep apnoea.

Polycythaemia is the complication most frequently encountered when a patient receives TRT for LOH. The mechanism of action is not yet fully explained (Grech *et al.*, 2014:197). A combination between direct and indirect stimulation of TRT induced haematopoiesis leading to an increase of erythrocytes above the normal limit, also known as polycythaemia per definition of this study, has been explained in section 2.7.2.1.2. Testosterone replacement therapy induces polycythaemia via direct stimulation of the red bone marrow, which induces mRNA synthesis and initiates the

increase in number of erythrocytes (Shanani *et al.*, 2009:706). The indirect stimulation of testosterone is also needed to stimulate erythropoietin and acts as a catalysed for the synthesis of Hb that is incorporated in the erythroblast, leading to a fully developed erythrocyte (Ridley *et al.*, 1994:130). Direct- and indirect haematopoiesis mechanism of actions has been proposed and consensus of the exact mechanism of action still needs to be published. What is clear is that TRT induces polycythaemia and that it is researched as a dose and route of administration-related complication of TRT (Davidiuk & Broderick, 2016:830). Ideas to reduce the risk of polycythaemia include reduced doses of TRT or longer dosage intervals, which will give ample time for the supra-physiological TT levels to reduce to a mean TT level before the next dose.

This study concludes that the parental TRT option is the route most frequently associated with polycythaemia. Furthermore, the aging male is statistically more prone to TRT-induced polycythaemia than their younger counterparts. Testosterone-induced polycythaemia is known to occur during treatment initiation and frequent monitoring for this side effect should be done by measuring the Hb levels during the first few weeks to months of treatment initiation.

This literature review also investigated TRT-induced, or associations in relation to prostate cancer, lower urinary tract symptoms and sleep apnoea and found that literature regarding the following was inadequate. To date, TRT has not been linked as the cause of new prostate cancers, yet the European Association of Urology advocates against TRT during or even after the successful treatment of prostate cancer (see section 2.7.2.2). The effect of TRT on lower urinary tract symptoms to date appears to be contradicting (see section 2.7.2.3). When one keeps the physiology of the prostate in mind related to TRT, it should be noted that TRT might lead to the growth of the prostate that might well increase the side effects associated with lower urinary tract symptoms. Finally, sleep apnoea was found to be worsened by TRT via a central nervous system mechanism of action and not a physical mechanism of action (Bassil *et al.*, 2009:438). Again, as with Hct-associated TRT-induced polycythaemia, it was also conclusive that sleep apnoea was worse during the treatment initiation phase compared to later stages of treatment.

4.3   Empirical investigation objectives

The empirical investigation was done in an article format (refer to Chapter 3) and its objectives were achieved by using the retrospective observational data as obtained in section 1.3.4. These objectives included the effect that TRT had on Hct and the TT values of diagnosed LOH patients. Furthermore, the prevalence of polycythaemia was determined in patients who presented with increased Hct values and an investigation was launched to determine whether the percentage

variance of TT levels following treatment can be used to predict the change in observed Hct levels. Conclusions of the empirical investigation objectives are summarised in sections 4.3.1 to 4.3.3.

4.3.1 Retrospectively observing the effect of testosterone replacement therapy, if any, on the haematocrit- and total testosterone values of patients diagnosed with late-onset hypogonadism

This study found that TRT has a statistically significant impact on both Hct and TT levels. The mean Hct rose with 3.49% over the three-month study period and the TT mean increase was 4.21 nmol/L. Even though the TT levels of this specific LOH cohort of South African male patients have been statistical significant, the TT mean for South African LOH patients remained lower compared to their international counter parts (see section 1.1 and 2.3). This was also noted by (Bornman & Reif, 2007:62) in their study that strengthened the evidence that South African males might qualify sooner for TRT than the rest of the world. Nevertheless, one should not ignore the fact that a greater risk for polycythaemia has been found according to Chapter 3 of this mini-dissertation. Accordingly, emphasis should be placed on guideline adherence regarding the screening intervals for polycythaemia in especially South African LOH diagnosed male patients.

4.3.2 Determining the prevalence of polycythaemia in patients with an increased haematocrit currently diagnosed with late-onset hypogonadism and treated with depot-testosterone undecanoate

With regards to determining the prevalence of TRT induced polycythaemia in LOH patients over the study period, it was found that the prevalence for polycythaemia was 34% (n = 50). This prevalence might seem extremely high and was found to be higher than other prevalence rates (see section 2.7.2.1.2). Possible reasons for the higher prevalence in this study might be the fact that in most to all of the compared studies, the primary outcome was not to determine the prevalence of polycythaemia in the LOH patient, but other primary end-points were measured. To date, those are the only studies available. On the other hand, this study reported on the crude prevalence of TRT-associated polycythaemia of treatment naïve LOH patients in South Africa. The conclusion can therefore be made that larger cohort studies of similar primary end-points are needed to review this novel finding more comprehensively.

4.3.3 Determining, if possible, whether the percentage variance in total testosterone level per patient can be used to predict the change in the haematocrit levels measured

A weak association was found between the variance of TT levels over the treatment period of three months as a forecaster to predict the change in Hct for the same time span. Another study conducted by Haider *et al.* (2009:353) was also not able to use the change in TT levels as a forecaster to predict the change in Hct.

4.4 Limitations

The retrospective nature of this study is a limitation in-itself, as de-identified data was received from the clinical data manager to exclusively answer the empirical investigation objectives. As a result, no information was obtained from the clinical data manager to draw conclusions and make assumptions regarding the haematopoietic status of patients see section 1.9. Lastly the empirical investigation was not stratified according to risk factors and co-morbidities as listed in section 2.5.4.

4.5 Strengths

The strengths of this study included:

- Only treatment naïve, diagnosed LOH patients were used in the study

- Only patients diagnosed by a urologist were used

- Retrospective, depersonalised data from a private urology practice were used

- This was a medium-risk study that presented with medium-risk ethical implications.

The indirect benefits of this study can be classified as strengths and were met by describing the side effect, polycythaemia, which is most prevalent in patients who receive TRT.

4.6   Recommendations

Larger and different clinical TRT study designs can be conducted under the treatment-naïve LOH South African population, as not many studies exist at this time. By embarking on such a venture, one might well be able to answer questions related to genetic differences between sub-populations and measure the cause and effect thereof. Co-morbid conditions and risk factors should be grouped in accordance with TT levels to minimise the masking effect as explained by Haider *et al*. (2009:353) in section 3.2.3. These type of studies might provide more insightful information on polycythaemia, known as the side effect most prevalent when a patient receives TRT.

4.7   Chapter summary

This chapter highlighted the aim of the study and has drawn conclusions from literature and empirical objectives. Study limitations and strengths were addressed, and recommendations followed to complete this chapter.

4.8   Study reflection

This study was done in retrospect to investigate the prevalence of polycythaemia among LOH males who received TRT in the form of depo-testosterone undecanoate. The prevalence of TRT-associated polycythaemia for the South African population is not known. This study has found a polycythaemia prevalence that was higher compared to international studies. These findings should alert prescribers to monitor South African patients for polycythaemia closely as per published recommendations.

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ANNEXURE A: DATA SOURCE

Number of patients	TT (nmol/l) month zero	TT (nmol/l) at 3months	Haematocrit % (HCT) month zero	Haematocrit % (HCT) at 3months	Polycythaemia No= 0; Yes= 1
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
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26					
27					

ANNEXURE B: GOODWILL PERMISSION
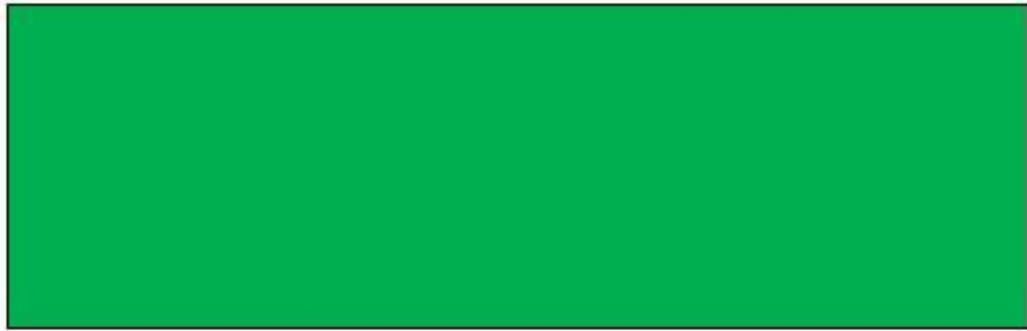
To whom it may concern

At The Male Clinic we see a lot of patients with late onset hypogonadism. Herman Bester informed us of a study he is currently interested in doing, titled "Testosterone undecanoate associated polycythaemia in males with late onset hypogonadism: private practice Emalahleni in males with late onset hypogonadism a retrospective cohort study".

We hereby give consent for him to use the relevant data from patients that we have collected, in his research project. The data can include, but are not limited to laboratory results and clinical examinations. All patient information will be removed prior to releasing the data to him, and it will thus be strictly anonymous.

If you have any queries, please do not hesitate to contact on

Thank you very much.

Warm regards,

The Male Clinic

Dr.

Dr.

Date: 22/09/2016

ANNEXURE C: PERMISSION TO USE PRACTICE NAME AND PERSONAL IDENTITIES

To whom it may concern

At the Male clinic we see a lot of patients with Late-onset hypogonadism. Herman Bester informed us of a study he is currently interested in doing, titled "Testosterone undecanoate associated polycythaemia in males with late onset hypogonadism: private practice Emalahleni in males with late onset hypogonadism a retrospective cohort study".

We hereby give consent to mention the practice and individual names for the protocol research study. Also mini-dissertation and article when published, with the anonymous data that was provided.

If you have any queries, please do not hesitate to contact on ▮▮▮▮▮▮▮

Thank you very much.

Warm regards,

The Male Clinic

Dr. ▮▮▮▮▮▮▮   Dr. ▮▮▮▮▮▮▮

Date: 22/09/2016

ANNEXURE D: AGING MALE SCORECARD (AMS)

AMS Questionnaire

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark "none".

Symptoms:	none	mild	moderate	severe	extremely severe
Score =	1	2	3	4	5
1. Decline in your feeling of general well-being (general state of health, subjective feeling)	☐	☐	☐	☐	☐
2. Joint pain and muscular ache (lower back pain, joint pain, pain in a limb, general back ache)	☐	☐	☐	☐	☐
3. Excessive sweating (unexpected/sudden episodes of sweating, hot flushes independent of strain)	☐	☐	☐	☐	☐
4. Sleep problems (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)	☐	☐	☐	☐	☐
5. Increased need for sleep, often feeling tired	☐	☐	☐	☐	☐
6. Irritability (feeling aggressive, easily upset about little things, moody)	☐	☐	☐	☐	☐
7. Nervousness (inner tension, restlessness, feeling fidgety)	☐	☐	☐	☐	☐
8. Anxiety (feeling panicky)	☐	☐	☐	☐	☐
9. Physical exhaustion / lacking vitality (general decrease in performance, reduced activity, lacking interest in leisure activities, feeling of getting less done, of achieving less, of having to force oneself to undertake activities)	☐	☐	☐	☐	☐
10. Decrease in muscular strength (feeling of weakness)	☐	☐	☐	☐	☐
11. Depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)	☐	☐	☐	☐	☐
12. Feeling that you have passed your peak	☐	☐	☐	☐	☐
13. Feeling burnt out, having hit rock-bottom	☐	☐	☐	☐	☐
14. Decrease in beard growth	☐	☐	☐	☐	☐
15. Decrease in ability/frequency to perform sexually	☐	☐	☐	☐	☐
16. Decrease in the number of morning erections	☐	☐	☐	☐	☐
17. Decrease in sexual desire/libido (lacking pleasure in sex, lacking desire for sexual intercourse)	☐	☐	☐	☐	☐

Have you got any other major symptoms?   Yes......... ☐   No.......... ☐

If Yes, please describe: _____

_____

THANK YOU VERY MUCH FOR YOUR COOPERATION

ANNEXURE E: INTERNATIONAL PROSTATE SYMPTOM SCORECARD (I-PSS)

International Prostate Symptom Score (I-PSS)

Patient Name: _____   Date of birth: _____   Date completed _____

In the past month:	Not at All	Less than 1 in 5 Times	Less than Half the Time	About Half the Time	More than Half the Time	Almost Always	Your score
1. Incomplete Emptying How often have you had the sensation of not emptying your bladder?	0	1	2	3	4	5	
2. Frequency How often have you had to urinate less than every two hours?	0	1	2	3	4	5	
3. Intermittency How often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Urgency How often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Weak Stream How often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Straining How often have you had to strain to start urination?	0	1	2	3	4	5	
	None	1 Time	2 Times	3 Times	4 Times	5 Times	
7. Nocturia How many times did you typically get up at night to urinate?	0	1	2	3	4	5	
Total I-PSS Score							

Score: 1-7: *Mild*   8-19: *Moderate*   20-35: *Severe*

Quality of Life Due to Urinary Symptoms	Delighted	Pleased	Mostly Satisfied	Mixed	Mostly Dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?	**0**	**1**	**2**	**3**	**4**	**5**	**6**

About the I-PSS

The International Prostate Symptom Score (I-PSS) is based on the answers to seven questions concerning urinary symptoms and one question concerning quality of life. Each question concerning urinary symptoms allows the patient to choose one out of six answers indicating increasing severity of the particular symptom. The answers are assigned points from 0 to 5. The total score can therefore range from 0 to 35 (asymptomatic to very symptomatic).

The questions refer to the following urinary symptoms:

Questions	Symptom
1	Incomplete emptying
2	Frequency
3	Intermittency
4	Urgency
5	Weak Stream
6	Straining
7	Nocturia

Question eight refers to the patient's perceived quality of life.

The first seven questions of the I-PSS are identical to the questions appearing on the American Urological Association (AUA) Symptom Index which currently categorizes symptoms as follows:

> Mild (symptom score less than of equal to 7)
> Moderate (symptom score range 8-19)
> Severe (symptom score range 20-35)

The International Scientific Committee (SCI), under the patronage of the World Health Organization (WHO) and the International Union Against Cancer (UICC), recommends the use of only a single question to assess the quality of life. The answers to this question range from "delighted" to "terrible" or 0 to 6. Although this single question may or may not capture the global impact of benign prostatic hyperplasia (BPH) Symptoms or quality of life, it may serve as a valuable starting point for a doctor-patient conversation.

The SCI has agreed to use the symptom index for BPH, which has been developed by the AUA Measurement Committee, as the official worldwide symptoms assessment tool for patients suffering from prostatism.

The SCI recommends that physicians consider the following components for a basic diagnostic workup: history; physical exam; appropriate labs, such as U/A, creatine, etc.; and DRE or other evaluation to rule out prostate cancer.

ANNEXURE F: VISUAL PROSTATE SYMPTOM SCORECARD (VPSS)

VPSS (Visual Prostate Symptom Score)

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Patient's Name:
Pasiënt se Naam: ....

Date:
Datum: ....

A:
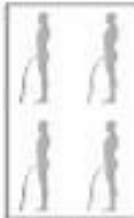
B:

1   2   3   4   5   6 or more

C:

1   2   3   4   5   6 or more

D:

0   1   2   3   4   5   6

A = ...   B = ...   C = ...   D = ...   A + B + C = ...

ANNEXURE G: INTERNATIONAL INDEX OF ERECTILE FUNCTION-5 SCORECARD (IIEF-5)

The IIEF-5 Questionnaire (SHIM)

Please encircle the response that best describes you for the following five questions:

Over the past 6 months:					
1. How do you rate your confidence that you could get and keep an erection?	Very low 1	Low 2	Moderate 3	High 4	Very high 5
2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	Almost never or never 1	A few times (much less than half the time) 2	Sometimes (about half the time) 3	Most times (much more than half the time) 4	Almost always or always 5
3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated your partner?	Almost never of never 1	A few times (much less than half the time) 2	Sometimes (about half the time) 3	Most times (much more than half the time) 4	Almost always or always 5
4. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	Extremely difficult 1	Very difficult 2	Difficult 3	Slightly difficult 4	Not difficult 5
5. When you attempted sexual intercourse, how often was it satisfactory for you?	Almost never or never 1	A few times (much less than half the time) 2	Sometimes (about half the time) 3	Most times (much more than half the time) 4	Almost always or always 5

Total Score: _____

1-7: Severe ED   8-11: Moderate ED   12-16: Mild-moderate ED   17-21: Mild ED   22-25: No ED

ANNEXURE H: LANGUAGE EDITING LETTER

To whom it may concern

Cecile van Zyl
Language editing and translation
Cell: 072 389 3450
Email: Cecile.vanZyl@nwu.ac.za

16 November 2018

Dear Mr / Ms

Re: Language editing of dissertation (Testosterone undecanoate associated polycythaemia in males with late-onset hypogonadism: Private practice Emalahleni)

I hereby declare that I language edited the above-mentioned dissertation by Mr Herman Bester (student number: 12136913).

Please feel free to contact me should you have any enquiries.

Kind regards

Cecile van Zyl

Language practitioner

BA (PU for CHE); BA honours (NWU); MA (NWU)
SATI number: 1002391

ANNEXURE I: TECHNICAL EDITING LETTER