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Anti-Müllerian Hormone Levels and Risk of Cancer in Women

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ABSTRACT

Objectives: To examine if age-specific anti-Müllerian hormone (AMH) levels are associated with cancer risk; and to investigate if age-related AMH trajectories differ between women who develop cancer and women who do not. More specifically, we examined associations with breast cancer, cancers in other tissues expressing AMH receptor AMHR2, and cancers in non-AMHR2-expressing tissues.
Study design: We included longitudinal data from 3025 women in the prospective Doetinchem Cohort Study. Cox proportional hazards models were used to assess the association of baseline age-specific AMH tertiles with cancer. We applied linear mixed models to compare age-related AMH trajectories between women who were diagnosed with cancer and women who were not.
Main outcome measures: Cancer (n = 385; 139 breast cancers, 112 cancers in other AMHR2-expressing tissues, 134 cancers in non-AMHR2-expressing tissues).
Results: Overall, baseline age-specific AMH levels were not associated with cancer risk, although in women ≤ 40 years an increased risk was suggested for breast cancer ($HR_{T2:T1}$ = 2.06, 95%CI = 0.95-4.48; $HR_{T3:T1}$ = 2.03, 95% CI = 0.91–4.50). Analysis of age-related AMH trajectories suggested that AMH levels were higher at younger ages and declined faster in women who were diagnosed with cancer compared with women who were not, but our results did not provide evidence for actual differences in trajectories.
Conclusions: Our results did not provide evidence for an association between age-specific AMH levels and age-related trajectories and risk of cancer. However, effect estimates for breast cancer were in line with risk-increasing effects found in previous studies.

1. Introduction

Higher circulating anti-Müllerian hormone (AMH) levels in women have been associated with increased breast cancer risk[1]. Although AMH is primarily known for its functions in sexual differentiation during embryogenesis[2] and ovarian follicle development[3], histologic evidence on the expression of AMH receptor type 2 (AMHR2) in different non-gonadal tissues[4–6] suggests responsiveness of a wide range of tissues to AMH.

This raises the question whether AMH levels are also associated with other forms of cancer, such as ovarian and lung cancer. A small number of studies examined circulating AMH levels in relation to different cancer types, but except for breast cancer results are inconsistent (see Verdiesen et al.[7] for a detailed overview). Furthermore, previous studies included a single AMH measurement per participant, although age-related AMH trajectories have been shown to vary between women [8]. Individual age-related AMH trajectories may therefore elucidate if, and how, circulating AMH levels affect cancer risk over time.

To provide more insight into the relation between circulating AMH levels and cancer risk, we examined the association of age-specific AMH

Abbreviations: AMH, anti-Müllerian hormone; AMHR2, AMH receptor type 2; BMI, body mass index; AFTP, age at first full-term pregnancy; OC, oral contraceptive; HRT, hormone replacement therapy; CG-LMS, Cole and Green, Lambda, Mu, and Sigma; IQR, interquartile range; HR, hazard ratio; 95% CI, 95% confidence interval.

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levels with the risk of cancer, using data from female participants of the Doetinchem Cohort Study. We further examined if age-related AMH trajectories were different for women who developed cancer compared to women who did not. More specifically, we aimed to confirm previous findings for breast cancer and to investigate associations between circulating AMH levels and risk of cancers in other AMHR2-expressing tissues, and cancers in non-AMHR2-expressing tissues.

2. Methods

2.1. Study population

We used data of female participants (median age 39 years, range 20-59) from the Doetinchem Cohort Study, an ongoing prospective cohort study of 3641 men and 4128 women, who were randomly selected from the municipal register of Doetinchem, The Netherlands, between 1987 and 1991[9,10]. Every 5 years, follow-up visits take place, during which physical examinations and questionnaires are completed. The study was approved by the Medical Ethics Committee of The Netherlands Institution of Applied Scientific Research. All participants signed informed consent prior to study inclusion.

This study included data from Round 1 (baseline; 1987-1991) to Round 5 (2008-2012). Women without any available AMH measurement (n = 802), and women whose data could not be linked to the cancer registry (n = 224) or who were diagnosed with cancer prior to their first AMH measurement (n = 77), were excluded, leaving 3025 women with at least one available AMH measurement for analysis (Fig. 1). The number of women with an AMH measurement per examination round was 2855, 2772, 2281, 2153 and 1909 for Round 1 through Round 5, respectively.

2.2. AMH measurements

Details on AMH measurements and sample storage conditions have been described previously[8,11]. Briefly, AMH was measured in all available plasma samples, collected from Round 1 to Round 5. Missing AMH measurements were the consequence of either non-attendance at certain examination rounds, no consent to blood draw at the particular examination, depletion of plasma samples because of other blood measurements, or an occasional unsuccessful AMH measurement. AMH was

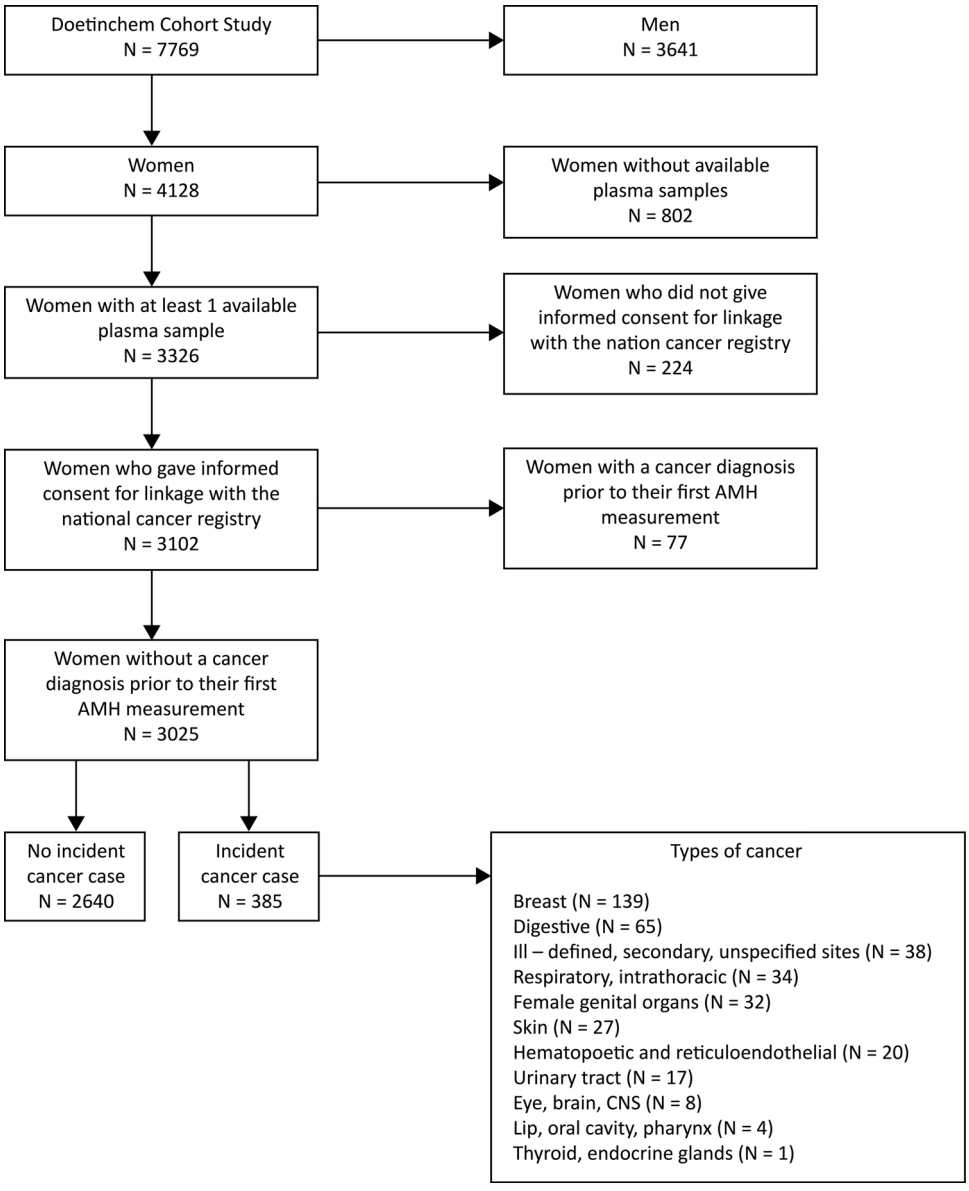

Fig. 1. Flow chart study population.

measured using the ultrasensitive picoAMH ELISA (Ansh Labs, Webster, Texas, USA) in the Ansh Labs laboratory. Because of its detection limit of 1.846 pg/mL (0.013 pmol/L), we were able to measure very low AMH levels in postmenopausal women in the Doetinchem Cohort Study[8]. The inter- and intra-assay coefficients of variation were 4.4 and 3.9%, respectively. There was no indication of plate drift, as all CVs within plate columns and rows of the picoAMH assay were below 5%[11]. AMH measurements below the detection limit were set to half the detection limit (0.923 pg/mL; 0.007 pmol/L).

2.3. Covariates

Information on potential confounders was collected through questionnaires and physical examinations. We included the following covariates in our analyses: age (years) at blood collection, age (years) at menarche, body mass index (BMI) (kg/m2), parity and age at first full-term pregnancy (AFTP) (nulliparous/ 1-2 children and AFTP $<$ 25 years/ 1-2 children and AFTP $\geq$ 25 years/ $\geq$3 children and AFTP $<$ 25 years/ $\geq$ 3 children and AFTP $\geq$ 25 years), current oral contraceptive (OC) use (yes/no), ever hormone replacement therapy (HRT) use (yes/no), menopausal status (premenopausal/postmenopausal), current smoking (yes/no), alcohol consumption (glasses/day), family history of breast cancer (yes/no) and educational attainment (primary education up to completing intermediate vocational education/up to higher secondary education/college degree or higher). A more detailed description of these covariates has been included in the Supplemental Methods.

2.4. Cancer outcomes

Through linkage of cohort data with the Dutch Cancer Registry, we identified 385 cases in registry data that were complete until 31 December 2014. Cancers were classified as "cancers in AMHR2-expressing tissues" based on previously published histological evidence[6] or data from the Genotype-Tissue Expression (GTEx) portal (www.gtexportal.org). As a result, the following tumors were defined as "tumors originating from AMHR2-expressing tissues": breast (n = 139), bronchus and lung (n = 32), hematopoietic and reticuloendothelial (n = 20), corpus uteri (n = 13), ovary (n = 11), kidney, except renal pelvis (n = 11), pancreas (n = 9), lymph nodes (n = 6), cervix uteri (n = 4), uterus, unspecified (n = 2), small intestine (n = 2), liver and intrahepatic bile ducts (n = 1), adrenal gland (n = 1). Breast cancer (n = 139; 127 invasive tumors and 12 with unknown behavior) and "cancers in other AMHR2-expressing tissues" (n = 112) were included as separate outcomes. We additionally included the outcome "cancers in non-AMR2-expressing tissues" (n = 134), which comprised tumors in the remaining tissues.

2.5. Statistical analyses

We calculated age-specific AMH tertiles at baseline (Round 1) using general linear modeling with CG-LMS[12] (Cole and Green, Lambda, Mu, and Sigma method; R package "gamlss"[13] version 5.1-2), as previously published[14]. Log-transformed AMH was modelled over age using splines, because of the non-linear decline of AMH with increasing age. Previous analyses showed that this model fits the AMH data in the Doetinchem Cohort Study well[8]. The CG-LMS method allows for estimation of the distribution of AMH at every age, and corresponding percentile values (for 33.3% and 66.7%) were used to create age-specific tertiles. Accordingly, women could be classified as having either low (1st age-specific tertile), normal (2nd age-specific tertile), or high (3rd age-specific tertile) AMH levels given their age.

Characteristics for women with an available AMH measurement at baseline (n = 2855) were described using mean (standard deviation), median [interquartile range (IQR)], or frequency (%). We summarized these baseline characteristics by age-specific AMH tertiles.

Missing information on most baseline and time-varying covariates was below 2%. Data on menopausal status was missing for up to 24.9% in Round 3, due to the relatively high proportion of OC users. Missing values for baseline age-specific AMH tertiles and baseline and time-varying covariates were imputed with multiple imputation (50 iterations, 10 imputed datasets) using the R package "mice" (version 3.3.0) [15] (Supplemental Methods). Subsequent regression analyses were performed in each imputed dataset; regression coefficients and standard errors of the mean were pooled according to Rubin's Rule of combination[16] using the pool function in "mice".

2.5.1. Baseline age-specific AMH tertiles and cancer risk

We investigated associations between baseline age-specific AMH tertiles and incident cancer, by estimating hazard ratios (HRs) and 95% confidence intervals (95% CIs) from Cox proportional hazards models. We used follow-up time in years as underlying time scale (t_0 = baseline examination, t_{max} = linkage of data with cancer registry; 31 December 2014), and adjusted models for known risk factors for cancer: age at baseline, age at menarche, current OC use, parity and AFTP, menopausal status, BMI, educational attainment, current smoking, alcohol consumption and family history of breast cancer.

2.5.2. Mean AMH trajectories in women who developed cancer compared to women who did not

To assess whether age-related AMH trajectories differed between women who were diagnosed with cancer and women who were not diagnosed with cancer during follow-up, we used linear mixed models (R package "nlme"[17] version 3.1-139). Measurements from examination Rounds 1-5 were used to construct AMH trajectories. In total, we analyzed 11,655 AMH measurements performed in the period from baseline until cancer diagnosis, censoring, or end of follow-up, whichever came first. Of these measurements, 4223 (36.2%) were below the limit of detection ($<$ 1.846 pg/mL). Missing AMH measurements were not imputed as this is not needed for linear mixed model analyses [18]. Imputed values were included for the covariates described below.

Linear mixed models included repeated log transformed AMH measurements as dependent variable and age in years, modelled with natural splines (2 knots: 36 and 45 years, upper boundary: 65 years), as the underlying timescale. To assess whether models including incident cancer status (yes/no) and interaction terms of this case variable and the spline terms were a better fit to the data compared with models without these variables, a global likelihood ratio test was applied [19] using the testModels function (method "D3") implemented in R package "mitml" (version 0.3-7 [20]). All models additionally included the following fixed effects: age at blood collection (time-dependent), current OC use (time-dependent), current smoking (time-dependent), BMI (time--dependent), menopausal status (time-dependent), alcohol consumption (time-dependent), age at menarche, parity and AFTP, educational level and family history of breast cancer. We also included random intercepts and random slopes for each woman. We used the estimated fixed effects from the fitted models to calculate predicted geometric mean AMH trajectories over age, which were adjusted for the described potential confounders. Predicted AMH trajectories and standard errors of the mean were also pooled using Rubin's Rule. All analyses were performed in R, version 3.4.3[21].

2.5.3. Sensitivity analyses

Because AMH is known to strongly decrease from age 40 and because less variation is found in AMH levels after this age[8], we performed sensitivity analyses restricted to women younger than 40 years at baseline (n = 1543). We additionally performed sensitivity analyses in which we excluded (1) AMH measurements within two years prior to diagnosis, (2) women who were current OC users at baseline of the cohort (n = 766, on average across 10 imputation sets), and (3) women who had ever reported hormone replacement therapy (HRT) use (n = 923, on average across 10 imputation datasets). Sensitivity analyses excluding current OC users at baseline were only performed for Cox

proportional hazards models, since current OC use was included as time-varying covariate in the linear mixed models.

3. Results

Baseline characteristics of women with an available AMH measurement at Round 1 are presented by age-specific AMH tertiles in Table 1. Women in the lowest age-specific AMH tertile were older than women in the middle and highest age-specific AMH tertiles. Women in the highest age-specific AMH tertile were more likely to be premenopausal, and less likely to be current OC user, ever HRT user or current smoker compared to women in the lowest age-specific AMH tertile. In addition, women in the highest age-specific AMH tertile were more likely to have attained a higher educational level and to have a positive family history of breast cancer. Alcohol consumption was also higher among women in the highest age-specific AMH tertile.

3.1. Baseline age-specific AMH tertiles and cancer risk

We observed no increased risk of cancer in women with higher age-specific AMH levels ($HR_{T2:T1} = 1.00$, 95% CI = 0.77 – 1.28 and $HR_{T3:T1} = 1.12$, 95% CI = 0.86 – 1.46; Table2). Restricting our analyses to breast cancer resulted in somewhat stronger risk-increasing effect estimates, but confidence intervals were wide and included the null (Table 2). Associations between age-specific AMH levels and risk of cancers in other AMHR2-expressing tissues were similar to those for risk of total cancer, whereas a risk-decreasing effect of higher AMH levels was suggested for cancers in non-AMHR2-expressing tissues ($HR_{T2:T1} = 0.74$, 95% CI = 0.49 – 1.14 and $HR_{T3:T1} = 0.96$, 95% CI = 0.62 – 1.49; Table2). Restricting analyses to women ≤ 40 years at baseline (n = 1543) resulted in stronger effect estimates for breast cancer, although corresponding confidence intervals still indicated considerable uncertainty: $HR_{T2:T1} = 2.06$, 95% CI = 0.95 – 4.48 and $HR_{T3:T1} = 2.03$, 95% CI = 0.91 – 4.50 (Table 2). Effect estimates for cancers in non-AMHR2-expressing tissues were also more extreme in women ≤ 40 age at baseline due to increased uncertainty (Table 2). Exclusion of AMH measurements within two years prior to diagnosis, exclusion of current OC users at baseline and exclusion of women that ever-used HRT did not change our conclusions (Supplemental Table 1).

3.2. Mean AMH trajectories in women who developed cancer compared to women who did not

On average, 3.9 AMH measurements were available per woman (see Supplemental Table 2 for details on repeated AMH measurements). Fig. 2 presents predicted geometric mean AMH trajectories in women who were diagnosed with cancer during follow-up and women who were not, averaged across the ten imputed datasets. These plots suggested that AMH levels were higher around age 30 and subsequently declined faster in women who were later diagnosed with cancer compared to women who were not, but our results did not provide evidence for an actual difference in trajectories (p-value global likelihood ratio tests > 0.05 for each outcome; Supplemental Table 3). Sensitivity analyses restricted to women younger than 40 age at baseline, exclusion of AMH measurements within two years prior to cancer diagnosis, and exclusion of women who reported ever having used HRT also did not provide evidence for differences in trajectories (p-value for each global likelihood ratio test > 0.05).

4. Discussion

This study found no evidence for associations between baseline age-specific AMH and cancer risk, although the risk-increasing effect estimates for breast cancer were in line with previously published findings. Examination of AMH trajectories indicated that AMH levels around age 30 may be higher, and may decline faster, in women who are diagnosed

Table 1
Baseline characteristics of women with an available AMH measurement at Round 1 of the Doetinchem Cohort Study (n = 2855) presented by age-specific AMH tertiles.

		Tertiles of age-specific AMH levels		
		1 st age-specific AMH tertile (n = 859)	2nd age-specific AMH tertile (n = 1048)	3rd age-specific AMH tertile (n = 948)
AMH (pg/mL)[a]		29.7 [0.9, 747.2]	1313.4 [154.9, 2734.6]	3796.3 [1036.8, 6405.0]
Age (years)[a]		42.1 [32.7, 51.5]	38.1 [31.6, 45.7]	39.0 [32.2, 46.0]
BMI (kg/m^2)[a]		23.7 [21.8, 26.3]	23.7 [21.6, 26.1]	23.3 [21.5, 25.8]
Educational attainment, % (n)[d]				
	primary education up to completing intermediate vocational education	71.5 (612)	68.3 (714)	63.8 (604)
	up to higher secondary education	16.9 (145)	18.9 (198)	20.9 (198)
	college degree or higher	11.6 (99)	12.7 (133)	15.2 (144)
Reproductive factors				
Age at menarche (years)[b,d]		13.4 (1.5)	13.4 (1.5)	13.4 (1.4)
Parity and age at first full-term pregnancy, % (n)[d]				
	Nulliparous	21.3 (183)	24.5 (256)	21.4 (203)
	1-2 children and <25 years	20.1 (173)	23.5 (246)	22.6 (214)
	1-2 children and ≥25 years	33.5 (288)	30.8 (322)	33.7 (319)
	≥3 children and <25 years	12.5 (107)	10.9 (114)	12.6 (119)
	≥3 children and ≥25 years	12.6 (108)	10.3 (108)	9.7 (92)
Premenopausal, % (n)[d]		72.9 (555)	84.0 (816)	95.6 (856)
Current OC use, % (n)[d]		28.5 (243)	27.5 (288)	19.1 (181)
Ever HRT use, % (n)[c,d]		35.3 (196)	25.5 (167)	27.4 (176)
Lifestyle factors				
Current smoker, % (n)[d]		35.0 (301)	35.1 (368)	29.5 (279)
Current alcohol consumption, % (n)[d]				
	No	20.8 (179)	19.8 (208)	17.3 (164)
	<1 glass/week	31.8 (273)	32.0 (335)	29.6 (280)
	≥1 glass/week	47.3 (406)	48.1 (504)	53.1 (503)
Familyhistory of disease				
Ever reported family history of breast cancer, % (n)[c]		12.2 (105)	14.8 (155)	17.0 (161)

Abbreviations: AMH, anti-Müllerian hormone; OC, oral contraceptive, HRT; hormone replacement therapy. [a] Median [interquartile range]. [b] Mean (standard deviation). [c] Ever variables are presented because of absent data on HRT use and

family history of breast cancer in Round 1. [d] Missing values (n): educational attainment (8); age at menarche (10); parity and age at first full-term pregnancy (3), menopausal status (228), current OC use (7), ever HRT use (1001), current smoking (1), current alcohol consumption (3).

with cancer compared to women who are not. However, our results did not provide strong evidence for an actual difference in age-related AMH trajectories.

The main strength of this study is that we were the first to investigate

Table 2
Associations between age-specific AMH tertiles and total cancer, breast cancer, cancers in other AMHR2-expressing tissues and cancers in non-AMHR2-expressing tissues in women of the Doetinchem Cohort Study (upper panel; n = 3025) and in women ≤40 years at baseline (lower panel; n = 1543).

	Total cancer[a,b]		Breast cancer[a,b]		Cancers in other AMHR2-expressing tissues[a,b]		Cancers in non-AMHR2-expressing tissues	
Age-specific AMH tertiles	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Total study population (n = 3025)	**385 cases**		**139 cases**		**112 cases**		**134 cases**	
1 st age-specific tertile	1	-	1	-	1	-	1	-
2nd age-specific tertile	1.00	(0.77, 1.28)	1.27	(0.83, 1.95)	1.05	(0.65, 1.70)	0.74	(0.49, 1.14)
3rd age-specific tertile	1.12	(0.86, 1.46)	1.27	(0.80, 2.01)	1.18	(0.71, 1.95)	0.96	(0.62, 1.49)
Women ≤40 years at baseline (n = 1543)	**131 cases**		**59 cases**		**34 cases**		**38 cases**	
1 st age-specific tertile	1	-	1	-	1	-	1	-
2nd age-specific tertile	1.02	(0.65, 1.59)	2.06	(0.95, 4.48)	1.10	(0.46, 2.66)	0.42	(0.19, 0.94)
3rd age-specific tertile	1.13	(0.71, 1.79)	2.03	(0.91, 4.50)	1.09	(0.43, 2.78)	0.66	(0.31, 1.42)

Abbreviations: AMH, anti-Müllerian hormone; AMHR2, anti-Müllerian hormone receptor type 2; HR, hazard ratio; CI, confidence interval. [a] Cox proportional hazards models adjusted for age at baseline (years), age at menarche (years), parity and age at first birth (nulliparous/ 1-2 children and AFTP < 25 years/ 1-2 children and AFTP ≥ 25 years/ ≥3 children and AFTP < 25 years/ ≥ 3 children and AFTP ≥ 25 years), menopausal status (premenopausal/postmenopausal), current OC use (yes/no), ever reported family history of breast cancer (yes/no), BMI (kg/m^2), educational attainment (primary education up to completing intermediate vocational education/up to higher secondary education/college degree or higher), current smoking (yes/no), alcohol consumption (g/day). [b] Cox proportional hazards models were not adjusted for menopausal status as only 1 woman of the 1543 women ≤40 years at baseline was classified as postmenopausal.

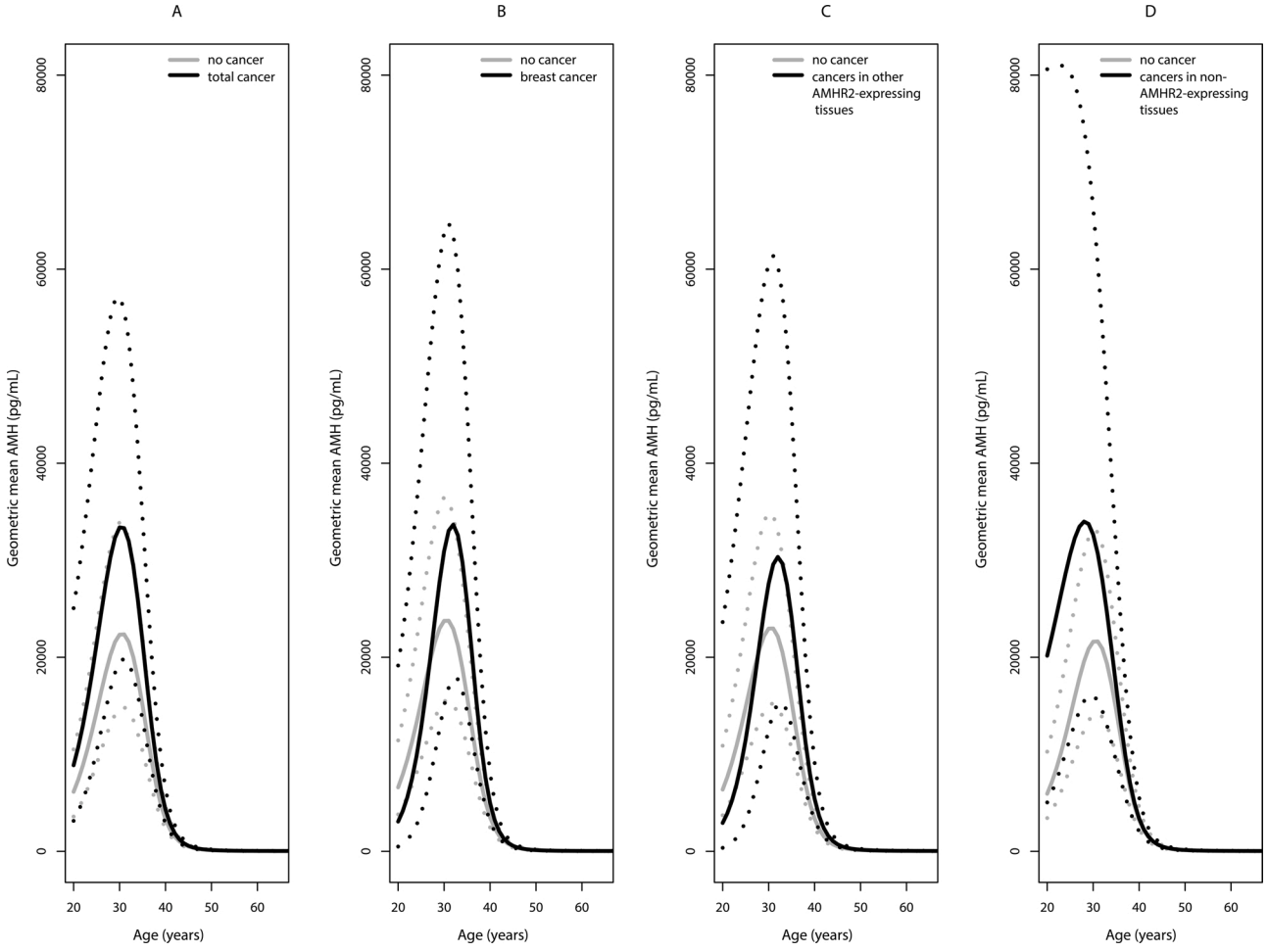

Fig. 2. Predicted geometric mean AMH trajectories (solid lines) and 95% confidence intervals (dashed lines) over age in women who developed (A) total cancer, (B) breast cancer, (C) cancers in other AMHR2-expressing tissues, and (D) cancers in non-AMHR2-expressing tissues compared to women who did not develop cancer during follow-up. Plots show average predicted AMH trajectories across 10 imputed datasets. Trajectories are adjusted for the time-varying covariates current oral contraceptive use, current smoking, body mass index, menopausal status, alcohol consumption; and the time-invariant covariates age at menarche, parity and age at first full-term pregnancy, educational level and family history of breast cancer.

the association between age-related AMH trajectories and risk of cancer, whereas previous studies included only one AMH measurement for each participant. Also, this is the first study to investigate the effect of AMH on the risk of total cancer, and on cancer types subdivided based on expression of AMHR2. Additional strengths of this study are its large study population, with a median follow-up period of 25 years, and time-varying information on risk factors for cancer. Nevertheless, the current analyses are mostly exploratory in nature because of the limited number of cancer cases, and the limited number of measurements at younger ages. As a result, we cannot rule out that age-related AMH trajectories do differ between women who later develop cancer and women who do not. Moreover, the heterogeneous nature across and within cancer types most likely also limited statistical power to detect associations.

Following our objective to investigate whether AMH trajectories differed for women who were or were not diagnosed with cancer during follow-up, we used linear mixed models in which AMH was included as dependent variable. An evident disadvantage of this approach is that time until cancer diagnosis is not taken into account. Although various methods that can model repeated measurements and time to event data are available (e.g. Cox proportional hazards models including a time-varying exposure or joint models), these approaches test whether AMH levels at, or near, the moment of diagnosis are associated with risk of cancer, whereas we were specifically interested in the complete AMH trajectory over time up to the moment at which women were diagnosed with cancer.

Even though not statistically significant, our finding for breast cancer is in line with a previous individual participant data meta-analysis, reporting that women in the highest AMH quartile were at a 60% increased risk of breast cancer compared to women in the lowest AMH quartile[1]. Interestingly, in this meta-analysis the relation of baseline circulating AMH levels with breast cancer was strongest in women aged 45-49 years at blood draw, whereas in our longitudinal analyses AMH levels were not different between future breast cancer cases and healthy women in that age range. A possible explanation for this difference may be the fact that the study by Ge et al. included a number of small studies in older women, which reported very large effect sizes. In contrast to previous studies on AMH and female specific cancers, we could not assess potential confounding of our results by estradiol and/or testosterone levels, as these were not measured in our study population. However, as correction for endogenous estradiol and/or testosterone did not influence results of previous studies[1,22–24], we do not expect a large confouding effect of these hormones in our study.

Our results provide no answer to the question whether AMH is merely a proxy for time until menopause, or whether AMH has a direct effect on tissues that express its receptor, AMHR2. Performing a formal mediation analysis for age at menopause was not feasible in the current study population, due to the limited number of cases that underwent the menopausal transition (for breast cancer only 72 cases; i.e. 52%). We hypothesized that if AMH regulates cell growth in AMHR2-expressing tissues, we would observe a stronger effect of high AMH levels on risk of cancers in AMHR2-expressing tissues than for total cancer, and absence of an association with cancers in non-AMHR2-expressing tissues. However, apart from supporting the association between high AMH and an increased risk of breast cancer, our results do not support an association with cancers in other AMHR2-expressing tissues. Due to the low number of cases in this latter group, we could not examine the association between AMH and individual cancer types, such as ovarian and endometrial cancer. Similarly, we were not able to investigate associations with different breast cancer subtypes.

In conclusion, plasma AMH levels were not associated with risk of cancer, although our findings are in agreement with previous evidence suggesting that higher circulating AMH levels are associated with an increased risk of breast cancer. Our longitudinal analyses suggested that AMH levels may be higher around age 30 and may decline faster in women who later develop cancer, but our results did not provide clear evidence for an actual difference in trajectories. Prospective studies with repeated AMH measurements in a larger population of young women are required to establish if, and at which age, AMH could be considered a risk factor for cancer, and specifically for breast cancer.

Contributors

Renée M.G. Verdiesen contributed to data analysis, data interpretation, preparation of first draft of the manuscript and critical revision of the manuscript.

Carla H. van Gils contributed to data interpretation and critical revision of the manuscript.

Rebecca K. Stellato contributed to data analysis, data interpretation and critical revision of the manuscript.

W.M. Monique Verschuren contributed to data collection and critical revision of the manuscript.

Frank J.M. Broekmans contributed to data collection and critical revision of the manuscript.

Annelien C. de Kat contributed to critical revision of the manuscript.

Yvonne T. van der Schouw contributed to data collection, data interpretation and critical revision of the manuscript.

N. Charlotte Onland-Moret contributed to data interpretation and critical revision of the manuscript.

Conflict of interest

FJMB has received fees and grant support from Merck Serono, Gedeon Richter, Ferring BV, and Roche. The other authors report no conflicts of interest.

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Ethical approval

The Doetinchem Cohort Study was conducted according to the principles of the World Medical Association Declaration of Helsinki and its amendments since 1964, and in accordance with the Medical Research Involving Human Subject Act (WMO). The Doetinchem Cohort Study received ethical approval from the Medical Ethics Committee of The Netherlands Institution of Applied Scientific Research and all study participants gave written informed consent prior to study inclusion.

Research data (data sharing and collaboration)

There are no linked research datasets for this paper. The full dataset, and statistical code are available on request, in liaison with the National Institute of Public Health and the Environment.

Provenance and peer review

This article was not commissioned. Peer review was directed by Martina Dören independently of Yvonne T. van der Schouw, an author and *Maturitas* editor, who was blinded to the process.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.maturitas.2020.10.017.

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