The Anti-Mullerian hormone and ovarian cancer

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The Anti-Mullerian hormone (AMH), which is produced by fetal Sertoli cells, is responsible for regression of Mullerian ducts, the anlagen for uterus and Fallopian tubes, during male sex differentiation. Ovarian granulosa cells also secrete AMH from late in fetal life. The patterns of expression of AMH and its type II receptor in the post-natal ovary indicate that AMH may play an important role in ovarian folliculogenesis. Recent advances in the physiological role of AMH has stimulated interest in the significance of AMH as a diagnostic marker and therapeutic agent for ovarian cancer. Currently, AMH has been shown to be a circulating marker specifically for granulosa cell tumour (GCT). Its diagnostic performance seems to be very good, with a sensitivity ranging between 76 and 93%. In patients treated for GCT, AMH may be used post-operatively as marker for the efficacy of surgery and for disease recurrence. Based on the physiological inhibitory role of AMH in the Mullerian ducts, it has been proposed that AMH may inhibit epithelial ovarian cancer cell both in vitro and in vivo. These observations will be the basis for future research aiming to investigate the possible clinical role of AMH as neo-adjuvant, or most probably adjuvant, therapy for ovarian cancer.

Key words: AMH/cancer therapy/diagnostic markers/epithelial ovarian cancer/granulosa cell tumour

Introduction

Ovarian cancer comprises a heterogeneous group of tumours of three major types: epithelial, germ cell and sex cord stromal.

Epithelial tumours arise from the coelomic mesothelium, which is capable of differentiating into both benign and malignant tumours. Epithelial malignancies represent about 82% of all ovarian malignancies. The predominant cell types are: serous, mucinous and endometrioid.

The malignant germ cell tumours are believed to arise from primitive germ cells in the ovary. These tumours represent about 5% of all ovarian malignancies. They include dysgerminoma, embryonal carcinoma, endodermal sinus tumour, choriocarcinoma and malignant teratomas.

The sex cord stromal neoplasms are derived from mesenchymal stem cells in the ovarian cortex and represent about 10% of all ovarian tumours. They include granulosa–theca cell tumours, granulosa cell tumours (GCTs) and Sertoli–Leydig cell tumours.

GCT constitutes 3% of all ovarian tumours. Two distinct types of GCTs have been described on the basis of clinical presentation and histological characteristics: the juvenile and the adult form. The more common adult form generally presents in pre- or post-menopausal women with a median age of 50 years during presentation (Pankratz et al., 1978; Bjorkholm and Silfversward, 1981).

A majority (two-thirds) of these GCT patients have endocrine manifestations as a direct consequence of hormone secretion by the tumour (Bjorkholm and Silfversward, 1981). In the majority of cases the effects are estrogenic in nature, though rarely androgenic effects have been documented (Nakashima et al., 1984).

In the pubertal female, these effects may manifest as precocious isosexual development, whereas in premenopausal patients, menstrual irregularities are seen (Biscotti and Hart, 1989). In older women, the most frequent symptom is post-menopausal bleeding. As a consequence of this hormonal secretion, an association between GCT with both endometrial hyperplasia and adenocarcinoma has been well documented (Salerno, 1962).

In GCT the routes of dissemination are not completely clear. These tumours grow locally and then spread to the adjacent intra-peritoneal organs and when systemic disease occurs it does so late in the course of the disease. The majority of GCT present as stage I and the 5-year survival rate is 90%.

The initial form of therapy for most patients should be a surgical procedure so that a histological diagnosis and appropriate staging may be obtained. In patients with disease localized to one ovary and those wishing to preserve fertility, conservative therapy seems to be indicated. In those with more advanced disease or in post-menopausal women, Total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH/BSO) would be an appropriate initial treatment. Chemotherapy should be considered in those patients with advanced, recurrent or metastatic disease. Although response rates to chemotherapy are high, the impact on both disease-free states and overall long-term survival is currently unknown.
The GCTs have the potential to secrete estrogen and inhibit. Hence, these products are used as markers for GCT.

In several studies, elevated serum levels of estradiol have been found in some patients with known disease. The levels generally fluctuate with response to or failure of treatment or with the bulk of the disease. Unfortunately, the cases that have been described are few and the marker itself lacks sensitivity. Although it has been shown that the granulosa cells produce estradiol, they do so only if testosterone is presented to them. Testosterone is produced by the adjacent theca cells, which are present in some ovarian tumours but not in extraovarian tumours (Lappohn et al., 1989).

A second marker, inhibin, has also been reported in patients with GCT. Inhibin is a dimeric glycoprotein secreted by the gonads to regulate pituitary FSH. It exists as a dimer of two subunits (α and βA or βB) to form inhibin A (αβA) or B (αβB) (Bilezikian et al., 2004). Studies investigating the specific assays for inhibin A and B have shown that almost all GCT have elevated inhibin B levels and the majority also have elevated inhibin A levels (Robertson et al., 1999a). Concentrations of immunoreactive inhibin and the specific dimeric inhibin in GCT are by far the highest seen in any form of ovarian malignancy or in other disease.

However, as a diagnostic test, neither inhibin A nor B are as effective as a total α subunit as measured by the inhibin radio-immunoassay (RIA) (Robertson et al., 1999a). Unfortunately, the inhibin RIA is limited as a diagnostic test due to its lack of practicability (5-day assay). A two-site immunofluorometric assay (αC IFMA) was subsequently developed (Robertson et al., 1999b). However, the αC IFMA is not appropriate for wide-spread diagnostic use as it has proved difficult to maintain the assay reliability between different batches of antisera. In conclusion there is controversy surrounding the use of different antisera and methodologies, which have resulted in conflicting results.

More than 10 years ago, an alteration in anti-Mullerian hormone (AMH) (also called mullerian inhibiting substance) secretion was described in a patient affected by GCT (Gustafson et al., 1992). After this first observation, several studies investigating the dynamics of serum AMH in patients with ovarian malignancies have been published.

In the present article, the main findings and possible clinical implications of these studies are explored.

AMH and AMH receptors

AMH is a member of the transforming growth factor (TGF) superfamily. AMH is strongly expressed in Sertoli cells from testicular differentiation up to puberty and to a much lesser degree in granulosa cells from birth up to menopause (Munsterberg and Lovell-Badge, 1991; Lee and Donahoe, 1993; Josso et al., 1998; Durlinger et al., 1999; Durlinger et al., 2001; Ikeda et al., 2002).

AMH seems to act only in the reproductive organs (Lee and Donahoe, 1993). The most striking effect of AMH is its capacity to induce regression of the Mullerian ducts, the anlage of the female internal reproductive organs. In the absence of AMH, Mullerian ducts of both sexes develop into the uterus, Fallopian tubes and the upper part of the vagina (Munsterberg and Lovell-Badge, 1991). During fetal life, only the male testis expresses the hormone (Munsterberg and Lovell-Badge, 1991).

In the female, AMH expression begins post-natally and acts in modulating follicular growth (Durlinger et al., 2001; Ikeda et al., 2002) and preventing recruitment of non-dominant follicles (Durlinger et al., 1999; Josso et al., 1998).

Granulosa cells of primary follicles show homogeneous AMH expression, whereas in larger follicles, AMH is mainly produced in cells near the oocyte and in a few cells surrounding the antrum. AMH continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which they are to be selected for dominance by the action of pituitary FSH.

In mice this occurs at the early antral stage in small growing follicles (Durlinger et al., 2002), whereas in human it is in antral follicles of size 4–6 mm (Weenen et al., 2004). Thus, AMH is expressed in follicles that have undergone recruitment from the primordial follicle pool and have not been selected for dominance. AMH is not expressed in atretic follicles and theca cells (Rey et al., 2000) (Figure 1).

AMH employs a heteromeric receptor system consisting of a single membrane spanning serine threonine kinase receptors of types I and II, respectively. The type II receptor imparts ligand-binding specificity and the type I receptor mediates downstream signalling when activated by the type II receptor (Figure 2). The AMH type II receptor (AMH-RII) is localized to the mesenchyme around the Mullerian duct in the urogenital ridge of both the male and female rat and mouse. It is interesting to note that loss of function mutations in the AMH-RII as well as the AMH ligand itself, are causes of persistent Mullerian Duct Syndrome in humans (Imbeaud et al., 1994). In the ovary of rats, the AMH-RII is expressed both in granulosa and theca cells (Ingraham et al., 2000).

Role of AMH in ovarian physiology

AMH is considered as a negative regulator of the early stages of follicular development (Themmen 2005). Earlier studies have shown that AMH inhibits the first meiotic division of diplotene oocytes in immature rats (Ueno et al., 1989), but this has not been confirmed by others (Tsafri et al., 1988). In addition, AMH blocks the proliferation of human granulosa–luteal cells in vitro (Kim et al., 1992) and the concentration of AMH in follicular fluid is inversely proportional to the mitotic indices of granulosa cells in vivo (Seifer et al., 1993). Thus, AMH appears to have an autocrine role in maturation of normal follicles (Figure 3).

Based on this observation it has been hypothesized that AMH could be one of the factors involved in determining the responsiveness of ovarian follicles to FSH during cyclic recruitment (Skinner, 2005). It has been recently shown that oocytes up-regulate AMH expression in granulosa cells in a fashion that is independent upon the developmental stage of the oocyte (Salmon et al., 2004). This observation leads to the hypothesis that oocytes in the pool of growing follicles could control the pool of primordial follicles by modulating the expression of the inhibiting factor AMH.

Circulating AMH in women

In males, AMH levels after birth are low, but rapidly rise to peak values by late infancy, then slowly decrease to the adult range at puberty (Josso et al., 1999; Lee et al., 1995). In women, AMH
serum levels are almost undetectable at birth (Rajpert-De Meyts et al., 1999), with a subtle increase noted after puberty (Hudson et al., 1990).

Serum AMH levels have been measured at different time-points during the menstrual cycle, suggesting minimal fluctuation (La Marca et al., 2006a). Minimal fluctuations in serum AMH levels may be consistent with continuous non-cyclic growth of small follicles.

Hence, AMH is relatively convenient to determine, especially as it seems to exhibit a relatively stable expression during the menstrual cycle, making it an attractive determinant of ovarian activity (Gruijters et al., 2003).

Recent studies indicate the AMH as a novel measure of ovarian reserve. Serum AMH levels have been shown to decrease over time in young normovulatory women (de Vet et al., 2002), and to correlate with age, FSH and the number of antral follicles. In a recent study, a group of women was studied twice and the interval between the two visits ranged from 1.1 to 7.3 years. A reduction in mean AMH levels of about 38% was observed, whereas the number of antral follicles and the levels of FSH and inhibin B did not change (de Vet et al., 2002). The decrease in AMH with advancing age may occur before changes in the

![Figure 1. AMH immunostaining in normal human adult ovary.](image1)

**Figure 1.** AMH immunostaining in normal human adult ovary. (A) Primordial follicles are negative (arrowheads), whereas a weak positive reaction can already be observed in the cytoplasm of a few cells in a primary follicle (arrow). (B) All granulosa cells of pre-antral follicles are AMH-positive. (C) AMH-positive and AMH-negative cells are intermingled in the cumulus oophorus of a pre-ovulatory follicle. (D) Only a few granulosa cells surrounding the antrum (ANT) are AMH-positive. Thelcal cells (TC) are not immunoreactive (original magnifications—A and B: ×200; C: ×100; and D: ×300) (Reprinted from “Anti-Müllerian hormone is a specific marker of sertoli- and granulosa-cell origin in conadal tutorius” by Rey R. et al. (2000) Human Pathology, 31:1202-1208, with permission from Elsevier).

![Figure 2. AMH receptor binding](image2)

**Figure 2.** AMH receptor binding. Upon ligand binding to the type II receptor, the type I receptor is recruited into the receptor complex and becomes phosphorylated by the type II receptor. Activation of the type I receptor results in the phosphorylation of the downstream Smad proteins. Phosphorylated receptor-specific Smads translocate to the nucleus where they regulate gene expression.

![Figure 3. Role of AMH in human folliculogenesis.](image3)

**Figure 3.** Role of AMH in human folliculogenesis. In women, AMH expression can first be observed in granulosa cells of primary follicles, and expression is strongest in pre-antral and small antral follicles (≤4 mm). AMH expression disappears in follicles of increasing size and is almost lost in follicles larger than 8–10 mm, where only very weak staining remains, restricted to the granulosa cells of the cumulus. This expression pattern suggests that AMH may play a role in initial recruitment and in the selection of the dominant follicle: AMH seems to be a negative regulator of the early stages of follicular development. AMH prevents recruitment of non-dominant follicles and reduces the responsiveness of ovarian follicles to FSH during cyclic recruitment by Stephen et al. (2002) Highly purified Müllerian inhibiting substance inhibits human ovarian cancer in vitro. Clin Cancer Res. 8, 2640-2646.
currently known ageing-related variables and this indicates that serum AMH levels may be the best marker for ovarian ageing and menopausal transition.

**AMH and AMH receptor in ovarian cancer**

*Granulosa cell tumour*

In the first description of increased AMH in serum from a patient with GCT (Gustafson et al., 1992), the AMH gene expression in neoplasm specimens was also investigated. RNA was extracted and analysed by northern blotting showing that a 2.0 kb AMH RNA transcript was present.

In a small series of ovarian tumours, AMH expression was detected in all of the GC tumours but not in the seven epithelial carcinomas (Matias-Guiu et al., 1998). Using paraffin-fixed sections and antibody directed to AMH, authors demonstrated that AMH immunoreactivity was always present in the GC tumours but was not as strong or diffuse as alpha-inhibin. The finding of AMH expression in both juvenile- and adult-type GCT of either ovarian or metastatic localization has been recently confirmed (Rey et al., 2000) (Figure 4).

Dutertre et al. (2001) developed a murine transgenic model of GCT of the ovary. They showed that driving the expression of the simian virus 40 (SV40) oncogene by the AMH promoter induces gonadal tumorigenesis in transgenic mice (Peschon et al., 1992; Dutertre et al., 1997). They also showed that granulosa cell tumours of less than 100 mg (10-fold the size of the normal ovary) were positive for both AMH and AMH-RII mRNA and proteins. Moreover, expression was not found in larger tumours (Dutertre et al., 2001). They also showed that AMHR-II was expressed on the cell surface and was able to bind to its ligand AMH. They concluded that GCT of transgenic mice produce AMH and secrete it into the blood when the tumour is relatively small. In advanced stages, the GCT invades organs and develops metastases and loses both AMH and AMH-RII expression. Considering the inhibitory effect of AMH on GCT growth (Kim et al., 1992), this phenomenon could indicate a counter-selection to favour further tumour progression.

*Epithelial ovarian cancer*

More recently, the AMH-RII expression has been found in human epithelial ovarian cancer (Masiakos et al., 1999). Human epithelial ovarian cancer cells and ascites cells isolated from patients with ovarian papillary serous cystoadenocancer were processed and analysed by RT–PCR for the detection of AMH-RII mRNA. AMH-RII transcripts were detected in cell lines from 10 of the 15 patients. In 89% of the cases, the presence of AMH-RII mRNA was predictive of the binding to AMH. In the solid tumours from four patients AMH-RII immunostaining was also demonstrated.

**AMH as circulating tumour marker for GCT**

Gustafson et al. (1992) have demonstrated abnormalities of AMH secretion in relation to ovarian malignancy in stored sera from 18 patients. Elevated serum concentrations of AMH was present in four women with GCT. Serum AMH levels were normal in six women who had undergone resection of GCT 2–45 months before and who were disease-free. AMH was undetectable in serum from women with metastatic epithelial ovarian cancer and in three women with previously resected ovarian carcinoma. Similarly, AMH was undetectable in serum from one woman with Fallopian tube carcinoma and from a woman with mesonephric adenocancer. Two women with bilateral gonadoblastoma or bilateral dysgerminoma AMH did not exhibit increased serum AMH levels.

Hence, based on these findings it seems that AMH is increased only in the serum from women with GCT.

One patient with GCT was followed in the clinical course for about 6 years and the results of hormonal evaluation demonstrated that serum AMH levels decreased after each of the subsequent major tumour resections. Furthermore, elevations of AMH preceded or accompanied recurrences of disease.

A successive study confirmed that AMH is a serum marker limited to GCT. Rey et al., 1996 showed normal AMH levels in 93.3% of patients with non-gynaecologic cancer or gynaecologic cancer other than GCT. In eight of nine patients with progressive GCT, AMH levels were above the normal range, and in one patient with clinical remission of the disease, AMH was below the detection limit.

In their longitudinal study of 11 patients with recurrent GCT (Rey et al., 1996), AMH showed a good correlation with evolution.
of the disease during follow-up in nine cases. Serum AMH fell to undetectable values after successful treatment or rose early in cases of recurrence of the disease. These findings indicate that AMH can be used to evaluate the efficacy of the treatment. Interestingly, comparing AMH and alpha-inhibin, it has been shown that serum alpha-inhibin fluctuated between normal and high values more frequently than did AMH. AMH has also been measured intraoperatively in cystic fluid from two children with juvenile GCT, showing elevated concentrations in comparison with circulating ones, thus indicating the local production (Rey et al., 1996).

Globally, AMH serum levels seem to be elevated in 76% (Lane et al., 1999) to 93% (Long et al., 2000) of GCT patients (Table I). The mean AMH level was 190.3 ng ml\(^{-1}\) (range 2–1124 ng ml\(^{-1}\)) in patients with GCT in one study (Lane et al., 1999). In patients in whom serial measurements were made following initial tumour resection, the length of time that an elevation in AMH levels preceded clinical detection of a recurrence was 3 months (Lane et al., 1999).

An ultrasensitive ELISA assay for AMH was developed some years ago (Long et al., 2000). The new ultrasensitive ELISA assay is a sandwich-type assay where sensitivity is as low as 0.1 ng ml\(^{-1}\) (in comparison with 2 ng ml\(^{-1}\) of the traditional assay used in the studies published before the year 2000). Using the new assay, Long et al. (2000) found that sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the presence of GCT were 93, 96, 93, 96%, respectively.

The relevance of using an ultrasensitive assay is demonstrated by the fact that in 6 of 31 patients the new and the traditional assays gave discordant results. Indeed in these patients, AMH was undetectable using the traditional assay but readily detectable with the ultrasensitive AMH ELISA (Long et al., 2000).

**AMH as a chemotherapeutic agent for epithelial ovarian cancer**

About two-thirds of the new cases of epithelial ovarian cancer diagnosed every year are at an advanced stage (Greenlee et al., 2001). Once the tumour has spread beyond the confines of the ovary, surgery alone is unlikely to provide adequate therapy.

After aggressive debulking surgery, primary chemotherapy with curative intent with platinum and taxanes is the standard of care. Carboplatin and cisplatin are equally effective, but carboplatin has less toxicity and is the most common platinum compound used as first line in combination with paclitaxel.

In patients with advanced epithelial ovarian disease, the overall response to primary platinum-based therapy is 70–80%, with one-half of these patients achieving complete clinical response and one-quarter reaching complete pathological response. One-half of these patients with complete pathological response will relapse. It is expected that 70–80% of all patients with advanced ovarian cancer will need second-line therapy (Kalil and McGuire, 2002). The object of second-line therapy is to reduce symptoms, minimize toxicity and prolong the progression-free interval, with palliative intent.

AMH may also be applicable as a chemotherapeutic agent for epithelial ovarian cancer. AMH induces regression of the Mullerian ducts, which arise from a common embryological precursor to the surface epithelium of the ovary, from which epithelial ovarian tumours originate (La Marca et al., 2006b). The inhibitory effect of the hormone is believed to be consequent to its binding to the AMH-RII. Indeed, expression of AMH-RII has been demonstrated at the transcriptional and translational level in a series of human ovarian cancer cell lines (Masiakos et al., 1999).

Masiakos et al. (1999) demonstrated that the growth of five of six epithelial ovarian carcinoma cell lines expressing AMH-RII was inhibited by exogenous recombinant AMH. Moreover, 56% of primary ascites cells seem to bind AMH (Masiakos et al., 1999). Using the colony inhibition assay, it has been demonstrated that AMH binding to its receptor is followed by inhibition of the cell growth (Chin et al., 1991; Masiakos et al., 1999; Ha et al., 2000; Stephen et al., 2001).

The growth inhibition correlates with a block in cell cycle progression and is characterized by apoptosis. These events are mediated by induction of the cyclin-dependent kinase inhibitor p16, which results in the up-regulation of the E2F family of transcription factors that are regulated by members of the pocket protein family (Ha et al., 2000).

In a recent study human ovarian cancer cells were implanted in immunosuppressed mice and treated with recombinant AMH (Stephen et al., 2002). Two to three weeks of AMH treatment was followed by a significant reduction in the graft size ratio indicating that AMH delivered parenterally may cause inhibition of human cancer cell lines in vivo (Figure 5).

**Conclusions**

Ovarian cancer is one of the most frequent gynaecological malignancies. Three major types are recognized: epithelial, stromal and germ cell. Epithelial ovarian tumours are the most common human ovarian carcinomas accounting for 82% of all the human ovarian cancers. The majority of these cancers present at III or IV stage, hence the 5-year mortality rate for the disease is about 70% (Cannistra, 1993).

GCT constitutes 3% of all ovarian tumours. GCT is divided in to the adult and the juvenile form. Of women with adult GCT, 90%
present with stage I and the survival rate at 5 years remains very high (Bjorkholm and Silfverswaard, 1981). The recurrence for adult GCT is late occurring: 4–6 years after the first treatment. In contrast, a large subset experience recurrence of juvenile GCT occurs within one year, with a mean of 6 months (Calaminus et al., 1997).

In recent years, the advances in the physiological role of AMH has stimulated interest in the significance of AMH as diagnostic marker and therapeutic agent for ovarian cancer. At this moment, AMH has only been shown to be a circulating marker for GCT. Its diagnostic performance seems to be very good, with a sensitivity ranging between 76 and 93%. AMH seems to be superior to alpha-inhibin and estradiol in the early diagnosis and in the follow-up of GCT.

It is largely recognized that AMH and alpha-inhibin exhibit a higher degree of sensitivity than estradiol in progressive GCT. Indeed, estradiol production by GCT is widely variable (Lappohn et al., 1989) while AMH seems to be a marker of comparable value to alpha-inhibin. However, AMH is elevated only in sex cord stromal tumours, while

Figure 5. Effects of AMH on ovarian cancer. OVCAR 8 is a human ovarian cancer cell line, which expresses the AMH-R1I. OVCAR 8 tumours were implanted under the renal capsules of nude mice after 3 weeks. (A) Control tumour shows neovascularization and minimal necrosis or inflammation. (magnification: x 40). (B) AMH-treated tumour (10-μg day⁻¹×3 weeks delivered i.p.) is about one-third the size of the control tumour and has minimal necrosis or inflammation (magnification: x 40) (Reprinted from “Highly purified Mullerian inhibiting substance inhibits human ovarian cancer in vivo” by Stephen et al. Clinical Cancer Research 2002, 8: 2640–2646, with permission from American Association for Cancer Research).
inhibin may be elevated in different types of cancer (Healy et al., 1993).

Doubts remain on the possible role of AMH in the pathogenesis of GCT. Indeed, AMH is considered a powerful inhibitor of granulosa cell proliferation. Hence, the high serum AMH levels found in patients with GCT, are in contrast with the progressive characteristic of the disease. One possible explanation is that the binding of AMH-RII by its ligand in tumour cells is not followed by the inhibitory signal. Otherwise from animal studies it has been hypothesized that the inhibitory signal is functional when the tumours are limited (Dutertre et al., 2001). Successively, when the tumours become progressive and reach a large dimension, they lose AMH and AMH-RII expression. This could be a counter selection to favour further tumour progression (Dutertre et al., 2001).

In patients treated for GCT, AMH may be followed post-operatively as a marker for efficacy of surgery and disease recurrence. Following surgery, AMH decreases to normal values within days to weeks. The AMH half-life is estimated to be 48 h (Vigier et al., 1983). Hence, reduced levels of AMH should be detected 72 h after successful surgery. Indeed, bilateral ovariectomy performed for benign disease is followed by undetectable AMH levels 3–5 days after surgery (La Marca et al., 2005).

In the past, anti-AMH antibodies were not commercially available. Only few laboratories in the world performed the assay and this has precluded the diffusion of AMH as a marker for GCT. The recent availability of ultrasensitive commercial ELISA will permit its clinical application (Rey et al., 1999; Long et al., 2000). This ELISA, which is valid both for males and females, is a sandwich-type assay where sensitivity is as low as 0.1 ng ml⁻¹ (in comparison with 2 ng ml⁻¹ for the traditional assay used in the studies published before 2000). More recently, a new double-antibody ELISA for AMH has been developed, with a detection limit of <0.078 ng ml⁻¹ (Al-Qahtani et al., 2005).

The specificity of the commercially available ultrasensitive assay was tested by assaying samples containing known concentrations of most of the AMH-related members of a large family of growth and differentiation factors: TGF-β, inhibin A and B, inhibin pro-αC subunit and activin A. No cross reactivity was observed in any of the cases (Long et al., 2000).

Based on the published studies it is possible to elaborate the following recommendations for the use of AMH as a marker of GCT:

(i) High serum AMH levels are found in 76–93% of patients with GCT.
(ii) Serum AMH levels should normalize within few days following surgery.
(iii) Persistent AMH levels are indicative of residual disease.
(iv) When bilateral ovariectomy is performed AMH must be undetectable in serum. Even very low levels may be suggestive of residual disease.
(v) A post-operative rise in serum AMH levels may precede the clinical recurrence. Hence, the clinical examination and imaging should be anticipated.
(vi) AMH levels should be obtained every 6 months for at least 5 years after the surgery. However, it should not be ignored that a late recurrence (up to 30 years after initial treatment) has been reported in up to 33% of patients (Stenwig et al., 1979).

(vii) Recurrences in juvenile GCTs occur within a year of initial diagnosis at a mean of 5–6 months. Hence, in juvenile GCT, AMH serum levels should be determined every month following the surgery.

At this moment, data on the role of AMH measurement in monitoring the response to chemotherapy is lacking.

These recommendations are based on a limited number of studies. Hence, they should be validated in clinical trials.

Several authors have also hypothesized a role for recombinant AMH in the treatment of epithelial ovarian cancer (Stephen et al., 2001, 2002). Based on the physiological inhibiting role of AMH on the mullerian ducts, researchers have demonstrated that AMH inhibits epithelial ovarian cancer cell in vitro (Fuller et al., 1982, 1985; Stephen et al., 2002; Masiakos et al., 1999).

In a small series, it has been shown that 56% of ovarian cancer ascite cells express the receptor for AMH, and when tested in solid tumours from patients with epithelial ovarian cancer, in each case AMH-RII was expressed (Masiakos et al., 1999). The administration of recombinant AMH in immunosuppressed mice with human epithelial ovarian cancer implants has been followed by reduced dimensions of the graft (Stephen et al., 2002).

This of course will be the basis for future research aiming to investigate the possible clinical role of AMH as neo-adjuvant or most probably adjuvant therapy for ovarian cancer.

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