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AMH assessment five or more years after an initially low AMH level

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INTRODUCTION

Premature ovarian insufficiency (POI) is defined by: age younger than 40 years, irregular menstrual cycle (amenorrhea or oligomenorrhea for at least 4 months), follicle-stimulating hormone (FSH) levels greater than 25 IU/L and low estradiol, on 2 assays at least 4 weeks apart according to the European Society of Human Reproduction and Embryology (ESHRE) (1). The causes of POI can be genetic, autoimmune, or iatrogenic (2) but in more than 80% of cases, it is idiopathic (3). POI is often confused with a diminished ovarian reserve (DOR). There is currently no consensual definition of DOR. The National Assisted Reproductive Technology Surveillance System Guidelines of USA define it as a reduction in fertility associated with a diminution in ovarian function, including elevated FSH or high estradiol at the start of the follicular phase or associated with a reduction in ovarian volume of a congenital, medical, or surgical origin (4). In the Federal Register Notice of USA, it is defined as a reduction in fertility related to a diminution in ovarian function characterized by $FSH > 10$ mIU/mL or $AMH < 1$ ng/mL (or 7.1 pmol/L) (5). Although it is more prevalent than POI, less is known about its aetiology. It is probably multifactorial, with a genetic component (6). Ovarian reserve can be assessed quantitatively by measuring plasma AMH(7). In the literature, studies have focused on AMH and prediction of the age of physiological menopause. A case-control study published in 2018 showed that for AMH levels of 1.5 ng/mL (10.7 pmol/L), 1 ng/mL (7.1 pmol/L) and 0.5 ng/mL (3.6 pmol/L), the risk of menopause before 45 years was respectively 2.6, 7.5 and 23 times higher than for women with an AMH level of 2 ng/mL (14.3 pmol/L) (8). The risk of POI (before 40 years) for low AMH concentrations has rarely been explored. The principal objective of this study was to determine the prevalence of POI 5 to 10 years after occurrence of a serum AMH level < 8 pmol/L. This threshold of 8 pmol/L was selected on the basis of earlier studies (5) (9). As secondary objectives, the factors associated with POI were studied and the annual percentage of AMH decrease was calculated. Finally, we assessed the percentage of pregnancies 5 to 10 years after an AMH < 8 pmol/L.

ABBREVIATIONS : AFC Antral follicle count ; AMH Anti-Müllerian hormone ; ART Assisted reproductive technologies ; BMI Body mass index ; DOR Diminished ovarian reserve ; ESHRE European Society of Human Reproduction and Embryology ; FMR1 Fragile X Mental retardation ; FSH Follicle-stimulating hormone ; POI Premature ovarian insufficiency ; UHC University Hospital Centre

MATERIAL AND METHODS

This is a cross-sectional observational, single-centre study conducted in the department of Endocrine Gynaecology, in a University Hospital Centre (UHC). This study was approved by the Institutional Review Board of the University Hospital.

The study initially included (Time T0) all the infertile women younger than 36 years who underwent a workup for infertility during day hospitalisation between January 2008 and December 2013, and who had a serum AMH < 8 pmol/L. The workup for infertility took place between the second and the fifth day of the cycle. We did not include women who were older than 40 years of age in 2018, had a history of endometriosis, ovarian surgery, endometriosis, cancer (treated by chemotherapy or radiation therapy), a genetic disease including or similar to Turner syndrome, pre-mutation FMR1, or another mutation known to be responsible for DOR. The exclusion criteria were: women for whom no telephone number could be found; or who did not respond after three calls at intervals of 2-3 days; or who expressed opposition to participating in the study when contacted by telephone; or for whom any of cancer treated by radiation or chemotherapy or radiation therapy, discovery endometriosis, or surgery of the ovaries or for endometriosis had taken place since the day hospitalization workup including AMH assay (T0); and women using a hormonal contraceptive that made it impossible to determine POI.

Data were collected during telephone interviews which took place from November 2018 to January 2019 using a pre-established questionnaire (Time T1) the patients agreed; these interviews took place at T1, 5 to 10 years after T0. At the end of the telephone interview, women were asked if they would be willing to come in for a consultation to test their ovarian reserve (estradiol, FSH, LH, AMH). The following data were collected for the study: age, age at menarche, BMI, smoking, cycle regularity, contraceptive use, signs of low oestrogen, the FSH assay, the antral follicular count (AFC), serum AMH level, and any pregnancy — spontaneous, by ART, or by donation.

AMH assays

Before 2016, serum AMH was measured by manual ELISA using the EIA AMH/MIS kit (A11893 Immunotech, Beckman Coulter, France). The limit of quantification (LoQ) of the assay was 2.5 pmol/L. The intra-assay coefficient of variation varies from 3.7% to 9.5% for a 151.4- to 5.8-pmol/L concentration range. The inter-assay coefficient of variation varies from 7.8% to 21.1% for a 140- to 2.2-pmol/L concentration range. From January 2016, ELISA has

been replaced by a fully automated immunoassay on the Access Dxi automatic analyzer (B13127, Beckman Coulter). The LoQ of the assay was 0.57 pmol/L. The within-run and between-run precision varied between 0.9–3.6% and 2.9–10.7%, respectively (10). The dosages of AMH prior to January 2016 were recalculated to compare them to with those performed in 2018 and after. The conversion formule used was « AMH (dxi) = 0,77 x AMH (ELISA) + 0,12 », in pmol/L, as previously shown (11).

Ultrasonography

The antral follicle count (AFC) was evaluated between day 2 and day 5 of a spontaneous cycle (off any hormonal contraception). The ultrasound device used was a General Electric Voluson E8 (vaginal probe frequency 5-9 MHz). All follicles between 2 and 9mm, on both ovaries, were included in the assessment of the AFC.

Statistics

This study is a preliminary study and there was no estimate of sample size.

The results are expressed as the median and first and third quartiles (Q1 and Q3) for the quantitative variables and by the number of individuals and percentages for the qualitative variables. We used Mann-Whitney's U test to compare the groups with and without POI according to the quantitative indicators. Fisher's exact test was used to compare the qualitative indicators. Significance was set at 5%. The statistical analyses were performed with SAS (SAS Institute, Cary NC 25513) software, version 9.4.

The principal endpoint was a diagnosis of POI at T1, 5 to 10 years after an AMH level < 8 pmol/L. It was defined by secondary amenorrhoea or severe cycle irregularity, signs of low œstrogen, FSH > 25 IU/L, and unmeasurable estradiol. Note that patients who still have regular cycles even without ovarian reserve test have been considered non POI.

RESULTS

The study included 92 women selected based on their records and contacted by phone between November 2018 and January 2019. In all, 59 women answered the questionnaire, and 47 finally had clinical assessments, including 21 who agreed with a biological testing of their ovarian reserve (FIGURE 1).

Table 1 summarizes the characteristics of the population at T0 and then at T1, 5 to 10 years later. In our population, the prevalence of POI at least 5 years after the diagnosis was 8/47 women, or 17% [8%; 31%]. The median time to onset of POI after an AMH assay < 8

pmol/L was 5.1 years [2.9; 7.3]. Table 2 compares the features of patients with or without POI at time T1. No differences occurred for age at diagnosis of AMH < 8 pmol/L (T0), age at menarche, BMI, age at test and/or diagnosis (T1), or smoking. On the other hand, the groups differed significantly for the regularity of cycles at T0. For laboratory results, the two groups did not differ significantly for either FSH or AFC at T0, but their serum AMH levels differed significantly then. An ovarian reserve test with a new AMH assay was performed in 21 women, 13 without POI group and 8 with POI. The percentage of annual decrease of serum AMH differed significantly between the no-POI group (10.2% [7.3; 11.8]) and the POI group (20.8% [13.8; 33.3]) with $P = 0.003$.

At least 5 years after the low AMH diagnosis, 40% of the women had not become pregnant, 32% had had spontaneous pregnancies, 18% had ART, and 10% an oocyte donation.

COMMENT

Some women present POI ("menopause before 40", a term that is no longer used). The identification of markers able to predict the onset of POI would be valuable in preventing the long-term risks of an early oestrogen deficiency and especially for fertility and its preservation (12). In our study, the prevalence of POI in women with low AMH was clearly greater than that observed in the general population (1%)(3). AMH < 8 pmol/L before the age of 36 years may thus be a first risk factor for POI. It is useful for clinical practitioners to know that there is probably an excess risk of POI when AMH < 8 pmol/L is discovered in women younger than 36 years. A more accurate threshold could not be determined, for example by ROC analysis, due to the small sample size in our study.

We also noted that women with AMH < 8 pmol/L and irregular cycles (long or short) were at higher risk of developing POI. In clinical practice, therefore, fertility follow-up is particularly justified for women with irregular cycles.

An interesting element is the percentage of decrease per year of AMH. The study by Victoria et al. of women aged 21 to 41 years calculated that serum concentration of AMH fell by 5.6% per year (13). It appears to decrease faster in women with AMH < 8 pmol/L than in the general population. One possible explanation for this phenomenon may be the low AMH level itself. That is, AMH is involved in the inhibition of follicle recruitment. It prevents progression from the primordial follicle to the primary follicle stage and thus enables preservation of the follicle supply (14). As AMH can be described as a guard of primordial

follicles, we might conclude that at some particular low AMH threshold, this role of suppression and follicle pool preservation is no longer performed correctly, so that the pool decreases faster.

Increasingly often, in our consultations, women would like an estimate of their ovarian reserve. They wonder about their fertility and their ability to become pregnant. Pregnancy planning becomes essential for some women (15). It might therefore be tempting to use AMH assays to assess their ovarian reserve. Nonetheless, while serum AMH is a good marker predictive of ovarian response to stimulation(16), it is not a good marker for spontaneous fertility. It has been shown that its variability is too great for it to be able to serve as a good indicator of fertility (9). In our study, in a population of infertile women with AMH < 8 pmol/L, 16 women (32%) had a spontaneous pregnancy. We must note that all of the women wanted to become pregnant at the time of the work-up. It is therefore difficult to extrapolate these results to the general population. In the general population of women of child-bearing age with no fertility problems, no correlation between serum AMH level and the probability of spontaneous pregnancy has been observed (17). Accordingly, it is not recommended for use in the general population to evaluate spontaneous fertility.

When a young women who does not want to become pregnant immediately has had an AMH assay for another reason and its result is low, a monitoring protocol may be set up, with assays at regular intervals to assess the rate of decrease. Fertility preservation may be envisioned if follicle loss appears accelerated. It is important to monitor and manage women with DOR but still capable of conception with their own gametes or with ART, unlike the women with POI.

Among our preliminary study's limitations is that our principal endpoint is the diagnosis of POI. Nonetheless, our definition does not fully correspond to that of ESHRE, because a laboratory follow-up test four weeks later was not performed in most cases. We observed a POI prevalence 17 times greater in our population than in the general population. To know if this difference is significant, it would be necessary to monitor a control group of women with identical initial characteristics but a normal AMH level (8 to 35 pmol/L) (18). Moreover, the use of two different AMH assays is acknowledged as a limitation. Our study probably underestimates the prevalence of POI. That is, after 5 to 10 years of follow-up, not all of the women have reached the age of 40 years. Moreover, women older than 40 years old in 2018 were not included to limit memory bias, but we can assume that some of these women had POI.

CONCLUSION

In conclusion, low AMH, that is, < 8 pmol/L before the age of 36 years, may be a risk factor for POI, especially in women with irregular cycles. The relative low power of our study requires that these results be confirmed in prospective cohort studies.

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FIGURE 1 : FLOW CHART

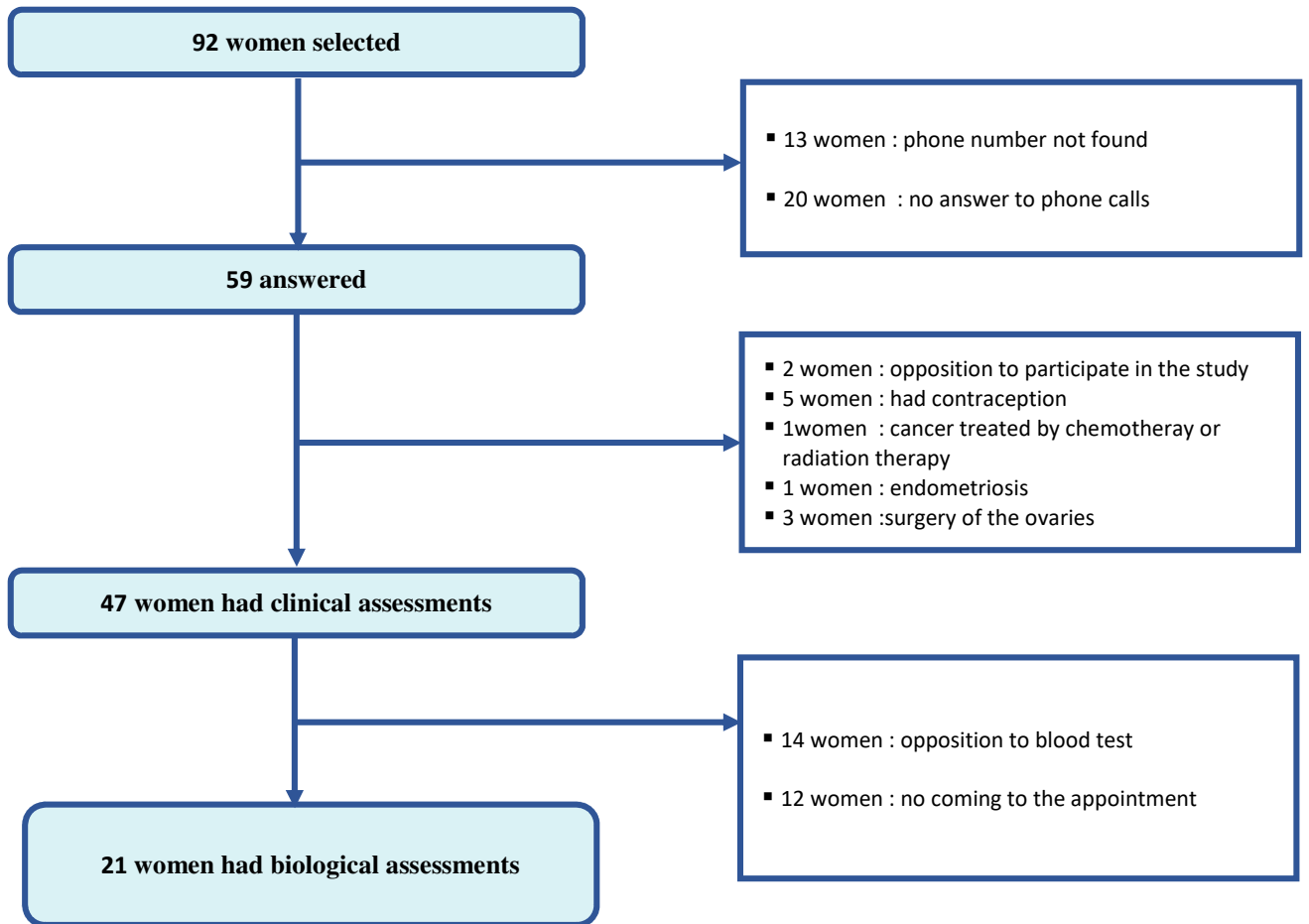


TABLE 1: Population characteristics at T0 (initial AMH assay < 8 pmol/L) and T1 (5 to 10 years later)

		Initial (T0)	5 to 10 years later (T1)	
n = 47	Age (years)	29.0 [27.0; 31.0]	36.0 [34.0; 38.0]	
	Cycles	Short	12 (25.5%)	12 (25.5%)
		Regular	25 (53.2%)	20 (41.2%)
		Long	10 (21.3%)	7 (14.9%)
	FSH (IU/L)	6.8 [5.1; 10.5]		
	AFC	10 [7; 13]		
	Age at menarche (years)	12.0 [11.0; 13.0]		
	BMI (kg/m ²)	24.2 [20.6; 30.0]	25.0 [21.0; 31.0]	
	AMH (pmol/L)	5.6 [4.5; 6.7]	1.1 [0.5; 2.7]	
	Smoker	21 (44.7%)	22 (46.8%)	
	POI	0 (0%)	8 (17%)	
	Time to POI diagnosis (years)		5.1 [2.9; 7.3]	
n = 21	Time to second AMH assay (years)		5.0 [5.0; 6.0]	
	Annual decrease in AMH level (pmol/L)		0.6 [0.5; 0.8]	
	Percentage of annual decrease in AMH level		11.8 [9.4; 15.0]	

The results presented are medians [Q1; Q3] with their first and third quartiles and the number of individuals, with their percentages.

AMH Anti-Müllerian hormone; AFC Antral follicle count; FSH Follicle-stimulating hormone; BMI Body mass index; POI Premature ovarian insufficiency

TABLE 2: Clinical and laboratory factors predictive of POI

		No POI n = 39	POI n = 8	P
Age at T0 (years)		29.0 [27.0; 31.0]	29.5 [28.0; 32.0]	0.69
Cycles at T0	Short	8 (20.5%)	4 (50.0%)	0.024
	Regular	24 (61.5%)	1 (12.5%)	
	Long	7 (17.9%)	3 (37.5%)	
Age at menarche (years)		12.0 [11.0; 13.0]	12.5 [10.5; 14.5]	0.80
Age at T1 (years)		36.0 [34.0; 38.0]	35.5 [31.0; 38.0]	0.42
BMI (kg/m ²)		24.0 [21.0; 32.0]	26.5 [24.0; 28.5]	0.67
Smoker		18 (46.2%)	3 (37.5%)	0.72
FSH at T0 (IU/L)		6.4 [5.1; 10.0]	8 [5.3; 12.2]	0.52
AFC at T0		10.0 [8.0; 13.0]	8.0 [6.0; 12.5]	0.32
AMH at T0 (pmol/L)		6.0 [5.2; 6.7]	4.2 [2.7; 4.9]	0.002
Pregnancy		24 (61.5%)	3 (37.5%) (1 spontaneously, 1 per ART, 1 per donation)	0.26
		No POI n = 13	POI n = 8	P
AMH at T1 (pmol/L)		2.3 [1.9; 3.7]	< 0.5	< 0.001
Annual decrease in AMH level (pmol/L)		0.6 [0.3; 0.8]	0.7 [0.5; 1.2]	0.25
Percentage of annual decrease in AMH level		10.2 [7.3; 11.8]	20.8 [13.8; 33.3]	0.003

The results of the quantitative data are expressed as medians [Q1; Q3] and those of the qualitative data as the numbers of individuals and percentages. The statistical tests used were Wilcoxon's test for the qualitative values and Fisher's exact test for the quantitative values.

AMH Anti-Müllerian hormone; AFC Antral follicle count; ART Assisted Reproductive Technology; FSH Follicle-stimulating hormone; BMI Body mass index; POI Premature ovarian insufficiency