Use of AMH in the Differential Diagnosis of Anovulatory Disorders Including PCOS

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Since the historical use of gonadotrophin and estradiol levels to define the different anovulatory disorders has shown some limitations, the use of other markers such as anti-müllerian hormone (AMH) has been proposed. This review addresses the role of AMH in the differential diagnosis of anovulatory disorders, especially focusing on its value in the prognostic characterization of their severity. Current limitations and future clinical applications are discussed.

Keywords: anovulatory disorders, polycystic ovary syndrome, amenorrhea, anti-Müllerian hormone, premature ovarian insufficiency

INTRODUCTION

Anovulatory disorders in women can be various. The main classification in use is adapted from the one proposed by the World Health Organization (WHO) and by the 1995 ESHRE Capri workshop group (1). The possible causes of anovulation are here categorized into three groups on the basis of serum gonadotrophin and estradiol levels. Besides having diagnostic purposes, such classification also aims at guiding the therapeutic approach, since each anovulation subsumes different long term health consequences and ovulation restoring strategies. WHO 1 anovulatory dysfunction, which accounts for 5–10% of all anovulatory disorders, is characterized by low gonadotrophin and low estradiol serum levels (2). The underlying cause of this dysfunction is usually a hypothalamic suppression, which occurs in association with weight loss and negative energy balance, such as in patients suffering from anorexia nervosa and endurance athletes. WHO 2 anovulatory dysfunction, which accounts for the 80% of all the ovulatory disorders, presents normal gonadotrophin and low estradiol serum levels (2). Polycystic ovary syndrome (PCOS), which is diagnosed on the basis of the Rotterdam consensus criteria (at least two between oligo-anovulation, clinical or biochemical hyperandrogenism and polycystic ovarian morphology, PCOM), represents its most frequent example (3). At last, WHO 3 anovulatory dysfunction is characterized by an ovarian reserve depletion with high gonadotrophin and low estradiol levels. If the patient is younger than 40 years old, this may indicate premature ovarian insufficiency (POI) (4).

Gonadotrophin and estradiol levels, however, are often overlapping in the various forms of anovulation, with no clear discriminatory thresholds established. Apart from their use, which can be limited by the above mentioned vagueness, several other markers have been proposed for the differential diagnosis of anovulatory dysfunctions. In particular, serum anti-Müllerian hormone (AMH) levels have been indicated as a potential tool in the discrimination of the various
AMH function in the ovary and at the central level

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor-beta (TGF-β) superfamily (5). In men, AMH is secreted by the Sertoli cells of the testes, inducing the regression of Mullerian ducts (6, 7). In adult life, AMH is exclusively produced in the ovary by the granulosa cells surrounding the growing follicles, from early antral to small antral follicles phase (8–10). It is therefore thought that its serum levels are a reflection of a cohort of small growing follicles (11, 12), which reflects the number of residual primordial follicles, or the ovarian reserve (13).

AMH is a key-regulator of ovarian function. It is considered a local growth factor and acts in the cellular differentiation, since it has been demonstrated to have a paracrine inhibitory effect on the activation of folliculogenesis (5). In vitro and in vivo studies on mice were the first to show that in AMH knockout animals the transition from primordial into growing follicles with subsequent early depletion of the primordial follicle pool was enhanced (13). In humans, AMH causes a decrease in the follicle-stimulating hormone (FSH)-stimulated aromatase expression (14), and also reduces FSH receptor messenger RNA (mRNA) expression (15), with a consequent modulation in the ovarian follicular responses to gonadotrophins. Moreover, in vitro studies proved a modulation of the response to luteinizing hormone (LH) induced by AMH (5). A central action of AMH on GnRH neurons has also been hypothesized in mice observing the increased LH pulsatility in many cases of PCOS, in which circulating AMH levels are also often elevated (16).

AMH in WHO 1 ovulatory dysfunction

In WHO 1 patients, either low, normal or slightly elevated AMH levels have been described.

Normal serum AMH levels have been reported in most of the studies on women with central secondary amenorrhea (17–20). La Marca et al. showed that in women with functional hypothalamic amenorrhea, AMH serum levels were similar to those found in normal controls. Moreover, there were no significant differences between the two groups in the number of 2–6 mm follicles, suggesting that initial follicle recruitment is not abolished in hypogonadotropic hypogonadism, with a stagnation of small antral follicles (17). No statistically significant difference of AMH serum levels between women with hypothalamic amenorrhea and anorexia nervosa and control group was observed in another case-control study conducted by Luisi et al. (18). Levels of AMH were within the normal range for age in a further study of van Elburg et al. on patients suffering from anorexia nervosa, confirming unaffected gonadotropin-independent growth of small preantral and early antral follicles in these patients. Under the same conditions of initial body weight, premorbid weight, duration of amenorrhea, duration of study participation and amount of prescribed medications, in patients suffering from anorexia nervosa the higher the AMH levels, the higher the probability of ovarian function recovery, indicating a possible prognostic role for AMH (21). The same evidence was observed in a group of seven patients with hypopituitarism during the years of adolescence, whose AMH serum concentrations were in the age-specific reference range, while in three out of four patients diagnosed in the infancy AMH serum concentrations were on or below the age-specific 25th percentile, with worse prognostic implications (22). On the contrary, in patients with long term profound gonadotrophin deficiency such as isolated hypogonadotrophic hypogonadism and Kallmann syndrome, AMH levels were significantly lower when comparing affected patients to healthy controls. The subgroup of patients with the lowest FSH levels showed also the lowest AMH levels, showing the correlation between AMH levels and the severity of gonadotropin deficiency (23).

In a recent study by Alemyar et al., median AMH values were significatively higher in a population of patients with hypothalamic hypogonadism compared to healthy controls, but lower than AMH levels in the PCOS population. We hypothesize that the increase in AMH levels in the group of patients with hypothalamic hypogonadism might be due to the presence of a relatively large pool of antral follicles smaller than 2 mm in diameter, which are not counted during transvaginal ultrasonography although they secrete AMH (24).

AMH in WHO 2 ovulatory dysfunction

Polycystic ovary syndrome (PCOS) represents the most frequent clinical manifestation of WHO 2 anovulatory dysfunction. Serum AMH is consistently higher in PCOS women (17, 25–30). For such reason, since serum AMH levels reflect the excess of small follicles which ultrasonography cannot detect, AMH has been proposed as a better marker in the diagnosis of PCOS than antral follicle count (AFC) (31–34). In a meta-analysis conducted by Iliodromiti et al., the specificity and sensitivity in diagnosing PCOS in the symptomatic women were of 79.4 and 82.8%, respectively, for a cutoff value of AMH of 4.7 ng/mL (31). Dewailly et al. separated asymptomatic women with PCOM to those with normal ovarian morphology in order to better calibrate the cutoff for the AMH value to distinguish patients with PCOS from normal women. A higher specificity (97 vs. 92%) and a better sensitivity (92 vs 81%) were demonstrated for a cutoff value of AMH of 4.9 ng/mL compared to AFC (32). Nevertheless, a universal diagnostic threshold for serum AMH in the diagnosis of PCOS has not yet been reached, and its use as an alternative for detecting PCOM in the diagnosis of PCOS has...
not been recommended by the new European Society of Human Reproduction and Embryology 2018 guidelines (35).

The reasons behind the elevated AMH levels of these categories of patients are different. In PCOS women there is a stagnation of AMH-producing follicles, with a stockpiling of transitional and classic primary follicles whose differentiation in the subsequent development phases is disrupted (36). Besides the elevated number of AMH-producing follicles, in these patients an increased production of AMH per single follicle has also been observed, with the mean level of AMH four times higher in granulosa cells from ovulatory PCOS and 75 times higher in granulosa cells from anovulatory PCOS patients in a study by Pellatt et al., demonstrating a correlation between AMH values and the severity of the syndrome with the possibility of ovulation restoration (37). Besides being a marker for the diagnosis of PCOS, AMH has also been thought to have a role in the pathogenesis of the disease. Both Visser and Homburg stated that the high AMH concentrations present in women with PCOS could play an integral role in causing anovulation due to AMH’s inhibitory influence on the actions of FSH that normally promotes follicular development from the small antral stage to ovulation (38, 39). According to Cimino et al., AMH increases GnRH-dependent LH pulsatility and secretion, with the consequent dysregulation of follicle growth (16). Moreover, AMH seems to correlate with the severity of the syndrome. Higher levels of AMH have been shown in amenorrheic than in oligomenorrheic women with PCOS, reflecting a more evident impairment in follicular development and granulosa cell function in the ovaries of amenorrheic than in those of oligomenorrheic PCOS women (17). According to Tal et al., increased AMH levels correlate more with PCOS severity in women with ultrahigh AMH (> 10 ng/mL) having greater prevalence of polycystic ovarian morphology, oligomenorrhea and amenorrhea than in women with AMH 5–10 ng/mL (40). Nevertheless, AMH has also been proposed as a marker for treatment monitoring in PCOS women. PCOS obese and overweight patients who showed improvements in reproductive function after weight loss had lower baseline AMH levels compared with those who did not respond (41). In a study evaluating response to treatment, the group who responded less well to induction of ovulation was the one with higher AMH levels (42).

**AMH IN WHO 3 OVULATORY DYSFUNCTION**

WHO 3 anovulatory dysfunction is characterized by low or undetectable AMH levels (17, 43, 44). Being connected to the loss of ovarian function, this condition is also known as primary ovarian insufficiency (POI), premature ovarian failure (POF) or premature menopause (44) when this condition occurs before age 40. In such dysfunction, the follicle pool is depleted, with a consequent ovarian insufficiency whose cause can be various: genetic, autoimmune, and iatrogenic (2). With respect to other markers such as AFC, FSH, inhibin B and estradiol, AMH seems to better reflect the continuous decline of the oocyte/follicle pool with age (15). AMH levels showed to be significantly different between incipient ovarian failure (IOF), with regular menstrual cycles and elevated FSH, and transitional ovarian failure patients (TOF), with oligomenorrhea and elevated FSH, permitting the identification of the clinical degree of follicle pool depletion (45). AMH values are also reported to perform as a predictor of follicle presence in ovarian biopsies performed on patients with a premature ovarian failure (46). Moreover, in patients diagnosed with steroidogenic cell autoimmunity (SCA-POI), since the depletion of follicles begins from the antral follicle stage, a preserved ovarian follicle pool producing AMH can be found for several years after an ovarian insufficiency diagnosis (47). The maintenance of AMH levels in women with autoimmune POI is important since it has been observed that in the initial stages of the disease, characterized by the persistence of antral follicles despite amenorrhea and high serum gonadotropin levels, strategies of fertility preservation such as in-vitro maturation (IVM) can be performed before the inevitable follicular depletion (48).

**DISCUSSION**

The Anti-Müllerian hormone (AMH), given its relationship with the follicular ovarian pool, is a reliable marker of ovarian reserve and its clinical use has recently been extended and emphasized. In particular, it has been proposed by different authors as a potential marker in the differential diagnosis of the various forms of anovulatory dysfunctions: it is usually normal in patients with hypogonadotropic anovulation (even if it can be also low or slightly elevated), high in normogonadotropic anovulations and low in hypergonadotropic anovulations (2, 7, 15). Due to its significantly elevated values in PCOS it has also been proposed as a marker for the diagnosis (31–34), even if a universal diagnostic threshold for serum AMH in the diagnosis of PCOS has not yet been reached (35).

Its use as a single marker in differentiating the different anovulatory forms, however, has been discussed. Since its levels can be increased in patients with hypothalamic hypogonadism whilst there is no increase in AFC, Alemyar et al. warned to avoid its use as a single biomarker for the characterization of an anovulatory disorder, highlighting the importance of measuring gonadotrophins and estradiol in order to avoid the misdiagnosis with WHO 2 anovulations (24). Nevertheless, AMH has proven to be insufficient in characterizing alone some anovulations such as WHO 1 anovulation disorder, since in such anovulation its values can vary widely. However, the underlying causes (congenital, functional or iatrogenic) of WHO 1 anovulation are not always specified in the literature, which can represent an important limitation. The evaluation of the differences in serum AMH between long-term vs. short-term etiologies could in the future be helpful in guiding the evaluation of AMH in WHO 1 anovulations.

Several evidences from the literature show that, rather than a diagnostic biomarker, AMH could have an important role as an index of severity and a guide for treatment of the various
anovulatory disorders. A correlation between AMH values and the probability of ovulation restoration has been described, even if, to date, no predictive values in terms of ovulation and pregnancy for any ovulation-inducing treatment have been demonstrated for this assay.

An important limitation in the use of AMH as a single diagnostic marker in the differential diagnosis of anovulations is represented by different numerical calibration of the existing AMH assays. An international standard is needed to standardize the existing assays before diagnostic cutoffs are meaningful. The optimal performance and stability of the automated AMH assays now in use compared to previous manual assays is well recognized (49, 50), with many clinicians assuming that the values derived from the two most common automated assays, the Elecsys AMH assay and the Access AMH assay, are interchangeable (51). Nevertheless, a certain debate is still present in literature (51, 52). Another issue is the change of its content of the work. All authors contributed to the article and approved the submitted version.

AUTHOR CONTRIBUTIONS

MC did the bibliographic research and wrote the article. ALM revised the manuscript. All authors agree to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.
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