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SUMMARY

Because of major improvements in the treatment of childhood cancer, the numbers of long-term survivors are increasing. This increased life expectancy however, comes at a cost for many survivors. As a result of their previous anti-cancer treatment, many CCSs experience late effects. This thesis has focused on the influence of chemotherapy and radiotherapy on the female reproductive system.

In **Chapter 2** we summarized the existing literature in a systematic review on the effects of chemotherapy on the incidence of ovarian dysfunction (i.e. age at menopause, prevalence of amenorrhoea, and/or elevated FSH levels). In addition, we evaluated the relationship between type and dose of chemotherapy, age at time of treatment, and time since treatment on the one hand and ovarian function on the other hand.
We found that median age at menopause was 33.5 years in young Hodgkin's lymphoma survivors and 44 years in the total group of CCS. The most important risk factors for a reduced ovarian function that were identified in more than one study were: (1) alkylating agents, specifically procarbazine and busulfan; and, (2) older age at treatment. In addition, etoposide was found to be an independent risk factor in one large well-conducted study in Hodgkin's lymphoma survivors. Another essential finding of this review was that the methodology of the included studies often suffered from many limitations. This was caused by small and heterogeneous study populations, insufficient follow-up time, and multimodal cancer treatment (combining both chemotherapy and radiotherapy). Because synergism may occur in multimodal treatment, multivariable regression analysis alone might not sufficiently correct for other treatments given simultaneously. Few studies were sufficiently powered to assess the effects of these treatments separately. Furthermore, treatment protocols have changed extensively over the last decades, making comparisons between study groups with the same childhood cancer type difficult. In our review, most included studies have relied on predictive models derived from cross-sectional data. Because the event of premature menopause after childhood cancer treatment requires a long follow-up time, many studies used soft end-points, such as (transient) amenorrhoea or ovarian reserve markers to assess ovarian function. Only studies that collect longitudinal data until menopause can reliably interpret endocrinological and ultrasound values of ovarian function, since they are stongly influenced by age. It is therefore likely that the finding in our literature review, that older age at diagnosis is associated with higher incidence of ovarian dysfunction, is due to this pitfall.

Since we concluded in our review that most studies conducted to date have methodological limitations, we aimed to minimize these flaws in the DCOG LATER VEVO study. First, we critically reviewed our own design as well as our control group, and reported on the pitfalls and challenges in **Chapter 3**. We formulated recommendations in order to control bias and to validate our instruments for data collection (Table 1).

Table 1 Recommendations formulated as a result of a critical appraisal of the design of our nationwide DCOG-LATER VEVO study

Recommendations

Study population	• Keep non-response or loss to follow-up to a minimum • Characterize non-responders or those lost to follow-up • Control for extent and direction of bias in final data analysis • In case the number of controls is insufficient: incorporate other types of control subjects • Choose types of controls that are representative of the source population from which the study population • was derived • Characterize and control for differences of potential confounders between survivors and controls
Data collection	• Compare self-reported data with a more objective source, such as medical records or registries • Conduct reliability studies to account for inter- and intra-observer variation • If possible, use data collection instruments that allow for one investigator to analyze collected data • (observer bias) • Assess exposed and unexposed in same manner • Blind observer to exposure status

Our first recommendation was to keep non-response or loss to follow-up to a minimum. To achieve this, we adhered to a strict protocol of reminders to make sure we contacted all patients. Due to earlier DCOG-LATER efforts, the total nation-wide cohort had already been identified and dedicated data managers had tracked many of the patients. In the final analysis of the DCOG-LATER VEVO-study we conducted a non-response analysis to control for the extent and direction of bias due to selective participation.

Between participating and non-participating CCSs a few statistically significant, but not clinically relevant, differences were found. However, there appeared to be some differences between those CCS who participated in the clinical part, and those who only consented to the questionnaire. Clinical CCSs appeared significantly younger than questionnaire-only CCSs while, in contrast, clinical controls were significantly older than questionnaire-only controls. In addition, birth rate was lower among both the clinical CCSs and controls compared to questionnaire-only CCSs and controls. This could indicate that our study has been subject to some degree of participation bias, i.e. subjects who have proven to be fertile were less inclined to participate in the clinical part of the study. Overall, this could have led to an overestimation of ovarian dysfunction in our study population. However, since this trend appeared to be present in both the CCSs and control group, our results concerning differences between both groups can be validly interpreted.

Because the number of sibling controls seemed insufficient and since we preferred a control group of a similar size as the CCS group, we included an additional source of controls. These controls were recruited via general practitioners of cases and matched for age with the

CCSs. The selection and comparison between the two subgroups was discussed in **Chapter 4**. The participation rate in the sibling controls was much higher than of GP controls, whereas considerably more effort was involved in recruiting GP controls. There were differences between the control groups with regard to education and age (GP controls were significantly older and higher educated than sister controls), but no significant differences were detected regarding fertility-related characteristics, suggesting minimal bias due to selective participation. We also evaluated the possible introduction of bias due to non-participation of the GP-controls. Participants, refusers and non-responders were compared on the following characteristics: current age, parity, age at birth of first child, having experienced fertility problems in the past, and having consulted a gynecologist for these problems. No significant differences between the response groups were present regarding current age, parity, age at birth of first child, having had fertility problems in the past, and having consulted a gynecologist in the past, although the proportion of parous women was slightly lower among participants compared to refusers.

For the validation and assessment of bias of our instruments for data collection, we conducted several methodological substudies, which are reported in **Chapters 5 to 9**.

Questionnaire data

Most studies derived from the Childhood Cancer Survivor Study [45 53], as well as other large studies (study population >200) that chose amenorrhoea as an endpoint [46 75 80 101 103], have used self-reported questionnaires to assess menopause, which may bias results. Since amenorrhoea may occur for multiple reasons (pregnancy, breastfeeding, use of hormones, PCOS, surgical interventions such as hysterectomy), assuming amenorrhoea is caused by a hypergonadotropic state as a consequence of previous cancer therapy might lead to a non-differential misclassification and thus a attenuation of the effect. In contrast, those women that are postmenopausal might report a regular bleeding pattern when on hormonal substitution, with a dilution of the effect as a consequence. For this reason, in the DCOG-LATER VEVO study we decided to focus on clinical ovarian function markers, rather than on questionnaire data for the evaluation of diminished ovarian function.

With regard to the self-reported pregnancy outcomes in the questionnaire, we were able to validate those by comparing records registered in the Netherlands Perinatal Registry (PRN) to self-reported outcomes. We found that self-reported pregnancy outcomes of CCSs agree well with registry data and that outcomes reported by CCSs agree better with registry data than do those of controls. Unfortunately, we were not able to validate other variables in the questionnaire, such as medical or surgical history, cycle history or medicine use.

Since we previously identified the method of invitation as a possible pitfall for introducing bias, we also compared the method of invitation (mixed invitation (paper-based together with Web-based questionnaire) vs. Web-only invitation (Web-based questionnaire only). We concluded that sending a mixed invitation rather than a web-only invitation resulted in a similar overall response as well as participation rates. However, women who were older, had a higher level of education, or were students, were more likely to have filled out the Web-based version of the questionnaire.

Ovarian function markers

To date, the perfect ovarian function test does not exist. Although elevated FSH levels are specific as to predicting imminent menopause, FSH will only be elevated shortly before the deep plunge in fecundity rates will occur. AMH and AFC share the characteristic that they gradually decrease with age, therefore allowing earlier predictions on chances of pregnancy and age at menopause. In this thesis, we critically evaluated the clinical measures of ovarian function.

AMH

We found that AMH varies significantly during the cycle in a group of regularly menstruating healthy women, and that younger women had significantly larger fluctuations in AMH levels than older women. We warned that in young patients with, commonly, a high ovarian reserve, fluctuations of AMH may have an impact on the discriminatory capability of diagnostic and predictive tests, respectively. This finding has since been replicated in other studies [351-353].

In our study we focused on the variability within one cycle, but what about long-term variability? The long-term variability in adult women in the general population has mainly been studied in cross-sectional studies, with some of them including as many as 10–15 thousand patients [258 354-357]. Overall, the studies are in good agreement that AMH declines with advancing age according to a pattern that is similar to the exponential decay of the primordial follicular pool [358 359], which is best described by a quadratic equation [258]. It was shown that of all markers, AMH can significantly aid in the prediction of menopause in the general population in a model next to age, and does so better than AFC and FSH. The prediction model failed, however, to predict extreme menopausal ages, either very young or very old. In addition, prediction intervals remained broad, rendering it unfit for use in a clinical setting [31].

There are no studies available in which AMH is measured longitudinally in a sufficiently powered group of CCSs. The decline of AMH might be different in CCSs in comparison to healthy women, due to the treatment they received. Longitudinal studies with multiple AMH measurements in which healthy women are followed throughout their reproductive life might overcome the very wide confidence intervals due to the large inter-individual differences.

Subsequently, it should be validated whether the age-related rate of decline in AMH is comparable between CCSs and controls, before implementing it in screening protocols [360]. Hypothetically, the previous gonadotoxic treatment may influence the turn-over velocity of the oocyte pool or change endocrinological thresholds, inducing a different rate of atresia in CCS in comparison to population controls [51]. Of note, an unmeasurable low AMH in the general population does not exclude the possibility of pregnancy [361].

Discussion remains whether or not AMH can predict live birth. The EAGer Trial concluded that lower and higher AMH values were not associated with fecundability in unassisted conceptions in a cohort of fertile women with a history of one or two miscarriages [362]. However, Reijnders et al. found that, in a group of infertile women, ≥36 years of age or when showing clinical signs of diminished ovarian reserve, a very low AMH did decrease the

risk of a live-birth when pregnancy did indeed occur [363]. These conflicting findings elucidate that AMH always needs to be interpreted in the context of age and (sub)fertility and that live birth rates are dependent on numerous variables other than AMH.

AFC

In this thesis we described a study in which we compared antral follicle counts using real-time 2D and stored 3D data in 50 childhood cancer survivors compared to 50 healthy sibling controls, randomly drawn from the DCOG-LATER VEVO study.

In terms of methodology, the use of the 3D equipment holds several benefits: it is possible to perform ultrasounds by multiple ultrasonographists and store the data, so the interpretation of the ultrasound data can be done by a single assessor, minimizing inter-observer variation. It should be noted, however, that a significant proportion of our childhood cancer survivor study population had never had sexual intercourse in comparison to healthy controls (14% vs 4%). In these women, it did not seem ethical to perform transvaginal ultrasound and therefore these women did not undergo ultrasound measurements.

Overall, we observed that with the 3D technique in comparison to the 2D technique fewer follicles were counted. It remains unclear whether this discrepancy is caused by an underestimation of the follicles in 3D-mode or an overestimation of the follicles in 2D-mode. Our data indicate that the higher the AFC values, the more likely it is that AFCs differ (either measured by two different persons, on two different time points or with two different techniques).

Image quality was associated with lower antral follicle counts and higher BMI, but not with older age. In our methological substudy we could not rule out the confounding effect of image quality on the incidence of low AFCs. Poor image quality was found significantly more often among survivors (35%) than controls (19%). This is also reflected in the relatively low between-method CCC (concordance correlation coefficient) in survivors compared to controls. We speculated that lower image quality might be caused by cancer treatment, as it is known that chemotherapy and radiotherapy can lead to ovarian atrophy, injury to the blood vessels and focal ovarian cortical fibrosis. However, image quality may also have been influenced by a higher BMI.

We found in this study that the BMI of survivors was significantly higher than the BMI of controls. Van Dorp et al. found that in their study of 191 female childhood cancer survivors, AMH and follicle counts were reduced in those with obesity and insulin resistance [364]. In addition, they found that AFC and AMH were not correlated as strongly as described in the general population. However, their analyses may have been hampered by the fact that AFC values were available in only five obese women.

More research should be done on image quality of ultrasonography before the test characteristics, that were defined on subfertile IVF patients, can be applied to childhood cancer survivors. In studies evaluating the predictive value of antral follicle counts (measured in 2D) on age at menopause in the general population, AFC performs worse than

AMH. AFC lost its predictive value when female age at baseline was introduced into the model [31 365]. These studies were never performed in CCSs.

FSH and inhibin B

FSH is produced by the pituitary in response to pulsatile GnRH, and is necessary for recruitment of a new cohort of follicles [323]. Historically, it has been used to detect menopausal transition. Models in which FSH is validated for menopause prediction in the general population are scarce. In available models it seemed that FSH did no longer have any predictive value when either AMH [366] or female age [365] was added to the prediction model. Inhibin B is secreted from granulosa cells in FSH-dependent growing follicles and levels are correlated with the number of developing antral follicles seen on ultrasonography during the early follicular phase [11]. Its levels fall in parallel with the number of ovarian antral follicles [12]. However, no specific concentration of inhibin B has been shown diagnostically discriminatory in the general population [13]. Robertson et al. proposed the FSH: inhibin B ratio as a possible alternative of measuring the menopausal transition and predicting the onset of cycle irregularity and the possibility of impending menopause in the general population [353]. However, no studies to evaluate this measure have been performed since. Very few studies have assessed the role of inhibin B for ovarian reserve in CCSs [59 130 367 368].

Which marker should be used then?

In the DCOG LATER-VEVO study the correlation coefficients between AMH and AFC were highest. Reciprocal relationships between AMH and the other three markers were investigated among the group of CCSs. Results showed that having low AMH levels in many cases was not associated with abnormal AFC, FSH or inhibin B values. However, a normal AMH was rarely associated with abnormal other markers. We showed that these discordances occurred most frequently in the youngest CCSs. The discordancies between the markers urge us to warn against the use of only one marker to assess ovarian function, until a gold standard has been firmly established. Nevertheless, our results show that AMH seems to be the first marker to be influenced by gonadotoxic therapy and thus likely to be the most sensitive marker to assess ovarian function in CCSs. Our results also show that it is more sensitive than other markers in women under 35 years of age.

Oral contraceptives

The use of oral contraceptives might influence ovarian function tests. We found overall significant decreases in FSH and inhibin B and significant increases in AMH, AFC and ovarian volume values after discontinuation of hormonal contraception in general population women. Since our research was published, several other studies on the subject have been performed. Some found no difference in markers after contraceptive use [369-371], but these studies likely suffered from methological errors and low power. In prospective studies with larger study groups, as well as in a recent RCT, especially AMH values were found to drop from 13 to 50% after a prolonged course of contraceptives [372-374]. This evidence is indicative of a suppressive effect of hormonal contraception on circulating AMH levels, at least when considering long-term use. Thus, serum AMH concentration may not retain its accuracy as predictor of ovarian function in long-term hormonal contraceptive users.

For this reason, we asked all participants in the DCOG LATER-VEVO-study to refrain from contraceptives for at least two months; however, some refused. The rate of contraceptive use was 42% for childhood cancer survivors and 34% for controls. Of CCS, 56% indicated that they were willing to temporarily refrain from contraceptives, whereas 52% of controls consented to stop. We have performed sensitivity analyses with respect to the use of contraceptives. When women using hormonal contraceptives were excluded, all multivariable results were comparable to those in which women using hormonal contraceptives were included.

Assets and novel findings of the DCOG LATER-VEVO study

In **Chapter 10** we described the main results of the DCOG LATER-VEVO study. With the DCOG LATER-VEVO study we endeavored to fill the gaps in knowledge described in the introduction of this thesis.

Methodologically, the DCOG LATER-VEVO study is unique in several aspects. In contrast to other studies in the field, we invited a well-defined nationwide cohort with long-term follow-up data and assessed the ovarian function of the majority of this cohort with clinical measures, in addition to questionnaires. Furthermore, the number of population controls that were recruited for this study, and for whom also clinical measurements were available is unprecedented. This does not only benefit the results of the DCOG LATER-VEVO study, but will also yield valuable data for further research in the field of reproductive medicine. Our methods have been subject to thorough validation. All these factors minimize the amount of bias.

Overall, the results of the DCOG LATER-VEVO study showed that the proportion of CCSs (<age 35 years) with abnormal ovarian function was remarkably low (7.0-17.7%, depending on the marker used), even after treatment with alkylating CT (2.7-8.8%). Moreover, within the CCS group only 5% reported to have a self-reported premature menopause and birth rates of CCS and controls were more or less similar (except in oldest age group). However, compared to controls, the proportions of women with reduced ovarian function increase steadily and more rapidly after age 35. Therefore, we recommend that these women should be counselled to pursue pregnancy timely as their reproductive lifespan may be shorter than anticipated.

Moreover, specific groups of CCSs seem to be at high risk of a decreased ovarian function, regardless of age (i.e. those treated with procarbazine, busulfan, melphalan, chlorambucil lomustine, lower abdominal/pelvic RT, or TBI). These CCSs should be counselled adequately and new patients receiving such treatments should be referred to a reproductive specialist for fertility preservation counselling.

Due to the large number of participants we were able to establish reliable doseeffect relationships for several types of treatments. Abdominal and/or pelvic RT appeared to affect all ovarian function markers at almost any dose, but a clear dose-effect relationship was found when ovarian function was assessed by AFC and inhibin B. For procarbazine increasing doses were associated with increasing risks of low AMH and high FSH values. Remarkably, we found no clear effect of cyclophosphamide on ovarian function. A reduced

ovarian function after treatment with cyclophosphamide was demonstrated only by AMH, although not in a dosedependent way. Cyclophosphamide administered during childhood may, therefore, not be as detrimental as previously thought. The (absence of) dose-response effects are important for the design of future childhood oncology protocols in which the curative effect of the treatment is balanced with the risk of gonadotoxicity.

Remaining limitations of the DCOG LATER-VEVO study

Although this study was preceded by several methological substudies and has been conducted with rigorous methods, there remain some limitations to the design with regard to the aims posed beforehand.

For our DCOG LATER-VEVO study, it seemed that many chemotherapeutic agents were associated with poorer outcomes of ovarian reserve markers in a univariate model. Due to the multi-modal treatment, combining different types of chemotherapy and radiotherapy, it remains very difficult to disentangle the separate effects.

Another limitation was that the population studied and the treatments administered were so heterogeneous that division into subgroups lead to insufficient power to detect associations, resulting in very broad confidence intervals. Since this was a nationwide study, the only solution to increase the power is to develop collaborations with other countries and join efforts.

Alike many other studies that have been performed to date, the follow-up time was long, but not nearly long enough (22.9 years (median 8.2 years). More than ninety percent of our CCS had not reached menopause at time of assessment, which makes it difficult to assess the influence of time since diagnosis on this event.

We have used AMH, AFC, FSH and inhibin B levels as a surrogate for ovarian function. Likewise, actual fertility could not be evaluated since a proportion of the participants has not yet pursued pregnancy.

The DCOG LATER-VEVO study was a cross-sectional study, rendering it impossible to evaluate the inter-individual variance in markers. For this, we need longitudinal assessment of ovarian function and associate these markers with age at menopause and pregnancy rates. Of course, predicting age at menopause and actual fertility are the outcomes that a childhood cancer survivor is really interested in. Because we were not able to evaluate age at menopause reliably in our young cohort, we are not able to shed more light on the prognostic and diagnostic value of the different ovarian reserve markers with regard to premature ovarian insufficiency, one of the gaps of knowledge that was formulated in the recommendations of the IGHG.

Hopefully, longitudinal follow-up of the DCOG LATER-VEVO study participants will provide this information in the future. In addition, future research should focus on the value of ovarian function markers to predict the chance of an actual pregnancy in CCSs, also taking into account a possible role for genetic factors. The DCOG LATER-VEVO study is uniquely positioned to examine this in a few years.

Lastly, other determinants of gonadal function after childhood cancer have been described, that were not assessed in the DCOG LATER-VEVO study. These include baseline gonadal function (before start of treatment), genetic factors, lifestyle factors and fertility preservation.

Baseline gonadal function

It has been shown that AMH levels are reduced in adults and young girls with newly diagnosed cancer even before the cancer treatment has started [375 376]. Since we did not have pre-diagnosis AMH levels available for the DCOG LATER-VEVO study, this could not be tested in our study. It would be wise to initiate measurement of baseline AMH levels before treatment for childhood cancer in all childhood cancer survivors. Although its value is still uncertain, this may provide more insight into the baseline gonadal function of girls before and after treatment.

Genetic factors

One of the most powerful predictors of age at menopause is family history. Twin studies have shown that 44% to 85% of the variance in age at natural menopause is inherited [323]. Mutations in genes functioning in hormonal regulation, DNA repair, and immune function pathways, have been associated with the age at natural menopause in genome-wide association studies (GWAS) [323]. In addition, van Dorp et al. found that SNP rs 1172822 was associated with impaired ovarian reserve and lower predicted age at menopause in adult female survivors of childhood cancer, independent of what treatment they had received [377]. In addition, genes involved in drug metabolism or DNA repair may also be of influence in the amount of overall damage the chemo- and radiotherapy do in a patient's body.

Lifestyle factors

In the general population, smoking has been shown to result in lower age-specific AMH [378 379] and AFC levels [380], higher FSH levels [380] and an earlier age at menopause [381 382]. Obesity is associated with subfertility in the general population, but also in childhood cancer survivors. Obese CCSs were at greater risk of lower follicle counts and AMH levels [383]. This finding is in contrast with recent results of the St. Jude Lifetime Cohort, in which patients with a BMI ≥30 kg/m2 at the time of assessment were less likely to have a diagnosis of premature ovarian failure [48]. We have corrected all results of the DCOG LATER-VEVO study for BMI as well as current smoking behavior in our regression model of treatment related effects on ovarian function to take these factors into account.

Fertility preservation

Many reports have been published over the last five years on the revolutionary development of live births after transplanted ovarian tissue. A known concern of ovarian tissue transplantation is the presence (and possible re-implantation) of malignant cells in ovarian tissue. However, other problems may also arise. It is known that ovarian reserve is slightly reduced after unilateral oophorectomy in both childhood cancer survivors [384], as well as in adult patients [385]. To our knowledge there is no information on the decline of ovarian function due to surgery with the aim of fertility preservation. It is of pivotal

importance that fertility preservation is only undertaken when the risk of an impaired future fertility is certain, and that the patient will benefit, rather than be harmed by the procedure. After all, an important part of the doctor's ethical mores is 'primum non nocere'.

In **Chapter 11** we reported the results of a survey in pediatric oncologists regarding practice, attitude and knowledge with regard to fertility and fertility preservation after cancer treatment. Although pediatric oncologists seemed to be well aware of the effect that cancer treatment may have on female fertility and their responsibility to counsel their patients and/or the parents on this issue, they stated that they did not possess the knowledge to sufficiently counsel these patients and, if needed, did not frequently refer them to a fertility specialist.

CLINICAL IMPLICATIONS

From the research described in this thesis, several implications for clinical practice can be formulated. These are summarized in Table 2.

Table 2 Clinical implications from the DCOG LATER-VEVO study

All childhood cancer survivors should be counselled to pursue pregnancy timely as their reproductive lifespan may be shorter than in healthy controls, even though ovarian reserve markers may be normal before the age of 35.
CCSs treated with procarbazine, busulfan, melphalan, chlorambucil or lomustine, lower abdominal/pelvic radiation, or with TBI are at highest risk of a reduced ovarian function. Physicians must be aware of these effects and inform CCSs and future patients treated with these types of treatment about fertility preservation options and refer them to a reproductive specialist timely.
Although AMH seems the first marker to decline with increasing age, measuring a full panel of ovarian function markers is still encouraged given the differences between these markers regarding their ability to detect a treatment effect.
AMH always needs to be interpreted in the context of age and (sub)fertility and it should be noted that live birth rates are dependent on numerous variables other than AMH.

RECOMMENDATIONS FOR FUTURE RESEARCH

This was the first report of the DCOG LATER-VEVO study, showing solid results on the gonadotoxic effect of cancer treatment on ovarian function in female childhood cancer survivors.

In this thesis we have focused primarily on ovarian function. However, through questionnaires, we have gathered extensive data on fertility, the need for fertility treatment

and pregnancy outcomes. These data will be analyzed and presented in the near future. We furthermore aim to prospectively follow-up this cohort to collect longitudinal data. This will not only allow us to eventually reach the endpoint in which all participants have reached menopausal age, but also to report on the predictive value of endocrine and ultrasonographic markers of ovarian function on pregnancy and menopause in childhood cancer survivors and controls. We asked all women participating in the DCOG LATER-VEVO study at what age their mother had reached menopause by means of a questionnaire, since age at menopause is highly heritable. In the planned follow-up study, (in which the incidence rate of menopause will be higher), we will be able to analyze this factor more thoroughly. Since age at menopause is highly heritable, testing of the genome should be included in future studies. In addition, future studies on childhood cancer survivors should also focus on pharmacogenetics and -genomics, since genetic variation may also influence the way and the rate at which chemotherapy and radiotherapy are metabolized and thus the amount of damage they may cause. We are currently in the process of preparing a GWAS analysis of all DCOG LATER-VEVO participants.

We recommend that longitudinal studies should be performed with significantly longer follow-up time, in analogy with the Framingham study or Women's Health Initiative [386 387]. In the meantime, pooling of the results of previously conducted studies or a meta-analysis based on individualised patient data (IPD) could be performed. IPD meta-analysis is a specific type of systematic review. Rather than extracting summary (aggregate) data from study publications or from investigators, the original research data are requested directly from the researchers responsible for each study. These data can then be re-analysed centrally and combined, if appropriate, in meta-analyses. IPD meta-analyses can improve the quality of data and the type of analyses that can be done and produce more reliable results [388]. Recently, the PanCareLIFE initiative was launched, a 5-year European programme that studies the impact of treatment regimens on the long-term health of childhood cancer survivors. PanCareLIFE will evaluate the risks of impairments in female fertility, in hearing, and in quality of life. The results of the DCOG LATER-VEVO study, and at a later time the PanCareLIFE results, in combination with the data of the large cohort studies in the USA and Europe may be the basis of an IPD meta-analysis, which will provide more robust answers on the (dose-related) effects of all types of chemotherapy on ovarian function. Nevertheless, the impact of time and ever changing therapeutic protocols should still be encountered.

IDEALLY…

So, what do clinicians really need in order to adequately counsel their patients?

First, they would need the perfect ovarian reserve test that reliably describes the entire remaining follicle pool and that is able to predict age at menopause with small confidence intervals. This will enable clinicians to better determine the remaining reproductive window, and prevent menopause-associated conditions, including osteoporosis, cardiovascular diseases, and psychosexual dysfunction, in the long run. In addition, they would be able to counsel patients at a younger age.

Second, they would need a database containing threshold doses for gonadotoxicity of chemotherapeutics agents and radiotherapy with corresponding odds ratios for premature menopause. The DCOG LATER-VEVO study has provided important new data that may be used in such an endeavour. However, longer follow-up of the DCOG LATER-VEVO study population is needed to estimate risks for premature menopause, as we have focussed on ovarian function markers for now.

Third, they would need a prediction model in which the chance of pregnancy can be calculated, taking into account age at treatment and previous treatments and possibly genetic factors. Such a model might be a utopia, as semen quality, tubal patency and endometrial receptivity also play a large role. In the Netherlands, reproductive specialists use the Hunault model to predict the chance of spontaneous ongoing pregnancy within one year in subfertile couples [389]. This model accounts for female age, duration of subfertility, parity and sperm motility. Hypothetically, one might use an adapted Hunault model, in which a corrected age (i.e. a modelled age higher than the woman's calendar age depending on the amount of gonadotoxic chemotherapy and radiotherapy she has received) is used. Or better yet, a prognostic model in which influencing factors (such as type and dose of therapy, age at therapy, use of contraceptives, baseline gonadal function, genetic factors, and lifestyle factors) are included.

Hopefully these models can be created in the future when more research has been done and when solid evidence as to who is at severe risk of involuntary childlessness and premature menopause has been established. New patients must be offered fertility preservation options and be given tailored advice as to when to pursue their wish for children when a shorter fertile life span is evident. When a physician would know the odds of a diminished ovarian function associated with the treatment that he/she is about to prescribe, it will become easier for pediatric oncologists to counsel, and for parents and young girls to decide on fertility preservation options.

From our PAK-study, it seemed that pediatric oncologists mainly felt restricted to discuss fertility preservation due to insufficient time, lack of knowledge and lack of scientific data on the effectiveness of fertility preservation options. The last two factors may be improved with the DCOG LATER-VEVO study and future studies that will follow from this large nationwide cohort study.