General discussion
Only two generations ago, not many would have imagined the challenges we are struggling with today regarding childhood cancer survivorship. Survival rates used to be so low that essentially all efforts were focussed on survival. Since then, research has made an incredible journey, resulting in dramatic increases in survival rates after cancer treatment. However, with increasing survival rates it became clear that “cure is not enough” – words first coined by Gulio D’Angio, since most cancer survivors will also suffer from secondary diseases as a consequence from their previous cancer treatment. This realization made current research focus’ grow beyond survival, and into a fully grown and mature field of late effects of childhood cancer.

This thesis builds further on many previous endeavours that have been undertaken to identify late effects, assess risk factors and that started outlining ways in order to minimize, screen or counsel for these late effects. Reproductive health, being one of the health factors at risk, is the topic of this thesis.

FROM CROSS-SECTIONAL TOWARDS LONGITUDINAL DATA

Ovarian function can be assessed in several ways, some of which are dependent on an active hypothalamic-pituitary-ovarian axis, which is not present yet in prepubertal children. Anti-Müllerian hormone (AMH) is a useful marker of the presence of gonadotropin-independent small growing antral follicles in the ovaries, and therefore constitutes the best marker of ovarian function, like inhibin B is for gonadal function in boys. As was previously reported in girls with newly diagnosed cancer and adults with type 2 diabetes mellitus, metabolic syndrome and systematic lupus erythematosus, we show that boys with newly diagnosed childhood cancer have decreased gonadal function markers at the moment of their diagnosis, indicating that their serious disease already has an impact on the physiology of their gonads. We have also shown that various treatment modalities, specifically abdominal radiation and high dosages of alkylating agents, reduce the gonadal function even further in a large number of the children. Nonetheless, the gonads are resilient as well as dynamic, and our results indicated that around half of the children with low gonadal function directly after the end of their treatment show recovery within the first year. While general markers of gonadal function remain relatively low in long-term adult female cancer survivors, we have also shown that survivors are not at increased risk of a late or sudden drop in their AMH levels. So although their AMH levels declined considerably during treatment, thereafter they might recover and then decline along the normal percentile lines until menopause. Long-term longitudinal data will be needed to assess at which time gonadal function should best be assessed in order to inform survivors about their gonadal function and their expected fertility window.
EXTRAPOLATION OF DATA

This thesis presents two studies with longitudinal data on gonadal function markers, where currently mostly cross-sectional research results have been reported. Further long-term longitudinal data may also help us to better understand the implications of a low AMH level. Even within a healthy population, predicting the cessation of fertility remains challenging based on individual AMH levels\textsuperscript{17,18}, specifically as for instance a pregnancy can be established with only a severely reduced number of remaining follicles\textsuperscript{19,20}. The latter also indicates that although the number of remaining follicles is low, the quality of these remaining follicles are not necessarily low\textsuperscript{21}. This is significantly different from women of advanced reproductive age as reduced quantity and quality go hand in hand in ageing women. Hence, ovarian reserve is not a proper term for ovarian capacity and ovarian function is a more adequate description in childhood cancer survivors.

AMH, as a surrogate of remaining ovarian function, has proven to be a valuable predictor of menopause, apart from age\textsuperscript{4,22-25}. However, current prediction models have not been designed to predict the extremes of menopausal age\textsuperscript{25,26}, while the prediction of extreme young menopausal age is exactly what would be of interest to the population of cancer survivors. In addition, the prediction intervals on an individual level remain wide with a variation of around 10 years\textsuperscript{25}. Future studies including data on repeated AMH measurements and age at diagnosis in childhood, adolescent and young adult (CAYA) cancer survivors or similar populations with relative low AMH levels, may improve prediction models of age at menopause in these populations.

CHEMOPROTECTANTS

Several courses of action can be undertaken to minimize the impact of childhood cancer treatment on reproductive health. Targeted treatment strategies may include the least amount of radiation and alkylating agent dosage that is still safe for survival. Many steps have already been taken in this regard, and we have shown the reduced impact of a cancer diagnosis on perinatal risks\textsuperscript{27}. In young adult women treated for breast cancer, a protective effect of GnRH analogues has been observed\textsuperscript{28-31}. Similarly, there are also data suggesting that the use of the oral contraceptive pill might protect the gonads from damage induced by chemotherapy\textsuperscript{32}, although types of administered estrogen and active cancer increases the risk of venous thromboembolism\textsuperscript{33,34}. A recently published mice study showed that administration of AMH resulted in a complete arrest of folliculogenesis, and that AMH prevented chemotherapy-induced overactivation, protecting the ovarian reserve from the burn-out phenomenon\textsuperscript{35}. If AMH could be used as a chemoprotectant for ovarian gonadotoxicity, therapeutic indications of AMH could even extent to delaying ovarian aging in
the general population, in analogy to patients with polycystic ovary syndrome with high AMH levels who are known to enter menopause at a relatively late age. To what extent the administration of AMH may be feasible or effective in humans, let alone children, remains speculative at this moment.

**OVARIAN TISSUE CRYOPRESERVATION**

The administration of chemoprotectants is only one possible course of action in the rapidly growing arena of fertility preservation. For women in their reproductive life, options such as embryo cryopreservation or oocyte vitrification are available. Oocyte donation can be a last resort for women with primary ovarian insufficiency (POI) after gonadotoxic treatment. Recent research has indicated that even in women with POI, harvesting the remains of the exhausted ovary and reimplanting it in the pelvis after it has been dissected and cultured, might rejuvenate follicles and result in pregnancies. Success rates of this procedure may be even higher in women with chemotherapy-induced POI, as the quality of the remaining follicles may be better than its quantity suggests. Embryo cryopreservation and oocyte vitrification cannot be offered to prepubertal girls with cancer, mainly due to an inactive hypothalamic-pituitary-ovarian axis and physical restraints. Fortunately, ovarian tissue cryopreservation (OTC), is maturing into an established option for young patients with childhood cancer.

Since the first successful pregnancy after OTC was reported in 2004, over 130 live births have been reported after harvests in young adults. The first live births after OTC during childhood have also been reported. Beside pregnancies, restoration of ovarian activity with an adequate function of the hypothalamic-pituitary-ovarian axis and restoration of ovulation has been reported, with renewed ovarian endocrine function in 95% of women receiving ovarian tissue transplantation with frozen/thawed tissue. In addition, transplantation of cryopreserved ovarian tissue could potentially induce puberty and this practice has been reported, although given the scarcity of the tissue and the possibility of standard hormonal induction of puberty this may not be the prioritized designation of the valuable cryopreserved ovarian tissue at this moment.

The various laparoscopic procedures of OTC are considered a reasonable safe procedure, although the benefits need to be balanced against the potential risk of complications, such as bleeding and anaesthetic risks that may occur. Women with transplanted tissue have not been shown to be at increased risk of a relapse, although it is generally accepted sensible to be cautious with cancers with a high risk of ovarian involvement. Promising steps have also been reported regarding in vitro maturation of primordial follicles as an alternative to reimplantation of the ovarian tissue, to circumvent the risk of recrudescence of the original haematogenous malignancy.
JOINING CLINICAL AND SCIENTIFIC POWER IN THE NETHERLANDS

In the Netherlands, paediatric oncologic care has recently been centralized at the Princes Máxima Center for paediatric oncology in Utrecht. In the context of reproductive health in children treated for cancer, this centralization taps into new potential to evaluate the effects of fertility counselling and monitoring the safety of the OTC procedure in a large, controlled clinical setting. In addition, the influence of a cancer diagnosis, cancer treatment and ovarian tissue harvest on the gonadal function can be monitored and longitudinal data can be collected prospectively, a crucial step in the advancement of knowledge and understanding of gonadal function markers, as discussed in the second paragraph of this chapter.

GENETIC DETERMINANTS OF OVARIAN FUNCTION IMPAIRMENT

Determinants of ovarian function impairment include baseline patient characteristics, type of treatment and life-style factors. Groups with low, moderate or high risk of gonadal function impairment after cancer can be identified\(^{62,63}\), but variation in the extent of gonadotoxicity remains in these groups. In the second part of this thesis, we consider genetic determinants as another factor of ovarian function after childhood cancer treatment\(^{64}\). We show that chemotherapy-induced gonadal impairment in female CCS was significantly modified by the \textit{BRSK1} gene. Female CCS who carry the G allele of rs11668344 and received high doses of alkylating agents, were at an increased risk of a low AMH level. To further investigate the modifying effect of genetic variation on the impact of chemotherapy on gonadal impairment, more research is needed, including large childhood cancer survivor cohorts and independent replication cohorts. The design of such a large international cohort, PanCareLIFE, is described\(^{64}\). Eventually, this information can help to improve individualized counselling on both fertility preservation prior to cancer treatment and counselling after cancer treatment and may aid future individualized treatment strategies.

COLLABORATION IN SCIENCE

Medical scientific research has historically been based on competition. In the field of genetics, competition typically results in underpowered studies with a high chance on false positive results and reports with little value for the scientific community. The large international collaboration within PanCareLIFE has resulted in the largest European cohort of childhood cancer survivors with genetic data and data on gonadal impairment. Within this endeavour, we have collaborated with research groups from the St. Jude Lifetime Cohort Study, building transatlantic research bridges and improving scientific knowledge.
with combined forces. Major recommendations can be drawn from genetic studies in large international collaborations. Firstly, false-positive results will increasingly occur where multiple independent tests are carried out. Failure to correct for multiple testing results in findings that may look ‘interesting’ and easy to publish, but are worthless to the scientific community in the long run. Therefore, correction for multiple testing should become common standard in all research fields. Another measure against false-positive findings is replication of results in an independent cohort prior to publication, increasing the likelihood of reporting an actual association. Finally, all research data is valuable and scarce. Combining efforts and forming consortia such as within PanCareLIFE can improve power tremendously, meanwhile building bridges between different research groups that may enable knowledge exchange as a valuable spin-off effect. Again, this requires not competition, but trust and collaboration.

Unfortunately, barriers for collaboration can include linguistic, cultural or modus operandi differences, and concerns about ownership of outputs. Another drawback of collaboration may be the dilution of the definition of authorship. Projects in physics can have hundreds of members, all of whom are listed as authors as a mark of membership of the team – without requirements of writing or revising the paper. Papers in medical science tend to follow suit, with increasing long author lists and shared authorships. According to the International Committee of Medical Journal Editors, authors should meet all four Vancouver criteria for authorship (playing a part in designing or conducting experiments or processing results, help to write or revise the manuscript; approve the published version; take responsibility for the article’s contents), a requirement that can hardly be expected from such large author lists. The large gap between ‘project membership’ and the Vancouver Criteria, while authorships are so highly rewarded in the current scientific field, calls for new definitions and standards of authorship, and new ways of academic achievement evaluation.

PERINATAL MANAGEMENT AND COMPLICATIONS

Perinatal risks such as premature birth and postpartum haemorrhage are higher in CAYA cancer survivors compared to control groups, and risks seem to increase in survivors who have been treated with abdominal radiotherapy. In a large population-based analysis, we have evaluated the risks of cancer survivors diagnosed before their forties, and show that they are at increased risk of premature delivery and postpartum haemorrhage, but not of giving birth to children small for gestational age or with congenital abnormalities. It was also shown that the risk of an operative delivery and postpartum haemorrhage diminished in the more recent cohorts compared to older ones, resulting in equal risks for those diagnosed in the most recent cohort. The reduced impact of a cancer diagnosis on the risk of
an intervention during delivery may be a result of better targeted treatment strategies, and of a reduction of therapeutic exposures known to be associated with organ toxicity, e.g. radiotherapy in Hodgkin lymphoma. This observation is also in line with decreased late mortality among survivors of childhood cancer as a result of reduced radiotherapy and chemotherapy exposure. It is reassuring that the impact of a cancer diagnosis on postpartum haemorrhage and mode of delivery has been greatly reduced in most recently diagnosed cohorts of survivors, although heightened alertness and careful management in cancer survivors remains appropriate. Evidence-based clinical guidelines may facilitate this careful management by identifying the specific perinatal risks and risk groups.

Perinatal risks in CAYA survivors are generally noted in published clinical practice guidelines by North American and European groups, but without comprehensive assessment of the risk features of women who may benefit from high-risk obstetrical follow-up. In collaboration with the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG), we formulated recommendations for consistent and evidence-based clinically effective counselling and care, with regard to obstetrical and perinatal risks for female childhood cancer survivors. Based on the IGHG cardiomyopathy guideline, cardiomyopathy surveillance is reasonable prior to pregnancy or in the first trimester for all female survivors treated with anthracyclines and/or chest radiation. We further recommend that healthcare providers counsel female CAYA cancer survivors treated with radiation to fields including the uterus on the increased risks of miscarriage, premature birth and low birth weight, and reassure survivors that there is no indication of an increased risk of congenital abnormalities. Healthcare providers should be aware of the risk of premature birth and low birth weight during the entire course of pregnancy in CAYA cancer survivors treated with radiation to fields including the uterus (Figure 1). These recommendations will need to be translated and imbedded into national protocols. In the Netherlands, these findings need to be addressed in the continuing dialogue between midwives and obstetricians concerning medical indications and place of birth for specific risk groups.

**CONCLUSIONS**

This thesis presents new insights in trends of gonadal function markers. In particular, it shows that gonadal function is already compromised at diagnosis, but also indicates that the ovary has the capacity to recover shortly after cessation of treatment and shows no accelerated decline in the subsequent years as compared to healthy peers. It also shows that although follicle numbers are reduced, the remaining follicles are healthy and perfectly capable to produce vital and largely uncomplicated pregnancies. The latter indicates that ovarian reserve markers generally measured in ageing women in whom quantity as well as quality of follicles are compromised are to be interpreted with caution in CCS. Future
Preconception consultation:
- Folic acid supplements
- Lifestyle and nutrition
- Medical history
- Family history

If no preconception consultation took place, this consult can be planned in the first trimester.

Health care providers remain alert:
- After uterine radiation:
  - for premature birth and adequate fetal growth
- After anthracyclines or chest radiation:
  - for cardiomyopathy

* Information can also be discussed upon Graduation to Adult Survivorship or as it becomes relevant to the survivor.

Figure 1. IGHG recommendations for preconception counselling and surveillance during pregnancy.
research is needed to determine the best time to evaluate gonadal function damage, and to extrapolate our knowledge of low AMH levels to prediction of fecundity, fertility and age at menopause. The inter-individual variability in gonadotoxicity is for some part influenced by genetic determinants. We have shown that a polymorphism in the BRSK1 gene is associated with the inter-individual variability of reduced ovarian function as a result of chemotherapy. These findings may be used to develop a prediction model for ovarian function.

Most childhood cancer survivors who become pregnant can expect a normal pregnancy risk. However, we have identified some determinants of high-risk pregnancies. The clinical guideline recommendations offered in this thesis will aid careful and proportional management in cancer survivors. For survivors treated with radiotherapy potentially exposing the uterus, antenatal and postnatal care should be offered in a specialised medical centre to anticipate and deal appropriately with the possible complications.

Future studies should focus on the development of risk prediction models, combining evidence from this thesis and other valuable research. These models could aid health care providers in not only assessing their patients’ risk, but also the need for fertility preservation and cycle restoration in order to establish a normal hormonal environment in female CCS. All future research calls for collaboration within research groups, nationally, internationally and globally, to maximize the quality and validity of its results.
REFERENCES


15. van der Kooi AL. Changes in anti-Müllerian hormone and inhibin B in children treated for cancer. Journal of Adolescent and Young Adult Oncology. 2019.


47. Elbourn C, Parry R, Davies J, Lane S, Lakhoo K, editors. Future fertility: giving hope to young people. 49th congress of the International Society of Paediatric Oncology; 2017; Washington DC, USA.


60. von Wolff M, Donnez J, Hovatta O, Keros V, Maltaris T, Montag M, et al. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy--a technique in its in-


