Evolution of serum Anti-Müllerian Hormone (AMH) level in young women treated with chemotherapy for breast cancer according to basal AMH level

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Running head: AMH level during breast cancer treatment

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Abstract

Objective: Serum AMH level has been shown to decrease in women treated for breast cancer in several studies. However, whether basal AMH status affects AMH dynamics during chemotherapy remains to be clarified. The objective of this study was to compare serum AMH dynamics in young women with either low, normal or high basal serum AMH level at diagnosis, during and after treatment with chemotherapy for breast cancer.

Study Design: In this secondary analysis of a prospective cohort study, serum AMH was measured during and after chemotherapy in 239 women of reproductive age diagnosed with breast cancer and treated with chemotherapy. The association between AMH dynamics throughout chemotherapy and during follow-up and basal AMH status, i.e. low AMH (<1µg/l, <7pmol/l), normal AMH (1-4.9 µg/l, 7-36 pmol/l) and high AMH (≥5 µg/l, >36 pmol/l), was evaluated. Menses occurrence was also recorded.

Results: A total of 21 women had low, 154 had normal and 64 had high basal AMH level. Serum AMH rapidly decreased during chemotherapy in all groups, and its variation during chemotherapy and follow-up was not significantly different between the 3 groups.

Conclusion: No association was found between AMH variation during chemotherapy and follow-up, and basal AMH level at diagnosis. However, women with high basal

AMH levels have significantly higher AMH levels throughout chemotherapy and follow-up than women with normal or low basal AMH levels at diagnosis.

Trial registration number: ClinicalTrial.gov identifier NCT01114464

Keywords: Breast cancer; fertility preservation; anti-Müllerian hormone; ovarian reserve

Word count: 2 306

1. Introduction

Breast cancer is the most common malignancy in women of reproductive age (1). The large majority of them are treated with adjuvant or neoadjuvant chemotherapy, based on gonadotoxic drugs such as anthracyclines and cyclophosphamide, and taxanes, potentially leading to impaired ovarian function, amenorrhea and infertility. We (2), and others (3),(4) , previously showed that serum Anti-Müllerian Hormone (AMH), also known as Müllerian-Inhibiting Substance (MIS), the most relevant hormonal marker of ovarian reserve, rapidly fell to low or even undetectable levels in young women with breast cancer treated with chemotherapy with a high prevalence of chemotherapy-related amenorrhea (2),(4), (5).

The modulation of AMH expression has recently been proposed as a promising therapeutic way to control ovarian physiology with clinical applications for ovulation induction, contraception or fertility preservation (6). Indeed, it was recently reported that AMH administration could protect ovarian reserve during chemotherapy in mice (7). Therefore, it can be postulated that basal AMH levels could account for intra-individual variability in terms of ovarian sensitivity to gonadotoxic treatment. However, and to the best of our knowledge no study on the potential association between AMH evolution during chemotherapy and post-chemotherapy follow-up and basal AMH level has been reported up to now.

The main objective of our study was to compare serum AMH dynamics in young women with either low, normal or high basal serum AMH level, during and until 24 months after treatment with chemotherapy for breast cancer.

2. Materials and Methods

2.1Patients

This is a subgroup analysis of our previously reported work conducted on the RESOVA cohort (2). Briefly, this observational and prospective study was conducted in 250 eligible patients, aged 18-39, diagnosed with breast cancer and about to be treated with chemotherapy in adjuvant/neoadjuvant setting. Patients were included at diagnosis in 11 French cancer centers, and followed up until 24 months after chemotherapy completion. A medical interview and blood sampling were performed for each patient at each visit, from diagnosis (sample S0),and before each of the 6 chemotherapy cycles (samples S1 to S6) and 6, 12 and 24 months after completion of chemotherapy treatment (samples S7 to S9). Patients were also asked to register all menstruations occurrences throughout the study.

2.2AMH assay

All assays were performed in the same centralized laboratory with 1st generation AMH/MIS EIA Immunotech Beckman Coulter™ method (Marseille, France) according to the manufacturer instructions. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) coefficients were below or equal to 12.3% and 14.2% respectively. The value of 0.42ng/mL was arbitrarily assigned to every values <0.42ng/mL (lower limit of quantification). Patients were grouped according to basal AMH level at diagnosis, i.e. low basal AMH level, defined as <1 ng/mL (<7 pmol/L), normal basal AMH level, defined as 1-4.9 ng/mL (7-36 pmol/L) and high basal AMH level, defined as ≥5 ng/mL (≥ 36 pmol/L). These thresholds were chosen arbitrarily but might reflect consensual values found in the existing literature on AMH reference

values (8), (9), (10). The variation of serum AMH level between 2 visits (S1 and S6 for instance) was calculated (variation S1S6 = delta S6S1/ S1-0.42).

The main endpoint of our study was the absolute (in ng/ml) and relative (in % of basal serum level) variation of serum AMH levels during chemotherapy and follow-up as compared to basal AMH level at diagnosis.

Subgroup analyses were carried out to evaluate the putative association between female age and AMH variation according to basal AMH levels, and between the prevalence of chemotherapy-related amenorrhea (CRA) and basal AMH level.

2.3 Statistical analysis

Mann –Whitney and Kruskall-Wallis tests were used for group comparison and Spearman coefficient for correlation studies. GraphPad Prism™ (version 5) software was used. A *p* value <.05 was considered statistically significant.

2.4 Ethical approval

This observational and prospective study (ClinicalTrials.gov identifier NCT01114464) has been approved by the local ethics committee (GNEDS). All patients provided written informed consent before starting study procedure.

3. Results

3.1 Demographic characteristics and basal serum AMH

AMH results could be analyzed in 238 women among the 250 initially included in the study. The demographic and treatment characteristics of these 238 women are presented in table 1. Basal AMH level at diagnosis was low in 21 patients (8.9%) (mean 0.59±0.34 ng/ mL; IC95% [0.45-0.72]; median 0.59 ng/ mL), normal in 153

patients (64.3%) (mean 2.71±1.19 ng/mL; IC95% [2.52-2.90]; median 2.46 ng/mL), and high in 64 patients (26.9%) (mean 9.28±7.06 ng/mL; IC95% [7.51-11.04]; median 7.44 ng/mL) (Figure 1). No significant correlation between basal AMH level and age was found in high and low basal AMH groups, whereas a significant negative correlation was found between age and basal AMH in the normal basal AMH group (r=-0.076, p=0.0049) (supplementary figure 1).

3.2 Comparison of AMH variation throughout study according to basal AMH level

Serum AMH level rapidly decreased during chemotherapy cycles, before slightly increasing during the 2-year follow-up in the low (Fig.2A), normal (Fig.2B) and high basal AMH group (Fig.2C). The calculated decrease in AMH level was -50.5% (-0,1 ng/ml), -52.5% (-1.2 ng/mL) and -41.5% (-4.2 ng/mL) ($p<0.0001$) after the 1st chemotherapy cycle (sample S2), -82.6% (-0.28 ng/mL), -85.29% (-1.95 ng/mL) and -81% (-6.92 ng/mL) ($p<0.0001$) after 2 chemotherapy cycles (sample S3), and -97.7% (-0.35 ng/mL), -96.9% (-2.22 ng/mL), and -98.9% (-8.08 ng/mL), (n=19, n=141 and n=54 respectively, $p<0.0001$) after chemotherapy completion (sample S6), in low (Fig 2A), normal (Fig.2B) and high basal AMH groups respectively (Fig 2C).

The final AMH level at the end of the study (24 months after chemotherapy completion, sample S9) was 0.35±0.23 ng/mL, 0.76±0.67ng/mL and 1.83±2.11 ng/mL in the low, normal and high basal AMH groups respectively ($p<0.0001$). The average increase in serum AMH level during the 2-year follow up after chemotherapy completion was not statistically different in the low, normal and high basal AMH groups (+11.9%, +10.3% and 16.1% respectively, $p>0.05$). However, the proportion of patients with increasing AMH level during the 24-month follow-up post-chemotherapy was significantly higher in the high basal AMH group (65%) than in the

normal (40%) or in the low basal AMH group (33%) (p=0.005). The respective AMH decrease during chemotherapy and increase during follow-up did not differ according to age (supplementary figure 2).

Finally, the cumulative decrease of serum AMH during the whole study (i.e. chemotherapy and 24 month follow-up) was -89.8%, -86.2% and -83.7% in the low, normal and high basal AMH groups respectively (p>0.05). It was significantly associated with age in women with normal basal AMH (fig. 3B), but not in the high and low basal AMH groups (fig.3A and 3C). The proportion of patients with undetectable AMH level after chemotherapy completion reached 95% in low basal AMH group vs 75% in normal basal AMH group and 81% in high basal AMH (p>0.05). After 24-month follow-up, the proportion of patients with undetectable AMH was significantly lower in the high basal AMH group than in normal and low basal AMH groups (33% vs 58% and 76% respectively, p=0.0003).

3.3 Prevalence of chemotherapy-related amenorrhea (CRA) and pregnancy

The prevalence of self-reported CRA according to basal AMH level is presented in figure 4. The prevalence of CRA was comparable in the 3 groups at the end of chemotherapy and after 12-month follow-up. However, it was significantly lower in the high basal AMH group than in the normal or low basal AMH groups after 6-month follow-up (75% vs 83 and 100% respectively, p=0.004). Two patients with high basal serum AMH became pregnant during the study, aged 27.2 and 27.7 years. Basal AMH level was 39.70 and 6.38 ng/mL, falling to undetectable level after chemotherapy cycles in both cases and further increasing to 1.82 and 0.72 ng/mL respectively.

4 Discussion

In this prospective study, we compared serum AMH evolution in young women treated for breast cancer with chemotherapy, from diagnosis to 2-year follow-up after chemotherapy, according to basal AMH level. We found that AMH dynamics and variation throughout chemotherapy and follow-up were not significantly different in women with high, normal or low basal AMH level. These results suggest that ovarian reserve status does not condition the intensity of the chemotherapy-induced gonadotoxic effect.

We recently reported that serum AMH level decreased rapidly and significantly in young unselected women treated with chemotherapy for breast cancer, with a slight increase observed after 2-year follow-up in 45% of them (2). Although other recent studies have also evaluated serum AMH levels before and after chemotherapy in women with breast cancer, this subgroup analysis of the RESOVA cohort (2) is to our knowledge the first study focusing on the evolution of serum AMH in women treated for breast cancer according to basal ovarian reserve. As AMH expression modulates follicular recruitment and maturation, its pharmacologic control has recently been proposed as a potentially relevant strategy for fertility preservation in order to limit chemotherapy-induced gonadotoxic effects in young women treated for cancer (6), with promising results obtained in animal models (7). In this respect, the influence of basal AMH levels on individual ovarian sensitivity to gonadotoxic treatment can be questioned. More specifically, whether the pool of growing preantral and antral follicles in women with high basal AMH level are less sensitive to chemotherapy-induced degeneration than in women with normal or low basal AMH level is not known yet. Our results suggest that high basal AMH level is not associated with a lower follicular sensitivity to the gonadotoxic effect of

chemotherapy. Some further work should focus on the mechanisms involved in ovarian follicle toxicity, for example using animal models.

After 24-months follow-up post-chemotherapy, a significantly higher frequency of partial AMH recovery was observed in women with high basal AMH than in patients with normal and low basal AMH level. However, the average increase in serum AMH was comparable in both groups. This should obviously not lead to exclude women with high basal AMH levels from fertility preservation program. Moreover, the clinical relevance of serum AMH as a predictor of pregnancy, either spontaneous or after ART cycle, has been largely questioned (11), (12) (13). In our study, only 2 patients out of 64 with high basal AMH became spontaneously pregnant during follow-up. However, we do not know whether other women tried to became pregnant in the cohort, preventing from drawing conclusions on this topic.

Amenorrhea has been frequently reported as an indicator of chemotherapy toxicity on ovarian function (14). However, its relevance was largely questioned, as its association with ovarian reserve markers has been shown to be very weak (15). In our population, we observed a lower prevalence of Chemotherapy-related amenorrhea (CRA) after 6-month follow-up in the high basal AMH group than in the normal and low basal AMH groups, suggesting that higher residual AMH level after chemotherapy in the high basal AMH group might partly protect ovarian function, although AMH cumulative decrease was not significantly different than in the normal and low basal AMH groups. However, these results should be interpreted with care. Indeed, this difference in CRA between the groups was not significant any more after 12-month follow-up. Moreover, the prevalence of CRA is difficult to evaluate in these women with high AMH owing to the suspected very high prevalence of PCOS, which is generally associate with menstrual cycle irregularity. Finally, tamoxifen use might

interfere with menstrual cyclicity in many patients (16), thus limiting the relevance of this marker.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 10-25% of the women of reproductive age (17). Numerous studies evaluated whether an association existed between PCOS and breast cancer, with conflicting results. While Kim et al reported a 3-fold increase in the risk of breast cancer in young patients with PCOS, this was not confirmed in a large case-control study conducted in more than 9000 women aged 20 to 54 (18), (19). Moreover, one meta-analysis did not retrieve any significantly higher risk of breast cancer in women with PCOS than in women without PCOS (20). The current diagnosis classification is based on hyperandrogenism, oligoanovulation, and polycystic ovarian morphology (PCOM) at ultrasound (21). Serum AMH level has been proposed as a surrogate for PCOS diagnosis (22). It could thus be postulated that most women with high basal AMH level in our study would have PCOS. However, we did not have access to all Rotterdam criteria. We could thus not determine precisely the proportion of women with PCOS and could not study a potential specific pattern for AMH evolution in these women.

BRCA status has been shown to be associated with ovarian reserve in some recent studies (23). However, the number of patients with BRCA mutation is too small in our population to yield relevant conclusion on the association between BRCA status and AMH dynamics during and after chemotherapy.

Although its prospective design, with long-term follow-up in a well-designed population can be considered as strengths for this study, we acknowledge a few limitations. First, the issue of AMH assay performance could eventually be raised. We used 1st generation AMH/MIS EIA Immunotech Beckman Coulter^{TM} method, which is

being progressively replaced by automated AMH assays with better sensitivity. Second, the thresholds used for the definition of groups were chosen arbitrarily. We tried to choose the most consensual ones, but this might obviously be discussed. Finally, the real prevalence of self-reported chemotherapy-related amenorrhea was difficult to interpret.

In conclusion, this longitudinal study describes for the first time serum AMH kinetic during chemotherapy and 24-month follow up in young women of reproductive age treated with chemotherapy for breast cancer according to basal AMH levels at diagnosis. Although women with high basal AMH levels have significantly higher AMH levels throughout chemotherapy and follow-up than women with normal or low basal AMH level, AMH dynamics and cumulative variation is comparable in all the groups. Therefore, AMH variation during chemotherapy for breast cancer and follow up does not appear to be associated with basal AMH level at diagnosis. Pre-treatment fertility preservation and post-treatment ovarian insufficiency follow-up should obviously be offered to all women of reproductive age diagnosed with breast cancer, whatever their basal ovarian reserve.

5 Acknowledgments

None

6 Conflict of interest

All the authors confirm that they have no conflict of interest related to this work.

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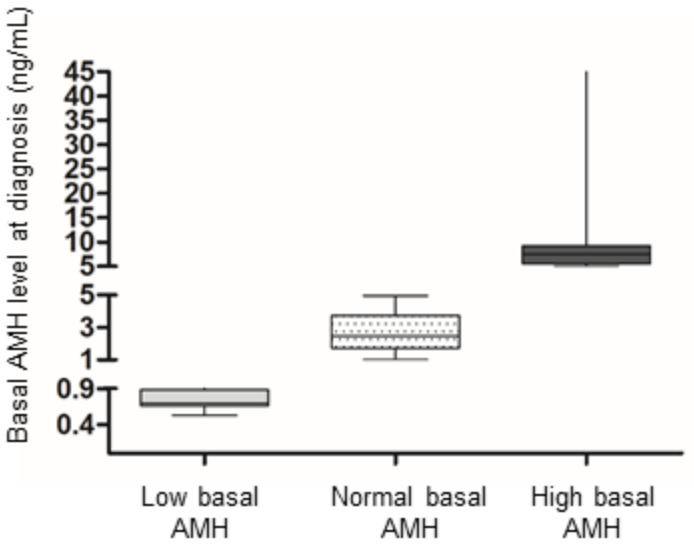
9. Figure legends

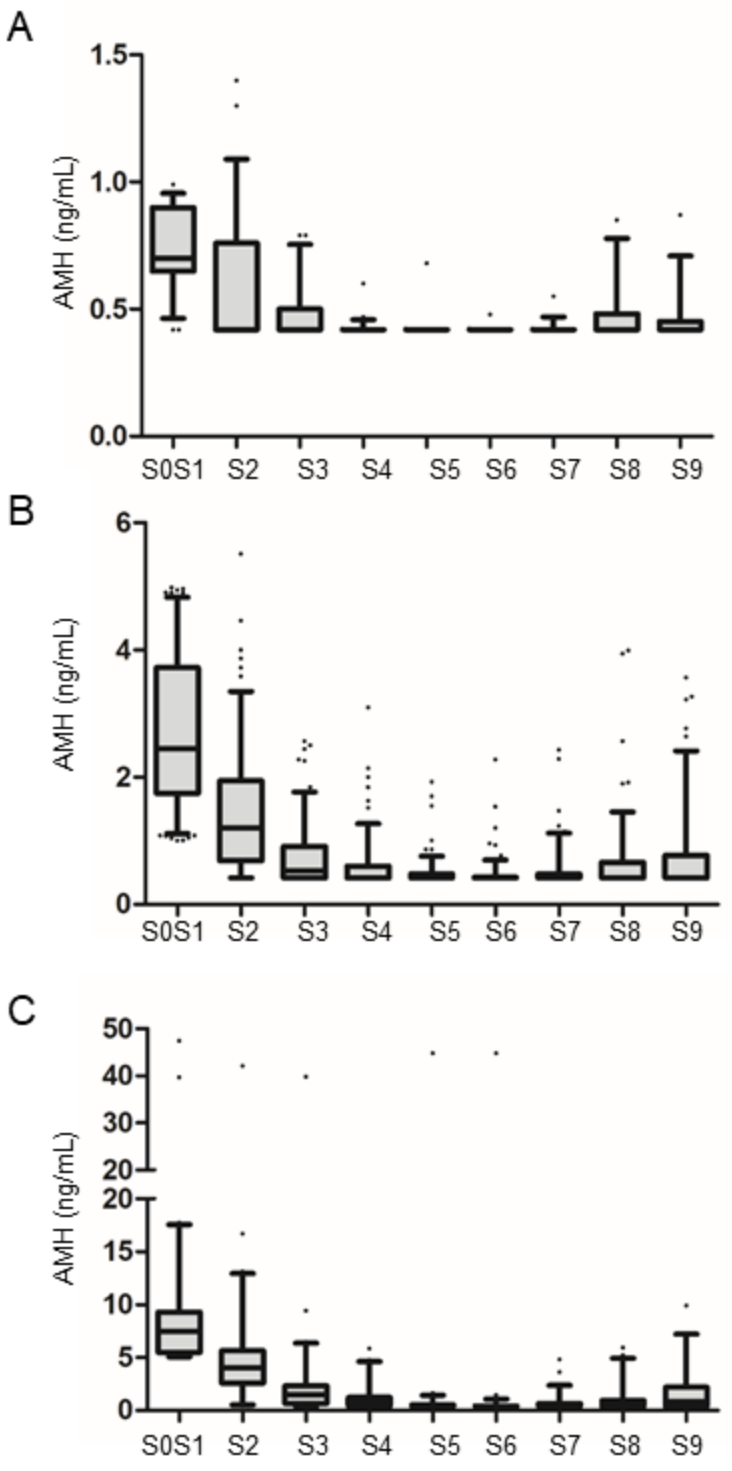
Figure 1: Basal AMH level at diagnosis in the group of patients with low basal AMH level (*n=21*), normal AMH level (*n=153*), and high basal AMH (*n=64*)

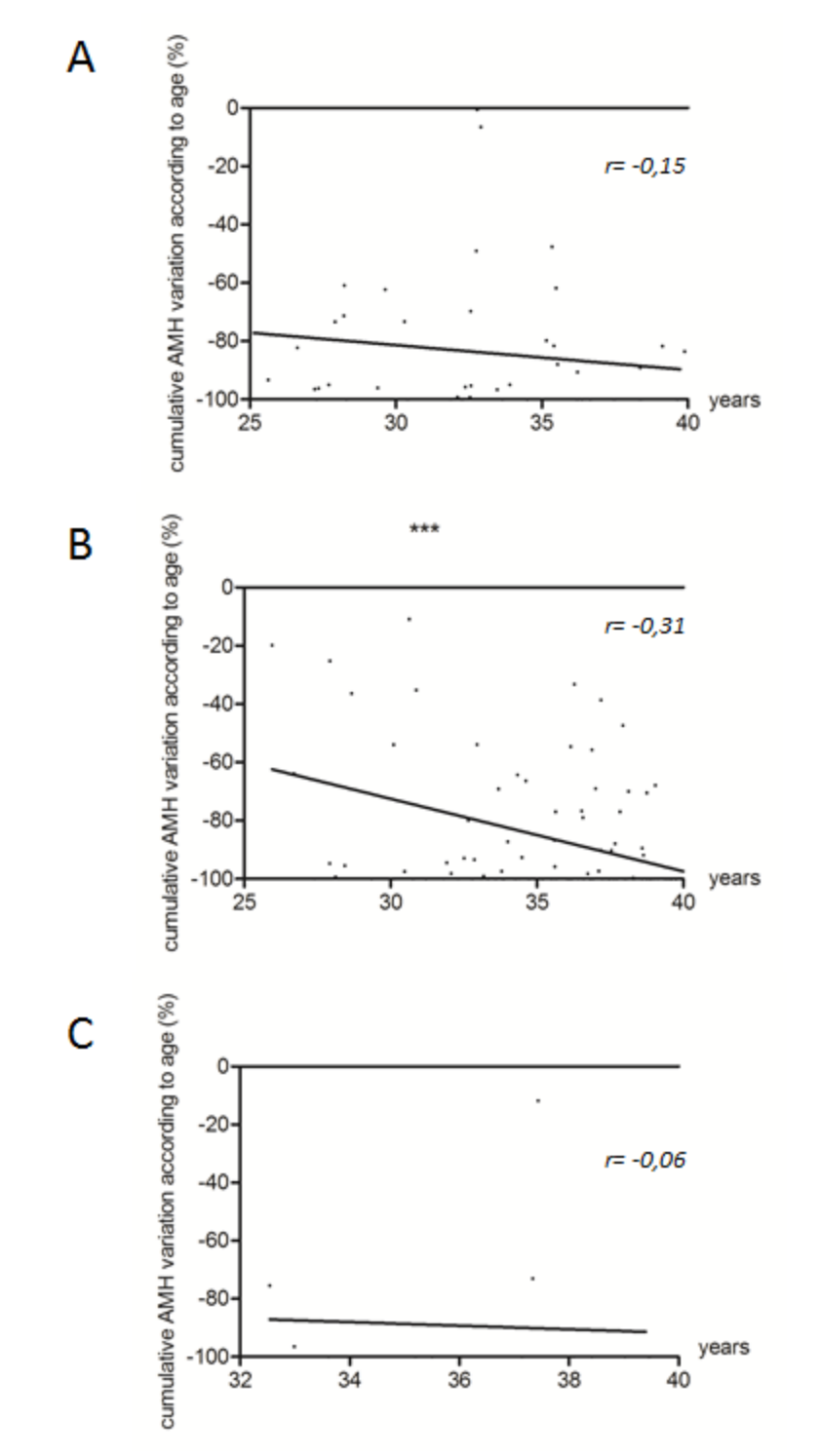
Figure 2: Evolution of serum AMH level during chemotherapy and during 24-month follow-up after chemotherapy completion in women with low (A), normal (B) and high basal AMH (C).

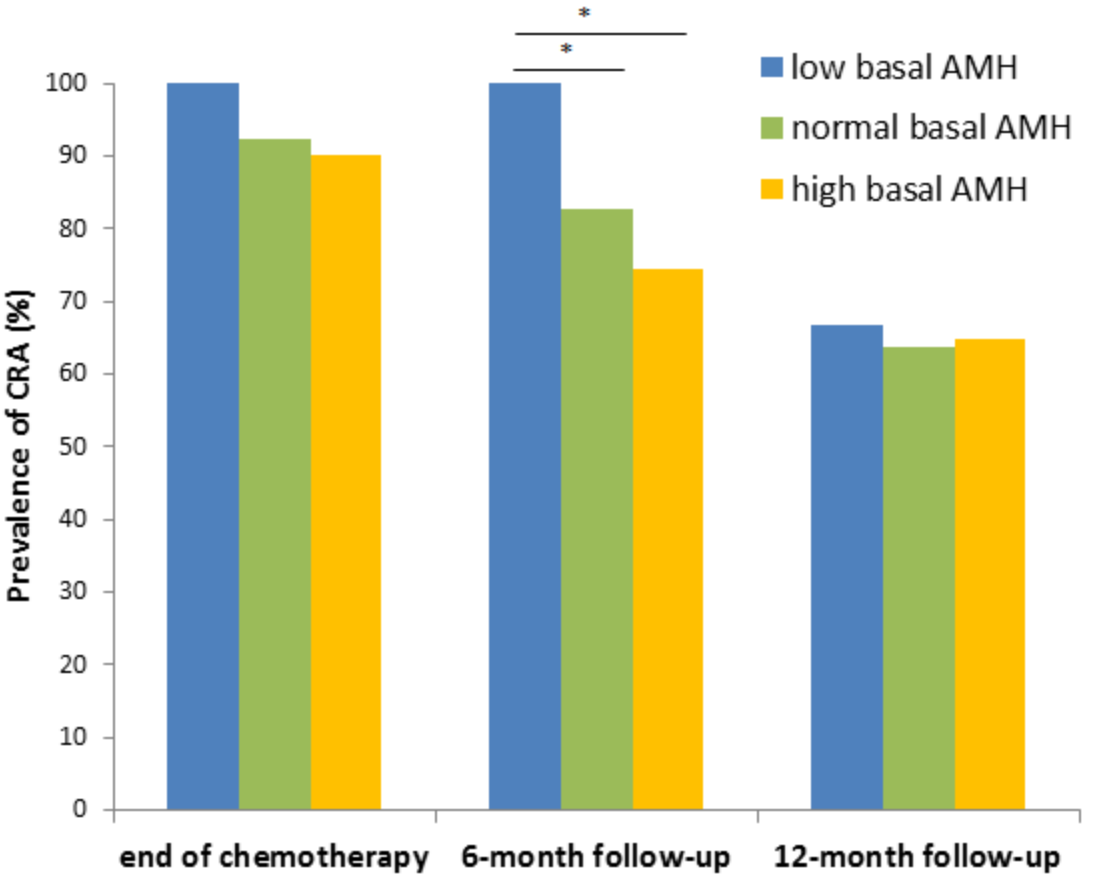
Figure 3: Cumulative variation of AMH between diagnosis and 24-month follow-up according to age at diagnosis in patients with high (A), normal (B) and low basal AMH level (C).

Figure 4: Prevalence of self-reported chemotherapy-related amenorrhea (CRA) in patients with high, normal and low basal AMH level at the end of chemotherapy cycles, after 6-month and 12-month follow-up.


Basal AMH level at diagnosis (ng/mL)
45
40
35
30
25
20
15
10
5
5
3
1
0.9
0.4
Low basal AMH
Normal basal AMH
High basal AMH


A
AMH (ng/mL)
1.5
1.0
0.5
0.0
S0S1
S2
S3
S4
S5
S6
S7
S8
S9
B
AMH (ng/mL)
6
4
2
0
S0S1
S2
S3
S4
S5
S6
S7
S8
S9
C
AMH (ng/mL)
50
40
30
20
20
15
10
5
0
S0S1
S2
S3
S4
S5
S6
S7
S8
S9


A
cumulative AMH variation according to age (%)
0
-20
-40
-60
-80
-100
r= -0,15
25
30
35
40
years
B
***
cumulative AMH variation according to age (%)
0
-20
-40
-60
-80
-100
r= -0,31
25
30
35
40
years
C
cumulative AMH variation according to age (%)
0
-20
-40
-60
-80
-100
r= -0,06
32
34
36
38
40
years


*
*
low basal AMH
normal basal AMH
high basal AMH
Prevalence of CRA (%)
100
90
80
70
60
50
40
30
20
10
0
end of chemotherapy
6-month follow-up
12-month follow-up

	Patients with low basal AMH level (< 1ng/mL) (n=21)	Patients with normal AMH level (1-4.9 ng/mL) (n=153)	Patients with high basal AMH level (>5 ng/mL) (n=64)
DEMOGRAPHIC CHARACTERISTICS			
Age (years)	37.1 ± 1.8*	35.4 ± 2.9*	32.4 ± 3.5*
< 30 years, % (N)	0 (0)	8.5 (13)	31.2 (20)
30-34 years, % (N)	14.3 (3)	30.1 (46)	35.9 (23)
35-39 years, % (N)	85.7 (18)	61.4 (94)	32.8 (21)
Women with children, % (N)	85.7 (18)	77.1 (118)	68.7 (44)
Number of child before diagnosis	1.8 ± 0.6	2.6 ± 0.8	1.7 ± 0.7
Time to have a child >6 months, % (N)	5.5 (1/18)	15.3 (18/118)	25 (11/44)
BMI (kg/m2)	23.6 ± 3.6	23.7 ± 4.8	23.8 ± 6.0
Current smoker, % (N)	33.3 (7)	32.7 (50)	29.7 (19)
Familial history of breast cancer, % (N)	38 (8)	47.7 (73)	45.3 (29)
BRCA 1/2 mutation carriers, % (N/number of women tested)	7.7 (1/13)	22.3 (17/74)	10.3 (4/39)
HISTOLOGICAL CHARACTERISTICS			
Invasive ductal carcinoma, % (N)	95.2 (20)	91 (139)	93.7 (60)
Invasive lobular carcinoma, % (N)	4.7 (1)	8.4 (13)	1.6 (1)
Other histological category, % (N)	0 (0)	1.3 (2)	4.7 (3)
Grade II-III, % (N)	95.2 (20)	94.1 (144)	93.7 (60)
Positive for hormone receptors, % (N)	61.2 (13)	71.2 (109)	59.4 (38)
HER2 positive, % (N)	38 (8/21)	22.9 (35/153)	38.0 (24/63)
Node-positive, % (N)	57.1 (12)	46.4 (71)	57.8 (37)
Breast-conserving surgery, % (N)	52.4 (11)	52.9 (81)	59.4 (38)
TREATMENT CHARACTERISTICS			
Neoadjuvant chemotherapy, % (N)	33.3 (7)	28.1 (43)	35.9 (23)
FEC, % (N)	4.8 (1)	0.6 (1)	3.1 (2)
FEC-T, % (N)	76.2 (16)	89.5 (137)	85.9 (55)
TAC, % (N)	0 (0)	0.6 (1)	0 (0)
Epirubicin T, % (N)	0 (0)	0.6 (1)	1.6 (1)
T, % (N)	9.5 (2)	5.8 (9)	7.8 (5)
Taxotere cyclophosphamide, % (N)	9.5 (2)	1.3 (2)	4.7 (3)
Anthracycline doses (mg/m2)	294 ± 43	277 ± 73	272 ± 64
Taxanes doses (mg/m2)	297.6 ± 66	304.8 ± 61.2	297 ± 43.2
Number of chemotherapy cycles	4.7 ± 1.4	4.3 ± 1.4	4.5 ± 1.5
Trastuzumab co-treatment, % (N)	9.5 (2)	5.8 (9)	9.3 (6)
GnRH analog administration during chemotherapy, % (N)	4.7 (1/21)	1.9 (3/153)	6.4 (4/63)
Ovarian cryopreservation before chemotherapy onset, % (N)	0 (0/21)	0.65 (1/152)	6.4 (4/63)
Tamoxifen administration, % (N)	61.09 (13)	67 (103)	54.7 (35)

Abbreviations: FEC: 5-Fluorouracil, epirubucin, cyclophosphamide – T: Taxanes – AC: Adriamycin, cyclophosphamide – BMI: Body Mass Index.

Table 1: Demographic, histological and treatment characteristics in patients with low, normal or high basal AMH levels. Results are presented as mean ± standard deviation or proportion (number) when appropriate. *$p<0.05$ when each group compared to the 2 other ones