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Added value of anti-Müllerian hormone in prediction of menopause: results from a large prospective cohort study

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STUDY QUESTION: What is the added value of anti-Müllerian hormone (AMH) on top of patient characteristics for predicting the risk to enter menopause within 10 years?

SUMMARY ANSWER: For women who did enter menopause, the risk of entering menopause within 10 years assigned by the model with AMH was on average 3% higher than that assigned by the model without AMH, and in the subgroup of young women with regular cycles, this increase was 14%.

WHAT IS KNOWN ALREADY: Prediction of age at menopause may be useful in predicting the end of female fertility. AMH may be useful for this, but the current evidence is based on small studies or specific subgroups, and does not take into account predictors other than age.

STUDY DESIGN, SIZE, DURATION: This was a retrospective cohort study among 1163 premenopausal women participating in the second follow-up round of the Doetinchem Cohort Study with follow-up assessments of menopausal status and age after 5 and 10 years of follow-up.

PARTICIPANTS/MATERIALS, SETTING, METHODS: This study included premenopausal women from the general population with a mean age of 41 (SD 7) years. A Cox proportional hazards' model without AMH was fitted using variables selected based on Akaike's information criterion. Performance of the prediction rule was assessed with C-statistics and compared with a model additionally including AMH and to a model with age only. The added value of AMH was assessed with Net Reclassification Index and change in absolute predicted risk. Performance of these three models was compared in subgroups based on age and reproductive characteristics.

MAIN RESULTS AND THE ROLE OF CHANCE: The final model included age, BMI, packyears of smoking and menstrual cycle status (regular, irregular, pregnant or taking oral contraceptives). This model had a C-statistic of 0.89 (0.01 SD), compared with 0.88 (0.01 SD) for the model including age only. Addition of AMH increased it to 0.91 (0.03 SD). In a subgroup of 25–43 year olds with regular menstrual cycles, the model with age only had a C-statistic of 0.79 (0.04 SD) and for the models without and with AMH the C-stastic was 0.79 (0.04 SD) and 0.87 (0.03 SD), respectively. The risk of entering menopause within 10 years assigned by the model with AMH was on average 3% higher than that assigned by the model without AMH, for women who did enter menopause. In the subgroup of young women with regular cycles, this increase was 14%.

LIMITATIONS, REASONS FOR CAUTION: Longer follow-up would have resulted in more of the young women becoming menopausal, improving the precision of the predictions for these women.

WIDER IMPLICATIONS OF THE FINDINGS: This study clearly shows the added value of AMH in predicting time to menopause on top of clinical predictors, in particular for younger women. New studies in specific target populations in clinical practice are needed to develop a prediction model for use in that target population.

STUDY FUNDING/COMPETING INTEREST(S): The Doetinchem Cohort Study is carried out by the National Institute for Public Health and the Environment which works under the authority of the Ministry of Health, Welfare and Sport of The Netherlands. F.J.M.B. declares to have received fees and grant support from the following companies (in alphabetical order); Ferring, Gedeon Richter, Merck Serono, MSD and Roche. The remaining authors declare no conflict of interest.

Key words: menopause / epidemiology / anti-Müllerian hormone / prediction

Introduction

Menopause, the only noticeable mark of the end of the female reproductive lifespan, occurs on average at the age of 51 years. However, age at menopause shows considerable individual variation between the ages of 40 and 60 years, with ~10% of women becoming menopausal before the age of 45 years. A fixed temporal relationship between the end of natural fertility, and menopause itself, is thought to be present, with the end of natural fertility preceding menopause by ~10 years (te Velde and Pearson, 2002; Broekmans *et al.*, 2007). This means that a woman with menopause at age 43 may reach the end of their natural fertility at age 33 without any noticeable changes such as cycle irregularity. Considering current trends of delaying first child birth, it is conceivable that specifically these women are confronted with infertility and require assisted reproductive techniques (ART) to fulfil their child wish (de Graaff *et al.*, 2011). If individualized indications of the remaining fertile life span could be derived from appropriate prediction of age at menopause, steps towards primary prevention of age-related infertility can be made by counselling women towards early child bearing or possibly towards cryopreservation of their oocytes (Stoop *et al.*, 2011).

Anti-Müllerian hormone (AMH) is a hormone that is secreted solely by ovarian follicles, and which has consistently been shown to reflect the age-associated depletion of the follicular pool (Lee *et al.*, 1996; van Rooij *et al.*, 2002; Gruijters *et al.*, 2003; Broekmans *et al.*, 2006; Broekmans *et al.*, 2007). As the onset of menopause is incited by exhaustion of the follicle pool and considering that AMH is a reflection of the size of the remaining follicle pool, age-specific AMH values have been used to predict the age at which a woman will become post-menopausal in both retrospective and prospective cohort studies (van Disseldorp *et al.*, 2008; Broer *et al.*, 2011a; Tehrani *et al.*, 2011, 2013; Freeman *et al.*, 2012; Dolleman *et al.*, 2013a). Lifestyle and environmental factors, such as smoking and body mass index (BMI), have also been studied as determinants of age at menopause (Gold, 2011; Dossus *et al.*, 2012; Morris *et al.*, 2012). Although studies on AMH unanimously agree that AMH is associated with the timing of menopause, it has never been studied what the true added value is of AMH on top of easily obtained information such as female age, and other environmental and lifestyle determinants of menopause. Furthermore, their retrospective design, small cohort sizes or short duration of follow-up, and selected populations of proven fertile women mean that substantiation in a large long-term population-based prospective cohort study is warranted.

The aim of this study was to assess the added value of AMH in the prediction of time to menopause (TTM) in a large population-based prospective cohort study. Furthermore, as AMH has been shown to be influenced by factors such as oral contraceptive (OC) use and menstrual cycle regularity, the added value of AMH is presented in clinically relevant subgroups of women.

Materials and Methods

Participants

We used data from the Doetinchem Cohort Study (DCS), an ongoing multipurpose prospective study, initially carried out in a random general population sample of men and women aged 20–59 years (1987–1991) in Doetinchem, the Netherlands (Verschuren *et al.*, 2008). The aim of the DCS was to study the impact of (changes in) lifestyle factors and biological risk factors on various aspects of health, such as the incidence of chronic diseases, physical and cognitive functioning, and quality of life. The cohort is re-examined every five years with questionnaires and a physical examination at the local health service. Three follow-up examination rounds were completed during 1993–1997, 1998–2002 and 2003–2007. All participants gave written informed consent, and the study was approved according to the guidelines of the Helsinki Declaration by the Medical Ethics Committee of the Netherlands Organization of Applied Scientific Research. Details on the DCS have been extensively described elsewhere (Verschuren *et al.*, 2008). For the current study, we applied a prospective design with the second examination round as the baseline, and follow-up was for ten years until the fourth examination round.

For the current study, 2075 women participating in the second examination round of the DCS (1993–1997) were eligible. Women were excluded if they were post-menopausal at the start of the study ($n = 59$), if they had undergone hysterectomy or (uni-or bilateral) oophorectomy ($n = 196$), if information on their reproductive status ($n = 5$) or AMH was missing at baseline ($n = 41$), or if they did not participate in the third and fourth examination round ($n = 614$, of these 614 women, 3 were already excluded on above-mentioned grounds), leaving 1163 women for analysis.

Outcome

Age at natural menopause, defined according to the World Health Organization as the age at which a women had amenorrhoea for at least 12 consecutive months without other obvious reasons (hysterectomy and/or unilateral or bilateral ovariectomy), was derived from reproductive history questionnaires.

Candidate predictors

All variables pertaining to patient characteristics and laboratory measures recorded in the DCS were critically reviewed for their potential relationship with menopause using up to date literature and clinical expertise. The following characteristics were considered to be possibly predictive for menopause: AMH, age at inclusion (packyears of) smoking, BMI, socioeconomic status (SES), age at menarche, parity, menstrual cycle status (whether the female had regular cycles, irregular cycles, was pregnant or taking HRT or OCs), and duration of OC use.

At baseline, blood samples for AMH were collected on a random day of the menstrual or OC cycle. Serum was frozen on the day of vena cubiti puncture and stored in liquid nitrogen for future analysis. Prior to the AMH measurement each sample went through one thaw–freeze cycle on ice for 4 h; intermittent storage until AMH measurement was at −80°C for a maximum of 4 weeks. Serum AMH was measured with the AMH Gen-II ELISA (Beckman-Coulter, Sinsheim, Germany) in a single laboratory, by

the same experienced lab technician. The precision of assay results was validated with linearity-of-dilution assessment. The limit of detection for this assay is 0.08 ng/ml, and the limit of quantification is 0.16 ng/ml. The inter-assay and intra-assay coefficients of variation were 3.35 and 4.0%, respectively.

Reproductive history was assessed via extensive questionnaires at all examination rounds. The questionnaire included questions on age at the first menstrual period, period regularity and length, the number of menstruations in the 12 months prior to questionnaire, date of the last menstruation, current pregnancy and parity. Additionally, women were asked about current or previous OC use, hormone replacement therapy (HRT) and duration of use. Furthermore the occurrence of, and age at, any gynaecological operations was recorded. A regular cycle was defined as having regular cycles with a mean cycle length of 24–36 days. The number of years of OC use was stratified into 0 years, <1 year, 1–5, 5–10, 10–15, 15–20 and >20 years. From the reproductive history, we created a categorical variable 'Menstrual cycle status at start of follow-up', which was coded as regularly cycling, irregularly cycling, using OC, being pregnant or using HRT. All HRT-users at baseline could be defined as premenopausal because they either entered menopause during follow-up or were peri-menopausal at the last follow-up round.

Body weight and height were measured by trained staff. Body weight was measured to the nearest 100 g on calibrated scales with participants wearing light indoor clothing without shoes, with emptied pockets (Verschuren *et al.*, 2008). Ever smokers were identified based on the question 'did you ever smoke regularly'. For ever smokers, information on age at which the respondent started smoking, as well as the total number of years of smoking and average amount of cigarettes smoked was assessed, followed by a question on current smoking ('do you smoke at present'). Packyears of smoking were calculated and divided into seven strata with five year-intervals. Socio-economic status (SES) was classified into four categories according to the highest level of education that a woman had completed: primary school (SES level 1), lower secondary or vocational school (SES level 2), intermediate vocational or higher secondary school (SES level 3) and higher vocational or university (SES level 4) (Verschuren *et al.*, 2008).

Data analyses

Because there were only two missing values for duration of OC use, we applied no specific methods for dealing with missing values.

TTM was calculated as the time between inclusion and menopause (defined as the absence of menstruation for 12 consecutive months). Cox proportional hazards analysis was used to estimate the association between candidate predictors and TTM. Hazard ratios (HRs) derived from these models represent the risk of becoming naturally menopausal at a given time, with HRs less than 1 indicating a later menopause and HRs greater than 1 indicating an earlier menopause compared with the reference. For women who reported induced menopause or HRT use during follow-up, TTM was censored at the time of the last menses before menopause inducing treatment (surgical or medical) or HRT. Women who remained premenopausal were censored at the time of the most recent follow-up interview. The shape of the association for continuous factors was analysed with restricted cubic splines with three knots to identify those candidate predictors that would need to be added to the model with a simple spline transformation. A total of 1163 women were available for analysis. At follow-up, 70 women recalled an age at menopause that was slightly younger than their age at baseline (mean −2.5 years, SD 2.7), while their questionnaires at baseline indicated that they were premenopausal. For these women, a random number between 0 and 1 year was generated and entered as their TTM.

With TTM as the main dependent variable, age is expected to be a strong predictor (i.e. a 45 year old female is more likely to enter menopause during a 10-year follow-up period than a 25-year old female); accordingly age remained in all models. A univariable model with age, transformed with a restricted cubic spline, was fit first. Next, a multivariable model including all candidate predictors (apart from AMH) with appropriate transformations was fit. In this model, the number of candidate predictors in the model was reduced with a backwards selection procedure based on Akaike's Information Criterion (AIC), corresponding to a *P*-value of 0.157 for predictors with one regression coefficient. The regression coefficients in the final model were adjusted with a shrinkage factor which was estimated with bootstrapping. These coefficients were then transformed to HRs with 95% confidence intervals (95% CI). Interaction terms between candidate predictors were assessed but interaction was not present. In a third step, AMH was added as an extra candidate predictor to this model. The predictive values of the models were assessed and compared with Harrell's C-statistic for time-to-event data (Chambless and Diao, 2006). The C-statistic indicates how well the ranking of model predictions corresponds with the true ranking of women regarding their age at menopause.

To assess the added clinical value of a model with AMH compared with a model without AMH or a model based on age alone, a Net Reclassification Index (NRI) was calculated. An NRI quantifies the improvement offered by new markers by examining the extent to which a new marker reclassifies subjects at a higher or lower risk of having an event during follow-up (Pencina *et al.*, 2011). A continuous NRI (cNRI) was chosen as no established risk categories for the occurrence of menopause exist. The cNRI counts the number of individuals that change upwards and downwards instead of counting the percentage that crosses a particular risk threshold. Each patient is counted as +1 or −1 depending on whether the change in calculated risk was in the correct direction (higher for those with events, lower for those without events) (Pickering and Endre, 2012). The NRI is the sum of the 'event NRI' and the 'non-event NRI', where the event NRI is the net proportion of patients who did experience menopause during a 10-year follow-up who had an increase in calculated risk and the non-event NRI is the proportion of women without menopause who had a decrease in calculated risk. The maximum possible cNRI is 200% as, theoretically, all women with an event and all without an event can be reclassified in the correct direction. For ease of interpretation, we also reported the average of the two net percentages. In addition, we calculated the difference between the estimated probabilities of entering menopause during follow-up for the models with and without AMH.

The above methods were all applied to the entire cohort. Subsequently, the performance of the model that was ultimately best fitting in the entire cohort was assessed in clinically relevant subgroups. The following subgroups were constructed by sequentially removing cases according to relevant profiles:

Subgroup 1 ($n = 776$): All women aged 20–43 years (excluding women aged >43 years, or taking HRT)
Subgroup 2 ($n = 687$): Women aged 20–43 years with regular cycles or taking OC (thus additionally excluding women pregnant at baseline)
Subgroup 3 ($n = 396$): Women aged 20–43 years with regular menstrual cycles (thus additionally excluding taking OC at baseline from subgroup 2)

Data were analysed with SPSS version 20.0 (Inc., Chicago, Il, USA) and with R version 2.13 (http://www.r-project.org/).

Results

Baseline characteristics of women included in the analysis are displayed in Table I. At the time of inclusion, 15 were taking HRT, 161 had an irregular cycle, 27 were pregnant, 345 were taking OC and 615 had a regular cycle. Of these women, 169 had become post-menopausal within the first five years and 527 became post-menopausal within ten years of follow-up.

Table I Baseline characteristics.

Characteristic	Mean (SD) or *N* (%)
Whole group *n* =	1163
Age at start of follow-up (years)	40.8 (7.0)
Age at end of follow-up (years)	48.0 (5.1)
AMH (ng/ml)	1.1 (1.5)
BMI (kg/m^2)	23.8 (3.9)
Menstrual cycle status at start follow-up	
Regular	615 (52.9%)
Irregular	161 (13.8%)
OC	345 (29.7%)
Pregnant	27 (2.3%)
HRT	15 (1.3%)
Age at menarche (years)	13.2 (1.4)
Parity (*n*)	1.8 (1.1)
Years of OC use	
Never	72 (6.2%)
<1 year	52 (4.5%)
1–5 years	230 (19.8%)
5–10 years	349 (30.0%)
10–15 years	253 (21.8%)
15–20 years	149 (12.8%)
>20 years	56 (4.8%)
Missing	2 (1.7%)
Packyears of smoking	7.53 (9.32)
Number of current smokers at start follow-up	585 (49.1%)
Socioeconomic status	
1 (low)	48 (4.1%)
2	571 (49.1%)
3	316 (27.2%)
4 (high)	228 (19.6%)

The mean age at natural menopause at follow-up was 50.6 years (SD 3.77).

In the entire study population, the model with age alone required age to be transformed with a spline as the hazard of menopause increased in a linear fashion up to the age of 38 years after which the slope decreased slightly (Fig. 1B). The C-statistic of the model including age alone was 0.88 (SD 0.01). In the multivariate model, when all candidate predictors apart from AMH were introduced, backward selection according to AIC resulted in a multivariate model containing the following four predictors: age, BMI, packyears of smoking and menstrual cycle status. The C-statistic of this model without addition of AMH was 0.89 (SD 0.01). Addition of AMH to this multivariate model (age, BMI, packyears of smoking and menstrual cycle status) resulted in a higher C-statistic, of 0.91 (SD 0.03), compared with the same model without AMH. The HR of AMH in this multivariate model was significant ($P \leq 0.0001$). C-statistics are displayed in Table II, the HRs for each candidate predictor are listed in Table III and the shape of the associations are displayed in Fig. 1A–D.

In the subgroups, excluding women over 43 years of age, age did not have to be transformed with a spline. AMH did have to be transformed with a spine, as shown in Fig. 1A and Table III, as the hazard of menopause during follow-up decreased until an AMH of 2 ng/ml and then levelled off. In each subgroup, the HR of AMH when added to the multivariate model was significant (*P*-value for all <0.0001). Excluding women over 43 years at baseline (subgroup 1) resulted in a lower discriminatory accuracy of the model of age alone. The biggest increase in accuracy offered by AMH was in the subgroup of 20–43 year olds with regular menstrual cycles (subgroup 3).

Overall, the model with AMH in addition to four other predictors correctly reclassified an extra 60.4% of women who did become post-menopausal during follow-up to a higher risk category (event NRI) than the model without AMH. It further correctly reclassified an extra 12.7% of women who did not become post-menopausal to a lower risk level (non-event NRI). This corresponds to an average improvement of 36.5% (Table IV). The mean difference in predicted probabilities between the model with and without AMH was plus 2.5% for women who reached menopause during follow-up and minus 2.9% in women who did not reach menopause during follow-up (Table IV).

The cNRI was used to calculate the data presented in Table IV. It shows the mean assigned risk of menopause for women who did and did not enter menopause at follow-up. The mean change in risk assigned to women when AMH is added to the model is also shown. In each subgroup analysis, addition of AMH to the model amplified the predicted risk in the correct direction (higher for women who did enter menopause and lower for the women who did not enter menopause). Per subgroup the amount of change resulting from adding AMH to the model was greater for women who did enter menopause at follow-up than for the women who did not. The added value of AMH was largest in subgroup 3.

Discussion

Main findings

With this study we have shown that from all relevant lifestyle and reproductive predictors, age and AMH contribute most to the prediction of TTM. Age was the strongest predictor of TTM, which is a very logical finding as it is intuitively clear that older women have a shorter TTM than younger women. Most importantly though, we have provided evidence that AMH has additive predictive value for this prediction even when taking age, BMI, cycle regularity and smoking into account.

The shape of the association between AMH and TTM (Fig. 1A) suggests that a woman with an AMH of above 4 ng/ml or higher will have the same risk as a woman with an AMH of 3 ng/ml. At lower ranges, below 1.5 ng/ml however, AMH is directly related to TTM suggesting that this is where a women is not only at risk but also where her individual AMH levels will distinguish her personal level of risk. In fact, if we set the upper limit of AMH at 1.5 ng/ml, the HR of AMH is 0.19 (95% CI: 0.14–0.25), showing the strong relationship between a lower AMH and a higher risk of menopause. This study has shown that the amount of additive value of AMH in this instance is most prominent in the group of young women with regular menstrual cycles (subgroup 3). In the overall study population, the added effect of AMH was marginal. This can be interpreted as a reflection of the fact that ~30% of the study population is aged 45 years and above. These women, based on age alone, have an almost 100% probability of entering menopause in the next 10 years, leaving little room for improvement of menopause prediction by AMH. In subgroups 1 and 2, the effect of AMH was also smaller in comparison

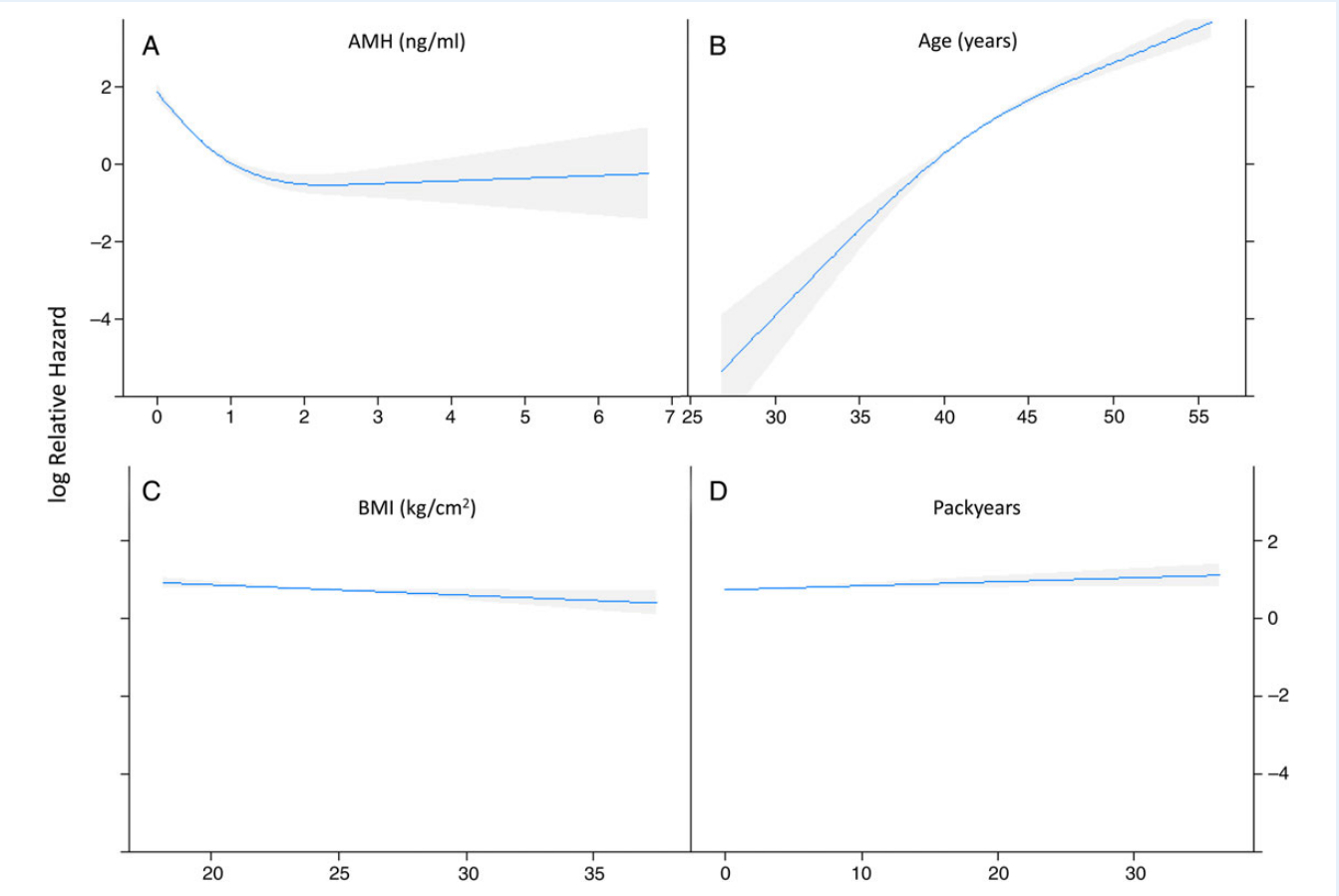

Figure 1 Hazard of TTM for the parameters included in the model. (**A**) AMH, (**B**) Age, (**C**) BMI and (**D**) packyears of smoking.

Table II C-statistics per model in the overall study population and per subgroup.

	Whole study			Subgroup 1 20–43 years			Subgroup 2 20–43 years (reg. OC)			Subgroup 3 20–43 years (reg only)		
	C-stat	SD	*n*	C-stat	SD	*n*	C-stat	SD	*n*	C-stat	SD	*n*
Age only	0.88	0.01		0.84	0.03		0.84	0.03		0.79	0.04	
Model without AMH*	0.89	0.01	1163	0.85	0.03	763	0.85	0.03	677	0.79	0.04	390
Model with AMH**	0.91	0.03		0.89	0.02		0.89	0.02		0.87	0.03	

C-statistics per model and per group are shown.
reg, regular cycle; irreg, irreg cycle; preg, pregnant; OC, oral contraceptives.
*Model with age, BMI, packyears of smoking and menstrual cycle status.
**Model with additional AMH. Subgroup 1: All women 20–43 years; Subgroup 2: 20–43 years with regular cycles or taking OC; Subgroup 3: 20–43 years with regular cycles.

to young, regularly cycling women. In the subgroups of women aged 20–43, it is likely that the predictive effect of AMH was influenced by the presence of determinants such as pregnancy, cycle irregularity and OC use. In these women AMH may not be a pure reflection of ovarian reserve but more a reflection of the amount of suppression of the pituitary and ovarian function induced by pregnancy, OC use or through ovarian dysfunction as in the case of cycle irregularity caused by polycystic ovary syndrome (PCOS) (Dolleman *et al.*, 2013b). Interestingly, the direction of the association between pregnancy and TTM was not as would be expected according to earlier publications by our group (namely that pregnancy shortens TTM), however this association is not significant aborting further speculation on this association. Furthermore, although previous studies indicated that current smoking most strongly affects age at menopause, in our analysis packyears of smoking was a better predictor than the variable smoking that was coded as 'current, previous or never smoker' (van Asselt *et al.* 2004; Kinney *et al.*, 2007).

Table III HRs per predictor in models with and without AMH.

Models without AMH	Whole group (n = 1163)			Subgroup 1 (n = 776)			Subgroup 2 (n = 687)			Subgroup 3 (n = 396)		
	HR	**95%CI**	***P***	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***
Age (years)	See Figure 1B		<0.0001	1.53	1.46–1.60	<0.0001	1.52	1.44–1.59	<0.0001	1.45	1.37–1.54	<0.0001
BMI (kg/m^2)	0.97	0.95–0.99	0.006	0.99	0.95–1.03	0.654	0.99	0.95–1.04	0.746	0.99	0.94–1.04	0.808
Packyears	1.01	1.01–1.02	0.001	1.03	1.01–1.04	0.000	1.03	1.01–1.04	0.000	1.02	1.00–1.04	0.026
Menstrual cycle												
Regularly cycling	*Reference category*			*Reference category*			*Reference category*			*Reference category*		
Irregularly cycling	2.36	2.12–2.60	<0.0001	2.65	2.14–3.16	0.000						
Pregnant	1.81	0.67–2.96	0.309	1.93	0.77–3.08	0.267						
Currently taking OC	0.87	0.63–1.11	0.255	1.03	0.66–1.40	0.875	1.03	0.66–1.40	0.874			
Currently taking HRT	1.63	1.03–2.22	0.109									
	Whole group (n = 1163)			**Subgroup 1 (n = 776)**			**Subgroup 2 (n = 687)**			**Subgroup 3 (n = 396)**		
Models with AMH	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***
Age (years)	See Figure 1B		<0.0001	1.38	1.31–1.45	<0.0001	1.36	1.29–1.44	<0.0001	1.29	1.21–1.38	<0.0001
AMH (ng/ml)	See Figure 1A		<0.0001	See Figure 1A		<0.0001	See Figure 1A		<0.0001	See Figure 1A		<0.0001
BMI (kg/m^2)	0.97	0.95–1.00	0.016	0.98	0.94–1.02	0.226	0.98	0.94–1.02	0.350	0.97	0.92–1.02	0.228
Packyears	1.01	1.00–1.02	0.019	1.02	1.00–1.03	0.015	1.02	1.00–1.03	0.021	1.01	0.99–1.03	0.558
Menstrual cycle												
Regularly cycling	*Reference category*			*Reference category*			*Reference category*			*Reference category*		
Irregularly cycling	1.91	1.67–2.15	<0.0001	2.15	1.63–2.67	0.004						
Pregnant	1.24	0.09–2.39	0.717	1.21	0.05–2.38	0.743						
Currently taking OC	0.53	0.28–0.77	<0.0001	0.63	0.25–1.01	0.018	0.63	0.24–1.01	0.017			
Currently taking HRT	1.24	0.65–1.83	0.471									

HRs per model and per group are shown.
Subgroup 1: All women 20–43 years; Subgroup 2: 20–43 years with regular cycles or taking OC; Subgroup 3: 20–43 years with regular cycles.
reg, regular cycle; Irreg, irreg cycle; preg, pregnant; OC, oral contraceptives.
*Model with age, BMI, packyears of smoking and menstrual cycle status.
**Model with additional AMH.

Table IV Mean predicted menopause risk for women who did and did not enter menopause within 10 years and change in risk according to addition of AMH.

	Women who DID become post-menopausal in 10 years			Women who did NOT become post-menopausal in 10 years		
	Predicted menopause risk without AMH	Predicted menopause risk adding AMH	Δ Predicted risk of menopause	Predicted menopause risk without AMH	Predicted menopause risk adding AMH	Δ Predicted risk of menopause
Overall population	82.6%	85.3%	2.7% (3.7)	18.5%	15.6%	−1.8 (2.1)
Subgroup 1	53.2%	61.9%	6.8% (6.4)	14.1%	11.7%	−1.0% (2.7)
Subgroup 2	50.7%	59.5%	6.8% (6.3)	14.5%	12.1%	−0.3% (4.0)
Subgroup 3	49.3%	63.3%	11.1% (7.9)	20.1%	15.6%	−1.6% (6.8)

Mean predicted menopause risk for women who did and did not enter menopause within 10 years and change in risk according to addition of AMH in the model as calculated by the cNRIs. Data were calculated using the cNRIs. Data are shown for the overall population and per subgroup.
Subgroup 1: All women 20–43 years. Subgroup 2: 20–43 years with regular cycles or taking OC. Subgroup 3: 20–43 years with regular cycles.

Clinical value

The accuracy with which the model including AMH could discriminate between women who do and do not enter menopause during follow-up was excellent, with an accuracy approaching 90% and small standard deviations. These C-statistics were similar to those reported in the study by Broer *et al.*, in which a model with age alone had a C-statistic of 87% and addition of AMH raised this accuracy to 90% (Broer *et al.*, 2011b). The added clinical value as measured by the cNRI was considerable with an average of 34–43% improvement added by AMH. Notably, however, this reflects only a marginal improvement of 3–8% as measured in the C-statistics. In terms of change in the risk assigned to women, addition of AMH to the model resulted in the assignment of an average higher risk of menopause by 2.5–11.1% in women who did enter menopause at follow-up and a mean lower risk assignment of 0.3–2.9% in women who did not enter menopause compared with the model without AMH. The additive effect of AMH was largest for young women with regular menstrual cycles, for whom the prediction of TTM is ultimately the most interesting as these women will benefit from knowing how many years of their fertile life still remain. This is further exemplified through assessment of the mean increased risk assigned to individuals who enter menopause during follow-up by addition of AMH to the predictive model. Whereas for the whole population, a mere 2.5% increase in risk is offered by addition of AMH, the estimated risk increases by 7–11% in younger women aged 25–43 years. With information on TTM, women with a high chance of an early onset of menopause (and thus potentially early subfertility) could be counselled towards not delaying pregnancy to a high age or towards cryopreservation of oocytes. Fertility prediction may have implications for the fulfilment of a child wish, on the individual level, and for creating large enough families for population maintenance and reducing expenditure for ART, on the societal level. Notably, however, no intervention studies have been performed to assess whether such measures actually result in fewer couples being involuntarily childless.

Strengths and weaknesses

Several studies have looked at AMH as a predictor of menopause in addition to age (Broer *et al.*, 2011a; Freeman *et al.*, 2012; Dolleman *et al.*, 2013a; Tehrani *et al.*, 2013). However, none have assessed the additive value of AMH on top of readily available patient characteristics with methods in accordance with the current state of art, even though a recently study suggested that smoking and BMI may improve AMH-based prediction (La Marca *et al.*, 2013). A major strength of our study was the population-based design of the study, enabling the study of different subgroups based on reproductive characteristics. Also, the size of the population in which other potential determinants of TTM could be assessed next to AMH was much larger than in previous studies. Another strength of the study is that a considerable number of young women were included, for whom the prediction of TTM is the most valuable. This study would have been even stronger if the duration of follow-up was longer so that more of these young women had become menopausal during follow-up as this would have made the predictions for these young women more precise. Also, unfortunately we were not able to properly distinguish between women with an irregular menses due to perimenopause and women with irregular menses due to PCOS. Therefore we could not look at short menstrual cycle length as a predictor of TTM, a factor which has previously been shown to be predictive of TTM. Furthermore, because we cannot be sure at which age a women taking HRT stops menstruating, we had to censor these women as premenopausal at the time they started HRT treatment. To check whether censoring these women as premenopasusal influenced our results, we checked whether the mean age at natural menopause changed if we coded these women as menopausal at HRT start. We saw that the mean age at natural menopause stayed the same with only a 0.02 change in the interquartile range. Recently, the stability of serum AMH measures have been questioned (Rustamov *et al.*, 2012), especially when AMH is stored at room temperature or −20°C. However, it is clear that AMH values are both reproducible, stable and reliable when appropriate sample processing is done (Fleming and Nelson, 2012). A strength of this study is that the AMH Gen-II assay (Beckman-Coulter Ltd) was used; this is currently the most reliable assay of AMH. Furthermore, samples were determined by a single experienced laboratory technician and assay result precision was validated using linearity of dilution assessment. These are all factors that support homogenous specimen sampling and the provision of both reproducible and reliable AMH measures.

Concluding remarks

This study has, with up to date statistical methods, justified AMH as an additive predictor of both TTM and the occurrence of menopause on

top of female age and other reproductive and lifestyle factors. However, the added value differs per subgroup of women and is largest in women who are young when AMH is measured and who have a regular menstrual cycle.

Authors' roles

W.M.M.V., F.J.M.B. and Y.T.v.d.S. conceived and designed the study. M.D. and M.J.C.E. analysed the data. M.D. wrote the first draft of the manuscript. All authors contributed to the writing of the manuscript and agreed with manuscript results and conclusions. M.D., W.M.M.V. and Y.T.v.d.S. are guarantors.

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Conflict of interest

F.J.M.B. declares to have received fees and grant support from the following companies (in alphabetical order); Ferring, Gedeon Richter, Merck Serono, MSD and Roche. The remaining authors declare no conflict of interest.

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