ANTI-MÜLLERIAN HORMONE LEVELS AS A PREDICTOR OF FEMALE GENERAL HEALTH STATUS: A CROSS-SECTIONAL STUDY

Antonio Quartucci[1], Enrico Papaleo[1], Laura Privitera[1], Agnese Rebecchi[1], Alessia Limena[2], Maria Longo[2], Eugenia Alleva[2], Eugenio Ventimiglia[1], Paola VIGANO[1], Massimo Candiani[1], Andrea Salonia[1], and Valeria Vanni[1]

[1]San Raffaele Hospital
[2]Università Vita Salute San Raffaele

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Abstract

Objective: To assess the correlations between clinical and hormonal parameters and comorbidity burden in Caucasian women presenting for fertility treatment. Design: Cross-sectional study. Setting: Single academic reproductive medicine center. Patient: Cohort of 3163 single-ethnicity women seeking medical help for fertility treatment, who underwent centralized lab testing for fertility-related hormonal assessment. Intervention: Complete clinical and laboratory data from the entire cohort of patients were retrospectively analysed. Main outcome measures: Assessment of i) the comorbidity burden scored via the Charlson Comorbidity Index (CCI; categorized as 0 vs. 1 vs. >2); and, ii) the potential associations between CCI and clinical and hormonal parameters. Results: Descriptive statistics and regression models tested the associations between clinical and laboratory parameters and CCI. Of 3163, CCI=0, CCI=1 and CCI>2 were found in 2977 (94.1%), 113 (3.6%) and 73 (2.3%) patients, respectively. Age, gravidity, Anti-Müllerian hormone (AMH) and thyroid stimulating hormone (TSH) values were found to be significantly different among CCI groups (all p[?] 0.01). At regression models, age at presentation and AMH emerged as independent predictors of CCI>1. Age at presentation <36 years (OR=1.742, 95% CI [1.284; 2.364]) and an AMH level <2.3 ng/ml (OR=1.864, 95% CI [1.29; 2.69]) were the most informative cutoff values for CCI >1. Conclusions: A younger age at presentation and lower AMH levels are significant predictors of decreased general health in women requiring clinical evaluation for fertility treatment. As observed for sperm parameters in men, AMH might serve as a proxy of women's general health status. Key words: AMH, comorbidities, health, infertility

INTRODUCTION

According to the World Health Organization (WHO) criteria, infertility is currently defined as the inability to conceive after at least one year of unprotected intercourse[1] and has a prevalence of around 15% among couples[2]. While genetic abnormalities are found in about 15-30% of infertile men and 10% of infertile women, in the majority of cases infertility is due to acquired factors[3,4]. Over the last years, increasing evidence has suggested that a number of either hormonal or immunological disturbances that cause infertility might not only be related to reproductive health but also to overall morbidity and mortality[5]. For this reason, fertility status also in women is increasingly appraised as "a proxy of general health", a "harbinger for future health"[6], or even as "the sixth vital sign"[5], thus representing a unique opportunity for developing preventive strategies and potential risk reduction.

Of importance, the causal pathways between health and infertility are not still definitively known[5], and fertility status is rather looked at as a potential early biomarker for risk stratification later in life.

In males, there are specific reproductive parameters that have been found to be associated with a decreased general health status as expressed by comorbidity scores[7] – indices that provide general health assessment and predict mortality by applying weights or severity ratings for each comorbid condition[8]. More specifically, low sperm concentration, low testosterone and high follicle-stimulating hormone (FSH) values in primary infertile men have been found to be inversely associated with the comorbidity burden expressed by the Charlson Comorbidity Index (CCI)[9,10].

Epidemiological data has suggested that also female fertility and health status are closely intertwined. In this context, infertility at any reproductive age emerged to be associated with later cardiovascular conditions[11], and an earlier decline in the ovarian follicular pool has been suggested in women with insulin resistance[12], type 1 diabetes[12,13], other immune disorders such as systemic lupus erythematosus[14] or Sjögren's syndrome[15], and cancer[16].

Nonetheless, no study has so far explored whether any female specific reproductive parameter might be associated with general health, as previously observed for men[10]. Therefore, we sought to assess potential associations between fertility-related clinical and hormonal features and comorbidity burden in a homogeneous cohort of white-Caucasian women presenting for fertility treatment.

MATERIALS AND METHODS

Patients

The study population of this cross-sectional study consisted of a cohort of 3163 Caucasian women presenting for fertility treatments between August 2005 and April 2018 at a single academic reproductive medicine center. Couple infertility and cause of infertility were diagnosed after a comprehensive diagnostic evaluation of the male and female partners. According to the World Health Organization (WHO) criteria, infertility was defined as not conceiving a pregnancy after at least 12 months of unprotected intercourse. Comorbidities were assessed with a thorough self-reported medical history (including, gravidity and parity) and scored with the Charlson Comorbidity Index (CCI), using the International Classification of Diseases modified 9^{th}version (ICD-9-CM) classification. For the specific purpose of this analysis, CCI was categorized as 0, 1 or [?]2. Gravidity was defined as the number of times that a woman had been pregnant and parity as the number of times that she had given birth to a fetus with a gestational age of 24 weeks or more. Age at menarche, menstrual frequency, smoking status and alcohol consumption were also recorded. Body mass index (BMI), defined as weight in kilograms by height in square meters, was measured for each patient.

All patients were admitted to the centralized laboratory of the same academic hospital to assess Anti-Mullerian hormone (AMH), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E_2), 25(OH)-Vitamin D, Thyroid Stimulating Hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and Prolactin (PRL). Follicle-stimulating hormone, LH and PRL were measured using electrochemiluminescent immunoassays (ECLIA, COBAS ROCHE). 17b-estradiol, 25(OH)-Vitamin D, TSH, fT3 and fT4 were measured with electrochemiluminescent immunoassays (COBAS C 8000). Anti-Mullerian hormone was measured with the generation I enzyme-linked immunosorbent assay (AMH Gen I ELISA; Beckman Coulter) until the 30^{th} of October 2013 (defined as Kit 1), with generation II enzyme-linked immunosorbent assay (AMH Gen II; Beckman Coulter) until the 23^{rd} of January 2017 (defined as Kit 2), and then with the electrochemiluminescent immunoassay (Elecsys(r) AMH Plus, COBAS ROCHE, defined as Kit 3).

Data collection followed the principles outlined in the Declaration of Helsinki and all patients signed an informed consent agreeing to deliver their own anonymous information for the study. The study was approved by the IRCCS San Raffaele Ethical Committee (BCGINEOS, July 12^{th} 2010).

Statistical analysis

Data are presented as n (%) for categorical variables and Mean (+- Standard Deviation) or Median (Range) for continuous variables, as appropriate. The statistical significance of differences in distribution was tested with Pearson chi-square test and non-parametric Kruskal-Wallis H test as appropriate. A 95% confidence interval was estimated for the association of categorical parameters. Either linear or logistic regression models

were applied to test the association between clinical predictors and continuously coded CCI or categorized CCI (defined as CCI = 0 vs. CCI > 1), respectively. Exploratory univariate analyses were initially applied to all variables, and variables that had significant association with comorbidity occurrence at univariate linear or logistic regression analyses were eventually included in the multivariate analyses. All variables showing a significant different distribution at non-parametric tests among subgroups of patients stratified by CCI score were then included for multivariate analyses. To identify the most informative cut-off value of age at presentation and AMH levels predicting CCI> 1, both maximization of Youden's index and minimum P value approach were used[17]. Further logistic regression model was used to test the association between categorized AMH concentration and CCI > 1. Statistical tests were performed using SPSS version 21 (IBM Corp.). All tests were two sided and a P value <0.05 was considered statistically significant.

RESULTS

Descriptive statistics of the whole cohort is shown in Table 1. Of all, CCI was 0, 1, and [?]2 in 2977 (94.1%), 113 (3.6%) and CCI 73 (2.3%) patients, respectively.

Table 2 details the comorbidities found in the entire population, according to diagnostic categories and ICD-9-CM codes.

Table 3 depicts patient characteristics according to comorbidity status (as defined by CCI scoring). Of variables studied, age, gravidity, AMH and TSH values were found to significantly differ among CCI groups (Table 3). No further differences have been observed among CCI groups. Distribution of causes of infertility differed among patients stratified by CCI score (p<0.001). Among others, healthy women (CCI=0) presented more often with male-only infertility compared to women with comorbidities (15.7%, 12.4% and 5.5% across groups with CCI=0, CCI=1 and CCI [?]2, respectively). Women with relevant comorbidities (CCI [?]2) were more often presenting for fertility preservation purposes (23.3% compared to 0.7% in group with CCI=0 and 1.8% in group with CCI=1).

Univariable and multivariable regression analysis are shown in Table 4. Among infertility-related factors, only age at presentation and AMH emerged to be independently associated with comorbidity burden, both at linear and logistic multivariate models (Table 4). Differences in terms of kit used for AMH assessment did not correlate with CCI score (p=0.33 and p=0.76, respectively at linear and logistic regression models). The most informative cutoff values predicting a CCI > 1 were age at presentation below 36 years (OR=1.742, p<0.001, 95% CI [1.284; 2.364]) and AMH levels below or equal to 2.3 ng/ml (OR=1.864, p=0.001, 95% CI [1.29; 2.69]).

DISCUSSION

We investigated whether fertility-related clinical and hormonal data might predict women's general health as it has been previously observed for sperm parameters in men[10]. The findings of this cross-sectional study showed that age at presentation and AMH levels are significant predictors of general wellbeing in infertile women. Conversely, BMI, FSH, LH, and other hormones such as PRL, TSH, fT3, fT4 or Vitamin D did not predict the presence of comorbidities, as scored for CCI [?]1. Indeed, to score patients' somatic general health[7], we used CCI, the most widely used scoring system to assess the rate and burden of comorbidities and predict mortality. While causal inferences cannot be drawn, strength of our study on a relatively-large homogenous same-ethnicity cohort of women, was the novel finding that AMH might be a marker of general female health, as observed for sperm concentration in males[10].

Anti-Mullerian hormone is produced by granulosa cells of pre-antral and antral follicles and its concentrations have shown to be proportional to the number of developing follicles in the ovaries[18,19]. Despite this study is the first to describe an association between circulating AMH and overall women's health status, an association between lower AMH levels and a greater risk of cardiovascular[20] and coronary heart[21] diseases had already been described. Likewise, AMH was also found to be decreased in women with cancer[16], liver disorders[22] or renal failure[23].

Therefore, our findings would confirm those previous data, providing the novel finding that a relative reduc-

tion in ovarian reserve - as expressed by lower AMH values - is associated with the wide variety of medical conditions, as classified by ICD-9-CM; thereof, it may indeed predict female global somatic health.

Hence, significant strenghts of our study are the relatively large homogenous population included and availability of several fertility-related hormones tested throughout the fertility work-up of every woman, among which only AMH emerged as a significant predictor of female general health. Thus, our findings are in line with recent evidence suggesting AMH to be related with systemic conditions and all-cause mortality in men[24,25] and deserve thorough consideration.

Altogether, while the observed associations suggest a complex interplay among conditions that may be connected to both female global health and reproductive functioning, in contrast they do not allow causal inferences about the role of AMH in women's general wellbeing. Common mechanisms might indeed underlie coexisting infertility and comorbidities[5] or – in contrast – comorbidities per se might have untoward effects on female ovarian function.

Nonetheless, our study seems to suggest that - as observed for seminal count in men[10] – there are also a few of specific reproductive parameters even in women which might be associated with general health status, thus serving as potential "proxy of general health".

This study might also have clinical implications for infertility treatments. Indeed, preliminary data suggested that treatment of comorbidities in infertile men may improve sperm motility[26,27]. Thus, further analysis on whether treatment of female comorbidities might affect assisted reproductive outcomes would be of interest.

Our study also has some limitations. First, it will be relevant to assess the generalizability of our findings in other populations, also considering and comparing fertile women. Second, comorbidities were assessed through self-reported medical history, possibly causing some degree of information bias.

CONCLUSIONS

This cross-sectional study suggests that younger age at presentation and lower AMH levels may be significant predictors of decreased general somatic health in women requiring fertility treatments.

Disclosure of interest

The authors have no conflict of interest to disclose.

Contribution to authorship

AQ, EP and VSV conceived and designed the study. AR, ML participated in literature search and data collection. EP, LP, VSV, AL, EA contributed to data collection and classification. AQ, VSV, EP performed statistical analyses and drafted the manuscript. EP, PV, EV, AS, MC participated in interpretation of the findings and critical revision the intellectual contents of the manuscript. All authors approved the final version of the manuscript.

Ethical approval

The study was approved by the IRCCS San Raffaele Ethical Committee (BCGINEOS, July 12th 2010).

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Table 1. Characteristics and descriptive statistics of the cohort of patients assessed at a single academic center (n=3163).

Variables
Age (y) Mean ± SD
Range
BMI (kg/m2) Mean ± SD
Range
CCI (continuously coded) Mean (median)
Range
CCI (categorized) CCI = 0 n (%)
CCI = 1 n (%)
CCI > 2 n(%)
Gravidity (n) Mean (median)
Range
Parity (n) Mean (median)
Range
FSH (UI/L) Mean (median)
Range
LH (UI/L) Mean (median)
Range
AMH (ng/ml) Mean (median)
Range
PRL (ng/mL) Mean (median)
Range

Variables
ΤΣΗ (μΙΥ/μΛ) Μεαν (μεδιαν)
Ρανγε
fT3 (nmol/L) Mean (median)
Range
fT4 (nmol/L) Mean (median)
Range
vitD (ng/mL) Mean (median)
Range
Keys: AMH =anti-mullerian hormone; BMI=body mass index; CCI =Charlson comorbidity index; FSH= f

Table 2. Diagnostic categories classified as the International Classification of Diseases modified 9th version (ICD-9-CM) and Charlson comorbidity index (CCI) weights found in the entire cohort of patients (n=3163).

Condition	CCI weights	N (%)
Myocardial infarction Old Myocardial infarction	1	2 (0.06)
Congestive heart failure	1	0 (0)
Peripheral vascular disease Peripheral vascular disease NOS	1	1 (0.03)
Cerebrovascular disease	1	7 (0.22)
Dementia	1	0 (0)
Chronic pulmonary disease Moderate or severe asthma COPD Genetic syndrome	1	49 (1.55) 1 (0.03) 1 (0.03)
Connective tissue disease Sjogren syndrome Systemic lupus erythematosus Undifferentiated CTD	1	4 (0.12) 5 (0.16) 12 (0.38)
Peptic ulcer disease	1	4 (0.12)
Mild liver disease Chronic hepatitis	1	14 (0.44)
Diabetes Diabetes mellitus	1	20 (0.63)
Diabetes with chronic complications	2	0 (0.0)
Renal disease Polycystic kidney	2	3 (0.09)

Condition	CCI weights	N (%)
Cancer (malignant neoplasm) Of	2	2 (0.06) 9 (0.28) 1 (0.03) 21 (0.66)
the colon		10 (0.32) 1 (0.03) 3 (0.09) 2 (0.06)
Of the thyroid		2 (0.06) 1 (0.03) 1 (0.03) 1 (0.03)
Melanoma		4 (0.12) 7 (0.22) 1 (0.03)
Of the breast		
Sarcoma		
Of the tongue		
Of the ovary		
Oligodendroglioma		
Of the pancreas		
Of the parothyd		
Choriocarcinoma		
Of parathyroid		
Leukemia		
Lymphoma		
Neuroendocrine tumor		
Moderate or severe liver disease	3	0 (0)
Metastatic solid tumor Of thyroid	6	1 (0.03)
HIV disease	6	0 (0)

Table 3. Characteristics and descriptive statistics of patients according to patients' comorbidity load.

Characteristic	CCI 0 (n=2977)	CCI 1 (n=113)	CCI[?]2 (n=73)	P value
Age (y)				
Mean ± SD	36.5 ± 4.4	36 ± 4.4	34.2 ± 5.9	**0.009**
Range	16.9-48.5	21.1-44.8	15.2-44.9	
BMI (kg/m^2)				
Mean ± SD	22.3 ± 3.8	22.9 ± 4.5	22.2 ± 3.1	0.99
Range	15.6-53.4	13.9-38.3	17.2-31.1	
Gravidity				
Mean (Median)	0.65 (0)	0.85 (0)	0.3 (0)	**0.001**
Range	0-8	0-5	0-4	
Parity				
Mean (Median)	0.1 (0)	0.11 (0)	0.05 (0)	0.158
Range	0-4	0-1	0-2	
Age at menarche				0.66
n (%)				
[?]9 y	39 (1.3)	2 (1.8)	1 (1.4)	
10-11 y	493 (16.6)	15 (13.3)	12 (16.4)	
12-13 y	1187 (39.9)	44 (38.9)	25 (34.2)	
[?]14 y	566 (19)	26 (23.0)	11 (15.1)	
NA	692 (23.2)	26 (23.0)	24 (32.9)	
Menstrual cycle				0.84
frequency n (%)				
Amenorrhea	29 (1)	1 (0.9)	0 (0)	
Irregular	43 (1.4)	1 (0.9)	0 (0)	
[?]25 d	177 (5.9)	8 (7.1)	5 (6.8)	
26-32 d	2308 (77.5)	85 (75.2)	56 (76.7)	

Characteristic	CCI 0 (n=2977)	CCI 1 (n=113)	CCI[?]2 (n=73)	P value
33-45 d	291 (9.8)	14 (12.4)	4 (5.5)	
>45	53 (1.8)	2 (1.8)	0 (0.0)	
NA	76 (2.6)	2 (1.8)	8 (11)	
N of smoked cigarettes/day n (%)				0.592
0	2226 (74.8)	83 (73.5)	48 (65.7)	
<5	318 (10.7)	14 (12.4)	9 (12.3)	
5-20	295 (9.9)	9 (8.0)	7 (9.6)	
>20	58 (1.9)	5 (4.4)	1 (1.4)	
NA	80 (2.7)	2 (1.8)	8 (11)	
Alcohol consumption/week n (%)				0.76
0 glasses	476 (16.0)	12 (10.6)	14 (19.2)	
1-7 glasses	470 (15.8)	19 (16.8)	10 (13.7)	
8-14 glasses	4 (0.1)	0 (0)	0 (0)	
NA	2027 (68.1)	82 (72.6)	49 (67.1)	
FSH (mIU/mL)				
Mean (Median)	8.9 (7.7)	7.7 (6.9)	10.7 (8.1)	0.067
Range	0.3-174	2-26	4.9-111	
LH (mIU/mL)				
Mean (Median)	6.3 (5.3)	6 .2(5.3)	6.8 (5.4)	0.688
Range	0-114	0.9-22	2.1-43	
AMH (ng/ml)				
Mean (Median)	2.16 (1.4)	2.17 (1.5)	1.29 (0.99)	**<0.001**
Range	0-19	0-17	0-7	
AMH kit n (%)				0.31
1	1622 (54.5)	65 (57.5)	33 (45.2)	
2	1077 (36.2)	36 (31.9)	29 (39.7)	
3	278 (9.3)	12 (10.6)	11 (15.1)	
PRL (ng/ml)				
Mean (Median)	29.5 (13)	36.1 (14)	19.5 (10.4)	0.114
Range	0.3-1147	0.2-741	4-295	
TSH (µIU/L)				
Mean (Median)	2.2 (1.8)	2.16 (2.11)	1.6 (1.5)	**0.003**
Range	0-305	0-6	0-5.6	
T3 (mIU/L)				
Mean (Median)	3.8 (2.9)	3 (3)	3.2 (3)	0.81
Range	0.3-145	2.1-5	2.5-5.2	
T4 (mIU/L)				
Mean (Median)	3.8(1.1)	3.8 (1.1)	4.8 (1.8)	0.51
Range	0.3-123	0.8-13	0.9-23	
Vitamin D (ng/ml)				
Mean (Median)	30 (23)	45 (25)	23.8 (21.1)	0.29
Range	3-602	6-329	9-41	
Cause of infertility n (%)				**<0.001**
Idiopathic	558 (18.7)	20 (17.7)	11 (15.1)	

Characteristic	CCI 0 (n=2977)	CCI 1 (n=113)	CCI[?]2 (n=73)	P value
Reduced ovarian reserve	977 (32.8)	34 (30.1)	26 (35.6)	
Male-only infertility	466 (15.7)	14 (12.4)	4 (5.5)	
Endometriosis	385 (12.9)	13 (11.5)	7 (9.6)	
Ovulation disorders	386 (13.0)	21 (18.6)	6 (8.2)	
Tubal factor	155 (5.2)	8 (7.1)	1 (1.4)	
Genetic	28 (0.1)	1 (0.9)	1 (1.4)	
Fertility preservation	22 (0.7)	2 (1.8)	17 (23.3)	

Table 4. Linear (beta; P value [95% CI]) and logistic (OR; P value [95% CI]) regression models predicting presence of comorbidities (CCI >1) in the whole cohort of patients (n=3163).

	Linear model	Linear model	Logistic model	Logistic model	Logistic model
Characteristic	**UVA model**	**MVA model**	**UVA model**	**MVA model/ continuous AMH**	**MVA model/ Categorized AMH**
Age	**-0.073; <0.001 [-0.009; -0.003]**	**-0.093; <0.001 [-0.011; -0.005]**	**0.956; 0.006 [0.926; 0.987]**	**0.941; <0.001 [0.91; 0.973]**	**0.94; <0.001 [0.91; 0.972]**
BMI	0.019; 0.295 [-0.002; 0.005]	-	1.029; 0.142 [0.991; 1.068]		
Gravidity	-0.024; 0.175 [-0.023; 0.004]	-0.015; 0.397 [-0.019; 0.008]	1.033; 0.887 [0.887; 1.204]	1.03; 0.707 [0.884; 1.2]	1.03; 0.704 [0.884; 1.199]
Parity	-0.024; 0.169 [-0.061; 0.011]	**-**	0.74; 0.23 [0.452; 1.21]		
AMH	**-0.048; 0.007 [-0.013; -0.002]**	**-0.076; <0.001 [-0.017; -0.006]**	0.93; 0.059 [0.862; 1.003]	**0.893; 0.007 [0.822; 0.97]**	
AMH categorized (< 2.3 ng/ml vs > 2.3 ng/ml)	**-0.056; 0.002 [-0.074; -0.017]**	-	**0.623; 0.01 [0.436; 0.892]**		**0.529; 0.001 [0.366; 0.766]**
AMH kit	0.006; 0.335 [-0.006; 0.018]		1.114; 0.335 [0.895; 1.386]		
VitD	-0.015; 0.749 [-0.001; 0.001]	-	1.003; 0.45 [0.996; 1.009]		
FSH	-0.008; 0.659 [-0.001; 0.002]	-	0.996; 0.764 [0.974; 1.02]		
LH	0; 0.981 [-0.002; 0.002]	-	1; 0.982 [0.969; 1.033]		
PRL	-0.001; 0.948 [0;0]	-	1; 0.667 [0.998; 1.002]		
TSH	-0.01; 0.56 [-0.003; 0.002]	-0.008; 0.642 [-0.003; 0.002]	0.94; 0.382 [0.813; 1.082]	0.936; 0.37 [0.809; 1.082]	0.938; 0.384 [0.812; 1.084]
T3	-0.008; 0.816 [-0.005; 0.004]	-	0.988; 0.802 [0.896; 1.089]		
T4	0.009; 0.788 [-0.002; 0.003]	-	1.004; 0.777 [0.975; 1.034]		