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«ΓΥΝΑΙΚΕΙΑ ΑΝΑΠΑΡΑΓΩΓΗ»

Μεταπυχιακή - Διπλωματικη Εργασια

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ΑΘΗΝΑ Νοέμβριος 2019

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Hyperandrogenism in post-menopausal women
Ο υπερανδρογονισμός στις μετεμμηνοπαυσιακές γυναίκες

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Υπερανδρογονισμος στις μετεμμηνοπαυσιακές γυναικες

Περίληψη

Η εμμηνόπαυση είναι η περίοδος στη ζωή της γυναίκας η οποία χαρακτηρίζεται από τη μόνιμη διακοπή της εμμηνόρροιας και που σχετίζεται με ορμονικές αλλαγές εκ των οποίων η πιο σημαντική είναι η μείωση των οιστρογόνων.

Μετά την εμμηνόπαυση μειώνεται επίσης, η συγκεντρώση των κυκλοφορούντων ανδρογόνων. Η εκκρισή τους ωστόσο, επάγεται από την αυξημένη συγκέντρωση της ωχρινοτρόπυ ορμόνης (LH), η οποία δρα στις ωοθήκες πιθανώς και στα επινεφρίδια. Η περιφερική μετατροπή των ανδρογόνων που οφείλεται σε βιοχημική μετατροπή, κυρίως στον λιπώδη ιστό, συμβάλλει επίσης στο προφίλ των κυκλοφορύντων ανδρογόνων στην εμμηνόπαυση.

Ορισμένες παθολογικές καταστάσεις συνδέονται με μεγαλύτερες συγκεντρώσεις ανδρογόνων μετά την εμμηνόπαυση σε σύγκριση με τους ομάδες ελέγχου, με πιο συχνό το σύνδρομο πολυκυστικών ωοθηκών (PCOS). Αυτές οι παθολογικές καταστάσεις μπορούν να χωριστούν σε δύο κύριες κατηγορίες: σε καταστάσεις, που δεν οφείλονται σε όγκους ή λειτουργικές και σε καταστάσεις που οφείλονται σε όγκους (καλοήθεις ή κακοήθεις μάζες, είτε στα επινεφρίδια ή στις ωοθήκες).
Εκτος από το PCOS που παραμένει το συχνότερο αίτιο του υπερανδρονισμού στις μετεμμηνοπαυσιακές γυναίκες, λιγότερο συχνές είναι: η παχυσαρκία, η μη κλασσική συγγενής υπερπλασία των επινεφριδίων (NCCAH), οι ενδοκρινοπάθειες, όπως το σύνδρομο Cushing ή η ακρομεγαλία, η υπερθήκωση των ωοθηκών, η χρήση ή η κατάχρηση φαρμάκων.
Αίτια υπερανδρογονισμού που οφείλονται σε όγκους περιλαμβάνουν τον καρκίνο του φλοιού των επινεφριδίων, τα αδενώματα των επινεφριδίων και τους όγκους

ωοθηκών όπως οι όγκοι του γοναιδικού στρώματος των ωοθηκών και οι μεταστάσεις άλλων καρκίνων στις ωοθήκες.

Η διάγνωση του υπερανδρογονισμού γίνεται με τη λήψη ιστορικού, την κλινική εξέταση και τις εργαστηριακές και απεικονιστικές εξετάσεις.
Η συνολική συγκέντρωση τεστοστερόνης 150 ng/dl μπορεί να χρησιμοποιηθεί για την αρχική διαφορική διάγνωση ενός αιτίου υπερανδρογονισμού που οφείλεται σε όγκο από ένα λειτουργικό αίτιο.
Η συγκέντρωση θειικής δεϋδροεπιανδροστερονής (DHEAS) μπορεί να υποστηρίζει την πηγή ανδρογόνων από τα επινεφρίδια.
Οι τεχνικές απεικόνισης χρησιμοποιούνται για τον εντοπισμό της πηγής των ανδρογόνων: η αξονική τομογραφία και η μαγνητική τομογραφία για τα επινεφρίδια και το διακολπικό υπερηχογράφημα ή η μαγνητική τομογραφία για τις ωοθήκες.

Σε αυτή την βιβλιογραφική ανασκόπηση περιγράφονται επίσης, η αιτιολογική και συμπτωματική θεραπεία και οι μακροπρόθεσμες επιδράσεις του υπερανδρογονισμού στις μετεμμηνοπαυσιακές γυναίκες.

Hyperandrogenism in post-menopausal women

Abstract

Menopause is the time in the life of women which is characterized from the permanent cessation of menses associated to hormonal changes, of which the most important is the decrease of estrogens.

Following menopause the concentrations of circulating androgens decrease. However, the increased concentrations of LH induce their secretion from the ovaries and presumably from the adrenal glands. Peripheral conversion of androgens due to biochemical transformations mainly in the adipose tissue, contributes to the circulating hormonal androgen profile.

Some pathological conditions are associated with greater concentrations of androgens after menopause than in controls, with polycystic ovary syndrome (PCOS) being the commonest. These pathological conditions can be divided in two main categories: non-tumorous or functional and timorous: benign or malignant masses, either in adrenals or the ovaries.
Apart from PCOS, which is the most frequent cause also in menopause, other non-tumorous causes of hyperandrogenism in post-menopausal women are: obesity, non-classic congenital adrenal hyperplasia, (NCCAH), endocrinopathies, such as Cushing syndrome or acromegaly; ovarian hyperthecosis, drug use or abuse.

Tumorous causes include adrenal cortical cancers, adrenal benign adenomas and even incidentalomas, or ovarian tumors, such as the sex-cord stromal ovarian tumors and metastases in the ovary.

The diagnosis of hyperandrogenism is made through the history, clinical examination, and laboratory tests.

Total testosterone concentration of 150 ng/dl can be used at first to distinguish a tumorous from a non-tumorous cause of hyperandrogenism.
DHEAS concentration may support adrenal source of androgens.

Imaging techniques are used to localize the source of androgens: computed tomography and MRI for the adrenals and transvaginal ultrasound or MRI for the ovaries. Treatment (etiologic and symptomatic) and long term effects of hyperandrogenism are developed in this overview.

Keywords

Menopause, menopausal transition, post-menopause, androgens, testosterone, DHEA, DHEAS, androstenedione, hyperandrogenism, hirsutism, virilizing tumors.

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Abreviations

ACC	Adrenocortical Carcinoma
ACTH	Adrenocorticotropic hormone
AFC	Antral Follicle Count
AMH	Anti Mullerian Hormone
A4	Androstenedione
A5	Androstenediol
BMI	Body Mass Index
CT	Computed Tomography
CVD	Cardiovascular Disease
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulfate
DHT	Dihydrotestosterone
FAI	Free Androgen Index
FDG PET	Fludeoxyglucose Positronic Emission tomography
FSH	Follicle Stimulating Hormone
GH	Growth Hormone
GnRH	Gonadotropin Releasing Hormone
hCG	Human Chorionic Gonadotropin
HU	Hounsfield Units
IGF-1 & 2	Insulin-like Growth Factor 1& 2
KS	Ketosteroids
LC-MS/MS	Liquid Chromatography tendem Mass Spectrometry
LH	Luteinizing hormone
LHRs	Luteinizing Hormone receptors
LA	Laparoscopic Adrenalectomy
LDDST	Low Dose Dexamethasone Supression Test
MRI	Magnetic Resonance Imaging
MS	Metabolic Syndrome
NAMS	North American Menopause Society
NCCAH	Non Classic Congenital Adrenal Hyperplasia
OA	Open Adrenalectomy
OAST	Ovarian Androgen Secreting Tumors
PCOS	Polycystic Ovary Syndrome
POI	Premature Ovary Insufficience
PSU	Pilosebaceous Unit
RA	Robotic Adrenalectomy
RCT	Randomize Controlled Trials
ROS	Reactive Oxygen Stress
SHBG	Sex Hormone Binding Globulin
STRAW	Stages of Reproductive Aging Workshop
SWAN	Study of Women's health Across the Nation
T	Testosterone
T2DM	Type 2 Diabetes Mellitus
17OHP	17-hydroxyprogesterone

1. Introduction

Menopause is the period of time in the life of a woman when menstrual cycles cease permanently, due to the lowering of concentrations of ovarian estradiol below a certain threshold. This physiological phenomenon reflects the decrease in number of oocytes in the ovaries and it is associated with the permanent cessation of ovulation. During post-menopause, the amount of androgens secreted by the ovary decreases gradually. In fact, the concentrations of circulating testosterone (T), as well as of androgen precursors, decrease with aging in post-menopausal women, even though transient increases have been reported, mostly during menopausal transition, as shown in the Study of Women's Health Across the Nation (SWAN), regarding mainly dehydroepiandrosterone sulfate (DHEAS), an androgen predominantly of adrenal origin [1].

Hyperandrogenism is a clinical syndrome which accompanies various pathologic conditions encountered in girls and women [2]. In menopause, it expresses a relative or an absolute excess of circulating androgenic hormones or androgens of adrenal or ovarian origin and it is characterized by dermatologic stigmata, such as acne, oily skin, seborrhea and skin inflammation, hidradenitis suppurativa, dystopic increase in terminal hair growth (hirsutism) or loss of hair (alopecia), virilization, as well as adverse metabolic phenomena. The latter are more intense when associated to insulin resistance. In some cases, although the circulating concentrations of androgens are found within normalcy, the increased sensitivity of the androgen receptor at the hair follicle might result to idiopathic hirsutism [2].

Polycystic ovary syndrome-related hyperandrogenism is the most frequently encountered condition during the fertile life of women. It persists after menopause, still representing the most frequent cause of hyperandrogenism [3]. It often manifests in puberty and is characterized by specific clinical, biochemical and morphological criteria [4]. During menopause, circulating androgens decrease in women diagnosed with PCOS before menopause, although remaining greater than those of non-PCOS

post-menopausal women (at least during the early years after menopause) [5]. Apparently stromal and thecal androgen production in post-menopausal PCOS women might still be responsive to the increased post-menopausal concentrations of the luteinizing hormone (LH) for some time [6].

Other causes of hyperandrogenism in post-menopausal women can be of non-tumorous (functional) or tumorous origin (adenomas and carcinomas). The former include obesity which is associated to insulin resistance-linked conditions, endocrinopathies, i.e. Cushing syndrome, acromegaly, hyperprolactinemia and hyperthyroidism, as well as pharmaceutical use- or abuse- associated conditions; while the latter are related to tumors, mainly of adrenal or ovarian origin [5].

This review of bibliography will present the causes of hyperandrogenism in post-menopausal women. There is followed the traditional didactic approach including physiology, presentation of clinical characteristics, causal pathophysiology (etiology) and diagnosis. There are suggested methods of management and treatment and an algorithm of diagnosis is proposed.

2. Menopause and staging

Menopause marks the permanent end of fertility in women. The diagnosis of menopause is based on the absence of menstruation for at least 12 consecutive months. The average age of menopause is around 51 years depending on various factors. These factors include geographic area and lifestyle. The influence of genetic and environmental factors on menopause age is still under investigation. Earlier menopause is observed in women living in developing countries (eg. Indonesia, Pakistan and Chile) and in women living in rural areas compared to those living in developed countries [7-10]. Also, women living at higher altitudes experience earlier menopause [11, 12]. Smoking is associated with earlier menopause (1 to 2 years earlier) compared to non-smoking habit [13, 14]. After menopause, an average life expectancy longer than 30 years is estimated for women in developed countries [15, 16].

The Stages of Reproductive Aging Workshop (STRAW) criteria of 2001, categorized, for the first time, the life of woman in three phases: reproductive, menopausal transition and post-menopause.

These criteria were revised in September 2011, during the Annual Meeting of North American Menopause Society (NAMS) and they were renamed STRAW +10 (Figure1) [17]. The criteria in this system mostly depend on the characteristics of the menstrual cycle, as a key indicator of ovarian age. Nevertheless, the hormonal criteria of ovarian aging, such as elevated follicle-stimulating hormone (FSH), low anti-Müllerian hormone (AMH) and inhibin B concentrations are also taken into consideration [17]. Menstrual cyclicity and circulating concentrations of early follicular phase FSH were employed as primary determinants of this categorization. Stages -2 and -1 refer to early and late menopausal transition; stages +1 (+1a, +1b, +1c) and +2 refer to early and late post-menopause. Stages -2, -1 and +1a include a period defined also as peri-menopause. The increased variability in menstrual cycle duration (over 7 days) is persistent in women with elevated FSH concentrations, low

AMH concentrations and low antral follicle count (AFC). The term persistence denotes the repeated occurrence (during 10 cycles starting from the first cycle) of variable cycle duration.

Stage	-2	-1	+1 a	+1b	+1c	+2
Terminology	**MENOPAUSAL TRANSITION**		**POSTMENOPAUSE**			
	Early	Late	Early			Late
	Perimenopause					
Duration	*variable*	1-3 years	2 years (1+1)		3-6 years	*Remaining lifespan*
PRINCIPAL CRITERIA						
Menstrual Cycle	*Variable Length* Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of >=60 days				
SUPPORTIVE CRITERIA						
Endocrine FSH AMH Inhibin B	↑ Variable* Low Low	↑ >25 IU/L** Low Low	↑ Variable Low Low		Stabilizes Very Low Very Low	
Antral Follicle Count	Low	**Low**	Very Low		Very Low	
DESCRIPTIVE CHARACTERISTICS						
Symptoms		Vasomotor symptoms *Likely*	Vasomotor symptoms *Most Likely*			*Increasing* symptoms of urogenital atrophy

Figure 1: Schematic representation of the stages from menopausal transition to postmenopause according to the Stages of Reproductive Aging Workshop +10 staging system for reproductive aging in women (modified from reference 17).

Although duration of stage -1 differs among women, it generally lasts 1 to 3 years. It is characterized by vasomotor symptoms and intervals of amenorrhea of 60 days or longer. Concentrations of FSH greater than 25 IU/L in a random blood sample, denote late menopausal transition. The menopausal transition ends with the final menstrual period (stage 0). It is recognized after 12 months of amenorrhea and corresponds to the end of stage +1a and peri-menopause. Follicle-stimulating hormone continues to increase, as estradiol continues to decrease for approximately two years, in the end of stage +1b. The period of stabilization of high FSH and low estradiol represents stage +1c and it lasts approximately 3-6 years. In total, early post-menopause lasts approximately 5-8 years [17].

The criteria of STRAW +10 are applicable in most women, irrespective of age, body mass index (BMI), demographic characteristics or lifestyle, while age is not used as a criterion. They are not easily applicable in some cases such as premature ovarian insufficiency (POI) or PCOS, for which amenorrhea is part of the pathologic manifestations [17].

3. Physiology of androgen production in menopause

3.1. Major androgens in menopause

The major androgens found in circulation are T, Androstenedione (A4), dehydroepiandrosterone (DHEA) and its sulfated form, DHEA sulfate (DHEAS). They are produced from the ovaries, the adrenals and peripheral tissues (hair follicles, the sebaceous glands, the external genitalia). (Figure 2).

Testosterone: During the reproductive life of a woman, T is derived approximately at equal quantities from adrenals and ovaries. These glands contribute about 50% of the plasma T concentration, with the remaining 50% coming from peripheral conversion of androgen precursors produced again at equal quantities from the adrenals and the ovaries (Figure 2) [18]. Total T concentrations decline with aging showing a 50% decrease and loss of mid-cycle pick in pre-menopausal women compared to 21 year old women. At post-menopause T concentrations decrease by 15% [19, 20]. Certain studies did not find changes of T concentrations during menopausal transition. Although T concentrations decrease at post-menopause, a state of relative hyperandrogenism is expressed clinically, due to the marked decline of estrogen concentrations at the same time [21].

Androstenedione: Production of A4 decreases with age. At post-menopause, ovarian production of sex steroids such as A4 decreases, although it appears to persist even 10 years after menopause [22]. This persistent ovarian androgens secretion represents probably the stimulation of ovarian interstitial cells by elevated post-menopausal LH concentrations [20].

DHEA: In post-menopause, DHEA is a unique source of sex steroids [23]. It is the major androgen synthesized in the adrenal gland, while DHEA sulfotransferase, an

enzyme which catalyzes the transformation of DHEA to DHEAS, is primarily

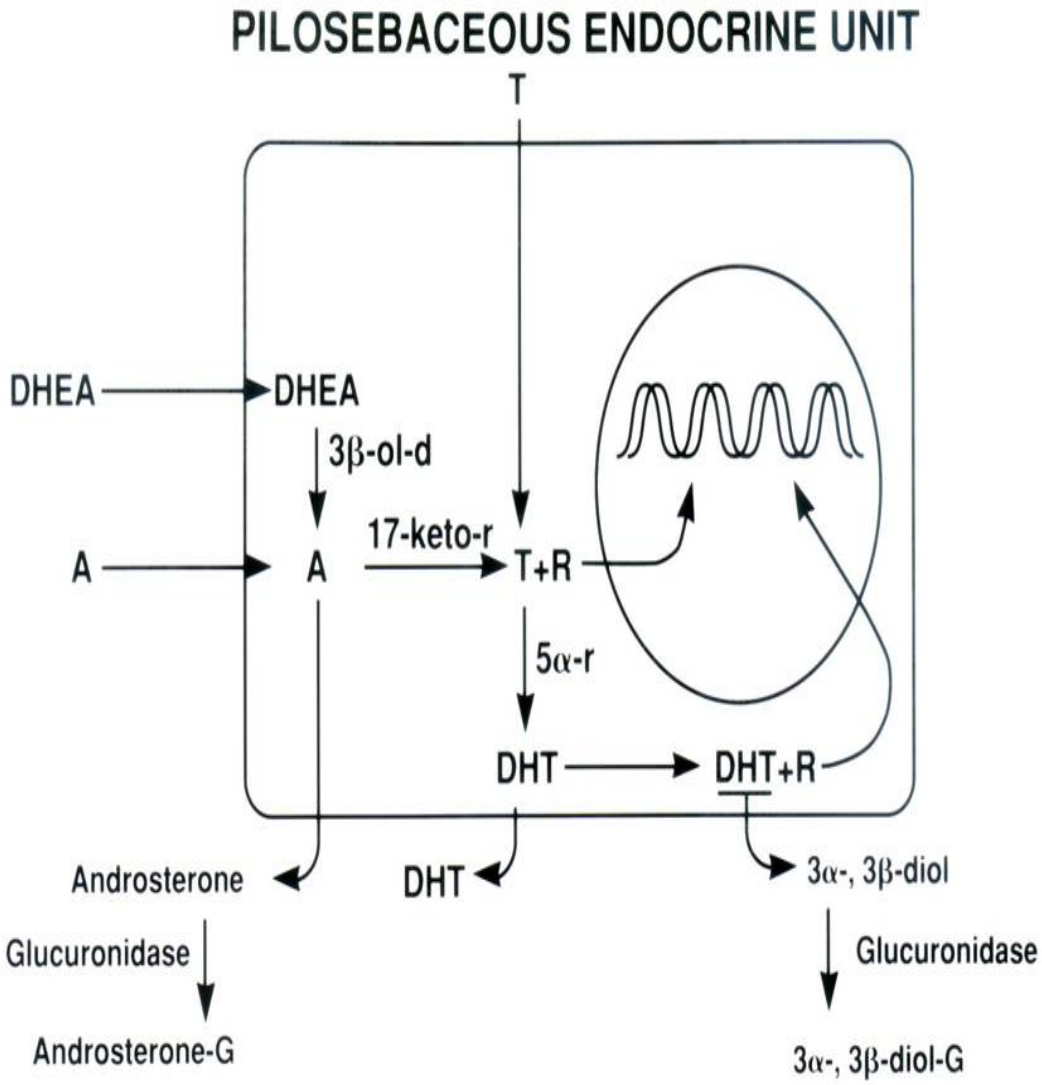

Figure 2: *Pilosebaceous endocrine unit*: DHEA stands for dehydroepiandrosterone; A stands for androstenedione; T stands for testosterone; R stands for androgen Receptor; DHT stands for dihydrotestosterone; 3α- and 3β- diol stand for alpha- and beta- androstendiol, respectively; G stands for glucuronide; 3β-ol-d stands for 3β-ol dehydrogenase; 17-keto-r stands for 17-ketosteroid reductase; 5α-r stands for 5α reductase.

expressed in the adrenals. Because ovaries do not possess this enzyme, ovarian DHEA is not transformed to DHEAS. The latter is a stable molecule with a long half-life. Due to this characteristic its measurement is more frequent than that of DHEA in the routine clinical practice, because its concentrations in peripheral circulation do not fluctuate over the nycthemeral. Approximately 80% of circulating DHEA is of adrenal origin, whereas the ovaries are responsible for only 20% of circulating DHEA. Thus, it is a reliable indicator of adrenal androgens production in post-menopausal women. Despite their weak androgenic activity DHEA and DHEAS are the major precursors for more active androgens or estrogens to which they convert in peripheral tissues (Figure 3) [24].

The production of adrenal androgens decreases gradually with age. By the time of menopause, peripheral DHEA concentrations are reduced by 60% compared to their peak concentrations earlier in life. They are found only at 10-20% of their peak concentrations in the eighth and ninth decade of life marking at this point the adrenopause [25]. The latter occurs in both sexes at a similar period. This might be due to the reduction of 17,20-lyase activity in the adrenals with aging and the decrease of the volume of the adrenal reticular zone, a process that might be as a result of the decrease of insulin-like growth factors (IGF-1 and IGF-2) concentrations [5]. Dehydroepiandrosterone secretion is not controlled by any feedback mechanism and in this way the low circulating concentrations of DHEA will gradually create a deficit of sex steroids in post-menopausal women, if it remains untreated in menopause [28, 29]. Other studies have shown slight increase of SHBG concentrations associated to total T increase found in peripheral circulation in late post-menopausal women [20, 30].

Of note, the conversion of DHEA and DHEAS to other androgens or estrogens in peripheral tissues in post-menopausal women [31, 32] and the locally synthesized T do not require SHBG for transport. Because the biological effects of these hormones are exerted locally within the target tissues [33, 34], circulating T concentrations are not indicative of intracellular bioavailable T.

The circulating conjugated metabolites of dihydrotestosterone (DHT) (androsterone glucuronide, ADT-G; 3 alpha-androstendiol-glucuronide, 3α-diol-G; 3 beta-androstendiol-glucuronide, 3β-diol-G) are good markers of intracellular testosterone activity in peripheral tissues. Serum androsterone sulfate (ADT-S) concentration, is also reported to be a useful marker reflecting total androgen pool. However, because different enzymes, such as ADT-glucuronyltransferase and steroid sulfotransferase, catalyze the conjugation of the various androgen metabolites to glucuronides or

sulfates, differential changes in the concentrations of ADT-G, 3α-diol-G, 3β-diol-G and ADT-S are possible and should be taken into consideration [35].

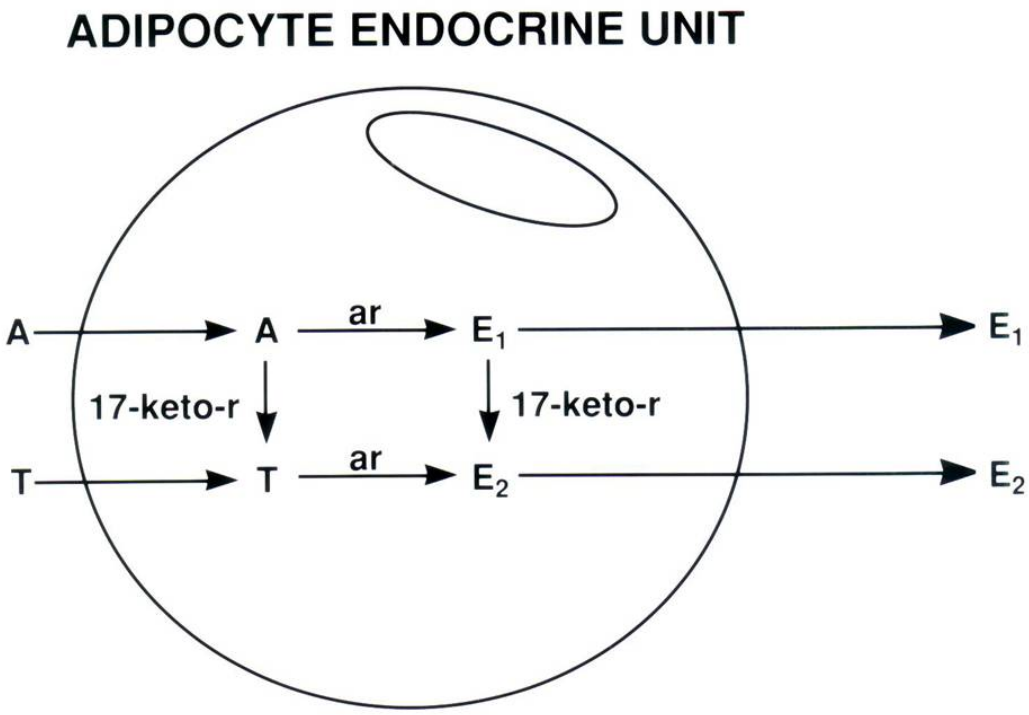

Figure 3: *Adipocyte endocrine unit*: A stands for androstenedione; T stands for testosterone; E_1 stands for estrone; E_2 stands for estradiol; ar stands for aromatase;

3.2. Androgens of ovarian origin in menopause

Following menopause, ovary turns from a reproductive to a non-reproductive gland, contributing however, to the hormonal *milieu* of the aging woman. Ovarian androgens are produced by stromal interstitial cells, under the influence of the increased menopausal LH concentrations.

In 1974, Judd et al measured the concentrations of sex steroids and their difference between the ovarian and peripheral veins in post-menopausal women, who did and did not undergo oophorectomy. They concluded that post-menopausal ovary continues to secrete a large amount of T and a moderate amount of A4, beside the only minimal ovarian estrogen secretion [36]. Later, in 1986, Longcope et al concluded that in about 50% of post-menopausal women ovaries continue to secrete some T, but little A4 or DHEA [18]. In 2000, in the Rancho Bernardo study, it was suggested that post-menopausal ovary remains a critical source of androgen production in older women. In women with intact ovaries, T concentrations were low around menopause, while an increase in total T (similar to pre-menopausal concentrations), but not that of bioavailable T, was reported in the 70-79 age group. In contrast, in women who underwent bilateral oophorectomy, a pronounced and sustained reduction, in both total and bioavailable T concentrations, was shown [37]. In 2002, H.G. Burger supported that post-menopausal ovary is an androgen-secreting organ and that the concentrations of T are not directly influenced by menopausal transition [38]. In post-menopausal oophorectomized women A4 concentrations decreased post-operatively by 30% while T concentrations did not [39].

Overall, it is believed that, at the time of menopause, the production of ovarian androgens decreases, while in post-menopause a relative increase of ovarian androgens of stromal origin takes place [1].

A decade after menopause circulate approximately half of T and A4 concentrations compared to those circulating at 40 years of age [16].

3.3. Androgens of adrenal origin during menopause

In adrenals, androgens are mainly produced in *zona reticularis*, in response to adrenocorticotropic hormone (ACTH). Interestingly, an increase in adrenal delta 5

steroids, DHEA and androstenediol (A5) and modestly of DHEAS concentrations was observed in almost 85% of women during menopausal transition [40]. It has been postulated that this increase could be attributed to the increased circulating LH concentrations during menopausal transition, forming the hypothesis of a gender specific difference in adrenal androgen production, but still, solid and direct evidence for this mechanism is missing [40]. Perhaps LH receptors (LHRs) are expressed in the human *zona glomerulosa* and other cortical zones as it has been described in the non-human primate animal model. Indeed, in experimental models with primates (either oophorectomized or treated with GnRH analogs), delta 5 steroids of adrenal origin have been shown to increase in response to an acute stimulation by human chorionic gonadotropin (hCG), evoking the presence of LHRs in adrenal cortices [41].

The contribution of adrenal glands in androgen production after menopause is confirmed indirectly from studies in oophorectomized post-menopausal women with primary adrenal insufficiency [42]. In these patients the concentrations of baseline circulating androgens were near or below the detection limit of the assays employed. Dexamethasone suppression test in post-menopausal women with intact adrenals also leads to a dramatic depression of circulating androgens [42, 43].

3.4. Androgens from peripheral conversion in menopause

Weak steroid precursors of ovarian and adrenal origin can be converted to potent androgens in peripheral tissues (Figure 2).
Major conversions are: A4 into T and T into DHT.
Major peripheral sites of androgen conversion are adipose tissue, hair follicles and sebaceous glands, in the pilosebaceous unit (PSU) and external genitalia.
During menopause, A4 from ovarian stromal cells and DHEA from the adrenal cortex, convert into T primarily in the adipose tissue [18]. In addition, in adipose tissue, active uptake of androgens and *in situ* estrogens synthesis occur due to high activity of the enzymes aromatase and 17-ketosteroid reductase (Figure 3). The former is responsible for the conversion of A4 and T to estrone and estradiol, respectively, while the latter converts A4 and estrone to T and estradiol, respectively. Glucocorticoids stimulate aromatase activity. Interestingly, different phenotypes of obesity are associated with different levels of hormonal conversions. For instance,

although both women with either upper or lower body obesity demonstrate increased aromatization of A4 to estrone and T to estradiol, greater androgen production rates and elevated free T concentrations are manifestated in the former, possibly due to increased insulin resistance and functional ovarian hyperandrogenism [44].

Menopause-associated changes in body composition include, amongst others, a progressive accumulation of intra-abdominal fat [45, 46]. Abdominal obesity is a risk factor for a multitude of pathologies, including cardiovascular disease (CVD), Type 2 Diabetes Mellitus (T2DM) and metabolic syndrome (MS) [47], thereby representing a serious threat to post-menopausal women's health.
Recent findings indicate that unbound free T concentrations are not associated with CVD or T2DM risk in menopausal women. Low SHBG concentrations, caused from the absence of estrogens in menopause, appear to be a strong predictor of these pathologies [48-50], questioning what has been previously reported for free T [51, 52]. Conversely, with respect to fat accumulation, recent data suggest that free T and DHEAS concentrations in early and late post-menopausal women, respectively, are positively associated with abdominal fat accumulation, whereas the latter cannot be predicted independently by SHBG concentrations [50]. Thus, SHBG correlation with development of CVD and T2DM, might be mediated by effects on insulin resistance [49].

Abdominal fat accumulation is found to be initiated during early post-menopause, in which T secretion is actively driven by ovarian stroma [53]. Of note, in the adipose tissue are expressed enzymes catalyzing the conversion of DHEAS to T [54]. This conversion might be of crucial importance, especially with aging, as the majority of T production is not SHBG-dependent, but is rather derived from DHEAS conversion in peripheral tissues, while T in its turn is aromatized to estrogens in the adipose tissue (Figure 3) [50]. Nonetheless, whether the observed changes in body fat distribution are the cause or consequence of changes in androgen production during menopause, remains elusive.

3.5. Intracrinology and enzymatic changes in menopause

Intracrinology was introduced by F. Labrie to support the idea that each cell in peripheral tissues makes a small and appropriate amount of estrogens and

androgens from the inactive precursor DHEA. The presence of 30 steroid-forming enzymes specific for each peripheral tissue, which catalyze the conversion of DHEA into the appropriate small amounts of estrogens and androgens, for a strictly intracellular and local action, independent from the rest of the body, has been documented [55].

On the other hand, humans, unlike species below primates, possess intracellular steroid-inactivating enzymes, especially glucuronyl transferases and sulfotransferases, which inactivate the estrogens and androgens at their local sites of formation, thus preventing the release of a biologically significant amount of estradiol and T in the circulation [56]. Thus, these authors postulate that, ultimately DHEA becomes the unique source of intracellular sex steroids after menopause.

4. Etiology and pathophysiology of female hyperandrogenism in post-menopause

There is proposed a classification for the etiologies of hyperandrogenism during menopause which is presented in Table 1.
At first, these etiologies can be distinguished in non-tumorous and tumorous. The former include functional hyperandrogenic conditions

4.1. Hyperandrogenism of non-tumorous etiologies

4.1.1 Endogenous etiologies

Obesity: Aging and specifically menopause are associated with increase in fat mass, particularly in the visceral area [43]. In obesity, supply outweighs demand and extracellular nutrients, hyperinsulinemia and glucocorticoids trigger intracellular stress. The latter leads to the increase of mitochondrial reactive oxygen species generation (ROS), which may be a physiological mechanism for glucose preservation (e.g. in fasting). Nonetheless, in presence of nutrient excess this process becomes pathological. Insulin resistance, on the other hand, reflects mostly a specific reduction in insulin-stimulated glucose uptake involving GLUT4 translocation, which is impaired by ROS. Genetic studies targeting GLUT4 have shown that disruption of glucose uptake in muscle or fat is sufficient to recapitulate the metabolic derangements observed in common insulin resistance, including unregulated hepatic glucose output [57]. In menopause, adipose tissue-related inflammation could be a relatively late contributor to insulin resistance which, in its turn drives systemic hyperinsulinemia. Insulin resistance and hyperinsulinemia in obesity can induce a state of relative androgen excess. Concentrations of DHEAS correlate positively with indices of insulin resistance and reduced high density lipoprotein (HDL) cholesterol concentrations [50], while SHBG and free T concentrations correlate negatively and positively with abdominal fat, respectively.

Hyperandrogenism of non-tumorous etiologies		
Endogenous etiologies	Obesity (PCOS-like)	
	Endocrinopathies	Acromegaly Cushing disease Hyperprolactinemia Hyperthyroidism
	Ovarian hyperthecosis	
	Inherited disorders	Polycystic ovary syndrome (PCOS) Non classic congenital adrenal hyperplasia (NCCAH)
Exogenous etiologies	Pharmaceutical use	Androgens (Testosterone, DHEA supplements) Antiepileptics (valproic acid, carbamazepine) Glucocorticosteroids
	Pharmaceutical abuse	Anabolic steroids
Hyperandrogenism of tumorous etiologies		
Adrenal Tumors	Androgen secreting adrenocortical adenomas Androgen secreting adrenocortical carcinomas	
Ovarian tumors	Sertoli-Leydig cell tumors (androblastomas) Granulosa theca cell tumors Metastatic (neuroendocrine/gastrointestinal) Cystadenomas	

Table 1. Classification of etiology of hyperandrogenism in post-menopausal women

The mechanisms by which hyperinsulinemia leads to androgen excess are complex. While, both insulin receptor and IGF-1 receptors are present in the ovary, most of the effects of insulin on steroidogenesis appear to be mediated through the former. A synergistic effect of insulin and LH, both of which are increased in obesity-associated menopause, lead to upregulation of the intra-ovarian enzyme CYP17 and subsequently to increased testosterone production [58].

4.1.2. Endocrinopathies*:*

In menopause some endocrinopathies can also cause hyperandrogenism. Such conditions are acromegaly, endogenous hypercortisolism (Cushing's syndrome), hyperprolactinemia and hyperthyroidism.

Growth hormone (GH) concentrations correlate negatively with SHBG concentrations, contributing to elevated free androgen concentrations. In addition, endogenous GH hypersecretion induces ovarian hyperandrogenism due to increased IGF-1 concentrations which directly affect the androgen-producing enzymes in the ovarian stroma.

Endogenous hypercortisolism correlates positively with free androgen concentrations, probably due to SHBG decrease. In addition, endogenous hypercortisolism in Cushing disease is sometimes accompanied by ACTH-driven adrenal androgens hypersecretion [5].

Hyperprolactinemia can also co-exist with post-menopausal hyperandrogenism of non-tumorous origin. Although relatively rare, prolactinomas can be observed in post-menopausal women and are frequently large. Moreover, hyperprolactinemia has been linked to weight gain and insulin resistance, which improve following medical treatment [59]. Hyperprolactinemic post-menopausal women may present with signs of chronic hyperandrogenism such as hirsutism and acne, possibly due to increased secretion of DHEAS from the adrenals, as well as reduced SHBG concentrations resulting to high free testosterone concentrations [60]. In cases with pre-menopausally existing hyperprolactinemia, the decline of estrogens in peri-menopause can improve galactorrhea, but also acne, and hirsutism [61]. Hypertheroidism and thyrotoxicosis may lead to an increase in total T and SHBG concentrations, as well as to a reduction in the concentration of non-SHBG-bound T. Little or no change is observed in free T concentrations [62]. Sex hormone binding globulin binds to T and DHT but not A4, and thereby the metabolic clearance rates of

testosterone and estradiol are reduced, whereas A4 and estrone clearance rates are within the normal range [63].

4.1.3. Ovarian hyperthecosis

Ovarian hyperthecosis represents the second most frequent non-tumorous cause of hyperandrogenism, (9.3%) in post-menopause [3]. It is characterized by stromal luteinization, high concentrations of gonadotropins (LH), hyperinsulinemia and insulin resistance. The pathophysiology of ovarian hyperthecosis remains unknown [64]. It is believed that elevated post-menopausal gonadotropins and especially LH may be involved in the process of stimulation of ovarian stromal cells. This is supported by the significant reduction in serum T concentrations after administration of gonadotropin-releasing hormone analogues (GnRHa), in post-menopausal women suffering ovarian hyperthecosis [65]. In menopause, ovary lacks granulosa cell aromatase activity and thus cannot convert testosterone to 17β-estradiol [66]. Nagamani et al. also observed in ovarian hyperthecosis a significant correlation between peripheral insulin concentrations and serum T concentrations, A4 and DHT, measured in ovarian vein samples, suggesting that the presence of hyperinsulinemia would act as a stimulating factor for the production of ovarian androgens, possibly through the induction of stromal luteinization [67]. Ovarian A4 aromatizes in estrone in peripheral tissues leading to hyperestrogenism, which can cause post-menopausal bleeding [68].

4.1.4. Inherited disorders

Polycystic ovary syndrome (PCOS) is usually diagnosed in women of early reproductive period, has a family segregation and represents the most common non-tumorous cause of hyperandrogenism in women during fertile life. The androgen production by ovarian stromal cells is still increased in post-menopausal PCOS women, as compared to non-PCOS healthy early menopausal women, under the stimulation of increased menopausal LH concentrations and/or the insulin resistance-associated hyperinsulinemia with insulin acting as a co-gonadotropin.

There is evidence that hyperandrogenism resolves only partially in women with PCOS in early post-menopause and that androgens of both ovarian and adrenal

origin are higher in post-menopausal women with PCOS than in healthy non-PCOS women. Elevated DHEAS in some women with PCOS is attributed to adrenal androgen excess [43]. These changes might influence proper insulin action [69].
In a retrospective review of 1205 women with hyperandrogenism in the United Kingdom, PCOS was the most common diagnosis in pre- (89%) and post- (29%) menopausal women [3]. Interestingly, there is evidence indicating earlier menopause for women with PCOS [70]. In fact, the severity of symptoms in PCOS depends on the presence of insulin resistance and obesity, which represent key factors in increasing the risk for metabolic abnormalities. Of note, A4 may be involved in the development of hirsutism in post-menopausal women with PCOS. However, data supporting this hypothesis are scarce.

Non-classic congenital adrenal hyperplasia (NCCAH) is an inherited non-tumorous condition associated with hyperandrogenism, that could be a cause even in menopause. Excessive androgen production is due to enzymatic deficiency, resulting in adrenal hyperplasia. In general, the deficiency of 21-hydroxylase is more common, whereas 3β-hydroxysteroid dehydrogenase and 11β-hydroxylase deficiencies are rare. The worldwide prevalence of NCCAH amongt women presenting with signs and symptoms of androgen excess is 4.2% (varying along different ethnicities) and is associated with elevated 17-hydroxyprogesterone (17OHP) and adrenal androgen concentrations [71]. While diagnosed overwhelmingly pre-menopausally, it could be present in post-menopausal women; there is evidence of one case diagnosed during post-menopause [72].

4.1.5 Exogenous etiologies

The iatrogenic hyperandrogenism can be caused by medications used for the treatment of specific pathologies (*pharmaceutical use*) such as androgens (testosterone or DHEA supplements), antiepileptics and glucocorticoids. Also, it can be caused by abuse of chemical substances or medications (*pharmaceutical abuse*) as it is the case with anabolic steroids (i.e. danazol). These medications lead to an increase in circulating androgens. The most commonly used drugs inducing hyperandrogenism are androgens, anabolic steroids, antiepileptics and glucocorticoids. Danazol historically used for treatment of endometriosis, was reported to cause hirsutism [73]. Testosterone or DHEA supplements themselves, may lead to hyperandrogenism, when used as treatments of menopausal and

androgen deficiency-related symptoms. Because valproic acid induces androgen production by thecal cells *in vitro* and it contributes to the development of a PCOS-like syndrome pre-menopausally, its use should be considered with caution in post-menopause [5, 74]. Exogenously received substances, including those employed for non medical purposes (e.g. anabolic substances used in athletic and sport training) can be associated with the clinical expression of hyperandrogenism.

4.2. Hyperandrogenism of tumorous etiologies

4.2.1 Adrenal tumors

Benign tumors of adrenals, called adenomas, are the most common type of adrenal tumor (including nonfunctional ones) [73]. Symptoms are virtually absent in non-secretory adenomas [5], but adrenal secretory adenomas can cause hyperandrogenism also in post-menopause. Interestingly, there have been described a few cases with unilateral adrenal adenoma, or with bilateral macronodular hyperplasia, in which adrenal androgens response to hCG or LH stimulation, due to the presence of aberrant LHRs. In most cases adenomas were secretory and pathology revealed adrenocortical tissue with characteristics of *zona reticularis,* alone or mixed with *zona fasciculata*. Some cases presented Reinke's crystals, which are characteristic of steroidogenic cells of ovarian hilus cells and Leydig cells [75].

Adrenal masses of incidental discovery, *incidentalomas* are usually found during examinations for other purposes. Thus, they are not characterized by clinical symptoms of hyperandrogenism as they are usually non-secretory, although in some of them a moderate increase of DHEAS is documented. Incidentalomas, sometimes, can harbor an adrenocortical carcinoma.

Adrenal cortical carcinomas (ACC) are highly aggressive malignant tumors, with an annual incidence of 0.7-2 cases per million, which mostly affect women in peri-menopause, but also at post-menopause [3, 76]. However, they represented a significant non-PCOS source of androgens in a cohort of British post-menopausal women (11 out of 75 cases) with increased concentrations of more than one

androgens [3]. Nearly all post-menopausal cases of ACC present with increased A4 while the majority of them shows combined increases of DHEAS, A4, and T. Sixty percent of cases with ACC manifest with increased DHEAS, which is often recommended as a biochemical marker for screening for ACC in large adrenal tumors [3]. Cortisol secretion (alone or in combination with androgens) is present in approximately 85% of patients with secretory ACC, being more frequent in women [76].

Adrenal rest tumors, are functional malignant and they present in some rare cases. They arise from an adrenal rest along the path of adrenal embryonic migration (eg, in the kidney, ovary, uterus, broad ligament, retroperitoneum) and produce adrenocortical steroids. They might manifest with a clinical image of Cushing syndrome and hyperandrogenism.. Although, such cases have not been described in post-menopausal women, they should be taken into consideration when evaluation of women with hyperandrogenism when the imaging of adrenals is not conclusive (negative adrenal imaging) [77].

4.2.2. Ovarian tumors

Ovarian androgen secreting tumors (OAST) may originate from sex cord cells which surround the oocytes (theca and granulosa) or from stromal cells. They are all described as sex cord-stromal tumors in the general World Health Organisation (WHO categorization of ovarian tumors [78]. They are relatively rare, representing 5-8% of ovarian tumors and are considered low grade malignancies as far as 70% of them present at stage I of disease [5, 73]. They are subdivided in the names of their cell of origin and less than half of them are androgen secreting. Among them, Sertoli-Leydig cell tumors (androblastomas) account for 0.5% of all ovarian tumors and 25% of them may occur after menopause. They consist of Sertoli, Leydig and androblastic cells. Their malignancy depends on the degree of differentiation and may manifest early recurrence after their surgical resection. Pure Leydig cell tumors are mostly androgen secreting, whereas pure Sertoli cell tumors secrete estrogens. Inhibin and AMH can be used as specific markers for Sertoli cell ovarian tumors. The hilus cell tumors are extremely rare and consist mainly of Leydig cells from the

ovarian hilus. They account for 0.02 % of all ovarian tumors with a mean age of onset of 58 years. They demonstrate significant steroidogenic activity with highly elevated T concentrations. Their malignant transformation is reported in very few cases [5].

Primary granulosa cell tumors account for 2-4% of all ovarian tumors and are mainly occurring during the sixth decade of life. These tumors are usually discovered at stage I, showing a good prognosis. Nonetheless, late recurrences and dissemination, which require systemic chemotherapy, have also been reported. Granulosa cells mainly secrete estrogens, inducing post-menopausal bleeding, endometrial hyperplasia and endometrial carcinoma. 10% may be accompanied by androgens secretion. Inhibin and AMH can be used as specific markers for granulosa cell ovarian tumors.

Serous cystadenoma is the most common benign epithelial tumor, with a peak of incidence in the 4th-5th decade, which in 20% of cases can be bilateral. It is not steroidogenic, but secretion of β-hCG can stimulate the steroidogenic cells *via* paracrine mechanisms [78]. In such cases, a common aftereffect of serous cystadenomas is surrounding stromal hyperplasia [5].

Metastases from other malignancies can mimic cystadenomas, such as Krukenberg tumors which are metastases of primary gastrointestinal tumors. They usually appear as bilateral solid masses in ultrasound, but they may be also cystic [73].

5. Clinical presentation of hyperandrogenism in post-menopausal women

The clinical signs of androgen excess in post-menopausal women may include hirsutism, acne, alopecia (a male pattern of baldness) and/or virilization with lowering of the voice, clitoromegaly, increased muscle strength and an anabolic appearance. Hirsutism and alopecia are the most common among them. These signs are usually associated with increased concentrations of androgens. Hirsutism may appear normally during menopausal transition, but the development of virilization suggests a specific source of androgen excess, such as androgen-secreting tumors. In these cases attention is required for diagnosis [79]. In some cases of hyperandrogenism and mostly those caused by a tumor, uterine bleeding can occur, caused by the peripheral aromatization of androgens to estrogens particularly in obese patients. Endometrial hyperplasia or endometrial carcinoma can develop as well in hyperandrogenic conditions.

6. Diagnosis

An algorithm of diagnosis is proposed in figure 4

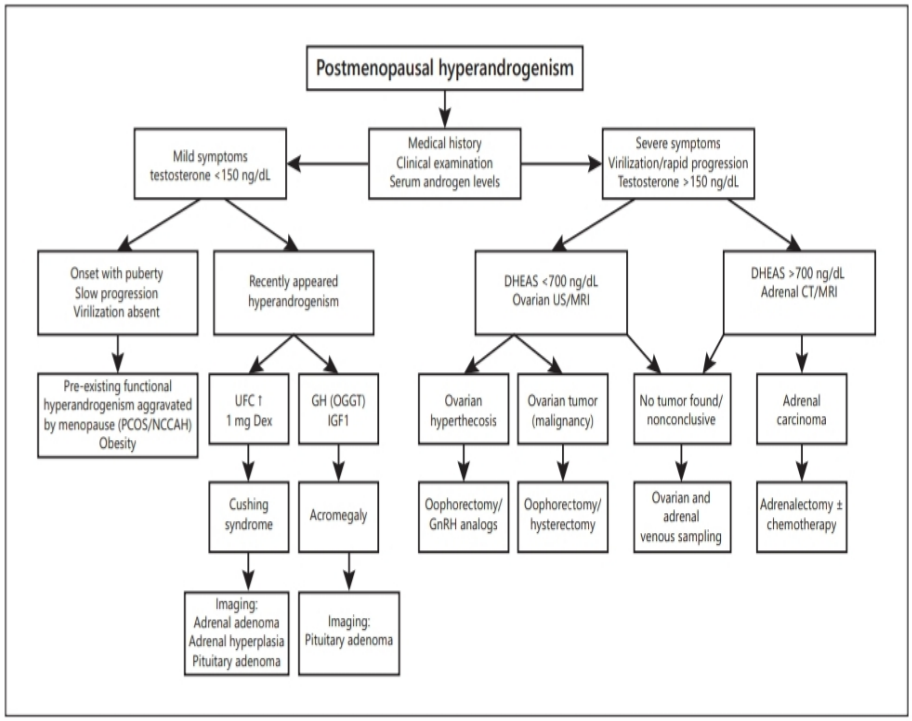

Figure 4. Algorithm of etiological diagnosis of hyperandrogenism in menopause

6.1 Diagnosis of hyperandrogenism

Diagnosis of hyperandrogenism includes medical history, physical examination, laboratory tests and imaging analyses.

A detailed medical history could determine the development of symptoms and signs over time, particularly if hirsutism starts developing pre-menopausally as in conditions such as PCOS or NCCAH. On the other hand, a rapidly progressive hirsutism, with a recent onset, during a short period of time (less than a year), strongly supports the presence of an androgen secreting tumor.
A drug history should exclude the use of anabolic steroids or exposure to exogenous androgens (eg.. transfer from a male partner using T gel) or synthetic glucocorticosteroids.

Clinical examination will evaluate the distribution of the hair which can be quantified by the Ferriman–Gallwey score, which assigns a score of 0–4 to describe hair in nine body areas. Even though this method for quantification of hirsutism has not been validated in post-menopausal women, a score >8 should be considered as positive in analogy to pre-menopausal women [80]. The presence of signs of virilization should also be evaluated. Of note, clitoromegaly is determined on the basis of the clitoral length or the clitoral index (length x width). Length >10 mm or an index >35 mm^2 is considered abnormal. The evaluation of percent body fat, a visual field testing for presence of pituitary tumor, examination for stigmata of hypercortisolism, galactorrhoea and symptoms of acromegaly are imperative [80].

The normal concentrations of androgens in post-menopausal women vary depending on the laboratory facilities and the type of assay employed. Generally they are within or near the following ranges:

- *total T*: 20 to 70 ng/dl (0,5 to 2,8 nmol/L)
- *A4*: 0,5 to 2,8 ng/ml (1,5 to 12,0 nmol/L)
- *DHEAS*: 18,5 to 185 µg/dl (0,5 to 5 µmol/L)
-

Concentrations higher than these are usually considered pathological, especially if accompanied by signs or symptoms of hyperandrogenism [73].

The most accurate laboratory test for the evaluation of hyperandrogenism, including that present in post-menopausal women and in particular in severe hyperandrogenism, is total T concentration, which is best measured by liquid chromatography - tandem mass spectroscopy (LC-MS/MS), a highly specific and highly sensitive method [81]. Free T measured by direct method is considered not accurate and should not be ordered. Serum DHEAS concentrations could help to distinguish adrenal from ovarian source of androgen excess, but they do not distinguish better than T malignant from benign androgen secreting tumors. In general, serum DHEAS concentrations above 700 μg/dl (18,9 μmol/L) should incite further evaluation. Ketosteroids (KS) measured in a 24 hours urine sample in patients with clinical hyperandrogenism without increased T and DHEAS concentrations, may provide evidence of an adrenal tumor, which does not produce DHEAS or T but androgen precursors. In post-menopausal women, most cases of severe DHEAS and A4 excess are associated with ACC while severe T excess is associated equally with ACC and ovarian hyperthecosis, meaning that the type of androgen and the severity of androgen excess is an important predictor of non-PCOS pathology and may be used to guide further investigations.Further routine testing comprises serum prolactin and 17OHP concentrations (evaluation for 21-hydroxylase deficiency-associated NCCAH).
In cases that the initial results are not conclusive, a general rule is to repeat measurements in order to create a clear opinion for the true levels [73, 81].
When other endocrinopathies are suspected further laboratory analyses are mandatory depending on the clinically suspected pathological entity (*e.g.* Cushing syndrome or acromegaly).

The imaging investigations are employed for differential diagnosis between adrenal or ovarian source of hyperandrogenism.

6.2. Etiological diagnosis

6.2.1. Differentiating benign from malignant causes

In general, total T assay is proposed as a first line approach for the differential diagnosis between benign and malignant causes of hyperandrogenism. [82] (Figure 4). Concentrations lower than 150 ng/dl (5.2nmol/L) in women without virilization or severe hyperandrogenism, support a benign cause, such as obesity, endocrinopathy (non cancer-associated Cushing syndrome, acromegaly, hyperprolactinemia or hyperthyroidism), inherited disorders (PCOS, NCCAH) or hyperandrogenism due to pharmaceutical use and abuse (Figure 4). Of note, no cut-off for total T concentration has been proposed for the distinction between benign and malignant causes [5, 73]. In PCOS or NCCAH the symptoms of hyperandrogenism start from adolescence (or even before in the case of NCCAH) and they persist even after menopause. In early menopausal PCOS, although the concentrations of androgens decrease, they remain greater than in healthy non-PCOS early menopausal women [5]. Deterioration of already present hirsutism or newly appearing signs of hyperandrogenism should prompt evaluation for causes other than pre-menopausally existing PCOS. Diagnosis of NCCAH is predominantly set well before the onset of menopause. Thus, the existence of this pathological entity should be known when patients are in menopause. Measurement of 17OHP concentrations is not recommended as part of the routine diagnostic testing in post-menopausal hyperandrogenism. When other endocrinopathy is to be ruled-out, the appropriate corresponding diagnostic work-up should be performed. The use of medications that can cause hyperandrogenism should always be excluded before any further investigation.

Virilization of recent onset with rapid progression and total T concentrations higher than 150 ng/dl (5.2 nmol/L) or DHEAS concentrations above 700 to 800 μg/dl (18.9 to 21.7 μmol/l), strongly support the suspicion of a malignant cause of hyperandrogenism. Serum concentrations of the adrenal androgens, DHEA or DHEAS, are often, but not always elevated in adrenal androgen secreting tumors.

Indeed, low DHEAS concentrations are generally proposed as a marker of benignity with a sensitivity and specificity calculated at 41% and 100%, respectively [83].

Of note, there are case reports of adrenal tumors that secrete exclusively T while some adrenal tumors may cause only a mild elevation of DHEAS [73]. Apart from virilization, women with androgen secreting adrenal tumors may present with symptoms of endogenous hypercortisolism (Cushing syndrome).

Tumors of ovarian origin are often accompanied with peripheral total T concentrations higher than 200 ng/dl while approximately 20 percent of ovarian androgen secreting tumors can be associated with total T concentrations lower than 150 ng/dl. Ovarian androgen-producing tumors of small size can be associated with fluctuating androgen concentrations. An exception to this rough rule is ovarian hyperthecosis in which, the symptoms of hyperandrogenism develop gradually, or may even have a more rapid course with severe hyperandrogenemia mimicking androgen secreting tumors. In this case, hyperandrogenism is usually combined with insulin resistance, with a slowly progressive acne and hirsutism or virilization.

Besides basal hormone concentrations, low dose dexamethasone suppression test (LDDST) is a simple dynamic test that could be useful also in post-menopausal women for distinguishing hyperandrogenism of tumorous from non-tumorous origin. Androgen concentrations in hyperandrogenism of non-tumorous origin are dramatically suppressed during this test in contrast to those of tumorous origin [84].

6.2.2. Localization

Suspicion of hyperandrogenism of adrenal origin: Adrenal imaging is suggested for the localization of an adrenal mass. Adrenal tumors including incidentalomas are best visualized by computed tomography (CT) scan, which will detect nodules >5 mm. Most of them appear as small, i.e. 2 to 2.5 cm, well defined from the homogenous surrounding mass, with attenuation on CT, depending on their lipid content. Measurement of Hounsfield units (HU) in an unenhanced CT is very useful in differentiating malignant from benign adrenal mass. A high density [greater than 10 HU] indicates a low fat content, giving strong evidence of malignancy, showing a

sensitivity and specificity that reach 71% and 98%, respectively. However, CT scan with delayed contrast media wash-out using a cut-off of 50% wash-out and an absolute value of 35 HU after 10-15 minutes, has superior diagnostic accuracy. Adrenocortical carcinomas are larger (4 to 25 cm) than adenomas. The former are heterogeneous, often with necrosis or calcification and they appear bright in magnetic resonance imaging (MRI), due to their high water content. They present iso-intense to liver on T1-weighted MRI images and have intermediate to increased intensity on T2-weighted MRI sequences [76].

Suspicion of hyperandrogenism of ovarian origin: in these cases a pelvic ultrasonography is indicated. In ovarian hyperthecosis, transvaginal ultrasound (TVUS) usually shows bilateral increase in ovarian stroma. The ovaries often exceed 10 cm^3 at a time that normal postmenopausal ovarian volume is approximately 2 to 4 cm^3, declining since the final menstrual period. Another critical parameter is the width-to-length ratio, which is greater than 0.80 in women with hyperthecosis. The ovarian stroma appears homogeneous on TVUS, even though a nodular pattern may be present, in which the luteinized cells aggregate into nodules of 1 cm or less. Ovarian tumors may be very small and the failure of ultrasonography to identify them does not rule out their presence, since small hilus cell tumors of the ovary that produce a large amount of T, may not be seen by ultrasonography, sometimes not even at the time of surgery. Other tumors such as androgen secreting sex cord stromal tumors are often not visualized. The ovaries may be described of normal size or with a slight asymmetry and may be detected in pelvic MRI. Ultrasound with color Doppler is helpful for diagnosing a tumoral cause of hyperandrogenism, as it can detect the vascular circulation within the tumor. In case reports, fludeoxyglucose positron emission tomography (FDG PET) imaging has been proposed for the identification of small ovarian tumors. All in all, the preferred imaging examination for ovarian tumors is TVUS, combined with color Doppler or MRI.

Leydig and Sertoli-Leydig cell tumors are solid tumors, without calcifications, generally smaller than 3 cm, often iso-echoic to the ovary. The signal intensity of these tumors on T2-weighted MRI sequences reflects the content of the stroma. Chemical shift MRI has been reported to successfully reveal the intra-cytoplasmic lipid stores, which are characteristic of steroid cell tumors. Intra-cytoplasmic crystals of Rinke found in histologic examination are suggestive but not diagnostic for Leydig cell tumor. Granulosa cell tumors are generally larger and more cystic than Leydig or Sertoli-Leydig cell tumors. They are typically iso-echoic on ultrasound. The imaging

characteristics of thecomas depend on their particular composition i.e. the amount of fibrosis and lipid content of the tumor. Krukenberg tumors are ovarian metastases of primary gastrointestinal tumors usually appearing as solid masses in ultrasound, but they may be cystic. They are usually found bilaterally.

Combined ovarian and adrenal vein sampling is performed occasionally for further evaluation in women with high serum total T concentrations (T>150 ng/dl), normal pelvic ultrasonography and adrenal imaging. In this setting, ovary is likely to be the source of androgen secretion, because adrenal tumors are almost always visualized on adrenal CT, while ovarian tumors are often too small to be seen on imaging studies. This scenario is far more common in post-menopausal women. The procedure includes selective catheterization of the ovarian and adrenal veins to demonstrate a left to right difference in androgen concentrations. This procedure is technically difficult and should only be performed by an interventional radiologist with experience. However, in a post-menopausal woman presenting with a gradual onset of hirsutism and/or virilization over the years and with a small adrenal incidentaloma, combined ovarian and adrenal vein sampling can be indicated [85].

7. Treatment of hyperandrogenism in post-menopausal women

7.1. Etiological treatment

The essential goal of the etiological treatment of hyperandrogenism in post-menopausal women is the elimination of the source of elevated androgens. The tumorous causes of hyperandrogenism in these women are treated mostly through surgery, with the resection of the tumor, especially the malignant ones. On the other hand, if surgery is not a safe option or the source of elevated androgens is not identified, the treatment choices are limited.

Historically, open adrenalectomy (OA) was considered the standard procedure for surgical excision of the majority of adrenal tumors; however, contemporary studies show that minimally invasive laparoscopic adrenalectomy (LA) performed with a transperitoneal or retroperitoneal approach, can be offered safely to carefully selected patients, as well as in resectable androgen secreting adrenal tumors. Robotic adrenalectomy (RA), which was first described in 2001, might be particularly useful for patients with high body mass index (BMI >30) and for large tumors (>5.5 cm) [86]. However, adenomectomy has been recently suggested in case of benign adenomas [87]. In unresectable carcinomas or in metastatic disease, other therapeutic modalities can be considered. Due to high recurrence rate and poor outcome of ACCs, adjuvant therapy is used, following surgical resection.
Mitotane is the standard medical treatment in cases of advanced or aggressive tumors. However, it requires close biochemical monitoring, since it is associated with adverse side-effects that may compromise its use [76].

For well□characterized ovarian hyperandrogenism, laparoscopic bilateral salpingoophorectomy may serve both as a diagnostic and therapeutic procedure [66]. This surgical procedure is often curative for ovarian hyperthecosis, if the

patient is a good surgical candidate. If ovarian hyperandrogenism-associated endometrial hyperplasia and/or carcinoma of ovarian origin, are not confirmed through TVUS (cancer is very unlikely if the endometrial thickness is <4 mm) or if an endometrial biopsy does not suggest hyperplasia or cancer, hysterectomy may not be necessary, although experts may disagree [73]. Nevertheless, women unable or unwilling to undergo ovarian surgery can be reassured that malignant OASTs are extremely rare and that long-term medical therapy with oral antiandrogens or GnRH analogues is safe and well□tolerated [66, 79]. The desirable effect of long-term suppression of T (eg. at least 3.5 years after the last dose of GnRH agonist) was achieved using 7.5 mg of depot leuprolide, once monthly for 3 months followed by a 3.75 mg decrease per month for another year. In another group this was achieved by using the same dose for 5 months and then administering two doses of 11.25 mg every 3 months, for a total of 11 months of treatment. Careful follow-up is needed during the use of GnRH agonists with periodic testing of androgen concentrations and ovarian imaging. If after several months of therapy the concentrations of androgens do not decrease, reconsidering surgery for histologic diagnosis is suggested [73].

The treatment of endocrinopathies should follow the corresponding treatment algorithms.

Following diagnosis of hyperandrogenism due to pharmaceutical use or abuse, the incriminated medication or chemical substance should be removed.

7.2. Symptomatic treatment

The symptomatic treatment is directed mostly towards the chronic effects of hyperandrogenism during post-menopause and is mostly used in cases of functional or non-tumorous hyperandrogenism.

Over the recent years, in western countries, the well-being of post-menopausal women has received a lot of attention. Symptoms such as vaginal dryness and dyspareunia affecting sexual function, are related to the low circulating estradiol concentrations in these women [88, 89]. Estrogens are considered a ‘gold standard’ in treating these menopausal symptoms but they have been questioned for their “off-target” effects such as their possible contribution to the development of hormone-

sensitive cancers [90-94]. Therefore, locally-acting vaginal treatments have been proposed including preparations with DHEA. After vaginal administration, DHEA is converted into active forms of estrogen and/or T acting locally on intra-cellular receptors [95, 96]. Hence, treatment of post-menopausal women with DHEA may restore intra-cellular androgenic and estrogenic activity without impacting systemic concentrations, and thereby enhance *libido*, ameliorate menopausal symptoms and ultimately promote well-being [97]. However, in a systematic review and meta-analysis of 23 RCTs, investigating the possibility of employing systemic administration of DHEA in post-menopausal women with normal adrenal function, no significant improvement in *libido* and sexual function was observed [98]. Similarly, the efficiency of systemic DHEA therapy for treatment of sexual dysfunction in post-menopausal women was not proven by RCTs [97]. Only one study has shown that vaginal DHEA exerts beneficial effects on sexual function (including enhanced desire/interest, arousal, orgasm, and decreased pain at sexual activity), as well as that it alleviates vaginal atrophy and dyspareunia in post-menopausal women [99]. Interestingly, more recent phase III prospective, randomized and placebo-controlled studies, provided a more solid evidence of beneficial effects of intra-vaginal DHEA on vaginal atrophy [100-102] and sexual dysfunction [103, 104]. A recent systematic review concluded that sexual interest, lubrication, pain, arousal, orgasm and sexual frequency improved following DHEA treatment in peri- and post- menopausal women [105]. Issues concerning the safety of DHEA treatment have been addressed by other studies. Even low doses of 6.5 mg of intra-vaginal DHEA can effectively treat vaginal atrophy without altering normal post-menopausal peripheral concentrations of estrogens, androgens or their metabolites, thereby ensuring safe menopause [106]. In another study, despite the fact that both a vaginal moisturizer and DHEA improved the severity of vaginal pain or dryness, the alleviation of the symptoms by the administration of 6.5 mg DHEA, was more rapid (significant difference at 8 weeks). This study was performed in post-menopausal women with a history of breast or gynecologic cancer, warranting further investigation [107]. However, small increases in systemic androgen concentrations after vaginal administration of DHEA were reported by the same research group a few months later [107]. Consequently, the issue of increasing androgens concentrations *vis-à-vis* hormone-dependent cancers, is not negligible. Highlighting the low concentrations of biologically active T and the absence of off-target effects of vaginal DHEA, the authors concluded that future studies should shed light on the potential risks of this treatment in cancer survivors. Importantly, the recent FDA approval of intra-vaginal administration of DHEA for treating symptomatic vulvo-vaginal atrophy, based on significant beneficial

effects, as well as the strictly local action of DHEA, suggest the absence of significant adverse effects of the drug [108].

Anti-androgens, such as cyproterone acetate, spironolactone and flutamide are used in combination with local therapies. In NCCAH patients, spironolactone is a good choice. In cases of post-menopausal women not responding to anti-androgens, improvement of the symptoms is achieved when these patients are treated with glucocorticoids but one should be extremely cautious regarding the development of exogenous hypercortisolism.

In cases with insulin resistance and hyperinsulinemia, especially in obese women or PCOS patients, insulin sensitizing agents such as metformin and thiazolidinediones (rosiglitazone and pioglitazone) can be used, in combination with lifestyle and diet modification (including prescription of exercise), to improve the deleterious metabolic profile associated with this condition [109].

8. Long term consequences of hyperandrogenism in post-menopausal women

8.1. The metabolic impact of androgens in post-menopausal women

Low DHEA and DHEAS concentrations have been implicated in a multitude of menopause and aging-related conditions, such as osteoporosis, muscle loss, fat accumulation, hot flashes, vaginal atrophy, T2DM and memory loss [83]. These findings have captured scientific attention, reflected in the plethora of studies aiming at the investigation of the beneficial effects (well-being and alleviation of age-related conditions) caused by the exogenously restored concentrations of DHEA and DHEAS [84]. While below 50 years of age metabolic syndrome is slightly more frequently encountered in men, after this age it becomes predominant in women due to sex-and-gender-related factors linked to activation of genetic and biological pathways driven by hyperandrogenism, insulin resistance and the menopause-associated increase of abdominal obesity and HDL-cholesterol reduction [85].

The risk for T2DM and CVD) increases. The available data so far indicate that coronary heart disease, as well as cerebrovascular disease, is more common in post-menopausal PCOS patients. The persisting high androgen concentrations through menopause, obesity and T2DM are proposed as the main factors, accounting for the increased risk of CVD in these post-menopausal patients [110]. Interestingly, the normalization of androgen concentrations in post-menopausal women achieved after surgical oophorectomy did not cause any significant change in body weight and insulin sensitivity. These findings may offer a different perspective on the duration-related impact of hyperandrogenemia on metabolism [111]. Of note, unbound T concentrations are not associated with CVD or T2DM risk in menopausal women, whereas low concentrations of sex hormone binding globulin (SHBG) resulting from the absence of estrogens in menopause, appear to be a

strong predictor of these pathologies [48-50, 112], questioning what has been earlier reported for free T. Conversely, with respect to fat accumulation, recent data suggest that free T and DHEAS concentrations in early and late post-menopausal women respectively, are positively associated with abdominal fat accumulation, whereas SHBG could not independently predict abdominal fat accumulation [50]. These findings suggest that SHBG correlation with CVD and T2DM, might be the reflection of insulin resistance on these pathologic entities [49]. One should corroborate to these findings the persistence of hyperinsulinemia in early post-menopausal PCOS as compared to healthy non-PCOS post-menopausal controls [5]. Non-alcoholic fatty liver disease (NAFLD) is twice as common in post-menopausal as compared to pre-menopausal women and hormonal replacement therapy is shown to decrease its risk. Insulin resistance, T2DM, sleeping apnea syndrome, cardiovascular disorders and non-alcoholic fatty liver disease are more frequent in post-menopausal PCOS [113]. Hyperandrogenemia, dyslipidemia, hyperglycemia, IR and low-grade inflammation, all of which characterize PCOS, are concomitant factors that generate and aggravate NAFLD [114]. Menopause *per se* has been correlated to a higher prevalence of NAFLD, possibly due to estrogen deficiency, relative androgen excess and decreased SHBG observed in post-menopausal women. These hormonal changes seem to be associated with increased abdominal adiposity, which is also observed in both post-menopause and aging. Importantly, the latter are closely related to severity and progression of NAFLD [115].

Hyperandrogenism can be associated with an increased risk of breast and endometrial cancer, possibly due to peripheral aromatization of excess androgens to estrogens [116]. Increased breast cancer risk and other gynecological malignancies are positively correlated with the androgens concentrations after menopause. This is more evident in estrogen receptor-positive breast carcinomas while in some studies, androgens concentrations have been correlated negatively with estrogen negative breast carcinomas [117]. A strong association between elevated A4 concentrations and risk for endometrial cancer independently of BMI and estrogen concentrations has been shown [118].

In general, androgen concentrations in women are positively associated with increased bone mass density (BMD), especially to those between 55 and 85 years, while the risk of hip fracture is higher in post-menopausal women with free T concentrations lower than the normal range, but PCOS post-menopausal women have similar BMD values compared to controls.

8.2. Future approach in improving health of the aging woman: beyond hormone therapy

Engaging to lifestyle changes that might be accompanied with increased DHEA concentrations is a recently introduced approach to the promotion of well-being during and after menopause. Several studies have investigated the impact of exercise on DHEA concentrations in post-menopausal women. A significant increase in DHEA concentrations has been reported in females up to 69 years old regularly performing resistance exercise, which was not the case for endurance exercise [119]. Of note, a combined endurance and strength training session was accompanied with a rapid increase in DHEAS concentrations (observed at 2 h after the training) in early post-menopausal females [120]. Nonetheless, there have been studies demonstrating no effect of sub-maximal exercise in DHEAS [121] or both DHEA and DHEAS concentrations [116]. The low number of participants and the lack of sampling during recovery might have accounted for these controversial findings. In fact, data from a recent study with larger sample size support the increase of DHEA and DHEAS concentrations immediately post exercise in post-menopausal women. Based on the results of this work, it was suggested that exercise can exert its effects upon DHEA and DHEAS concentrations in adults of older age irrespective of training status, which makes exercise a very attractive approach to effectively treat menopause- and age- related symptoms [117].

9. Conclusions

The endocrine system undergoes major alterations during aging, affecting the majority of body functions. Many of these changes have been so far well described, but their exact impact on health and disease is yet to be unraveled.

It is important to differentiate between aging-associated alterations which constitute beneficial, physiological adjustments of human body to environmental stimuli and those harmful to cell viability. This would further help the development of targeted medical interventions, with long-term hormonal replacement/blockade with one or more hormones in an effort to promote longevity. As life expectancy increases, it is important not to accept the brutal stereotype that aging is unavoidable, but rather delineate the pathophysiology of aging and take advantage of longevity pathways, in order to achieve longer life expectancy of acceptable quality.

Instead, concerning the pathological cauuses of hyperandrogenism it is important to distinguish between life threatening situations such as cancer and the benign situations, together with the localization of the source of elevated concentration of androgens. In order to do this, it is important to perform a structured diagnostic methodology, combining history, clinical examination and analyses, so as to conclude in treating decisions that will conserve or improve the quality of life of the patient. New interventional methods are being utilized and others have to be develooed in the future.

10. Bibliography

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