厚生労働科学研究費補助金(長寿研究事業)
分担 研究報告書

LOH症候群における遺伝子多型とARTの治療感受性遺伝子同定に関する研究

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【研究要旨】

LOH症候群における遺伝子多型(特に一塩基多型)の解析とARTに対する治療感受性遺伝子の同定によって,個人の発病リスクや予後推察,ARTの治療効果や副作用予測,新しい診断法,治療法や予防法の開発へと発展させるとともに,テーラーメイド医療への昇華を試みる.

【研究目的】

平均寿命の延長に伴う急激な高齢化という社会的背景のもと,高齢者の健康増進や予防医学への積極的な取り組みが国策の一つとなり,健康寿命の延伸を目指した長寿科学研究が待望される時代となった.近年,アンチエイジング医学の登場とともに,その治療法として,サプリメント摂取,抗酸化療法,ホルモン補充療法の必要性が過度にクローズアップされている.特にホルモン補充療法については,更年期女性に対してはエストロゲン補充療法が広く普及してきた一方で,中高年男性に対するそれは未だ発展途上にある.中高年男性のアンドロゲン低下は,本邦ではこれまでも"加齢に伴う一現象"とみなされる程度で,医療の対象として軽視されていた経緯があったが,アンドロゲンは男性における重要な生理的機能を有するため,その低下はED,認知症,筋力低下,骨粗鬆症,貧血,内臓脂肪増加(あるいメタボリックシンドロームへの進展)等の各種合併症を引き起こし,高齢男性のADL,QOLに多大な影響を及ぼす.そこで,LOH症候群に伴うこれらの症候の改善を目指した,アンドロゲンの補充による骨,筋肉,血管,脂質代謝等に対する臨床効果に関する強力なエビデンスを創出する必要がある.その際,あらかじめLOH症候群に対するARTの有用性を予測,予見できれば,テーラーメイド医療の構築によって,医療費削減につながることが期待される.以上のように,治療の有効性に関する遺伝的背景の解析として,治療感受性と副作用を規定する遺伝子多型の存在がなかでも,特に一塩基多型 single nucleotide polymorphism (SNP) の同定を試み,個人化治療への進展に進展させることを本研究の目的とする.

【研究方法】

LOH症候群と診断されARTが施行された対象症例より，血液7 ml 採取し，血清を使用してDNA を抽出する．ゲノムDNA増幅キットを用いて抽出したDNAを増幅し，後々の追加解析のためにストックサンプルを作成しておく．ARTにおける治療感受性遺伝子に照準を絞って，主として一塩基多型SNPを検索し，ART有効群を予測する．ターゲット遺伝子の同定に際し，以下の2つのアプローチを用いる．

1) 候補遺伝子アプローチ：先駆的な知識をベースに遺伝子を狙い打ちする方法で，遺伝子の選択方法は多種多様にわたる．今回我々はARTの有効例に絞って遺伝子多型を検索するため，テストステロン代謝に関わることが予想される以下の遺伝子を解析する．すなわち，チトクローム p450 酵素系であるCYP17 や CYP3A4，細胞増殖因子とその受容体である insulin-like growth factor-1 (IGF-1) と IGF binding protein-3 (IGFBP-3)，前立腺組織内でテストステロンをdihydrotestosterone (DHT)に変換する酵素である steroid 5α-reductase type II enzyme (SRD5A2)，における遺伝子多型を候補とする．(資料7) さらに，アンドロゲン受容体(AR)におけるCAG repeat 数が前立腺癌の発ガンリスクや予後因子に相関すると報告されているため，SNP以外に同反復多型も解析対象とする．

2) ゲノムワイドアプローチ：SNP をゲノム全域に渡って絨毯爆撃的に解析する方法で，症例対照群においてゲノム上のSNPをマーカーと考えて網羅的に調べていき，その近傍にあるSNPがマーカーとして検出されることによって疾患との関連性を見出す．さらに，DNAチップなどの遺伝子研究の革新的技術とコンピューターの情報処理能を駆使して，網羅的なSNPの解析を展開する予定である．なお，標的SNPの検出にはTetra-primer amplification refractory mutation system (ARMS)-PCR 法(資料8)を用いる．

【研究結果】

加齢男性性腺機能低下症候群における遺伝子多型の解析が，ようやく平成20年6月に金沢大学ヒトゲノム・遺伝子解析研究倫理審査委員会より承認された．(資料9) 倫理委員会での承認が遅れていたことが律速段階となっていた経緯があり，進捗状況は当初の予定より遅延している状況にある．

今年度は，症例のエントリーが始まったばかりで，採血のタイミングをART終了後あるいは，開始後半年という設定をしているため，現時点では数症例のサンプルしか集まっておらず，結果報告には至っていない．しかしながら，SNP解析のために，各種試薬，各種キットは既に購入済で，電気泳動ゲル装置，全自動核酸抽出・精製装置を準備すると共に，Tetra-primer ARMS-PCR に必要なプライマーの一部は既に注文済みである．

【考察】

各個人間におけるゲノムの塩基配列の差異は遺伝子多型といわれ，各個人に生来備わっている個性の多くは，この遺伝子多型よって説明されると考えられている．遺伝子多型は1）一塩基多型（SNP），2）挿入・欠失多型（insertion/deletion polymorphism），3）反復多型（repeat polymorphism）の3種類に大別される．

なかでも，SNPはある集団内で1%以上の頻度で認められるゲノム上の一塩基多型と定義され，その大多数が2種類の対立遺伝子（アレル）からなり，ゲノム上に平均100ー1,000塩基につき1個の割合で均等に存在するため，各個人の遺伝的背景を個別化するのに最適なマーカーである．SNPの生物学的な意義は，ゲノム上における存在部位によって異なり，大まかに4種類に分類される．すなわち，1）プロモーターなどの調節領域にあるもの（regulatory SNP: rSNP），2）エクソン内の翻訳領域にあるもの（coding SNP: cSNP），3）イントロンに存在するもの（intronic SNP: iSNP），4）その他の領域にあるもの（genomic SNP: gSNP）である．さらに，cSNPはアミノ酸置換を引き起こすもの（non-synonymous cSNP）とそれを伴わないもの（synonymous cSNP）に分類される．そのほかにも，イントロン内に存在しスプライシングに関与する多型や変異によって本来と異なる部位に終止コドンが形成される多型（missense SNP）などが知られている．また，このような1ー数十塩基の多型以外にも，数千ー数万塩基以上の大きな欠失や挿入，さらには遺伝子単位での重複（遺伝子のコピーの数的差異）が健常人においても認められることが明らかにされている．

さて，遺伝的な観点からみると，ヒト疾患は，単一遺伝子疾患，多因子疾患，および遺伝子が全く関与しない疾患に大別できる．現在の疾患ゲノム研究における最大のテーマは，多因子疾患であるcommon disease に関連する遺伝子を同定し，その機能を探ることにある．多因子疾患の発症には，遺伝要因，環境要因，そしてそれらの相互作用が関与しているが，疾患遺伝子の同定が成因解明に直結し，さらにはテーラーメイド医療とよばれる個人差に応じた医療の実現に向けた基盤となりうることが期待されている．疾患関連SNP の同定研究における初期目標は，強い有意差で関連し，かつ高いオッズ比を有するSNPを見つけ出すことにある．SNPが与える影響は，既述したように遺伝子発現量の変化，スプライシングの違い等が考えられ，実際にイントロンや翻訳領域にあるsynonymous SNPで遺伝子発現変化を伴うものの報告が散見される．

今回我々は，ステロイド合成・代謝に関わるCYP17，CYP3A4，SRD5A2を候補遺伝子とするとともに，細胞増殖因子ならびに受容体の遺伝子多型であるIGF-1とIGFBP-3も標的とした．特にCYP17はアンドロゲン合成に関わる酵素であるP450c17aをエンコードしている．転写開始部位から34bp上流にT/C多型が存在し，変異アレルであるA2は転写活性を上昇させると推測されている．

また，SRD5A2は前立腺組織内でテストステロンをdihydrotestosterone (DHT)に変換させ，アンドロゲン受容体との結合を可能ならしめる．SRD5A2の3'-UTRにはTA反復多型が，そしてcodon 49と89にはSNP(A49TとV89L)が存在し，いずれもSRD5A2の酵素活性に影響を及ぼすことが推測されている．

IGF−1は細胞の増殖や分化そしてアポトーシスに関与している増殖因子の一つであり，前立腺ガンではアンドロゲン非存在下での癌細胞の増殖を促す．プロモーター領域にはCA反復多型が存在し IGF-1の発現を調節していることが示唆されている．一方IGFBP-3はIGF-1と結合することによりIGF-1受容体への結合を阻害することから，IGF-1に対して抑制的に作用していると考えられる．セリンプロテアーゼであるPSAはIGFBP-3を消化するため，PSAレベルが高い環境下ではIGFBP-3濃度は減少し，相対的にIGF-1濃度が増加することが予想される．

ARに関しては，ARのエクソン1に存在するCAGとGGNの反復多型において，長い反復数を持つARはトランス活性能とアンドロゲン結合性が弱いため，前立腺ガンの発祥に対して抑制効果をもつものと推測されている．しかし，メタアナリシスではいずれの多型においても反復数の少ないアレルは前立腺ガン発症のリスクを高める傾向が認められるものの，正常コントロールとの差は極めて小さい．

従って，LOH症候群におけるSNPを遺伝マーカーとした患者・対照関連解析(アソシエーション・スタディ)は，遺伝要因に対するアプローチ法の1つとなりうると考えられる．また，ART における治療感受性遺伝子に照準を絞って，SNPを検索することによってART有効群の予測が可能になるかもしれない．

【結論】

本研究は，SNP解析を中心としたLOH 症候群における疾患関連遺伝子の解析とARTに対する治療感受性遺伝子の同定によって，個人の発病リスクや予後推察，ART の治療効果や副作用予測，新しい診断法，治療法や予防法の開発へと発展しうる大変意義深い成果が得られる可能性が高い．今後，症例の蓄積によって，LOH症候群とARTに関する新たなSNPの知見を集積していく次第である．

【健康危険情報】

該当なし

【研究発表】

1. 論文発表

該当なし

2. 学会発表

該当なし

【知的財産権の出願・登録状況（予定を含む。）】

1. 特許取得

該当なし

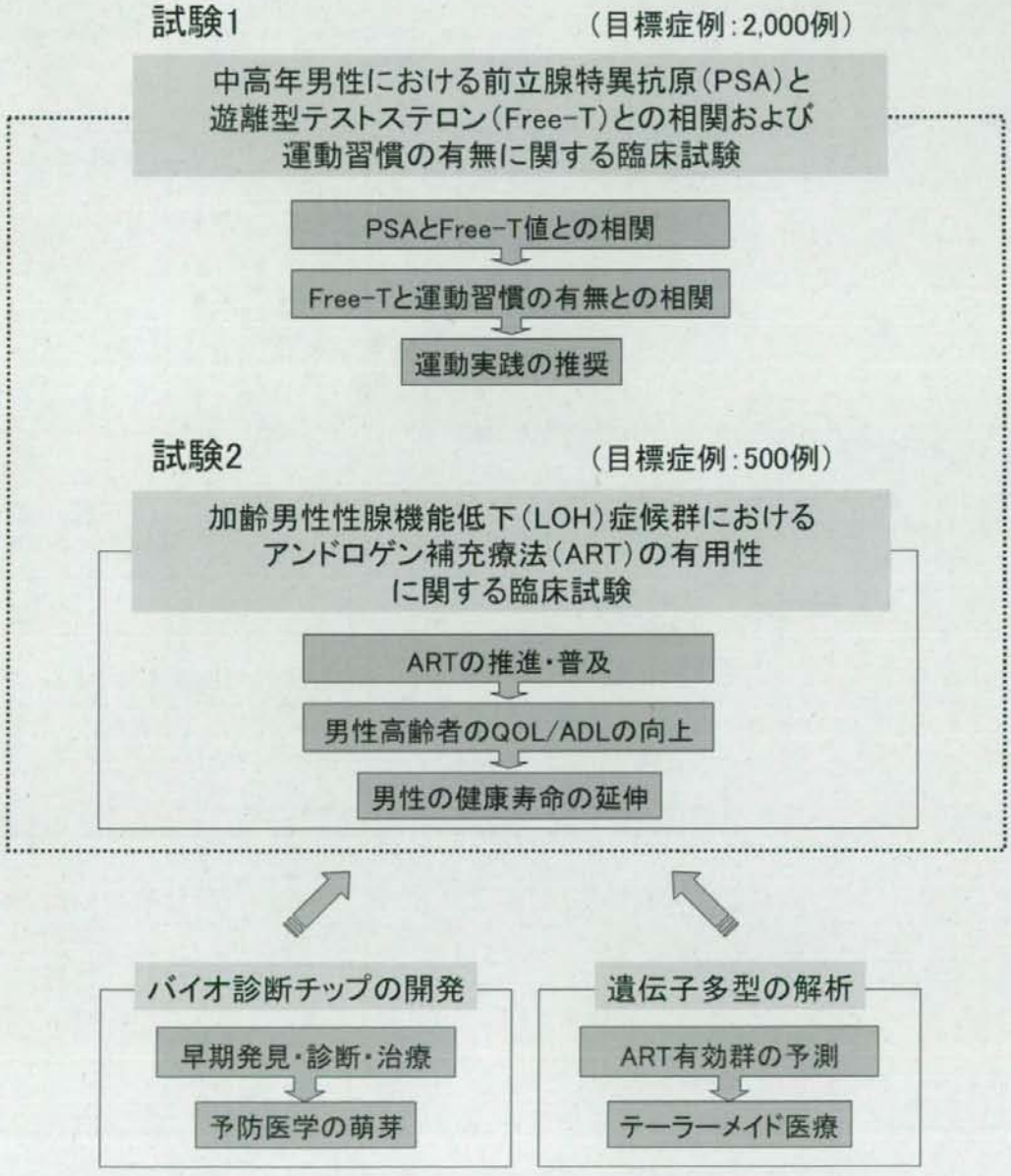
2. 実用新案登録

該当なし

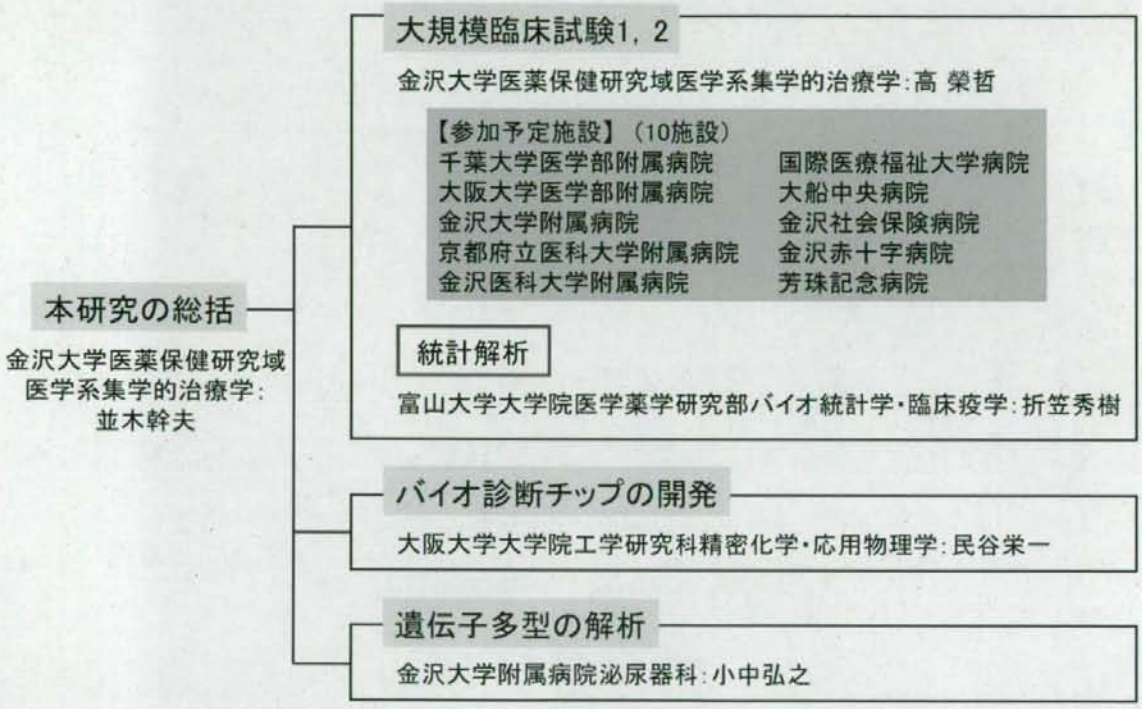
3. その他

該当なし

本プロジェクトの全体構想



平成21年度組織表


本研究の総括
金沢大学医薬保健研究域
医学系集学的治療学:
並木幹夫
大規模臨床試験1, 2
金沢大学医薬保健研究域医学系集学的治療学:高 榮哲
【参加予定施設】(10施設)
千葉大学医学部附属病院
大阪大学医学部附属病院
金沢大学附属病院
京都府立医科大学附属病院
金沢医科大学附属病院
国際医療福祉大学病院
大船中央病院
金沢社会保険病院
金沢赤十字病院
芳珠記念病院
統計解析
富山大学大学院医学薬学研究部バイオ統計学・臨床疫学:折笠秀樹
バイオ診断チップの開発
大阪大学大学院工学研究科精密化学・応用物理学:民谷栄一
遺伝子多型の解析
金沢大学附属病院泌尿器科:小中弘之

研究年次計画

実施項目　　年次	19年度	20年度	21年度
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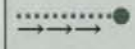
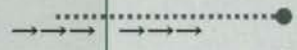
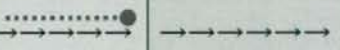
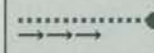
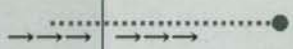
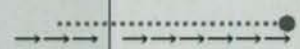
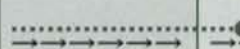
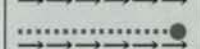
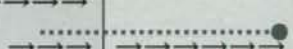
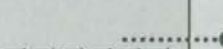
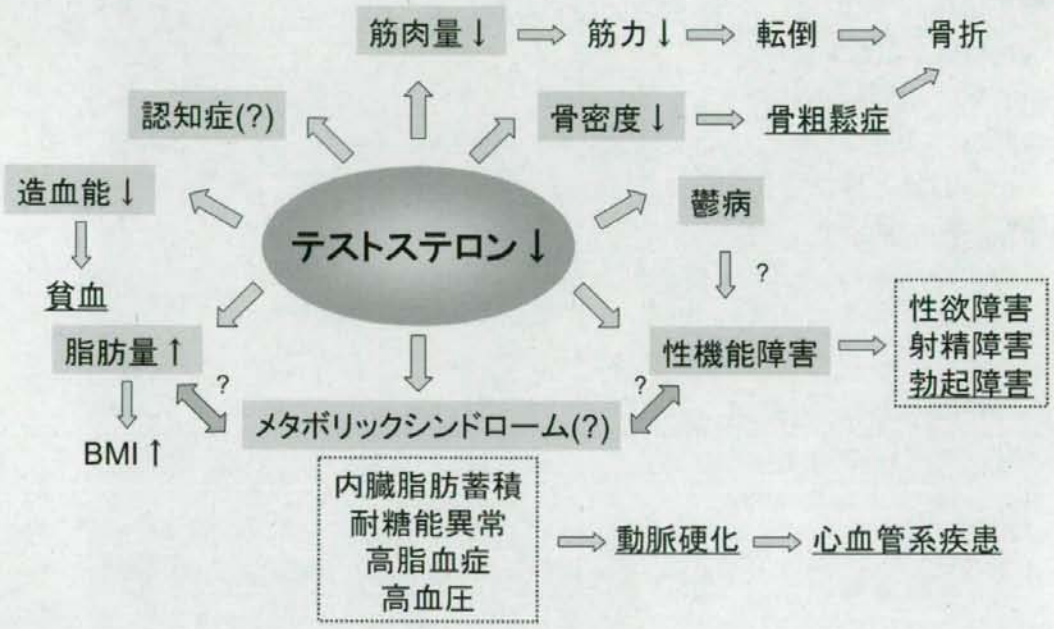
準備期間（各施設での承認・審査等）			
症例組み入れ			
データの解析			
大規模臨床試験2			
準備期間（各施設での承認・審査等）			
症例組み入れ			
治療期間（1年）＋追跡期間			
データの解析			
バイオ診断チップの開発			
イムノセンサーの設計・試作			
分子マーカー・抗体の選定			
臨床検体（血液・唾液）の収集			
イムノセンサーの評価			
遺伝子多型の解析			
標的遺伝子の探索			
臨床検体（血液）の収集			
データの解析			

現在までの進捗状況

LOH 症候群の病態


筋肉量↓
筋力↓
転倒
骨折
認知症(?)
骨密度↓
骨粗鬆症
造血能↓
テストステロン↓
鬱病
貧血
?
脂肪量↑
性機能障害
性欲障害
射精障害
勃起障害
?
?
BMI↑
メタボリックシンドローム(?)
内臓脂肪蓄積
耐糖能異常
高脂血症
高血圧
動脈硬化
心血管系疾患

International Journal of Urology (2008) 15, 377–388 doi: 10.1111/j.1442-2042.2008.02010.x

Guidelines

Clinical Practice Manual for Late-onset Hypogonadism Syndrome

Mikio Namiki, Hideyuki Akaza, Toru Shimazui, Naoki Ito, Teruaki Iwamoto, Katsuyuki Baba, Hiroaki Kumano, Eitetsu Koh, Akira Tsujimura, Kiyomi Matsumiya, Shigeo Horie, Osamu Maruyama, Ken Marumo, Toshihiko Yanase and Yoshiaki Kumamoto

Working Committee on Clinical Practice Guidelines for Late-onset Hypogonadism
The Japanese Urological Association/Japanese Society for the Study of the Aging Male

Introduction

With the aging of the population, the quality of life (QOL) of middle-aged and elderly men has come into question and it has been taken up from an interdisciplinary standpoint in recent years.

Partial androgen deficiency of the aging male (PADAM) or late-onset hypogonadism (LOH) is a syndrome consisting of symptoms caused by partial deficiency of androgens, but the time of onset varies and the epidemiological status is unclear. Therefore, in Japan to date, this syndrome has been considered as a general phenomenon associated with aging, the medical authorities have not reacted and patients are not being treated.

In Western countries however, this phenomenon has attracted attention in relation to geriatrics and reproductive endocrinology since the 1980s. In 1998, the International Society for the Study of the Aging Male (ISSAM) was founded to conduct basic and clinical research, to provide postgraduate education and to engage in publicity activities for the enlightenment of the public. The social background is characterized by the appearance of a very rapidly aging society with longer average life spans. The importance of improving the health of the elderly and preventive medicine as government policy has increased. Improving the health of the elderly not only promotes self-reliance of the elderly but also increases the work force. A high QOL is also possible.

The main topic for healthcare in the 21st century is how to maintain the QOL of the elderly. In women, hormone replacement therapy (HRT) is widely applied internationally, but specific healthcare for elderly men appears to be limited to the widespread use of phosphodiesterase type 5 (PDE5) inhibitors to treat erectile dysfunction (ED). Although the delay in healthcare policies for elderly men is not a direct reason, a large gap has appeared between the average life spans of men and women in recent years and in Japan, men have a shorter life span than women by about seven years. In response to this sense of crisis, the World Health Organization (WHO) issued the Geneva Manifesto in 1997 and 'healthy aging for men' became an international movement. ISSAM was established in 1998 with the goal of 'aging male research on gender specific issues in male health'.

The first meeting of the society in Asia was held in Malaysia in 2001 and this topic was adopted on an international level from an early stage. The reason appeared to be strong economic and social concern that Asian countries with a current pyramid-type population distribution will become aging societies with a lower birth rate than in developing countries. Japan has already become an aging society with a low birth rate. In the national census (summary) in 2005, the elderly population of 65 years and older accounted for about 21% of the total population, the highest in the developed world.

In Japan, scientific research on the aging male started at about the same time as in the rest of Asia, and the Japanese Society for the Study of the Aging Male (JSSAM) was founded in November 2001 with Yoshiaki Kumamoto, professor emeritus of Sapporo Medical University, and Hajime Nawata, professor of Kyushu University as representative facilitators. The goal of this society is 'undertaking basic, clinical and social research and surveys on policies for the diagnosis, treatment and prevention of male-specific medical problems, and contributing widely to men's health by development, promotion and spread of proper healthcare'.

As mentioned above, the concept of research on the aging male is being promoted as 'healthy aging for men' but almost no actual treatment for such patients has been performed. When the JSSAM was established, so-called 'male climacteric symptoms' or 'male menopause' was popular in the media, and when such treatment was started, many patients mainly with a chief complaint of climacteric symptoms appeared in medical practice. These patients included many with psychiatric problems such as depression and considerable confusion arose in clinics and hospitals.

Based on this background, the Subcommittee on Endocrinology, Reproductive Function and Sexual Function of the Japanese Urological Association asked the Scientific Committee to prepare a clinical practice guideline, and a working group was organized to prepare the guideline by a collaborative team from the Japanese Urological Association and JSSAM after a review by the Board of Directors. In this clinical practice manual, the term 'late-onset hypogonadism (abbreviation: LOH)' syndrome was adopted as the term that best expresses this condition medically. In order to recommend standard procedures for diagnosis, treatment, prevention and monitoring of adverse reactions due to androgen replacement therapy (ART) and post-treatment assessments, a literature survey of clinical papers was performed, but since treatment of LOH Syndrome has just started, almost all papers had a low recommendation rank. Therefore, the name was changed to 'Clinical Practice *Manual* for Treatment of Late-onset Hypogonadism (LOH) Syndrome' ('Manual' hereinafter) instead of the initially planned 'clinical practice *guideline*'.

Care of LOH Syndrome is in its initial stages and such treatment requires careful consideration. Since many men visiting medical institutions at present complain of 'climacteric symptoms', measures must be taken to have this disease recognized in the mental health field. In the future, it will be necessary to establish evidence for treatment of LOH Syndrome from the broad perspective of promotion of 'healthy aging for men.' This 'Manual' is the first edition aimed at gathering evidence through future diagnosis and treatment of LOH and it is hoped that it will serve as a reference for routine medical practice.

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This is an English translation of text originally published in Japanese in 加齢男性性腺機能低下症候群(LOH症候群)診療の手引き, 2007, Jihou

Received 29 January 2008; accepted 31 January 2008.

Table 1-1 Signs and symptoms of Late-onset Hypogonadism (LOH) Syndrome

1)	The easily recognized features of diminished sexual desire (libido) and erectile quality and frequency, particularly diminished nocturnal erections.
2)	Changes in mood with concomitant decreases in intellectual activity, cognitive functions, spatial orientation ability, fatigue, depressed mood and irritability.
3)	Sleep disturbances.
4)	Decrease in lean body mass with associated diminution in muscle volume and strength.
5)	Increase in visceral fat.
6)	Decrease in body hair and skin alterations.
7)	Decreased bone mineral density resulting in osteopenia, osteoporosis and increased risk of bone fractures.

Lunenfeld *et al. Aging Male* 2005; 8: 56–58.

[1] Definition of LOH

The term 'andropause' was used in the past for hypogonadism of the aging male, but internationally, the expressions androgen decline in the aging male (ADAM) or partial androgen deficiency of the aging male (PADAM) have been widely used to express a 'set of symptoms associated with androgen deficiencies due to aging.' In Japan, the PADAM concept has also become established.[1-3] However, the pathophysiology of men with the so-called 'climacteric symptoms' visiting medical institutions is complex. Patients in the stage of early male climacteric show a high percentage of stress-related psychosomatic symptoms and in many cases, androgen deficiency symptoms come to the fore in the mature stage after late male climacteric. Male climacteric shows a complex pathophysiology and it cannot be explained simply as a deficiency in androgens associated with aging in all cases. Since PADAM and male climacteric were considered to have the same meaning, it cannot be denied that this has resulted in confusion in medical practice.

In a joint recommendation by the International Society of Andrology (ISA), ISSAM and the European Association of Urology (EAU) in 2005, use of the term 'LOH' was recommended,[4-6] which was defined as 'A clinical and biochemical syndrome associated with advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels. It may result in significant detriment in the quality of life and adversely affect the function of multiple organ systems'.[7] The key words in this definition are deficiency in androgen levels, aging, detriment in the quality of life and multiple organ dysfunction. The basic concept of 'healthy aging for men' is the prevention of reduction in organ functions caused by a deficiency in androgen levels associated with advancing age by androgen replacement.

In this Manual, the LOH Syndrome is used to accurately express this pathophysiology medically in keeping with this concept. Table 1-1 shows the signs and symptoms included in this syndrome based on the above recommendation.

[2] Diagnosis

LOH Syndrome starts with the evaluation of gonadal functions. Hormone testing is centered on testosterone blood levels and it is necessary to analyze test values based on an adequate understanding of biochemical diversity and characteristics. General laboratory tests and urological tests are useful in deciding the indications of ART as well as in the screening of the underlying diseases, and in simplifying the differential diagnosis of LOH Syndrome. LOH patients are often examined for unidentified complaints and questionnaires are essential in the differentiation from mental diseases, especially depression. Since relatively young men are also examined for this disorder, diagnosis without any predictions based on age is necessary.

Fig. 1 Forms of testosterone. Vermeulen A. Diagnosis of partial androgen deficiency in the aging male. *Ann. Endocrinol.* 2003; 64: 109–114.

1 Hormone testing[8]

1) Gonadotropin and other pituitary hormones

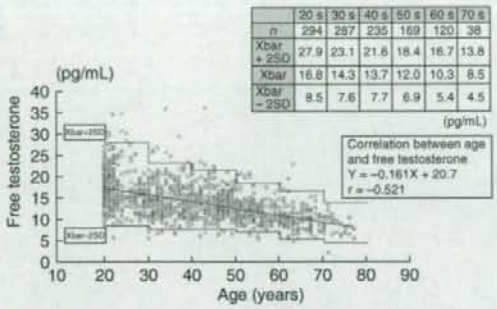
Sex hormones are controlled by precedence from the hypothalamus and pituitary gland and they can undergo changes caused by organic diseases such as tumors or inflammatory disease, aging or extrinsic factors such as drugs. The measurement of gonadotropin is useful in the differentiation between primary and secondary hypogonadism. Therefore, in diagnosis for LOH, it is necessary to measure pituitary hormones, the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). Prolactin (PRL) causes hypogonadism and it is recommended to measure PRL since hyperprolactinemia is caused by prolactin-producing tumors and by the adverse reactions of drugs such as sulpiride. Deficiencies in growth hormone (GH)/insulin-like growth factor (IGF-1) can explain reduced muscle strength, increased visceral fat and reduced bone density and their measurement is also useful.

2) Testosterone

The main androgen is the testosterone produced in the testes. However, the active testosterone in the blood is free testosterone, which makes up only 1–2% of total testosterone. Total testosterone consists of three fractions: the sex hormone binding globulin (SHBG)-bound testosterone, albumin-bound testosterone and free testosterone. Since albumin-bound testosterone can be easily separated from albumin, it is called bioavailable testosterone (BAT), which is biologically active, when combined with free testosterone (Fig. 1). SHBG however, is tightly bound to testosterone and this combination is biologically inactive. Since SHBG bound testosterone gradually increases with age, BAT is considered to show a relative decrease with no change in total testosterone. If total testosterone, SHBG and albumin are measured, it is possible to obtain calculated free testosterone and calculated BAT by such calculations (http://www.issam.ch/freetesto.htm).

3) Adrenal steroids

Because the adrenal androgens dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) gradually decrease with aging, they can serve as a senility indicator and might cause LOH signs and symptoms. Blood levels of cortisol basically show no changes throughout life, but



	20 s	30 s	40 s	50 s	60 s	70 s
n	294	287	235	169	120	38
Xbar + 2SD	27.9	23.1	21.6	18.4	16.7	13.8
Xbar	16.8	14.3	13.7	12.0	10.3	8.5
Xbar − 2SD	8.5	7.6	7.7	6.9	5.4	4.5

Fig. 2 Distribution of free testosterone with age.

they are known to be altered by stress, which makes these values useful in the differentiation of LOH and transient stress.

2 Standard values for ART indications

1) Overseas values

In the guidelines of the Consensus Committee of the Endocrine Society in the United States (2001),[9] the standard value for indication of ART is total testosterone of less than 2.0 ng/mL. For patients with values of 2.0–4.0 ng/mL, free testosterone and BAT are recommended as references. In the ISA, ISSAM and EAU recommendation of Lunenfeld *et al.* LOH standard value is a total testosterone of less than 8 nmol/L (2.31 ng/mL) and the normal value is not less than 12 nmol/L (3.46 ng/mL). Therefore, patients with values of greater than 8 and less than 12 nmol/L (2.31–3.46 ng/mL) are defined as borderline. In such patients, determination of calculated free testosterone is recommended and a diagnosis and treatment algorithm should be prepared.[7] According to the detailed policies of Nieschlag *et al.*,[10] total testosterone and SHBG are measured by a chemistry test using blood collected between 7 and 11 AM. Thus, the algorithm for diagnosis of LOH Syndrome overseas is based on total testosterone, not free testosterone.

2) Japanese values

From studies on healthy men in Japan however,[11] it was found that decreases in total testosterone with age are very slight, but free testosterone values decrease significantly with aging (Fig. 2). Total testosterone and free testosterone cannot be measured simultaneously for LOH Syndrome because of health insurance coverage. Therefore, the Working Committee on Clinical Practice Guidelines for Late-onset Hypogonadism recommends that free testosterone should be the diagnostic test for LOH Syndrome.

Free testosterone cannot be expressed uniformly as a mean value for the reason described previously. Therefore, the data in Figure 2 was used as the standard diagnostic criteria for LOH Syndrome and the normal lower limit was set at a mean−2SD value of 8.5 pg/mL for men in their twenties. Patients with values of greater than 8.5 pg/mL and less than 11.8 pg/mL, 70% of the mean value for men in their twenties (young adult mean: YAM) are recommended to be given ART as cases with a tendency toward low androgen levels (LOH borderline cases). The reason for applying the concept of the YAM percentage of the free testosterone value is that the mean values by age range only decrease to 80% from andropause through the mature stage, when LOH Syndrome occurs most frequently, using the YAM value of total testosterone. However, the YAM value of free testosterone shows a linear decrease with aging and drops to 50%, indicating that the effect of the decrease in the standard level with aging is more marked for free testosterone than for total testosterone. Even within the standard ranges (mean−2SD) of total testosterone and free testosterone, it is possible to detect an abnormal value with assessment using the YAM percentage. The YAM value is already applied in routine clinical practice for the evaluation of bone mineral density in osteoporosis based on evidence based medicine (EBM).[12]

The algorithm for diagnosis of LOH in Japan (Fig. 3) has been prepared for reference. Differences in the standard value of free testosterone in this Manual and the standard calculated free testosterone value for LOH recommended by ISA, ISSAM and EAU[7] are due to differences in the measurement and calculation methods and caution is required when making comparisons.[13]

3 Laboratory tests (Table 1-2)

1) General laboratory tests

There are currently no specific physical findings or test parameters for LOH Syndrome. At present, it is valid to use general parameters for excluding other serious diseases and prostate diseases and for assessment before treatment and during the course of treatment associated with ART. Table 1-2 shows the required minimum parameters for assessment as the essential parameters that can be measured routinely, and the optional values.

Androgens are known to act on erythrocyte production, glucose metabolism and lipids. In recent years, the metabolic syndrome based on visceral fat obesity has attracted attention and in consideration of the antiobesity effects of testosterone, LOH Syndrome might be complicated with the metabolic syndrome. Evaluation of the metabolic syndrome is performed using the BMI (height and weight) and the waist-hip ratio. Diagnostic criteria were published in April 2005 based on a consensus of eight societies including the Japanese Society of Internal Medicine.[14] A waist circumference of Japanese males of 85 cm or more, equivalent to a visceral fat area of 100 cm^2 or more on CT, was applied as the essential item in these diagnostic criteria.

2) Urological tests

A visual examination including the pudendal region is very important as an indicator of androgen deficiency of LOH.

1 Palpation of the testes and measurement of testicular volume

In palpation of the testes, epididymis, ductus deferens and spermatic cord are palpated in that order. The size and hardness or softness of the testes is especially important. Testicular volume is measured by a testicular ultrasound examination or a testicular volume meter.

2 Observation of body hair

It is important to observe changes in facial and pubic hair since they are often correlated with androgen concentration.

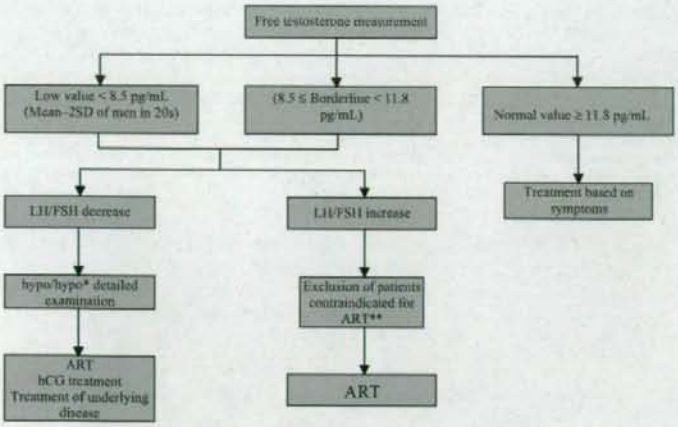
3 Evaluation of sexual function

(a) Sexual function is usually assessed by the International Index of Erectile Function (IIEF) or the simplified IIEF5. The results are useful in assessment of therapeutic effects.

(b) Nocturnal penile tumescence (NPT) and morning erection are simple and useful assessments of sexual function. The erectometer can also be used as a simplified method.

4 Prostate evaluation

(a) Evaluations of symptoms related to urination and voiding conditions are useful for differentiation from prostate dis-



*: Hypogonadotropic hypogonadism
**: Androgen replacement therapy

(Working Committee on Treatment Guidelines for Late-onset Hypogonadism)

Fig. 3 Algorithm of diagnosis of Late-onset Hypogonadism (LOH).

Table 1-2 Tests for Late-onset Hypogonadism (LOH)

Essential tests	
Physical findings	Height, weight, BMI, waist circumference (umbilical circumference), blood pressure, grip strength (both hands)
Examinations	Chest X-ray, ECG
Hematology	Especially hemoglobin, hematocrit, RBC count
Blood chemistry	Especially, TC, TG, HDL-C, LDL-C, GOT, GPT, ALP, γ-GTP, Ca, P
Urinalysis	Protein, glucose, occult blood
Glucose tolerance	FBS, HbA_{1c}
Tumor marker	PSA
Optional tests	
Bone mineral density	Dual energy X-ray absorptiometry (DEXA)
Body fat ratio	
Urological tests	
Physical tests	Testicular palpation, testicular volume measurement, pudendum (penis), body hair (facial hair, pubic hair), digital rectal examination of the prostate
Questionnaires	International Index of Erectile Function (IIEF) International Prostate Symptom Score (IPSS)

BMI, body mass index; DEXA, Dual energy X-ray absorptiometry; ECG, electrocardiogram; PSA, prostate specific antigen.

cases. The International Prostate Symptom Score (IPSS) is a diagnostic aid.

(b) A digital rectal examination of the prostate is important for diagnosis of prostatic hyperplasia and prostate cancer.

4 Questionnaires

1) Questionnaires used in LOH Syndrome diagnosis

Various symptoms are caused by reduced testosterone levels in the aging male, and questionnaires are widely used for screening. The most widely used questionnaire at present is the Aging males' symptoms (AMS) scale by Heinemann *et al.*[15,16] (Table 1-3). The self-rating questionnaire consists of five questions on psychological factors (questions 6–8, 11, 13), seven on physical factors (questions 1–5, 9, 10) and five on sexual function factors (questions 12, 14–17) for a total of 17 questions. Each question is answered in five grades: 'none', 'mild', 'moderate', 'severe' and 'extremely severe' and the grade is assigned one to five points. The AMS score was found to be effective for 116 men over the age of 40 and 992 German men over the age of 40 were tested for verification. It has now been translated into 14 languages and is useful in international comparisons of LOH symptoms.[17]

When this questionnaire was applied to urology department outpatients (without complaint for andropause), the overall severity was found to increase with aging, but it has often been reported that there is no clear correlation coefficient between the AMS score and blood levels of total testosterone.[18] No papers have appeared on the correlation with free testosterone to date and this point awaits further study. Since some questions show different nuances based on German culture such as Question 12 'Feeling that you have passed your peak' classified as a sexual factor, caution is required when using this questionnaire in Japan.

The Male Climacteric Symptom Scale by Kumamoto (MCS-K) (Table 1-4) is a questionnaire on male climacteric symptoms developed in Japan. In males with male climacteric symptoms, the total 'MCS-K' score and the AMS score show a significant correlation. However, this form has not been adequately validated for diagnosis and evaluation of LOH Syndrome and future studies are required.

2) Diagnosis of depression

The mental symptoms of LOH Syndrome are similar to those of depression and differentiation is difficult. As a mental disorder, depression is broadly classified into two types: major depressive disorders and dysthymic disorders. Diagnostic and Statistical Manual of Mental Disorders, the 4th Edition (DSM-IV), the diagnostic criteria of the American Psychiatric Association, is often used for diagnosis of depression, but structured interviews are recommended to increase the reliability of the results. For this reason, the Mini International Neuropsychiatric

Table 1-3 Aging males' symptoms (AMS) scale by Heinemann *et al.*

	Symptoms Points	None 1	Mild 2	Moderate 3	Severe 4	Extremely severe 5
1	Decline in your feeling of general well-being (general state of health, subjective feeling)	1	2	3	4	5
2	Joint pain and muscle ache (lower back pain, joint pain, pain in a limb, general back ache)	1	2	3	4	5
3	Excessive sweating (unexpected/sudden episodes of sweating, hot flushes independent of strain)	1	2	3	4	5
		1	2	3	4	5
4	Sleep problems (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)	1	2	3	4	5
5	Increased need for sleep, often feeling tired	1	2	3	4	5
6	Irritability (feeling aggressive, easily upset about little things, moody)	1	2	3	4	5
7	Nervousness (inner tension, restlessness, feeling fidgety)	1	2	3	4	5
8	Anxiety (feeling panicky)	1	2	3	4	5
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)	1	2	3	4	5
10	Decrease in muscular strength	1	2	3	4	5
11	Depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)	1	2	3	4	5
12	Feeling that you have passed your peak	1	2	3	4	5
13	Feeling burnt out, having hit rock bottom	1	2	3	4	5
14	Decrease in beard growth	1	2	3	4	5
15	Decrease in ability/frequency to perform sexually	1	2	3	4	5
16	Decrease in number of morning erections	1	2	3	4	5
17	Decrease in sexual desire/libido (lacking pleasure in sex, lacking desire for sexual intercourse)	1	2	3	4	5

Level of severity: 17–26 points, none; 27–36 points, mild; 37–49 points, moderate; more than 50 points, severe.
(Draft Japanese translation: Department of Urology, Sapporo Medical University School of Medicine)

Interview (M.I.N.I.) is widely used.[19,20] The M.I.N.I. was developed for the application of DSM-IV in routine practice and is designed to permit simple application in a short time.

Procedures for structured interviews using M.I.N.I. for major depressive disorders are shown in Table 1-5. With this procedure, the form is read out directly and an explanation is added if the meaning is not clear so that accurate replies can be obtained. First, there are two questions in a colored square. If the answers to both questions are 'No', it is judged negative for major depressive disorder and if the answer to either question is 'Yes', the diagnosis proceeds to the final stage based on the instructions.

Major depressive disorders are the most common form of depression and show a prevalence of 5–6% of the general population (3–4% of men). The test application of this questionnaire in 92 first-visit outpatients with andropause in a total of nine medical institutions nationwide in 2004 resulted in the diagnosis of 44 people (47.8%) with major depressive disorders. In people in their sixties, major depressive disorders were diagnosed in only about 20%, but in those in their forties and fifties, the figure was about 60%. Therefore, it appears that in addition to major depressive disorders as a complication of LOH, many patients, especially middle-aged ones, visit outpatient clinics for andropause among patients with major depressive disorders that are not associated with LOH.

Procedures for structured interviews using M.I.N.I. for dysthymic disorders are shown in Table 1-6. When the diagnosis of 'Major depressive episode – Current' is made as noted at the top of the page, this diagnosis is not considered.

The prevalence of dysthymic disorders is 10% of patients with major depressive disorders in the general population, but it is higher in middle-aged and elderly men. When testosterone levels were compared among groups with major depressive disorders, dysthymic disorders or healthy individuals in elderly men 60 years of age or older, it was reported that only the group with dysthymic disorders showed low levels. When the fact that major depressive disorders were few in men in their sixties visiting outpatient clinics for andropause as described above is also taken into consideration, it appears possible that dysthymic disorders are more closely related to LOH than major depressive disorders. Therefore, when symptoms such as a dejected mood occur and the diagnostic criteria for major depressive disorders are not met, it is necessary to consider a diagnosis of dysthymic disorders.

3) Severity assessment of symptoms of depression

The forms used for assessment of the severity of depression and changes in the severity of depression are basically divided into self-rating scales and observer rating scales. The self-rating scales include the Self-rating for Depression Scale (SDS), Beck Depression Inventory (BDI) and Hospital Anxiety and Depression Scale (HAD).[21,22] The most common observer-rating scale is the Hamilton Depression Rating Scale

Table 1-4 Male Climacteric Symptom Scale by Kumamoto (MCS-K)

	Symptoms	Almost none	Moderate	Severe	Very severe
A: Psychological factors	1. General physical condition not good, irritable	1	2	3	4
	2. Have trouble sleeping (insomnia)	1	2	3	4
	3. Feeling of anxiety, loneliness	1	2	3	4
	4. Often uneasy, depressed mood	1	2	3	4
B: Physiological factors	5. Hot flushes, light-headedness, excessive sweating	1	2	3	4
	6. Palpitations, shortness of breath, suffocating feeling	1	2	3	4
	7. Dizziness, nausea	1	2	3	4
	8. Tire easily (fatigue)	1	2	3	4
	9. Headache, head feels stuffy, neck feels stiff	1	2	3	4
	10. Lower back pain, joint pain in limbs	1	2	3	4
	11. Stiffness in limbs	1	2	3	4
	12. Numbness, tingling sensation, cold feeling in limbs	1	2	3	4
C: Sexual factors	13. Decrease in sexual desire	1	2	3	4
	14. Decrease in erectile power	1	2	3	4
	Symptoms	At least 2 or 3 times a fortnight	Once a week	Occasionally	Almost never
	15. Aware of morning erection	1	2	3	4
	Symptoms	At least 2 or 3 times a fortnight	1 or 2 times a month	Less than once a month	Almost never
	16. Frequency of sex	1	2	3	4
	Questions for reference				
	Symptoms	Almost none	Moderate	Severe	Very severe
Urination factors	Voiding difficulties, long time needed for urination	1	2	3	4
	Often has to urinate at night	1	2	3	4
	Cannot wait for urinary sensation, leakage	1	2	3	4

(HAM-D). None of these can be used for diagnosis and they should be used on the condition that they are applied only for assessment of the depressed state at the time of the replies. They are most useful in assessing changes in the depressed state associated with the course of treatment.

A depressed state is often only found when definite stress factors are present (usually persists for less than six months) and such cases are not very serious (they do not meet the diagnostic criteria for major depressive disorders). This is called a transient depressed state. When clinical problems arise in such patients, they are diagnosed as 'adjustment disorders with depressed mood' in DSM-IV. The symptom scores described here are useful in the evaluation of the severity of subjective symptoms.

4) Evaluation of ADL

Activities of daily living (ADL) are an important indicator of the mental and physical health status of the elderly. The Tokyo Metropolitan Institute of Gerontology (TMIG) Index of Competence (Table 1-7) is useful for evaluating independent activity levels in the elderly living in the community.[23] The Index consists of three factors: material self-reliance (questions 1–5), intellectual activeness (questions 6–9) and social role (questions 10–13). There are a total of 13 questions (5, 4 and 4, respectively). This is considered useful in assessment of delayed symptoms of LOH.

[3] Treatment

1 Usefulness of androgen replacement therapy (ART)

Androgens have many important physiological activities in men and they have affects on the muscle, bone, central nervous system, prostate gland, bone marrow and sexual function.

1. Actions related to sexual function include maintenance of sexual desire, ejaculation and erectile action.
2. A relation with maintenance of cognitive power and emotion is suggested but the actual relation remains unclear.
3. Reported actions on the muscles include enhanced muscle strength[24] and increased muscle mass and muscle strength.[25]
4. Actions on the bone include promotion of osteogenesis, and inhibition of bone resorption. Part of the bone mass maintenance action of testosterone occurs via the action of estrogen converted in the body. Many reports[24,26–30] have given an increase in bone mineral density as an effect of ART on bone.
5. The action on erythrocyte production involves a stimulation effect on erythrocyte production. In clinical studies, it was reported that hematocrit increased 2.0–5.0% during ART, above the normal value of 6–25%.[27,30–35] However, no significant increase in hematocrit was found when testosterone was administered percutaneously.
6. The effects on lipids and body fat include a decrease in body fat due to ART.[24,26,33] Total cholesterol and LDL cholesterol tend to decrease.

Table 1-5 Major depressive episode – The Mini-International Neuropsychiatric Interview (M.I.N.I), Japanese version 5.0.0 (2003), Modified

A1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	No	Yes
A2	In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?	No	Yes
	Is A1 or A2 coded Yes?	No	Yes
A3	Over the past two weeks, when you feel depressed or uninterested:		
a	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e. by ± 5% of body weight or ±3.5 kg for a 70 kg person in a month)? If yes to either appetite change or body weight change, code Yes.	No	Yes
b	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	No	Yes
c	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?	No	Yes
d	Did you feel tired or without energy almost every day?	No	Yes
e	Did you feel worthless or guilty almost every day?	No	Yes
f	Did you have difficulty concentrating or making decisions almost every day?	No	Yes
g	Did you repeatedly consider hurting yourself, feel suicidal or wish that you were dead?	No	Yes
	Are 5 or more answers (A1-A3) coded Yes?	**No**	**Yes**
		Major depressive episode, current	
	If a patient has a current major depressive episode, continue to A4. Otherwise, continue to **Table 1-6**, B1.		
A4			
a	During your lifetime, did you have other episodes of two weeks or more when you felt depressed, or uninterested in most things, and had most of the problems we just talked about?	No	Yes
b	In between two episodes of depression, did you ever have an interval of at least two months without any depression and any loss of interest?	**No**	**Yes**
		Major depressive episode, recurrent	

Diagnostic method

If either A1 or A2 are coded Yes and five or more answers of the nine questions from A1 to A3 are coded yes = major depressive disorder → **Major depressive disorders were handled by the departments of neuropsychiatry or psychosomatic medicine.**

If both A1 and A2 are coded No and less than 5 of the answers of the nine questions from A1 to A3 are coded Yes → **Move to 'Dysthymic disorders'** (Table 1-6)

7 In interventional research on coronary artery disease using testosterone, it was reported that electrocardiographic ST segment depression due to exercise was improved by ART in patients with coronary artery disease.[36-38] In short-term research, administration at the time of attacks was ineffective and had no effect on the number of attacks. It was also reported that the number of attacks could be decreased and the tolerated level of exercise increased. However, it is not clear if ART is connected with the prevention of the onset of coronary artery disease.

2 Indications for ART (Table 1-8)

1 ART is indicated for men over the age of 40 with signs and symptoms of LOH when there is a drop in the free testosterone blood level.
2 ART is first-line treatment when the free testosterone blood level is less than the 8.5 pg/mL, the mean–2SD of men in their twenties.[11]
3 ART should be considered for men with a free testosterone level of less than 11.8 pg/mL, 70% of the mean value (16.8 pg/mL) of men in their twenties (YAM value), i.e. greater than 8.5 and less than 11.8 pg/mL, which tends to be deficient but is within the normal range. Based on the severity of signs and symptoms, ART is a treatment option after the risks and usefulness of ART have been explained to the patient.
4 ART is not performed when the free testosterone blood level is 11.8 pg/mL or higher, and the following treatment is considered based on the symptoms. When sexual function symptoms are severe, a PDE5 inhibitor is administered. When psychological symptoms are severe, a neuropsychiatrist or psychosomatic physician is consulted and antidepressants or antianxiety agents are administered. When physical symptoms are severe, if osteoporosis is suspected, consultations are held with a specialist and drug therapy is considered, and for reductions in muscle strength, guidance is given on lifestyle improvements.

3 ART exclusion criteria

ART is not performed in patients with the diseases or conditions shown in Table 1-9.

4 ART protocols

1) Protocols

The following three ART protocols are recommended.

1 Testosterone enanthate is administered intramuscularly at 125 mg each time every two or three weeks or 250 mg each time every three to four weeks.

Table 1-6 Dysthymic disorders – The Mini-International Neuropsychiatric Interview (M.I.N.I), Japanese version 5.0.0 (2003), Modified

If the patient's symptoms currently meet criteria for a major depressive episode, do not explore this module.				
B1		Have you felt sad, low or depressed most of the time for the last two years?	No	Yes
B2		Was this period interrupted by your feeling OK for two months or more?	No	Yes
B3		During this period of feeling depressed most of the time:	No	Yes
	a	Did your appetite change significantly?	No	Yes
	b	Did you have trouble sleeping or sleep excessively?	No	Yes
	c	Did you feel tired or without energy?	No	Yes
	d	Did you lose your self-confidence?	No	Yes
	e	Did you have trouble concentrating or making decisions?	No	Yes
	f	Did you feel hopeless?	No	Yes
		Are two or more B3 answers coded yes?	No	Yes
B4		Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially or in some other important way?	No	Yes
		Is B4 coded Yes?	**No**	**Yes**
				Dysthymic disorder current

Diagnostic method

Dysthymic disorder (not major depressive disorder, but pathological depressed state continues for a long time)

→ **Handled by the departments of neuropsychiatry or psychosomatic medicine.**

If B1 is coded No, B2 is coded Yes, or less than two of the answers of the six questions from B3 is coded Yes = **Not dysthymic disorder**

If B1 is coded Yes, B2 is coded No, two or more of the answers of the six questions from B3 are coded Yes, and B4 Yes = **Diagnosed as dysthymic disorder**

Table 1-7 Tokyo Metropolitan Institute of Gerontology (TMIG) Index of Competence

These questions concern daily activities. Reply to the following questions by encircling either Yes or No. Please answer all questions.			
(1)	Can you go out alone using a bus or train?	1. Yes	2. No
(2)	Can you purchase your own daily necessities?	1. Yes	2. No
(3)	Do you prepare your own food?	1. Yes	2. No
(4)	Can you pay your bills?	1. Yes	2. No
(5)	Can you handle your bank or post office account by yourself?	1. Yes	2. No
(6)	Do you prepare documents concerning your pension, etc.?	1. Yes	2. No
(7)	Do you read the newspaper?	1. Yes	2. No
(8)	Do you read books or magazines?	1. Yes	2. No
(9)	Are you interested in articles or programs about health?	1. Yes	2. No
(10)	Do you visit the homes of friends?	1. Yes	2. No
(11)	Do you discuss matters with your family or friends?	1. Yes	2. No
(12)	Can you visit people who are sick?	1. Yes	2. No
(13)	Do you try to talk to young people?	1. Yes	2. No

Since the maximum testosterone blood levels are reached in about four to seven days after administration, caution is required because of the possibility that the serum testosterone levels will exceed normal values and reach a non-physiological level when the single dose is high. It is recommended that blood be collected once about four to seven days after administration and the concentration of free testosterone in the blood be measured.

2 Human chorionic gonadotropin (hCG) is administered intramuscularly at 3000–5000 units each time once or twice a week or every two weeks.

The hCG test is performed on patients with normal LH blood levels and if the response of testosterone in the blood is good, hCG is administered.[39] The advantage is that changes in blood testosterone are relatively small when compared with those of testosterone enanthate, and the disadvantage is that many administrations are required.

3 Testosterone ointment is applied at a dose of 3 g each time once or twice a day on the skin of the scrotum (equivalent to 3 mg of testosterone each time). Administration is easy and stable testosterone concentrations in the blood are obtained.[40]

2) Treatment period

With all methods, evaluation is performed every three months after the start of treatment. If effects are observed, treatment is continued with caution concerning adverse reactions.

Table 1-8 Indications of androgen replacement therapy (ART)

	Men over 40 years of age with the Late-onset Hypogonadism signs and symptoms and free testosterone blood levels as follows.
Less than 8.5 pg/mL	ART is first line therapy.
8.5 pg/mL to 11.8 pg/mL	Based on severity of signs and symptoms, ART is one treatment option after the risks and usefulness have been explained to the patient.
11.8 pg/mL and higher	ART is not performed and the following treatment is considered based on the symptoms. • Sexual function symptoms: phosphodiesterase type 5 (PDE5) inhibitor • Mental and psychological symptoms: consultation with a neuropsychiatrist or psychosomatic physician and administration of antidepressants or antianxiety drugs • Physical symptoms: consultation with a specialist on osteoporosis, drug therapy and guidance on life style improvements for reduced muscle strength

Table 1-9 Androgen replacement therapy (ART) exclusion criteria

• Prostate cancer	• Polycythemia
• Pretreatment PSA values of not less than 2.0 ng/mL	• Severe hepatic dysfunction
Treatment should be performed with caution when the PSA value is not less than 2.0 and less than 4.0 ng/mL	• Severe renal failure • Congestive heart failure
• Moderate to severe benign prostatic hyperplasia	• Severe hypertension
• Breast cancer	• Sleep apnea

PSA, prostate specific antigen.

3) Contraindicated concomitant medication

Contraindicated concomitant medication for male testosterone enanthate are warfarin potassium and other anticoagulants. Since the action of anticoagulants is intensified by concomitant administration, caution is required including reducing the dose of the anticoagulants.

[4] Adverse reactions of androgen replacement therapy (ART) and their monitoring

1 ART adverse reactions and complications

Androgens are steroid hormones that act on many organs and tissues. Risks that should be considered when performing ART include cardiovascular diseases, lipid metabolism disorders, polycythemia, fluid retention, prostatic hyperplasia, prostate cancer, hepatotoxicity, sleep apnea, gynecomastia, acne, testicular atrophy, infertility, and changes in behavior or mood.[3,41]

1) Adverse reactions

1 Cardiovascular diseases

It has been reported that the prevalence of coronary artery disease is high in patients with hypogonadism.[42,43] However, no effects of long-term ART on the cardiovascular system have been confirmed and cardiovascular testing is required depending on clinical symptoms.

2 Lipid metabolism

No adverse effects on lipid metabolism have been found with ART when blood testosterone levels do not exceed the physiological range during treatment.[44,45] Decreases in blood levels of HDL cholesterol have been observed at high doses.[44]

3 Polycythemia

Polycythemia that requires suspension of thrombectomy or cessation of ART is observed in 24% of patients with hypogonadism treated with ART.[46] During treatment, it is necessary to monitor polycythemia by periodic blood tests. The criteria for polycythemia are red blood cell (RBC) counts $6 \times 10^6/\mu L$ or more, hemoglobin of 18 g/dL or more and hematocrit of 53%. In such conditions, adjustment of ART intervals and consultations by a hematologist are needed.

4 Hepatotoxicity

Hepatic dysfunction has been found in about one third of patients administered testosterone orally,[47] but hepatic dysfunction due to oral administration of testosterone undecanoate or intramuscular administration of testosterone enanthate is rare.[48]

5 Sleep apnea

Since ART can exacerbate sleep apnea,[49,50] ART is contraindicated for patients with sleep apnea.

6 Other adverse reactions

Acne, increased body hair and flushing have been observed but they are not significant adverse reactions.[51]

2) Monitoring of ART adverse reactions (Table 1-10)

At baseline, physical examination and laboratory tests, an evaluation of voiding conditions using IPSS and history of sleep apnea should be ascertained. In hematology, hemoglobin, hematocrit and RBC count are especially important. In blood chemistry, TC, TG, HDL-C, LDL-C, GOT, GPT, ALP, γ-GTP, FBS and HbA_{1c} must be tested. Routine urinalysis including urinary sugar must be checked periodically. Blood tests should be checked two to four weeks, three months, six months and twelve months after the initiation of the treatment and once a year thereafter. Treatment must be discontinued or the dose is adjusted as required based on the test values. Voiding conditions and sleep apnea should be monitored and if abnormalities are found, treatment is discontinued or doses should be reduced, and then referred to specialists as required. Periodic cardiovascular examinations are not necessary, but when abnormalities are discovered by tests performed, ART should be discontinued and an evaluation by a specialist is needed.

2 ART and prostate disease

Caution is required in relation to prostate disease, which is a risk for ART. ART is absolutely contraindicated in cases of prostate cancer and relatively contraindicated for prostatic hyperplasia. ART and prostate disease are discussed from three standpoints: prostatic hyperplasia, prostate cancer and the serum prostate specific antigen (PSA) value.

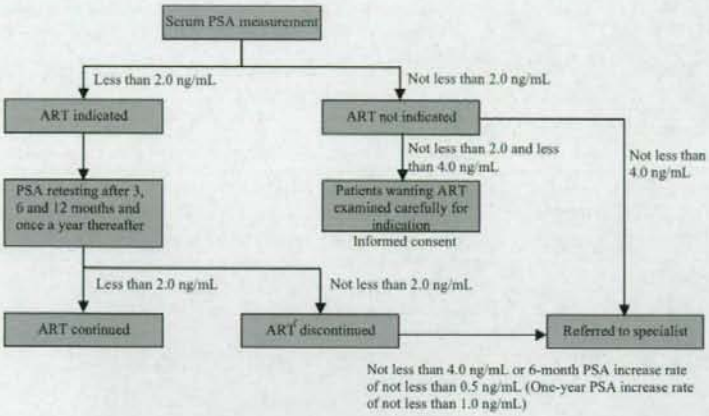


Fig. 4 Evaluation of PSA values at start of and during ART.

Table 1-10 Monitoring to avoid androgen replacement therapy (ART) adverse reactions

Course	Monitored items
Before treatment	• General examination and laboratory tests • Questionnaire on voiding conditions • Checking for sleep apnea • Serum PSA
After treatment	• Optimal dose set after 1–2 months based on clinical symptoms • Blood tests (after 2–4 weeks, 3 months, 6 months and 12 months and once a year thereafter) • Monitoring of voiding conditions and sleep apnea • Serum PSA

PSA, prostate specific antigen.

1) Effect of ART on prostatic hyperplasia and dysuria

In comparison with a placebo group, the ART group showed no worsening of voiding symptoms or complications due to prostatic hyperplasia.[24,30,52-57] In short-term ART (maximum of 36 months) in elderly men, no clear changes in prostate size, urinary flow rate or IPSS were reported.[58] However, the prostate is known to be androgen dependent[59] and prostate weight usually decreases with antiandrogen therapy. It is necessary to consider enlargement of the prostate due to ART.

2) Relation between ART and prostate cancer

There is little evidence that ART causes prostate cancer but there are case reports suggesting the progression from latent cancer to clinical cancer.[60,61] In prospective studies,[27,28,44,57,62] prostate cancer appeared in five out of 461 patients (1-%) given ART for 6–36 months, but this was the same as the general prevalence. However, the effects of long-term treatment for more than 36 months are unclear. Therefore, the serum PSA value must be carefully monitored as described below.

3) ART and Serum PSA values

Appropriate Serum PSA monitoring is important for early discovery of prostate cancer. The PSA criteria for ART are shown in Figure 4.

1 PSA of less than 2.0 ng/mL is recommended as the standard value for indicating ART. There is no evidence for this standard value. One report concluded that a standard value of 4.0 ng/mL is generally appropriate[41] while another report concluded that ART is not performed at 3.0 ng/mL. In addition, there are reports that latent cancer[63,64] and highly atypical cancer[64,65] are likely to occur in the low testosterone group. In this Manual, the PSA standard value was set at a low value.
2 ART should be considered after a careful investigation when PSA is greater than 2.0 and less than 4.0 ng/mL. In Western countries, ART is sometimes performed after prostate biopsies.[54]
3 When PSA is greater than 4.0 ng/mL or when other findings of suspected prostate cancer are obtained, a specialist should be consulted.
4 ART should be discontinued and a specialist consulted when PSA increases by greater than 0.5 ng/mL at six months or greater than 1.0 ng/mL at one year after start of ART.

[5] Assessment after treatment

Treatment of LOH is usually continued for a long time. When ART is performed, blood levels of testosterone basically increase in all patients. It is necessary to assess the signs and symptoms of LOH associated with improvements in blood testosterone levels as treatment effects. Since evidence has still not been established for the therapeutic effects on LOH Syndrome in particular, it is necessary to accumulate evidence by global evaluations. This is the objective of this Manual.

The therapeutic effects are usually evaluated at least every three-months at one year after the start of treatment and the intention of the patient, including continuing or discontinuing treatment, is confirmed. When treatment is performed for more than one year, it is important to observe variations in the symptoms periodically. In the official recommendation by ISSAM in 2002, it states 'Observation of the course during ART is the responsibility of both the physician and the patient. The necessity of periodic evaluations of patients by physicians must be stressed and the patients must agree to follow these requirements.' Thus, the importance of periodic evaluations by both physicians and patients is emphasized.[1] This recommendation includes the statement that 'Hormone replacement therapy is normally performed for life and observation of the course is also a lifetime duty.' However, time is required before this point is clearly understood in Japan. At present, it