Predictive Value of the AMH Level and Serum Estradiol for Ovarian Hyperstimulation Syndrome in the Assisted Human Reproduction

IRINA PACU[1,2], CRINGU IONESCU[1,2]*, SIMONA VLADAREANU[1], MIHAI BANACU[1,2], ADRIAN NEACSU[1], ALINA CALIN[3]

[1] University of Medicine and Pharmacy Carol Davila Bucharest, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[2] Clinical Emergency Hospital Sf Pantelimon Bucharest, Department Obstetrics Gynecology, 340-346 Pantelimon Str., 21591, Bucharest, Romania.

[3] Dunarea de Jos University of Galati, 47 Domeasca Str., 800008, Galati, Romania

The ovarian hyperstimulation syndrome (OHSS) is one of the major complications which occurs as a result of controlled ovarian stimulation (COS) in the assisted human reproduction. There are several factors including age, body mass index (BMI), plasma estradiol level, the anti-mullerian hormone (AMH) level and the antral follicle count (AFC), which can be used to identify the cases with high risk for this complication. The purpose of the study is to establish the predictive value of AMH for the development of OHSS before COS as well as its association with the plasma estradiol level during stimulation. The study group included 155 COS cycles using gonadotropin-releasing hormone (GnRH) agonist protocols, and analyzed the predictive value of the mentioned parameters for OHSS. The serum AMH level is superior to age and BMI for identification of patients with high risk for OHSS before starting the ovulation stimulation, and the cut-off level is 3.78ng/mL with 91.1% sensibility and 85.2% specificity.

Keywords: ovarian hyperstimulation syndrome, controlled ovarian hyperstimulation, antimullerian hormone, estradiol

At present, the assisted reproduction techniques are a part of the current practice for the treatment of couple infertility, and the use of controlled ovarian stimulation (COS) has become widespread within these programs. COS is carried out in the assisted reproduction according to various protocols (long, short, ultra-long or ultrashort) with different doses of gonadotropins, individualized according to each patient's response, and using different pharmaceutical preparations, namely highly purified FSH (follicle stimulating hormone) and/or LH (luteinizing hormone) preparations obtained from the urine of women at menopause or recombined preparations obtained in the laboratory through genetic engineering techniques. Pituitary inhibition is obtained by the use of GnRH (gonadotropin releasing hormone) antagonists or agonists, and the final oocyte maturation (ovulation trigger) by the use of chorionic gonadotropin hormone (hCG) (LH-like structure) or recombined LH. Irrespective of the stimulation protocol used in the assisted reproduction, the purpose of the stimulation is to obtain an increased number of mature ovarian follicles and, therefore, good quality oocytes which will provide a satisfactory number of good quality embryos, with a high rate for implantation and pregnancy occurrence. Under these conditions, a controlled ovarian hyperstimulation is obtained, which, according to the classification made by Navot et al [1], corresponds to the mild stage of ovarian hyperstimulation syndrome (OHSS), including a mild abdominal distension and discomfort, increase of the ovary volume, mild nausea, without modification of the biological constants.

OHSS is one of the most severe complications of COS, it is an iatrogenic possibly life-threatening complication[2]. From a pathophysiological point of view, several mechanisms are involved, but the most important is the secretion of a vascular endothelial growth factor (VEGF) by the hyperstimulated ovary, which causes the increase of the vascular permeability and vasodilation of all blood vessels, and the immediate consequence is the liquid transvasation from the intravascular space to the extravascular one, increase of blood concentration, hyponatremia, and effusion in the extravascular spaces (subcutaneous cell tissue, peritoneal cavity, pleura, pericardium) [2]. hCG seems to mediate the effect of VEGF, and higher hCG concentrations are associated with the increased severity of the syndrome, thus explaining why late OHSS (occurring over 7-9 days since the follicular puncture and associated with the pregnancy) is of the highest severity [3]. The occurrence of the syndrome is reduced (1-5%) but it is difficult to establish the real incidence because of the significant reporting differences [4]. The Practice Committee of the American Society of Reproductive Medicine established in 2016 that besides the mild symptoms described above, the moderate forms are associated with ultrasound signs of ascites with Ht>41% and leucocytes>15000/mL, the severe forms present ascites, hydrothorax, dyspnea, oliguria, nausea and incoercible vomiting, venous thrombosis, rapid weight gain (>1kg/24 h),Ht>55%, leucocytes>25000/mL, Na<145 mEq/L, K>5mEq/mL, increased hepatic enzymes. Anuria, pericardial effusion, massive hydrothorax, venous and arterial thrombosis occur in critical cases [5].

It is of high importance to predict the cases who will develop OHSS before initiating the COS treatment for establishing the optimal therapeutic scheme as well as during treatment to reduce the risk for a severe form of OHSS. There have been attempts to reach this goal through evaluation of the risk by reference to age, body mass index (BMI), the cause of infertility (polycystic ovary syndrome-PCOS), the plasma level of serum estradiol at the time of the ovulation trigger, and the antral follicle count (AFC) [6]. Regardless of the individual or associated assessment of these parameters, it seems not easy to predict with maximum accuracy before initiating the ovarian stimulation what patients will develop OHSS. It was identified the role of the anti-mullerian hormone (AMH) as a predictive factor of OHSS, starting from the idea that the majority of patients with poor response to COS are those

* email: antoniuginec@yahoo.com; Phone: (+40) 722344391 *All authors had an equal contribution with those of the main authors*

with low AMH at the onset of the follicular phase, therefore having a reduced ovarian reserve, often correlated with advanced age. At puberty, AMH starts to be secreted by the granular layer cells of the incipient antral follicles (and not by the primordial or dominant follicles), being an ideal marker for the estimation of the ovarian reserve [7]. The decrease of AMH with age precedes with a few years the obvious increase of FSH and, therefore, offers extremely accurate indications on the menopausal transition. AMH has a physiologic role at two moments of the folliculogenesis: the initial recruitment of the primordial follicles and the cyclic recruitment of follicles, dependent on FSH [8]. The AMH basal value will accurately express the number of follicles recruited among the primordial follicles at the beginning of every menstrual cycle or during COS. In the COS for IVF the number of recruited follicles that will increase as a result of the ovarian stimulation has a stronger predictive value for OHSS occurrence than the antral follicle count that will generate quality oocytes for collection and fertilization[7]. The estradiol is secreted in larger amounts from the granular cells of the antral and preovulatory follicles as opposed to the smaller follicles, which explains why the predictive value of AMH for the development of OHSS is higher than that of plasma estradiol on the day of hCG administration [8].

Experimental part

This study was a retrospective one made in Department Obstetrics Gynecology Clinical Emergency Hospital Sf Pantelimon and Medsana Genesis Clinic, were we analyzed a number of 155 cycles of controlled ovarian stimulation between January 2016 and December 2016. The stimulation protocols differed but the study included only the cases in which long or short protocols with GnRH agonists were used, namely 119 cases, excluding the ultra-long or ultra-short protocols (5 cases), GnRH antagonist protocols (22 cases), combined use of clomiphene citrate with gonadotropins (9 cases).

In the case of the short protocol (n=66 cases) the GnRH agonist was administered from day 3 of the menstrual cycle 0.1mg/day; the ovarian stimulation was carried out using various gonadotropin preparations starting with day 2-3 of the menstrual cycle. In the case of the long protocol (n=53 cases), the agonist was administered from day 21 of the menstrual cycle which precedes the stimulation cycle, and the administration of gonadotropins was similar. The gonadotropin dose was individualized and adjusted depending on the response of each patient, and the monitoring was done by combining the data showed by the transvaginal ultrasound (number and size of ovarian follicles) and the serum estradiol level. The hCG was administered when minimum two ovarian follicles reached 18mm in size, the dose was 250μg in all cases, and the collection of oocytes was carried out by transvaginal puncture 32-34 h after this moment. The GnRH agonist was administered until the day of hCG injection. The luteal phase was supported in all cases with 400-800mg/day dose of intravaginal progesterone. The embryo transfer was carried out in an individualized manner, between days 3 and 6 after the follicular puncture. The patients considered pregnant were those whose serum hCG values were higher than 50IU/l 14 days after the embryo transfer; the first ultrasound examination was done minimum 21 days after the embryo transfer.

As regards the ovarian hyperstimulation, for inclusion of patients within this diagnosis we used Navot classification [1,3], which also allows the differentiation of the severity of this syndrome. We did not consider the cases of mild hyperstimulation, which are inherent in any procedure of ovarian hyperstimulation for the assisted reproduction, but only the cases of moderate and severe hyperstimulation [3,7]. In the moderate hyperstimulation we included patients with abdominal distension, discomfort, nausea, vomiting, ascites, and ovaries of 8-12cm in size (fig 1). We considered it severe hyperstimulation in patients with voluminous ascites, pleural effusion, dyspnea, oliguria, incoercible vomiting, weight gain of over 1kg/24 h[8]. Paraclinically, we included in the cases of moderate hyperstimulation patients with a hematocrit level of over 41%, leucocytes over 15000/mL; the cases of severe hyperstimulation were considered those with hematocrit over 55%, leucocytes over 25000/mL, hyponatremia (<135mEq/L), hyperkalemia (>5mEq/L), elevated transaminases.

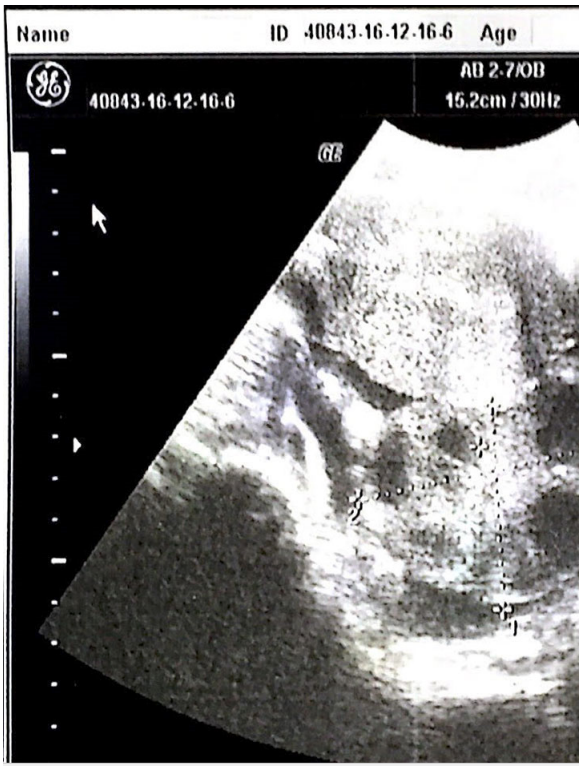

Fig 1. Ultrasound image of OHHS-ovary and ascites

During the stimulation treatment, the serum estradiol values were monitored in all cases starting with day 3 of the stimulation cycle, then on day 7-8, and on hCG day, together with the progesterone dosing. The estradiol was determined by competitive immunoassay using Immulite kit. The lower threshold for detection of estradiol was 15pg/ml (55pmol/L).

Statistically, we analyzed the parameters relevant for COS, which were presented as median value (SD) and levels (range). To estimate the predictive value of the measured and analyzed parameters we performed the analysis curve (ROC). The parameters analyzed for prediction of the occurrence of moderate and severe hyperstimulation syndrome were compared with their values under the ROC curve and with the value of 95% of the confidence interval (CI).

Results and discussions

This study included 119 cases of patients who underwent COS for assisted reproduction procedures, and the ovarian stimulation was performed according to the long protocol (53 cases) or short protocol (66 cases) with GnRH agonists. The goal of the work is to establish the predictive role of serum AMH level before initiating

Variable	Mean (SD)	Range
Age (years)	31.3 (4.7)	23-45
BMI (kg/m2)	20.3(3.5)	17-35.4
Basal serum AMH (ng/ml)	2.29(1.93)	0.02-8.96
Plasmatic estradiol on hCG day (pg/mL)	1543(1124)	78.2-4825
AFC on hCG day (>10mm)	10.9(6.5)	1-27
Number of retrieved oocytes	9.1(7.1)	0-24
OHSS rate (%)	12/119 (10.08)	3.3-12.5%
Pregnancy rate (%)	44/119 (36.97%)	31.2-40.1%

Table 1
PATIENTS CHARACTERISTICS, PARAMETERS ANALYZED

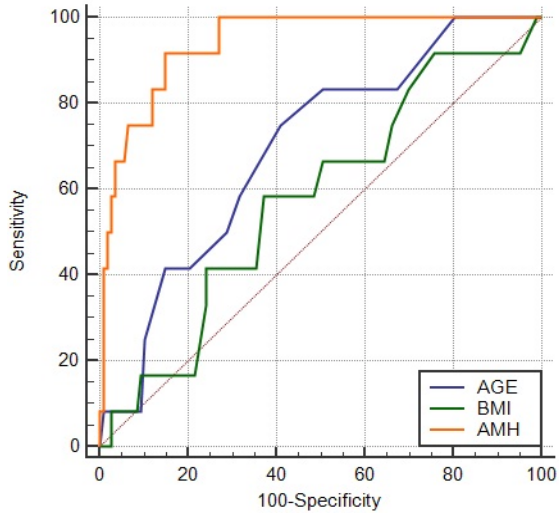

Fig. 2. Predictive values for age and AMH for OHSS

stimulation and of estradiol at hCG administration in the occurrence of the hyperstimulation syndrome in these patients.

The entire study group was analyzed according to age, body mass index (BMI), AMH values on day 3 of the cycle preceding the stimulation one, estradiol values on day 3 of the stimulation cycle and on hCG day, the number of ovarian follicles over 10 mm according to the transvaginal ultrasound from the day of the ovulation trigger, number of collected oocytes, pregnancy rate (clinically), and occurrence of moderate and severe hyperstimulation syndrome. These data are shown in table 1. One can observe that there are predictive factors which can be identified before initiation of COS, of which the most important are BMI, age, and basal AMH , AMH being obviously superior in this regard (fig 2).

As regards the ovarian hyperstimulation syndrome (OHSS), it was diagnosed in 12 cases (10.08%); there were 8 cases of moderate hyperstimulation (6.72%) and 4 cases of severe hyperstimulation (3.36%). Depending on the stimulation protocol, there were 3 severe hyperstimulation cases (5.67%) and 5 moderate (9.4%) within the long protocol, and one severe hyperstimulation case (1.5%) and 3 moderate (4.5%) within the long/short protocol, respectively. Among the risk factors for OHSS, the most important seems to be the polycystic ovary syndrome which was diagnosed in 7 of the 12 cases (58.3%).

In table 2 presents the predictive values of the risk factors for OHSS. One can observe that the predictive value of BMI is lower than that of the other factors and significantly lower than that of AMH, estradiol on the ovulation trigger day, number of ovarian follicles and number of collected oocytes. AMH has the highest level of specificity in the detection of patients with risk for developing OHSS due to ovarian stimulation therapies for the techniques of human assisted reproduction, the cut-off value being 3.78ng/mL determined according to ROC curve .

The serum estradiol value on hCG day has a weaker predictive value than AMH, but the difference between these factors is not statistically significant. Instead, combining the two factors causes a sensitivity of 54.7% and a specificity of 91.2%, higher than the specificity of AMH alone. As regards the relation with pregnancy, the only factor with statistically significant correlation is age, while the AMH basal value or the estradiol level on hCG day have no clinically significant predictive value (OR:1,1, P = 0.9120).

OHSS incidence in this study (10.8%) is higher than in the studies reported by others (7.7%) or (8.9%) [8,9]. This may be explained by the large number of patients with polycystic ovary syndrome (28 of 119) and by the median age of patients (31.3years), which is lower than in the above mentioned studies. The cut-off value of the estradiol on hCG day, which imposed known prophylactic methods (freeze-all embryos, coasting) was 3500pg/mL and the number of ovarian follicles over 20 in both ovaries, values which were chosen according to the data provided by the specialized literature [9,10]. These aspects somehow limit the predictive value of the estradiol level and of AFC in the prediction of OHSS using these isolated parameters.

The relation with polycystic ovarian syndrome is a certain one, as the study confirms that it remains the main pathology which increases the risk for OHSS in COS[11]. Moreover, these patients present significantly increased AMH and AFC basal values, and the ultrasound reveals an

Table 2
THE PREDICTABILITY OF DIFFERENT PARAMETERS FOR OHSS

Variable	Cut-off	Sensitivity 95%CI	Specificity 95% CI	Positive predictive value	Negative predictive value
Age (years)	30.2	72.4(51.8-89.7)	53.1(47.2-62.1)	12.3	95.1
BMI (kg/m^2)	17.22	34.7(12.8-52.4)	87.4(72.3-88.7)	26.8	91.2
Basal serum AMH (ng/mL)	3.78	92.9(69.5-98.6)	85.2(77.3-88.6)	21	99.2
Plasmatic estradiol on hCG day (pg/mL)	3800	92.3(72.1-99.7)	62.3(56.7-72.6)	19.3	96.2
AFC on hCG day (>10mm)	17	94.7(72.9-99.2)	60.2(56.1-68.5)	18.2	97.9
Number of retrieved oocytes	12	89.2(64.7-97.3)	63.2(57.5-70.9)	18.1	97.3

increased number of antral follicles at the beginning of the proliferative phase.

As previously shown, the AMH basal value has a similar predictive value, if not higher than the estradiol value on hCG day combined with AFC. Establishing the cut-off was difficult, the median value in patients with OHSS being 4.95ng/mL (SE 4.35ng/mL). Statistical data have shown that the best predictive value is that of the cut-off level of 3.78ng/mL (table 2). AMH has a physiological role in two moments of the recruitment: the initial recruitment of the primordial follicles and the cyclic recruitment of follicles, dependent on FSH[12]. The AMH basal value will accurately express the number of follicles recruited among the primordial follicles at the beginning of every menstrual cycle or during COS. In the COS for IVF the number of recruited follicles that will increase as a result of the ovarian stimulation has a stronger predictive value for OHSS occurrence than the antral follicle count that will generate quality oocytes for collection and fertilization [13]. The estradiol is secreted in larger amounts from the granular cells of the antral and preovulatory follicles as opposed to the smaller follicles, which explains why the predictive value of AMH for the development of OHSS is higher than that of plasma estradiol on the day of hCG administration [14]. The best predictive value is that of AMH after stimulation, on day 5, compared to basal AMH, but this value is not useful for establishing the used protocol, but only for adjusting doses.

As respects the estradiol value on hCG day compared to AFC, the result of our study shows the superiority of the estradiol level compared to AFC in predicting OHSS; an explanation would be the fact that we included in the study only GnRH agonist protocols with a particular pattern of follicular growth. It should be noted that the two parameters, the estradiol value and AFC are dependent on each other and cannot be analyzed independently to establish the risk for OHSS.

The combination of AMH with the estradiol level has a better predictive value than each parameter alone, with a reduced rate of false positive results, but the inconvenient is that AMH is useful in establishing the risk just before the beginning of stimulation, while the association with the estradiol value at hCG moment implies starting the protocol. The value of the estimation of the two factors is highly useful in establishing the preventive measures required in such cases in order to avoid severe OHSS cases. In our study there was a reduced number of cases with false positive results (3) of COS performed with milder protocols, obtaining a more reduced number of ovarian follicles and collected oocytes. However, the possibility of an inadequate ovarian response as a result of an ovarian stimulation with too low doses of gonadotropins in these patients with false positive results remains an issue; a better prediction can be achieved if, for each case, we associate the age factor before starting treatment, and during therapy the results will be obviously better by association with plasma estradiol levels measured repeatedly [15]. In order to reduce the number of false positive cases, the increase of the cut-off value can also be considered. This will nevertheless lead to a dramatic decrease of the predictive value of AMH for OHSS.

Conclusions

The role of the AMH basal value in the prediction of occurrence of the ovarian hyperstimulation syndrome is more significant than that of BMI and age, the cut-off value in our study being 3.78ng/mL. The practical clinical implication is to identify more accurately the patients at risk for OHSS before initiation of the stimulation and to personalize *mild* stimulation protocols for these patients with high AMH basal values. The value of this parameter is comparable with the determination of the serum estradiol on hCG day, and the combination of the two plasma constants seems to be the most useful way for prediction of moderate and severe OHSS during COS, using the long and short protocols with GnRH agonists for assisted reproduction. In the cases associating high AMH basal values with estradiol values of over 3500ng/mL on the ovulation trigger day, the specific measures known for avoiding severe OHSS should be used (coasting, freeze all embryos), and for the subsequent cycles, the protocols with GnRH antagonists using a different trigger are indicated.

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