The impact of reduced ovarian function after childhood cancer treatment

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 28 March 2025 at 10:00 in Belfragesalen, BMC, Lund, Sweden

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Title: The impact of reduced ovarian function after childhood cancer treatment

Abstract:

Background: Considering the increased number of childhood cancer survivors (CCSs), it is of great importance to identify females at risk of ovarian dysfunction early on to limit further complications. Highly gonadotoxic treatments such as alkylating agents, radiotherapy to the ovaries, and hematopoietic stem cell transplantation (HSCT) may induce accelerated follicular loss, consequently leading to premature ovarian insufficiency (POI). Women with POI are at risk of impaired quality of life, infertility, metabolic syndrome, cardiovascular disease, and osteoporosis.
Aims: To estimate the prevalence of POI and compare ovarian serum markers with antral follicle count (AFC) and ovarian volume (OV). To evaluate quality of life among CCSs with and without POI. To assess ovarian function and fertility using the Swedish and PanCareLIFE infertility risk classifications. To investigate the association between age at diagnosis, ovarian markers, and POI.
Methods: In this cross-sectional study, 167 adult CCSs were compared with 164 age-matched controls. Anti-Müllerian hormone (AMH), inhibin B, follicle-stimulating hormone (FSH), and oestradiol were collected. Transvaginal ultrasound was used to assess AFC and OV. Comprehensive data were gathered regarding cancer diagnoses and treatments, quality of life, and fertility.
Results: 13% of CCSs had POI. AMH, inhibin B, and FSH correlated significantly with AFC and OV in both CCSs and controls. AMH was the most accurate serum marker for detecting POI and low AFC. Health state and well-being were significantly decreased in CCSs compared with controls. Moreover, CCSs with POI reported the lowest well-being. CCSs exposed to highly gonadotoxic treatments based on the infertility risk classifications had significantly reduced AMH and AFC, higher POI prevalence, as well as a trend towards impaired fertility compared with controls. CCSs aged >8.4 years when diagnosed had significantly lower AMH compared with those aged ≤8.4 years at diagnosis. In addition, CCSs who were older when diagnosed were more often treated with alkylating agents and received a higher Cyclophosphamide Equivalent Dose (CED) compared with those who were younger at diagnosis. POI prevalence was higher for those aged >8.4 years when diagnosed.
Conclusions: AMH is a valid serum maker reflecting ovarian function in both CCSs and controls, and is thus valuable in follow-up programmes. CCSs, especially those with POI, should be identified early to receive adequate support. Fertility preservation counselling should be offered to females prior to highly gonadotoxic treatments (i.e., CED ≥6 g/m^2, radiotherapy to the ovaries, or HSCT), which are associated with reduced ovarian markers, POI, and a trend towards reduced fertility outcomes. Finally, younger age at diagnosis seems to be protective of the ovarian function.

Key words: childhood cancer survivors; anti-Müllerian hormone; premature ovarian insufficiency; quality of life; fertility; age at diagnosis

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The impact of reduced ovarian function after childhood cancer treatment

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To my dearest Elsa, Carl, Eric, and Joakim

Table of Contents

Populärvetenskaplig sammanfattning

Långtidsöverlevnaden för barn och unga under 18 år som idag insjuknar i cancer är 85 % i Sverige. Det är en fantastisk siffra men den har en baksida. Så många som 60–70 % får komplikationer till följd av sin cancerbehandling, så kallade seneffekter eller sena komplikationer. Dessa komplikationer som kan uppstå många år efter avslutad cancerbehandling kan drabba organ såsom hjärna, hjärta, njurar och skelett. En av de vanligaste komplikationerna är någon typ av hormonbrist som drabbar nästan hälften av överlevarna. Hormoner är ämnen i kroppen som transporteras med hjälp av blodet till olika organ. Deras uppgift är bland annat att styra vår tillväxt, pubertet samt fortplantningen. För att möjliggöra en god livskvalitet hos överlevare behöver vården därför ha stor kunskap om sena komplikationer för att erbjuda behandling i tid. Syftet med vår forskning var att studera äggstocksfunktionen, livskvalitet, fertilitet samt betydelsen av ålder vid insjuknandet hos vuxna kvinnor som överlevt barncancer.

Den mest pålitliga metoden hittills för att bedöma äggstocksfunktionen har varit att titta på antalet äggblåsor med hjälp av ultraljud via slidan, något som kan medföra obehag särskilt för unga kvinnor. Vi ville därför undersöka om anti-Mülleriskt hormon (AMH) som produceras i äggstockarna och mäts med blodprov var en likvärdig metod. Hos friska kvinnor har man sedan tidigare påvisat att dessa två metoder är likvärdiga, dock inte hos de som överlevt barncancer. Vår första delstudie visade att AMH var en pålitlig metod för att utvärdera äggstocksfunktionen hos både canceröverlevare och friska kontroller. Vidare visade våra resultat att 13 % av canceröverlevarna hade drabbats av ett för tidigt klimakterium före 40 års ålder, så kallad prematur ovariell insufficiens (POI), vilket ökar risken för lägre livskvalitet, infertilitet, hjärt-kärlsjukdom och benskörhet.

I vår andra delstudie kunde vi påvisa att kvinnor som överlevt barncancer hade nedsatt livskvalitet jämfört med friska kontroller. Särskild påverkan på livskvalitet hade överlevare med POI.

Den tredje delstudien syftade till att utvärdera äggstocksfunktionen samt fertilitet utifrån given cancerbehandling baserat på den svenska riskskattningen för infertilitet. Vi evaluerade även en internationell riskskattning från PanCareLIFE. Dessa riskskattningar används för att planera eventuella fertilitetsbevarande åtgärder före start av barncancerbehandling. De fertilitetsbevarande åtgärder som finns att tillgå är bland annat nedfrysning av utplockade ägg eller äggstocksvävnad. Vår studie som var den första att bedöma användarbarheten för den svenska riskskattningen visade att flickor som behandlats enligt hög riskskattning hade lägre AMH-nivåer i vuxen ålder, högre förekomst av POI samt tendens till nedsatt fertilitet jämfört med friska kontroller.

Vår fjärde och sista delstudie redovisar sambandet mellan ålder vid insjuknandet och äggstocksfunktionen. Tidigare studier har visat att flickor som insjuknar innan

pubertet har bättre bevarad äggstocksfunktion. I denna delstudie ville vi undersöka om detta samband kunde förklaras av eventuella skillnader i diagnoser och cancerbehandlingar för olika åldrar. Vi fann tämligen liten skillnad i cancerbehandlingar mellan de flickor som insjuknade i yngre och äldre ålder. Flickor som fick sin cancerdiagnos vid äldre ålder hade i större utsträckning blivit behandlade med cellgifter i form av så kallade alkylerare, som anses vara särskilt skadliga mot äggstockarna, dock var det ingen större skillnad i dosen flickorna fått. Vidare noterade vi lägre AMH-nivåer och högre förekomst av POI hos de flickor som fick cancerbehandling vid äldre ålder jämfört med de som var yngre vid insjuknandet.

I takt med att behandlingarna modifierats och förbättrats med tiden och överlevnaden ökat ligger idag fokus på att överleva barncancer till ett rimligt pris, inte längre till varje pris. Dagens höga överlevnadssiffror resulterar i en växande population av barncanceröverlevare som på sikt kan drabbas av sena komplikationer med påverkan på livskvalitet. Dessa sena komplikationer behöver därför identifieras tidigt för att minska lidande och följdsjukdomar. Denna avhandling visar att AMH är pålitlig för att utvärdera äggstocksfunktionen och kan således inkluderas i framtida uppföljningsprogram. Överlevare med POI har särskilt påverkad livskvalitet och dessa kvinnor behöver adekvat information, uppföljning och stöd. Både den svenska och PanCareLIFE riskskattningen för infertilitet är värdefulla redskap för att identifiera flickor med ökad risk för framtida infertilitet. Fertilitetsbevarande åtgärder bör erbjudas alla flickor som ska genomgå cancerbehandling med hög riskskattning för infertilitet. De flickor som genomgått sådan typ av behandling men inte erbjudits dessa åtgärder bör identifieras i tidig vuxenålder för bedömning av reproduktionsförmågan och eventuella åtgärder. Att insjukna vid yngre ålder verkar till en viss del skydda äggstocksfunktionen men även dessa flickor bör erbjudas fertilitetsbevarande åtgärder vid behandling som medför påtaglig infertilitetsrisk. Slutligen, med ökad kunskap om hormonella sena komplikationer efter barncancer kan patienter och familjer få tidigare information, optimerad behandling och en förbättrad uppföljning.

List of Papers

Paper I

Nyström A, Mörse H, Nordlöf H, Wiebe K, Artman M, Øra I, et al. Anti-müllerian hormone compared with other ovarian markers after childhood cancer treatment. Acta Oncol. 2019;58(2):218-24. doi: 10.1080/0284186x.2018.1529423.

Paper II

Hjelmér I, Gustafsson Kylberg A, Fridenborg A, Leijonhufvud I, **Nyström A**, Mörse H, et al. Quality of life among female childhood cancer survivors with and without premature ovarian insufficiency. J Cancer Surviv. 2021. doi: 10.1007/s11764-021-00987-y.

Paper III

Nyström A, Mörse H, Øra I, Henic E, Engellau J, Wieslander E, et al. Anti-Müllerian hormone and fertility in women after childhood cancer treatment: Association with current infertility risk classifications. PLoS One. 2024;19(8):e0308827. doi: 10.1371/journal.pone.0308827.

Paper IV

Nyström A, Mörse H, Øra I, Henic E, Elfving M. The impact of age at diagnosis on ovarian reserve after childhood cancer treatment. *In manuscript.*

Author’s contribution to the papers

Paper I

I am the first author of this paper. The first version of this manuscript emerged from a master's dissertation, written by a medical student. Later, I became involved in this PhD project in 2017. I then made major revisions and rewrote the manuscript after my main supervisor and I had reassessed the collected data. All the statistical analyses were reconducted by me and we chose to no longer use paired sample t-test as childhood cancer survivors (CCSs) and controls were not matched for the use of hormone replacement therapy and oral contraceptives. We also chose to present continuous variables as means, having previously presented medians. The tables and figures were produced by me with help from my main supervisor. For the final revision, it was suggested by one of the co-authors to add Receiving Operating Characteristic (ROC) curve, which was conducted by my main supervisor as I was on parental leave. Shortly after this minor revision, my main supervisor submitted the manuscript but kept me updated throughout the whole process and we worked together on the reviewer’s comments. After this paper was published, it came to our attention that follicle-stimulating hormone (FSH) methods had been updated during the study, i.e., 56 CCSs had FSH values analysed with a previous assay. We reanalysed the data with a transformation for those CCSs to the most recent FSH assay. When I performed these analyses, I noticed that the FSH data was somewhat skewed. Nonparametric tests were therefore added for FSH analyses. The erratum was written by me with suggestions from my main supervisor. These changes have now been incorporated into the published paper.

Paper II

For this paper, I am the fifth author. My contribution to this paper was to provide the clinical data regarding age at examination, age at diagnosis, cancer diagnoses and treatments, ovarian markers (i.e., FSH and oestradiol), as well as fertility data and treatments. In addition, questionnaires containing data on quality of life were supplied, but were not analysed by me. During the writing of this paper, we held regular meetings on how the results should be interpreted. The statistical analyses were not performed by me. However, I reviewed the manuscript several times and contributed comments. To be counted as a co-author, the Vancouver rules must be fulfilled. For this paper, I contributed data analysis and manuscript review, approved the final manuscript, and agreed to be accountable for all aspects of the work, thereby fulfilling the rules.

Paper III

This paper was prepared by me and my main supervisor from the outset, and I am the first author of this paper. I conducted all the statistical analyses, interpreted the results, prepared the tables and the figures, and wrote the original manuscript draft.

After comments from the supervisors and the co-authors, manuscript revisions were made by me. Furthermore, I submitted the manuscript and was the corresponding author. I wrote the reply to the reviewer's comments with help from my main supervisor, and I carried out the final revision.

Paper IV

For this paper, my contribution was the same as for *paper III*. In comparison to the former paper, I independently carried out all the statistical analyses, prepared the tables and the figure, and wrote the manuscript. When all aspects of the manuscript had been completed, I shared it with my supervisors and received valuable comments. This paper is still in manuscript, but will be submitted by me after final revision.

Abbreviations

AFC	Antral follicle count
ALL	Acute lymphoblastic leukaemia
AMH	Anti-Müllerian hormone
ART	Assisted reproductive technology
AUC	Area under the curve
CCSs	Childhood cancer survivors
CED	Cyclophosphamide Equivalent Dose
CI	Confidence interval
CNS	Central nervous system
E2	17β-oestradiol
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
GnRHa	Gonadotrophin-releasing hormone agonists
Gy	Gray
HPG axis	Hypothalamic-pituitary-gonadal axis
HRT	Hormone replacement therapy
HSCT	Hematopoietic stem cell transplantation
ICCC	International Classification of Childhood Cancers
IVF	In vitro fertilisation
LH	Luteinising hormone
OC	Oral contraceptives
OV	Ovarian volume
POI	Premature ovarian insufficiency
ROC	Receiving Operating Characteristic
TBI	Total body irradiation
TTO	Time trade-off
VAS	Visual analogue scale

Introduction

The words *cancer* and *oncos*

Hippocrates (460–370 BC), a Greek physician and the father of modern medicine, was the first person to use the words *carcinos* and *carcinoma* for non-ulcer and ulcer forming tumours, respectively. These terms are attributed to the crab, as cancer projections resemble the crab's legs [1]. Thereafter, Celsus (25 BC–50 AD), a Roman physician, converted the Greek words into the Latin word for crab: *cancer* [1, 2]. Another Greek word, *oncos*, reflecting tumour swelling was introduced by Galen (130–200 AD). This word was latter applied to oncology, which is the branch of medicine treating cancer [1].

Childhood cancer survival

Over the last 50 years, there has been a major improvement in childhood cancer survival due to advances in diagnostics, combinations of treatments, and supportive care (e.g., infusion of blood and blood products, infection treatments, and intensive care) [3]. The 5-year survival rate for all childhood cancers combined has now exceeded 80% in high-income countries, compared with only 30% in the 1960s [4, 5]. This success began with treating childhood leukaemia, the most common childhood cancer diagnosis, which was viewed before 1960 as incurable and fatal (i.e., untreatable with surgery) [3, 6]. Nowadays, the survival rate is about 95% for acute lymphoblastic leukaemia (ALL) in Sweden [7]. Moreover, the biggest achievements in survival came about in the 1970s and 1980s, thanks to intensified treatments combining surgery, radiotherapy, and chemotherapy, especially for children with ALL and non-Hodgkin lymphoma (Figure 1). Since 1995, survival rates have reached a plateau, but with the latest figures showing further improvements for diagnoses such as acute myeloid leukaemia and neuroblastoma [7, 8].

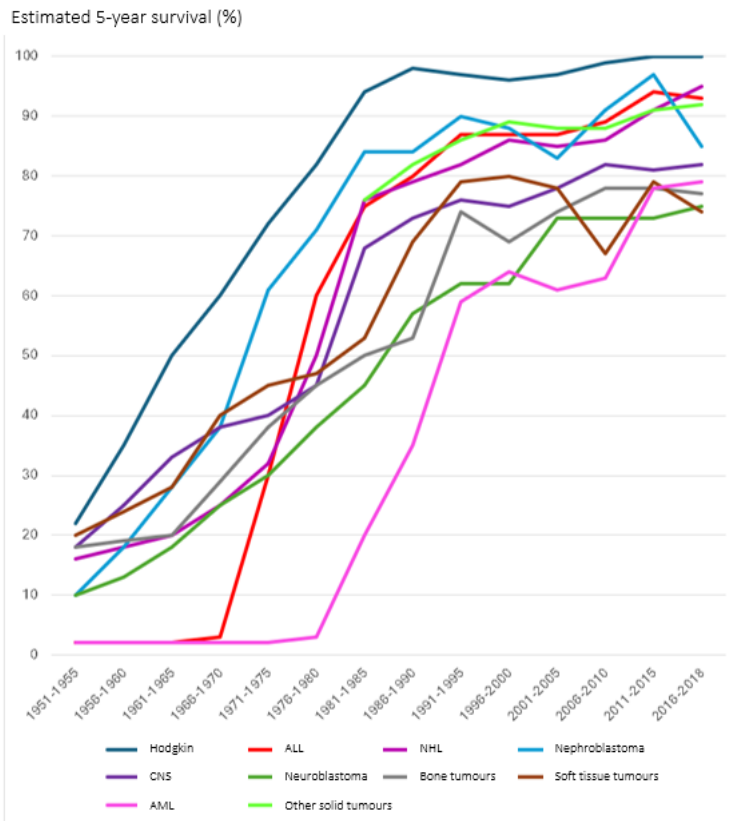

Figure 1. The 5-year survival rate over time for chilhood cancer diagnoses in Sweden [7].

Childhood cancer diagnoses

Diagnoses of childhood cancer can be divided into three large groups: leukaemia (30%), central nervous system (CNS) tumours (28%), and solid tumours (42%), and are generally categorised based on histology into 12 major groups according to the International Classification of Childhood Cancers (ICCC) [8, 9]. The distribution of childhood cancer diagnoses is presented in Figure 2. In Sweden, the annual incidence of childhood cancer is 16/100 000 children among those below 15 years of age [8]. This corresponds to 350 children diagnosed with primary cancer each year [10]. Childhood cancer diagnoses are often age-related, with ALL showing a peak incidence for children aged two to four. Neuroblastoma most often affects children younger than one year. Germ cell tumours have two peaks, one at ages zero to three and the other at adolescence. Bone tumours are predominantly seen in

teenagers, while brain tumours exhibit more consistent incidence during childhood. In addition, childhood cancer is most prevalent at the age of five to six years [8].

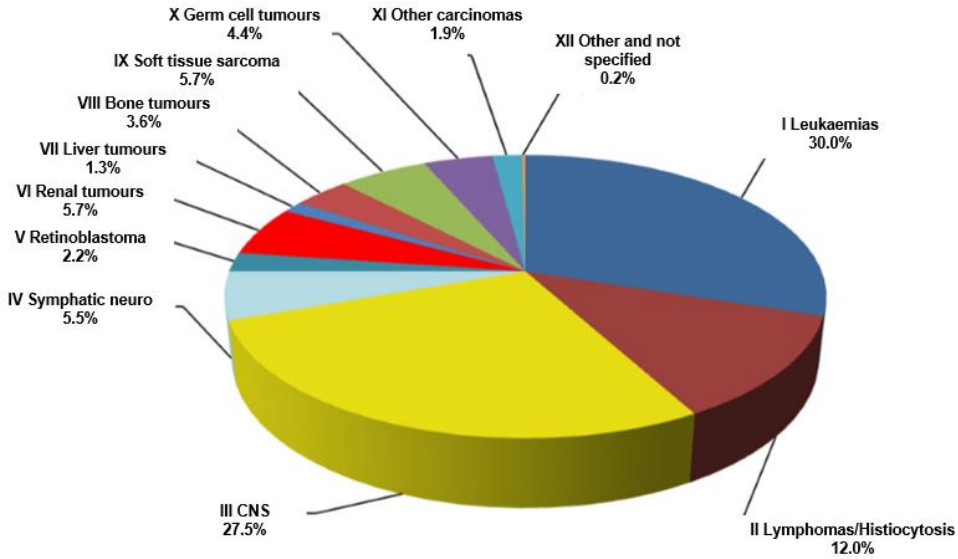

Figure 2. The distribution of childhood cancer diagnoses in Sweden 1984–2010, <15 years when diagnosed [8].

Cancer is somewhat more common among boys than girls (i.e., a male/female ratio of 1.17). However, both sexes present the same survival rates, and the prognosis is independent of age at diagnosis, with one exception for those aged over 10 when diagnosed showing poorer survival [8].

The distributions of cancer diagnoses and contributing factors are considerably different in adults. The most common cancers in adults are prostate cancer in males and breast cancer in females, representing 30% of all cancer cases for the sexes. Skin cancer is the third most common cancer diagnosis in adults (i.e., 10% of all cancers) [11]. It is estimated that approximately 40.5% of the adult population will develop a malignancy during their lifetime [12]. While childhood cancer is rare, it is still the leading cause of death for children >1 year of age in high-income countries [13]. In contrast to adults, it is rarely possible to identify the cause of childhood cancer. Advanced age and environmental or lifestyle factors are the main contributors for adult malignancy, but not for children [14]. Genetic predisposition has been shown in 9% of all children diagnosed with cancer [15]. The most common gene mutation was in the TP53 gene, associated with Li-Fraumeni syndrome as well as leukaemia, CNS tumours, and sarcoma. Other frequent mutations were in the following genes: APC associated with neuroblastoma and leukaemia, BRCA2

mainly increasing the risk of breast and ovarian cancer in adulthood, NF1 associated with CNS tumours, PMS2 associated with Ewing's sarcoma, and RB1 associated with retinoblastoma and osteosarcoma [15, 16]. Several other familial syndromes, such as Beckwith-Wiedemann syndrome, Fanconi anaemia, Noonan syndrome, and von Hippel-Lindau syndrome, are also known to increase the risk of childhood malignancy [17]. The above-mentioned cancer predisposition syndromes are suspected when a child is diagnosed with specific cancer types or bilateral cancers, or when there is a family history of the same cancer type or related cancers [18]. Genetic testing enhances the chances of early detection and treatment of childhood cancer. Although germline mutations add some pieces of the puzzle, the reasons why children develop cancer remain unknown in most cases.

Late complications

With the tremendous success in survival, the population of childhood cancer survivors (CCSs) has increased. In Sweden, there are approximately 11 000 individuals with a previous or current childhood cancer diagnosis [19]. Furthermore, there are currently 300 000–500 000 CCSs in Europe and about one in every 640 young adults has survived childhood cancer [20]. With these numbers in mind, it has been noted that the journey for children with cancer does not end with surviving the disease. The price of surviving childhood cancer can be high, and the so-called *late complications* affect as many as 60–80% of survivors [21, 22]. Late complications are defined as a late occurring or chronic condition, either somatic or psychosocial, which occurs later than five years after initial cancer diagnosis [9]. The risk of developing a chronic condition for survivors of childhood cancer was three times higher than for siblings, and for a severe or life-threatening condition the risk increased to eight-fold. In addition, a chronic condition among CCSs contributed to an incidence of 40% for severe or life-threatening disease or death [21]. Late complications were most often observed for those treated for bone tumours, and were least common for those surviving leukaemia or Wilms tumour [22]. Moreover, the prevalence of side effects increases with advanced age of CCSs [23].

Nearly all organs in the body can be affected by previous life-saving treatments [20]. The most frequent late complications are cognitive, psychological, endocrine, cardiovascular, and musculoskeletal, as well as those associated with a second cancer occurring later in life [9]. The wide spectrum of late complications is shown in Figure 3 [24]. Many factors contribute to the occurrence of late complications, such as disease severity, tumour location, age of survivors, surgery, cumulative doses of chemotherapy and radiotherapy, and organs exposed during treatment [9, 25]. Studies have reported that CCSs have an increased risk of second malignancy as they become older compared with the general population [26, 27]. Secondary

leukaemia generally appears within the first ten years after completion of primary cancer treatment, with treatments including high doses of alkylating agents, epipodophyllotoxins (e.g., etoposide), and anthracyclines increasing the risk. By contrast, secondary solid tumours more often occur ≥10 years after initial diagnosis, usually located in the CNS, thyroid gland, breast, bone, and skin. For secondary solid tumours, high doses of radiotherapy and low age at diagnosis are considered the main risk factors [28]. CCSs have a cumulative incidence of about 1% and 3% for a second malignancy within 10 and 20 years after primary cancer diagnosis, respectively [27]. The cumulative incidence increased to 16% at age 40–55 years, and CCSs had twice the risk of developing a second cancer after 40 years of age compared with the general population. In addition, female sex showed an association with developing a second cancer [26]. The following sections will address the late complications which are the most relevant to this doctoral thesis.

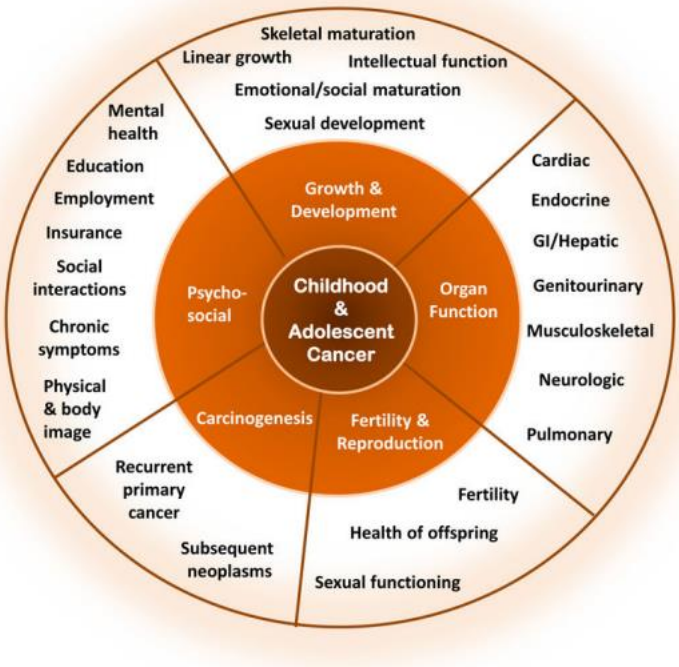

Figure 3. Late complications in survivors of childhood cancer [24]. Reproduced with permission.

Endocrine complications

One of the most common complications after childhood cancer treatment is endocrine disorders, affecting about 50% of CCSs [29, 30]. Gonadal dysfunction, hypothyroidism, and growth hormone deficiency are most frequently reported among survivors [29]. The main single risk factor for detrimental effects on the hypothalamic-pituitary axis, thyroid gland, and gonads is radiotherapy exposure [25, 31]. Other major risk factors reported were hematopoietic stem cell transplantation (HSCT) and older age when diagnosed [29]. In particular, the harm to the endocrine system is most notable with higher radiotherapy doses, as well as with increasing age of survivors. However, survivors also presented an elevated risk of thyroid disorders and diabetes, irrespective of treatments given, when compared with siblings without a previous cancer diagnosis [31]. Hence, the high prevalence of endocrine disorders with a debut sometimes several decades after cancer treatment, it is of utmost importance with long-term follow-up for CCSs at risk to reduce morbidity.

Female puberty

On average, girls begin puberty between the age of eight to 13 years. Considerable emotional and hormonal changes occur during puberty, together with physical changes including breast and pubic hair development (i.e., thelarche and pubarche, respectively), linear growth, and the occurrence of the first menstrual period (i.e., menarche) [32]. Adrenarche, contributing to pubarche, is a sign of androgen production (i.e., dehydroepiandrosterone, DHEA) in the adrenal cortex, and usually starts two years prior to puberty [32-34]. Puberty in girls begins with breast development according to Tanner stages B 2–5, accompanied by a growth spurt at approximately 11 years. Menarche normally appears at an average age of 13 years. After menarche, it can take several years before menstruation becomes regular. Moreover, pubertal timing is influenced by genetic factors, such as the parent's debut of puberty [10].

Puberty starts when the hypothalamic-pituitary-gonadal (HPG) axis becomes activated. In infancy, the HPG axis is transiently active, resulting in a so-called "mini-puberty of infancy", with elevated levels of steroidal hormones causing breast development in some cases. This activation later subsides during the first two years of life, and is regarded as benign [34]. Around one year prior to puberty onset, the dormant HPG axis gets activated when gonadotrophin-releasing hormone (GnRH) inhibition declines. Thereafter, GnRH is secreted in a pulsatile pattern from the hypothalamus, subsequently leading to follicle-stimulating hormone (FSH) and luteinising hormone (LH) release from the anterior pituitary gland. FSH stimulates

the granulosa cells in the ovary to produce oestrogen as well as oogenesis (i.e., the formation of oocytes from primordial germ cells), while LH contributes to progesterone production. Growth hormone also stimulates granulosa cells and the ovarian production of insulin-like growth factor 1, as well as reinforces the action of gonadotrophins (i.e., FSH and LH). The HPG axis is regulated by a negative feedback loop, with sex steroids inhibiting GnRH, FSH, and LH secretion (Figure 4) [32].

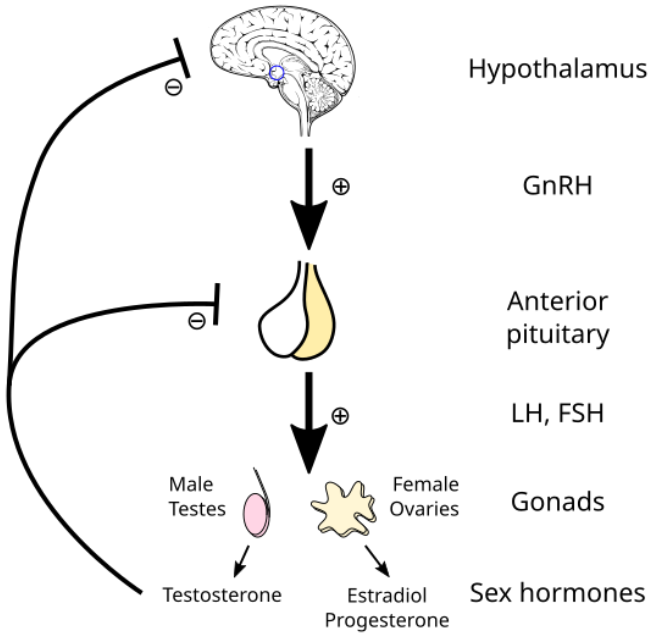

Figure 4. The hypothalamic-pituitary-gonadal (HPG) axis and the negative feedback loop exerted by sex hormones (https://creativecommons.org/licenses/by/3.0/deed.en, no changes were made) [35].

Precocious and delayed puberty

Precocious (early) puberty is defined as puberty debuting before eight years of age in girls [10]. A Danish epidemiologic study estimated the prevalence of some form of precocious puberty at 0.2% and <0.05% in females and males, respectively [36]. Another study also showed that precocious puberty is more common among girls, and that genetic inheritance from mothers was paramount [37].

The causes of precocious puberty range from benign to malignant disorders. Precocious puberty can be subdivided further into central and peripheral precocious puberty, according to the aetiology [34].

The most common causes of central precocious puberty are the following [34, 37]:

- Familial precocious puberty (i.e., genetic inheritance of precocious puberty)
- CNS tumours
- CNS injury – for instance cranial radiotherapy or surgery
- Syndromes – such as neurofibromatosis type 1 and tuberous sclerosis

Many children with a central cause of precocious puberty have, however, no underlying medical condition [38].

Peripheral precocious puberty is independent of gonadotrophins, and is caused by endogenous production or exogenous exposure of sex hormones. It is more rare than central precocious puberty, and is most frequently caused by [34]:

- Ovarian tumours
- Adrenal tumours
- Congenital adrenal hyperplasia
- McCune-Albright syndrome
- Exogenous oestrogen exposure

Treatment of precocious puberty should be directed towards the underlying condition. For central precocious puberty, GnRH agonists (GnRHa) are standard therapy. GnRHa can be administered in several ways, including subcutaneously or intramuscularly, and are either short or long acting. Treatment can be considered if the child is of young age, has fast puberty progression, and/or has advanced bone age with subsequent risk of reduced final height. During therapy, any side effects, pubertal development, linear growth, and bone age should be controlled [34]. The potential side effects of GnRHa are reversible and consist of headache, hot flashes, sweating, and injection site reactions [34, 39]. The purpose of therapy is to achieve optimised adult height, but also to relieve psychosocial stress. Regarding peripheral precocious puberty, treatment intends to remove the excessive production of sex hormones. Therapy for ovarian and adrenal tumours includes, among other things, surgery [34].

Delayed puberty is the lack of breast development in girls after 13 years of age [10]. The prevalence is approximately 2% among adolescents [40], and it has several causes such as [32]:

- Hypogonadotropic hypogonadism
- Hypergonadotropic hypogonadism
- Hypopituitarism
- Chromosomal abnormalities

- Hypothalamic dysfunction – secondary to other conditions

Hypogonadotropic hypogonadism is a result of GnRH, FSH, or LH deficiency. Such a deficiency can be due to tumour, surgery, injury, genetic defects, or an infection, involving the hypothalamus or pituitary gland. It can also be seen after long-term use of cortisone, pronounced stress, and malnutrition. In the case of hypergonadotropic hypogonadism, there is a deficiency of sex hormones, and gonadotrophins will be raised because of negligible negative feedback on the HPG axis. Ovarian failure due to autoimmune conditions can be a reason for the development of hypergonadotropic hypogonadism. In hypopituitarism the pituitary gland fails to produce gonadotrophins, and this can occur alongside hypothyroidism, growth abnormalities, as well as adrenal insufficiency. Chromosomal abnormalities can also delay puberty, such as the lack of one X chromosome in females known as Turner syndrome (45 XO). The clinal presentation in these females, in addition to delayed puberty, can also be short height and primary amenorrhea due to ovarian failure. Several conditions, including hypothyroidism, cystic fibrosis, and celiac disease, as well as malnutrition, can also cause delayed puberty [32]. However, in most cases delayed puberty is constitutional, accounting for 53% of all cases and predominantly observed among males (i.e., 63% and 30% of males and females, respectively) [41]. It accounts for an uttermost of the normal range of pubertal onset, and should be considered an exclusion diagnosis [42]. Functional hypogonadotropic hypogonadism, that is having an underlying disease but still spontaneous puberty onset, was shown in 19% of the cases. Permanent hypogonadotropic hypogonadism was observed in 12% of the cases, and hypergonadotropic hypogonadism was noted in 13% of those with delayed puberty [41].

The prevalence of hypogonadotropic hypogonadism is around 11% in CCSs treated with cranial radiotherapy [43], which is considerably higher than the total estimated prevalence of 2% in the general population for delayed puberty [40]. It may present as delayed puberty, arrested puberty (i.e., normal onset of puberty without further progress), or amenorrhea [25]. Amenorrhea (i.e., the absence of menstruation) is categorised as either primary or secondary amenorrhea. Primary amenorrhea is defined as the absence of menarche at 15 years of age or more than three years after breast development. The definition of secondary amenorrhea is no menstruation for ≥3 months in a female who has previously presented with regular cycles, or ≥6 months in a female who has previously had at least one menstruation [44]. Moreover, cranial radiotherapy doses of ≥22 Gray (Gy) were associated with a deficiency of gonadotrophins [43]. Lower doses of 18–24 Gy can also cause precocious puberty, which could be treated with GnRHa. Cranial radiotherapy doses of >30 Gy can often cause permanent deficiency of gonadotrophins, which can appear many years after childhood cancer treatment with a need for long-term follow-up [10]. Screening for a deficiency of gonadotrophins should also be carried out for CCSs with previous tumour localisation in the area of the hypothalamus or pituitary gland [25].

Females with either hypogonadotropic or hypergonadotropic hypogonadism require hormone replacement therapy (HRT), which includes oestrogen and progesterone, for the induction and sustainment of puberty. HRT for this purpose in female adolescents usually consists of transdermal 17β-oestradiol and micronised progesterone [45]. Untreated hypogonadotropic hypogonadism has shown associations with hypertension, dyslipidaemia, reduced bone mineral density, and obesity [43].

Importantly, radiotherapy to volumes that expose the ovaries (hereafter referred to as ovarian radiotherapy) and certain chemotherapeutic agents (i.e., alkylating agents) can induce ovarian failure, with consequences such as delayed puberty [10]. The childhood cancer treatments considered especially gonadotoxic are described in detail under the section *Gonadotoxic treatments*.

Female reproductive system

It is believed that the human ovary at birth consists of a finite number of primordial follicles, as the primordial germ cells are non-renewable after foetal life [46, 47]. This hypothesis, however, has been disputed based on the findings of animal studies indicating the potential renewal of primordial germ cells [48]. The formation of follicles begins at a gestational age of approximately four months, and shortly thereafter reaches the highest number of about 7 million primordial follicles. At birth, approximately 1 million primordial follicles remain with a further decline to about 300 000 by the age of menarche [49]. The decline thereafter occurs in a bi-exponential fashion with an acceleration at around 37 years of age, resulting in an estimated number of 25 000 remaining follicles. At the time of menopause, the number of primordial follicles left is only 1000 (Figure 5) [46, 49]. This estimated decline rate is based on the Faddy-Gosden equation using several mathematical models, commencing from studies that observed follicle number at different ages [46, 47, 49-51].

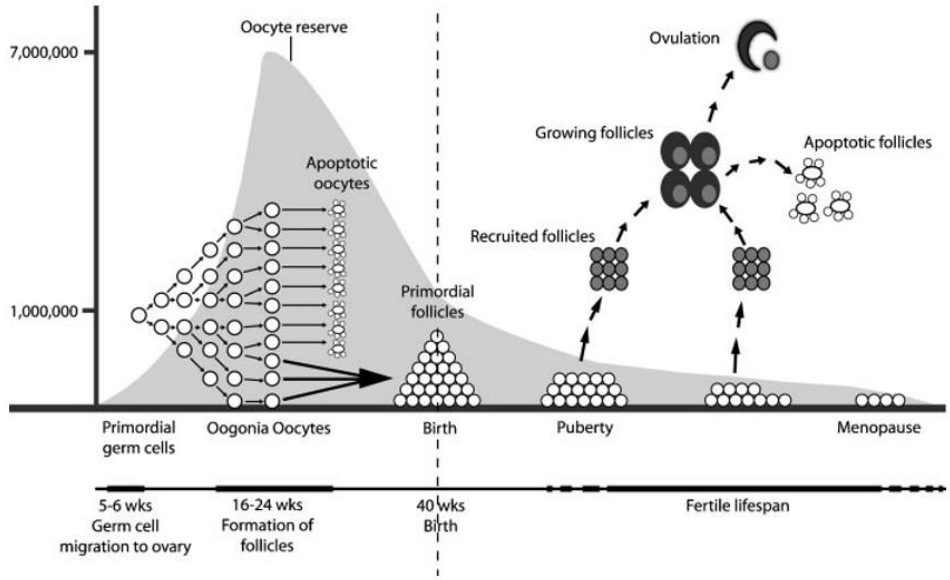

Figure 5. The approximated number of primordial follicles from foetal life to menopause. Across the reproductive lifespan, the majority of growing follicles undergo atresia [49]. Reproduced with permission.

Each primordial follicle consists of an oocyte that is surrounded by somatic granulosa cells. A woman will have approximately 400 ovulatory cycles through the reproductive lifespan [49]. At each cycle, one of the growing follicles will mature to become the dominant follicle, releasing an oocyte at ovulation. Thus, most growing follicles will be lost due to atresia and apoptosis [52]. At the onset of puberty, FSH begins to stimulate the granulosa cells of the growing follicles, which than start to proliferate and produce oestrogen [49]. LH has a midcycle surge during the menstrual cycle, and regulates ovulation. After ovulation, the corpus luteum produces progesterone, causing endothelial proliferation in the endometrium. This results in a thickened endometrium, where implantation of the fertilised egg can take place [49, 53].

Ovarian reserve

The term “ovarian reserve” traditionally refers to the reproductive potential of a female, which comprises both the residual number of oocytes (i.e., primordial follicles) as well as their quality at any point in time [54]. Several ovarian markers are used in the clinic to assess the ovarian reserve, including biochemical tests (i.e., anti-Müllerian hormone [AMH], FSH, oestradiol, and inhibin B), as well as ultrasonographic measures (i.e., antral follicle count [AFC] and ovarian volume [OV]) [55]. However, ovarian reserve markers such as AMH and AFC reflect the

quantity of oocytes rather than their quality. The age of the female is still considered the best predictor of oocyte quality. Hence, ovarian reserve should be used to describe the persisting oocyte quantity [56].

Another term, “diminished or decreased ovarian reserve”, applies to females with regular cycles, but with a poorer response to ovarian stimulation or fertility compared with those of similar age. The reason for diminished ovarian reserve is, in most cases, unclarified. However, it is known that cancer treatments as well as genetic disorders such as Turner syndrome or FMR1 premutations can compromise the ovarian reserve [54]. Lifestyle factors do not seem to affect the ovarian reserve, although ongoing smoking is associated with raised FSH levels [54, 57]. Moreover, it is unknown whether diminished ovarian reserve is due to an abnormal accelerated atresia of growing follicles with a normal primordial follicle pool initially, or whether it is due to natural atresia but with an innate reduced follicle pool. No evidence currently exists for the assumption of varying atresia rate in healthy females. However, there is histological support for variation in the number of primordial follicles at birth [51]. In addition, decreased ovarian reserve precedes menopause, which is considered the end of a female’s reproductive years [54]. The age of menopause occurrence varies widely between individuals. A Dutch study including over 4000 females found a mean menopause age of 50.2 years (24–62), with a standard deviation of ±4.2 [58]. Furthermore, the FSH cut-off level is typically 40 IU/L for menopause, but FSH by itself is of limited use in predicting when transition into menopause will occur [59].

Anti-Müllerian hormone

AMH is a glycoprotein belonging to the transforming growth factor-beta superfamily, with an important role in folliculogenesis. Its function is to inhibit the recruitment of primordial follicles from the dormant primordial follicle pool. AMH is produced by the granulosa cells of growing follicles, i.e., from the stage of primary to small antral follicle. The production of AMH in large antral follicles seems to vanish gradually (Figure 6) [60-62].

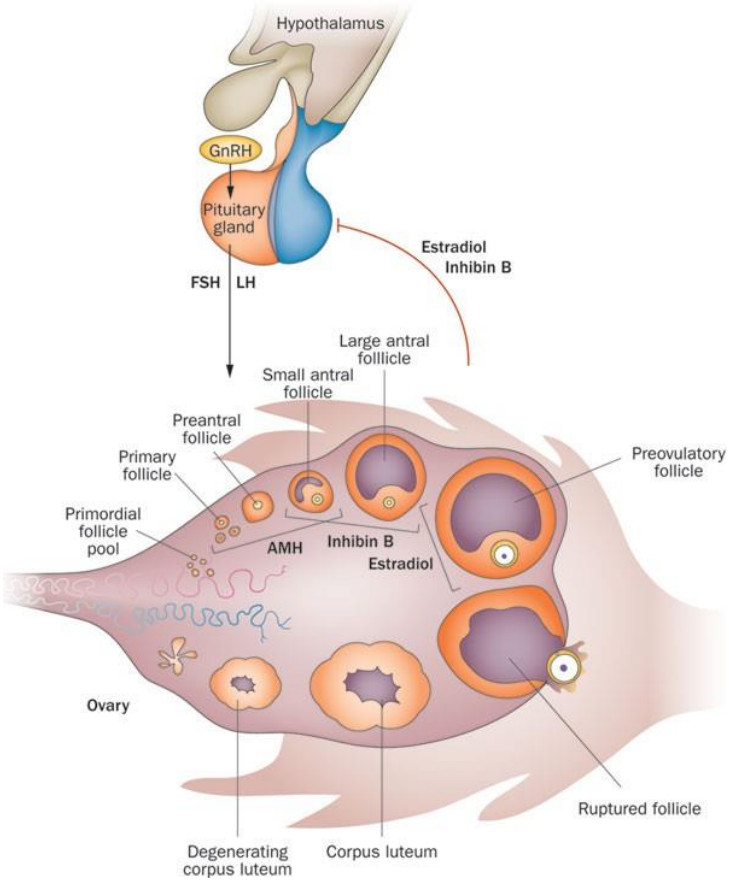

Figure 6. Illustration of folliculogenesis, the maturation from the primiordial follicles to one ovulatory follicle, regulated by several hormones [62]. Reproduced with permission.

AMH is a serum marker, which is already detectable at birth, with a rise during the first year of life followed by a small decline [63]. This agrees with the observed "mini-puberty of infancy", with a transient activation of the HPG axis [34]. It later increases during childhood with a temporary drop around puberty, whereafter a final peak occurs at the age of approximately 24.5 years. After this peak, AMH decreases until it becomes non-detectable at the time of menopause (Figure 7) [63].

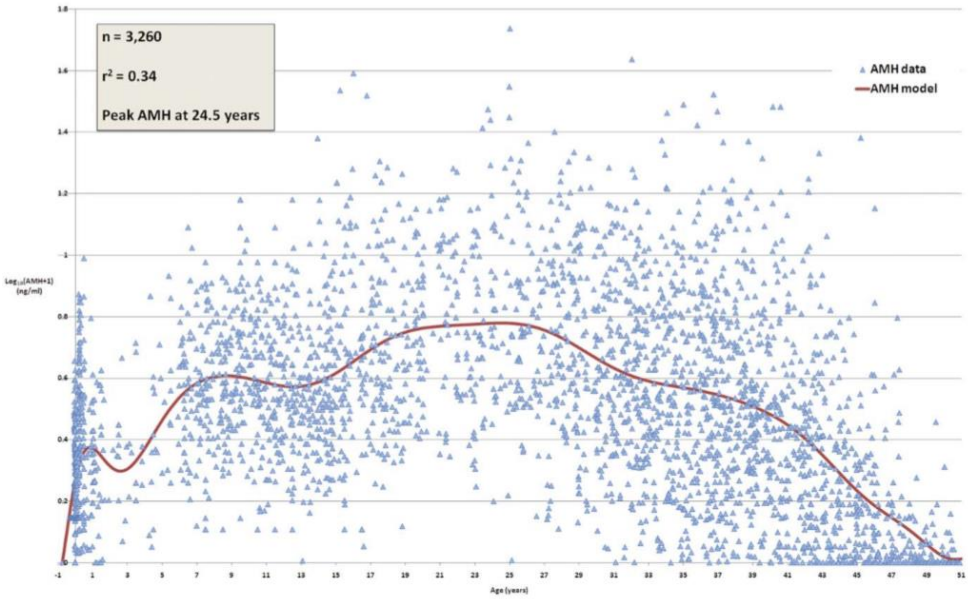

Figure 7. AMH values from conception to menopause, derived from 3260 healthy females. From Kelsey et al. 2011 [63].

AMH is considered a valid serum marker for ovarian reserve assessment in healthy adult females, with significant correlation to histologically verified primordial follicle count. AFC (i.e., the total number of growing follicles 2–10 mm in diameter in both ovaries) measured during the early follicular phase has been the gold standard method in the clinical setting for estimating the ovarian reserve, as it also correlates well with the remaining number of primordial follicles [64]. However, this method is assessed using transvaginal ultrasound, and is therefore unfeasible in children and can also cause discomfort for younger females. AMH and AFC are now considered equivalent methods. Besides AMH and AFC, several other methods are utilised as mentioned for evaluating ovarian reserve, including OV as well as serum levels of FSH, oestradiol, and inhibin B [55].

Inhibin B is also a glycoprotein hormone produced by the granulosa cells under FSH influence, exerting negative feedback on FSH secretion [65]. In contrast to AMH, it is secreted by larger follicles such as antral follicles from puberty, reflecting follicular growth (Figure 6). Inhibin B can therefore also be used to evaluate ovarian reserve [66]. The levels of inhibin B fluctuate during the menstrual cycle, with an increase in the follicular phase, a peak after the midcycle FSH surge, and a decrease during the luteal phase [65].

As AMH is not involved in the negative feedback system, it presents with little variation during the menstrual cycle, and is thus assessable at any time [67, 68]. On

the other hand, as mentioned earlier, FSH, oestradiol, and inhibin B are all involved in the feedback system and should therefore be measured during the early follicular phase (i.e., days 2–5 of the menstrual cycle) in females with regular cycles [32, 65]. Furthermore, there have been conflicting results regarding the AMH decrease using oral hormonal therapy, with one study finding a 20% reduction for users and other studies reporting no difference [69-71]. Regarding FSH and sex steroids, the use of HRT and oral contraceptives (OC) affect the hormone concentrations, which contribute to interpretation difficulties regarding ovarian reserve [72]. With advancing age, the depletion of primordial follicles leads to reduced levels of inhibin B and oestradiol, followed by increased FSH [73]. Moreover, another advantage of AMH is that it declines prior to the FSH increase, and is hence the preferable serum marker for detecting reduced ovarian reserve [74, 75].

Ovarian reserve markers are mainly used to predict which patients are eligible for in vitro fertilisation (IVF) [54]. IVF is the most common type of assisted reproductive technology (ART), a method where oocytes are stimulated and matured in vivo (i.e., the ovaries), but fertilised in vitro (i.e., in a petri dish). The fertilised egg (i.e., the embryo) is thereafter transferred into the uterus, where pregnancy evolves. The first live birth with the IVF technique was reported in 1978 in England. Today, IVF accounts for 5% of the total number of live births in Europe and 2% in the United States [76, 77]. Furthermore, most IVF clinics report AMH as the preferable ovarian reserve test for predicting the ovarian response to IVF. However, the best predictor of live birth was the patient's age [78]. Thus, AMH and AFC are considered good predictors of quantitative measures (i.e., ovarian responsiveness and oocyte retrieval), but not of qualitative measures (i.e., pregnancy and live birth rates) [55, 79]. Indeed, a Danish prospective study did not report decreased fertility in healthy females in their mid-20s with low AMH. Females in this study with high AMH levels presented with lower probability of conceiving [80]. Another study reported that most females with high AMH levels have polycystic ovarian syndrome [81], which presents with anovulatory infertility and affects about 7–15% of females of reproductive age [82]. Ovarian hyperstimulation syndrome (i.e., an IVF treatment complication) is also more common among females with elevated AMH [81].

Premature ovarian insufficiency

Premature ovarian insufficiency (POI), also referred to in the literature as primary ovarian insufficiency or premature ovarian failure, is a condition with decreased ovarian function before 40 years of age [54, 83]. The term premature ovarian failure is not preferable, as ovarian dysfunction may change over time. POI is diagnosed when a female fulfils the following criteria [84]:

- Amenorrhea ≥4 months

- Low oestradiol
- Two measures of serum FSH >40 IU/L obtained one month apart
- Below 40 years of age

During childhood, POI may manifest as delayed or absent puberty, or as a disruption in the progression of puberty [85]. As mentioned, natural menopause occurs at a mean age of 50 years [58]. POI can sometimes be transient or can ultimately progress into menopause before the age of 40 years, which is considered the end-stage of POI [84]. Hence, a female with POI still has a remaining ovarian function to some extent, while menopause presents depletion of primordial follicles and permanent cessation of menses [86]. It has been reported that 50–75% of females with POI have intermittent ovulation and 5–10% conceive spontaneously [87, 88].

Prior to POI diagnosis, other possible causes of secondary amenorrhoea must be excluded such as pregnancy, hypothyroidism, and genetic disorders (e.g., FMR1 gene premutation) [86, 89]. The process behind POI is not fully clarified, but is believed to be due to an inherently reduced number of primordial follicles, disturbances in the recruitment of primordial follicles, or an increased atresia rate. The aetiologies of POI include autoimmune, genetic, metabolic, infectious, and iatrogenic causes [83]. Unfortunately, for 90% of females diagnosed with spontaneous POI (i.e., normal karyotype), the cause will be unknown [90]. Iatrogenic causes such as childhood cancer treatments, including alkylating agents, ovarian radiotherapy, and conditioning regimen prior to HSCT, are considered highly gonadotoxic. These cancer treatments can accelerate follicular loss, consequently resulting in POI [91-93].

POI causes menopausal symptoms such as hot flashes, night sweating, vaginal dryness, and dyspareunia [90]. However, it is also associated with more severe health complications including reduced quality of life, depression, infertility, metabolic syndrome, osteoporosis, and cardiovascular disease [84, 90, 94, 95]. The POI prevalence among CCSs is about 8–11%, which is considerably higher than in the general population (i.e., 1–2%) [83, 93, 96, 97]. CCSs report that infertility has a negative influence on well-being and intimate relationships [98]. They also express doubt about having children because of their previous cancer diagnosis [98], with concerns regarding the biological child's health but also their own physical health [99-101]. CCSs are less likely to have a biological child compared with siblings without a prior cancer diagnosis [102]. Cancer survivors are also at greater risk of subfertility and delivery complications [103]. Nevertheless, they still express a longing for future biological children [101]. However, for CCSs with POI, the chance of conceiving spontaneously is limited (i.e., 5–10%), which is why egg donation with IVF is advised [87-89].

HRT is recommended for females with POI to ease discomfort from vasomotor symptoms and vaginal dryness, as well as to decrease the risk of the above-

mentioned associated comorbidities. The therapy consists usually of 50–100 μg transdermal oestradiol daily and, for those with a uterus, an additional 5–10 mg of medroxyprogesterone acetate for 12 days each month is advised. Contraception should be offered for those not desiring pregnancy. In such a case, combined oral contraceptives can be used as both contraception and HRT. However, for those preferring intrauterine devices or barrier methods, supplemental HRT is needed [83]. The duration of HRT is generally recommended up to the age when natural menopause occurs (i.e., 50–51 years) [58, 83]. There have been concerns regarding the elevated risk of breast cancer for those on HRT; however, studies did not find an association either for those aged 40–49 years in the general population or for those with POI on HRT [104, 105]. No different therapy approach is therefore needed for CCSs with POI unless there is a family history of breast cancer.

Considering the potential impact POI has on an individual's life, it is of the utmost importance to detect CCSs at risk in time to limit the morbidity and mortality associated with oestrogen deficiency.

Gonadotoxic treatments

It is well documented that alkylating agents, ovarian radiotherapy, and conditioning regimen before HSCT have a harmful effect on the ovarian function. These treatments are further detrimental with increasing doses or when combined [92, 93, 106, 107]. Alkylating agents such as cyclophosphamide, busulfan, and procarbazine are reported to be the most gonadotoxic of the chemotherapeutic agents used to treat childhood cancer [108-111]. There are two quantitative methods suggested for calculating cumulative alkylating agent exposure: the Alkylating Agent Dose (AAD) and the Cyclophosphamide Equivalent Dose (CED). The AAD score is obtained from each study population's drug dose distribution, with the disadvantage that it cannot be applied to compare the score between different populations because every population will have its own distribution. CED is recommended as it is independent of a specific distribution and can therefore be used to compare results from different cohorts [112]. Thus, we chose to use CED in our studies. The alkylating agents included in the CED are presented in Table 1, where each agent's cumulative dose is multiplied by the conversion factor and subsequently summed together.

Table 1. The Cyclophosphamide Equivalent Dose (CED) equation [112].

Alkylating agent	mg/m²
Cyclophosphamide	1.0
Ifosfamide	0.244
Procarbazine	0.857
Chlorambucil	14.286
BCNU	15.0
CCNU	16.0
Melphalan	40
Thio-TEPA	50
Nitrogen mustard	100
Busulfan	8.823

There are two possible theories on how chemotherapeutic agents induce follicular loss in the ovaries (Figure 8) [113]:

1. Direct damage to the primordial follicles
2. Indirect by damage to growing follicles, causing depletion of primordial follicles because of increased turnover

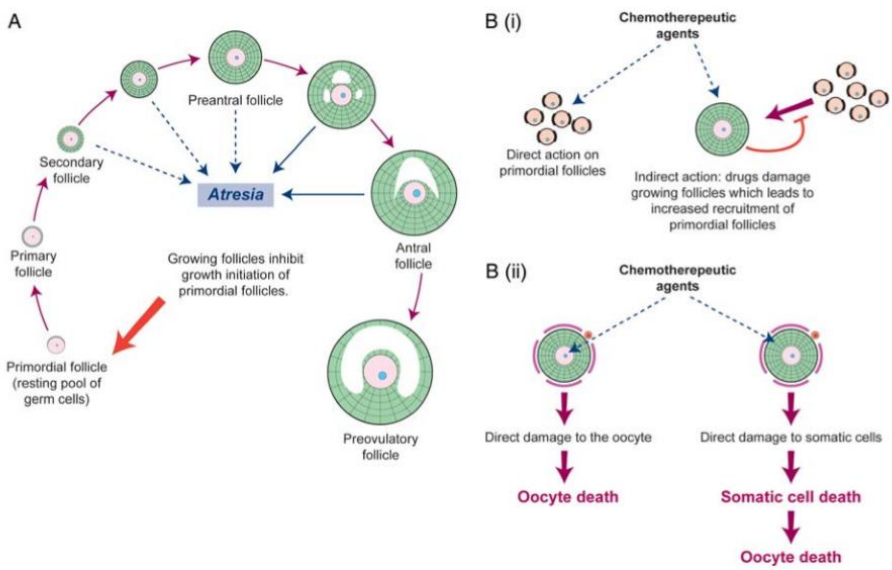

Figure 8. The potential mechanims of ovarian damage caused by chemotherapeutic agents [113]. Reproduced with permission.

Moreover, chemotherapeutic agents can cause harm to either the oocyte or the surrounding granulosa cells, contributing to oocyte death in both cases (Figure 8). The granulosa cells are highly proliferating and might therefore be primarily affected by alkylating agents, which inhibit cell division by forming deoxyribonucleic acid (DNA) cross-linking. On the other hand, oocytes are non-dividing, possibly making them less targeted [113]. Aside from inducing DNA damage by free radicals, ovarian radiotherapy also causes fibrosis as well as damage to the blood vessels in the ovarian tissue [114, 115].

To date, many studies have shown reduced ovarian markers in CCSs [74, 75, 109, 111, 116, 117]. Green et al. reported that CCSs treated with an ovarian radiotherapy dose >5 Gy were less likely to conceive, and the likelihood decreased even more with increased doses. The same was evident for higher doses of alkylating agents [107]. A more recent study by van den Berg et al. identified that CED of >7 g/m^2 and any radiotherapy dose to the lower abdomen were consistent with decreased fertility [118]. CCSs who received combined treatment of alkylating agents and lower abdominal radiotherapy had a POI prevalence of 30% [93]. Moreover, Wallace et al. have calculated the effective sterilising dose (i.e., a dose resulting in immediate POI), which is lower with increased age of the patient. The sterilising dose was 20, 18, 17, and 14 Gy at birth, 10 years, 20 years, and 30 years, respectively [119]. Kelsey et al. have also developed a predictive model for POI timing, incorporating the patient's age at cancer diagnosis along with the ovarian radiotherapy dose [120]. In addition, radiotherapy involving the uterus in the field carries risks such as preterm birth, babies small for gestational age (SGA), and congenital malformations because of affected myometrial elasticity and reduced uterine blood flow [121, 122].

It is difficult to define an exact toxic threshold dose of chemotherapeutic agents and radiotherapy due to interindividual differences among patients, which is most likely genetically determined [123]. The ovarian damage is dependent not only of the cumulative dose and type of cancer treatment used, but also of the patient's age. Several studies have reported better preserved ovarian function in CCSs treated before puberty [124-128]. This observation could be explained by the fact that younger girls have a larger primordial follicle pool to start with.

Fertility preservation

In 2021, the EU-funded PanCareLIFE, together with the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) (hereafter referred to as PanCareLIFE only), presented fertility preservation guidelines along with infertility risk classifications for females based on childhood cancer treatments

given [129]. The infertility risk classifications stated by PanCareLIFE and the Swedish guidelines are outlined in Tables 2 and 3, respectively [129, 130].

Table 2. The infertility risk classification for females based on the PanCareLIFE guidelines [129].

Group 1	Group 2	Group 3	Group 4
Other treatments	Unilateral oophorectomy	- CED <6 g/m^2 - Cranial radiotherapy	- CED ≥6 g/m^2 - Ovarian radiotherapy - HSCT

CED: Cyclophosphamide Equivalent Dose; HSCT: hematopoietic stem cell transplantation.

Table 3. The infertility risk classification for females based on the Swedish guidelines [130].

Low-risk	Moderate-risk	High-risk	Very high-risk
- Vincristine - Methotrexate - Actinomycin D - Bleomycin - Mercaptopurine - Vinblastine - 5-fluorouracil (5-FU)	- Cisplatin - Carboplatin - Cyclophosphamide <6 g/m^2 - Ifosfamide <60 g/m^2 - CCNU <360 mg/m^2	- Cyclophosphamide >6 g/m^2 - Ifosfamide >60 g/m^2 - Procarbazine - BCNU - CCNU >360 mg/m^2 - <10 Gy to the ovaries	- >10 Gy to the ovaries - Allogenic HSCT - Autologous HSCT

Gy: gray; HSCT: hematopoietic stem cell transplantation. This is the upcoming edition with reduced cumulative doses for cyclophosphamide (earlier, <9 g/m^2 for the moderate-risk group and >9 g/m^2 for the high-risk group).

The Swedish guidelines are more detailed, as they divide the PanCareLIFE group 4 (i.e., the group treated with the most gonadotoxic treatments) into high-risk and very high-risk groups. According to the PanCareLIFE guidelines, groups 2–4 are considered to have a potential infertility risk, but not group 1. In addition, the PanCareLIFE recommendations consider ovarian radiotherapy to be a highly gonadotoxic treatment, irrespective of the dose [129].

Until now, there are no existing methods for protecting the ovarian follicles in vivo during chemotherapy. A possible fertility preservation option for post-pubescent girls is oocyte cryopreservation. This established method is, however, not feasible for pre-pubescent girls because of immature oocytes. Pre-pubescent girls can therefore only be provided ovarian tissue cryopreservation, which was regarded as experimental up until relatively recently. Oocyte cryopreservation should be offered prior to highly gonadotoxic therapy, i.e., treatments according to group 4 as well as the high-risk and very high-risk groups, as stated in both guidelines. Regarding ovarian tissue cryopreservation, the guidelines vary slightly for pre-pubescent girls with the Swedish guidelines recommending this measure for those considered at

very high infertility risk, whereas the PanCareLIFE guidelines state moderate consideration for those in group 4 [129, 130].

The main possible risk associated with oocyte cryopreservation is the treatment delay, which might compromise the prognosis. The subcutaneous hormonal stimulation usually takes 10–14 days before oocyte retrieval can be performed (Figure 9) [130]. Moreover, oocyte retrieval is an invasive method, with the use of an aspiration needle either transvaginally or abdominally, guided by ultrasound. This procedure can be conducted under sedation or general anaesthesia [131]. One alternative to oocyte cryopreservation is embryo cryopreservation. However, most post-pubescent girls will choose the former, as they are less likely to be in a partnership and not show an interest in donor sperm [129]. Concerning the nature of this procedure, it can be accompanied by emotional distress, as observed in adult females undergoing IVF, as well as hormonal adverse effects (e.g., ovarian hyperstimulation syndrome) [132, 133].

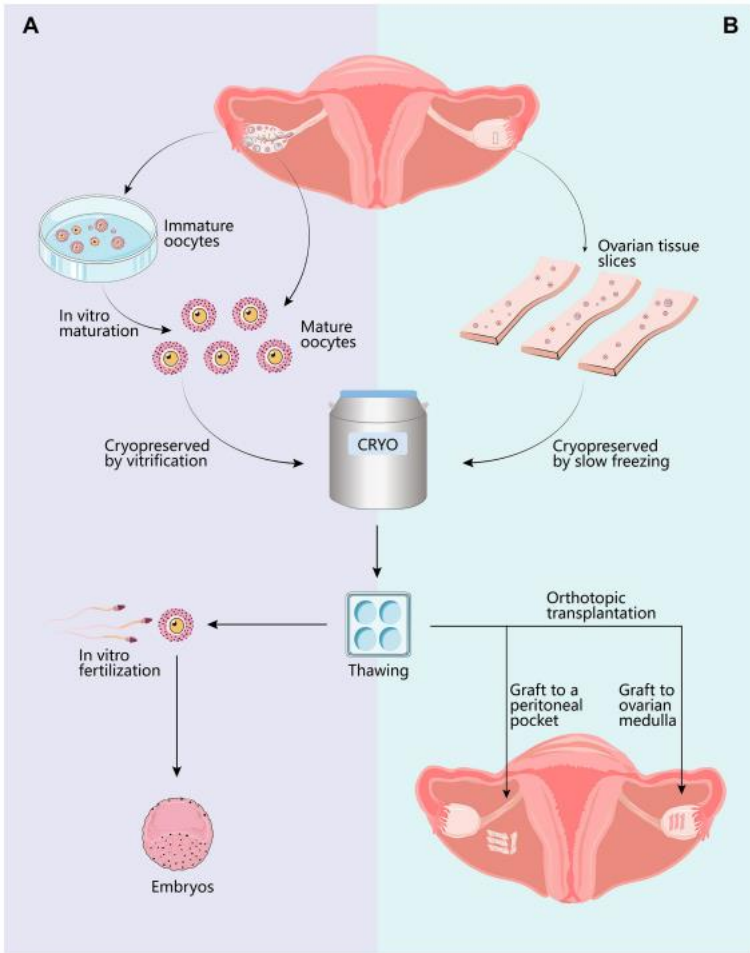

Figure 9. Fertiliy preservation options for childhood cancer patients. **A** Oocyte cryopreservation for post-pubescent girls. Thawed oocytes are later used for IVF. **B** Ovarian tissue cryopreservation for pre-pubescent girls or for post-pubescent girls in need of immediate cancer therapy. Cryopreservation by vitrfication and slow freezing are preferable for oocytes and ovarian tissue, respectively. Reproduced from Chen et al., Front. Endocrinol., 2023 [134].

In young children, ovarian volume is smaller, thus instead of performing multiple biopsies, a whole ovary is typically removed laparoscopically for cryopreservation. The ovarian tissue cryopreservation procedure is also invasive under general anaesthesia, but does not cause a delay to cancer treatment and can therefore also be used in post-pubescent girls requiring immediate treatment [130, 135]. This procedure is usually performed under anaesthesia for another reason, such as for diagnostic purposes or during cancer treatment. The site of future reimplantation of thawed ovarian tissue can be either orthotopic (i.e., in the peritoneum of the ovarian fossa or at the location of the remaining ovary) or heterotopic (i.e., subcutaneously in the abdomen or the forearm) (Figure 9). The first mentioned is favoured as it

provides the chance to restore ovarian function and spontaneous conception, while heterotopic transplantation necessitates subsequent ART [135]. For survival, the implanted ovarian tissue must undergo neovascularisation, a critical process with animal studies reporting extensive loss of follicles [136, 137]. Another major concern with this method is the possible reintroduction of malignant cells, while oocyte or embryo cryopreservation confer no such risk. The risk is particularly evident for CCSs with a prior diagnosis of leukaemia, non-Hodgkin lymphoma, or solid tumours with metastases [129, 135]. Before reimplantation, one of the cortical fragments can be histopathologically investigated to ensure the absence of malignant cells [135]. Furthermore, the pioneering transplantation of ovarian tissue was first conducted in 1999, and the first pregnancy resulting from this method was accomplished five years later [138]. In Sweden, the first live birth following transplantation of cryopreserved ovarian tissue was announced in 2013 [139]. Shortly afterwards in 2014, a successful transplantation of cryopreserved ovarian tissue resulted in a spontaneous pregnancy and the first live birth from a graft preserved before menarche at the age of 13 years [140]. To date, >200 live births have been described worldwide using this technique [141]. However, reports on puberty induction and achieved pregnancies from pre-pubescent transplants are still limited [142-144].

Oophoropexy (i.e., transposition of the ovaries) is another established method for patients undergoing ovarian radiotherapy. This method also requires laparoscopic surgery under general anaesthesia. Before planned ovarian radiotherapy, PanCareLIFE makes a moderate recommendation for this procedure after discussion with a radiation oncologist [129]. In the current Swedish guidelines, oophoropexy is not outlined as a recommended fertility preservation measure [130].

The use of GnRHa is still considered an experimental fertility preservation option for childhood cancer patients [129]. Due to findings that pre-pubescent girls have better preserved ovarian function after cancer treatment, it has been hypothesised that GnRHa can return the ovaries to a dormant stage by suppressing the HPG axis [124-128]. In this state, the ovaries could potentially be less susceptible to the gonadotoxic effect of chemotherapeutic agents. An animal study found that GnRHa reduce the ovarian vascular permeability as well as density, and could therefore possibly restrict the amount of chemotherapeutic agent reaching the ovary [145]. Current guidelines state that GnRHa might be recommended for young women with breast cancer, but not as a substitute for proven fertility preservation methods [146].

Cancer survivors experience emotional distress because of comprised reproductive potential [147]. CCSs also express concern about future fertility [148]. About 50% of CCSs reported no memory of receiving counselling regarding gonadotoxic effects on fertility. For those who were counselled, worrying about their offspring's cancer risk was less pronounced and they more often sought fertility investigation [149]. Another study found that survivors want to be informed about future fertility [150]. In addition, females who underwent infertility counselling as well as fertility

preservation prior to their cancer treatment had better quality of life [151]. This illustrates the ongoing need for healthcare providers to offer improved counselling, and all childhood cancer patients and their parents should therefore be informed about the potential future infertility risk [129]. There is also a need to give adequate support for the emotional concerns that survivors experience. Lastly, to provide appropriate counselling on fertility and fertility preservation, it is of great importance to identify treatment risk factors that could jeopardise future reproductive health.

Rationale

When we wrote *paper I*, it was well known that AMH correlates significantly with AFC in healthy females [64]. However, to the best of our knowledge, no studies had previously investigated whether this was also true for CCSs. As several studies indicated decreased AMH levels in CCSs compared with healthy controls [75, 109, 116], we hypothesised that AMH could be a useful serum marker for identifying reduced ovarian reserve in this population. Based on this knowledge gap, current guidelines do not include AMH as a routine ovarian reserve marker in follow-up programmes for assessment of POI in CCSs [10, 85]. Therefore, we wanted to explore whether AMH could serve as a marker for ovarian reserve in CCSs, as well as investigating its value in predicting POI and low AFC.

POI is associated with infertility, a state that is known to have a significant impact on well-being [98, 147]. When planning *paper II*, we wanted to investigate quality of life in all CCSs, and especially CCSs with POI, compared with healthy controls. Our hypothesis was that those CCSs with POI who longed for children but were unable to have them would have poorer quality of life than females who had the opportunity to have as many children as they wanted. Investigating quality of life among females with POI and infertility might not bridge a knowledge gap, but it strengthens the current knowledge within this field and potentially identifies the need for enhanced follow-up and support.

Regarding *paper III*, this study is the first of its kind known to us, to focus on both evaluating and comparing the Swedish and PanCareLIFE infertility risk guidelines. Based on these guidelines, we investigated ovarian markers, fertility, and POI prevalence according to the estimated infertility risk. Furthermore, we explored this in a study population treated before fertility preservation measures could be offered to childhood cancer patients.

It is hypothesised that the larger primordial follicle pool in younger cancer patients could reduce the overall gonadotoxic effect of cancer treatments. To date, several studies have reported an association between younger age at cancer diagnosis (i.e., pre-pubescent) and better preserved ovarian function [124-128]. However, this finding has been mostly reported as an association without making further efforts to seek an explanation in terms of cancer diagnoses and treatments that might differ depending on age when diagnosed. For this reason, in *paper IV*, we divided up CCSs

based on age at diagnosis and evaluated ovarian markers, POI prevalence, and cancer diagnoses and treatments between the groups.

Aims

The overall aim of this thesis was to investigate the late complications on ovarian function after childhood cancer treatment in an adult population of female survivors compared with healthy matched controls.

The specific aims were:

Paper I: To estimate the prevalence of POI and compare ovarian serum markers with AFC and OV.

Paper II: To evaluate quality of life among CCSs with and without POI.

Paper III: To assess ovarian function and fertility using the Swedish and PanCareLIFE infertility risk classifications. We also aimed to evaluate and compare these classifications.

Paper IV: To investigate the association between age at diagnosis, ovarian markers, and POI.

Subjects and methods

Subjects

The studies included adult female survivors who underwent childhood cancer treatment between 1964 and 2008 in southern Sweden. All CCSs were diagnosed below 18 years of age, and had been off their treatment more than two years before inclusion. We excluded those who were diagnosed with rare cancers (e.g., thyroid and skin cancer) and those treated only with surgery for solid tumours outside the CNS. CCSs that met the inclusion criteria were identified from the Swedish Cancer Registry. Healthy controls were recruited from the same geographical region (i.e., southern Sweden) using the Swedish Population Registry and matched for sex, birth date, and ethnicity. Later, upon enrolment, they were also matched regarding smoking habits. Eligible participants were recruited between October 2010 and November 2015.

Data retrieved from the questionnaires included age at menarche, occurrence of primary or secondary amenorrhoea, ongoing or previous HRT, and use of contraceptives. Fertility information included number of pregnancies, children born or adopted, desire for children in the future, and fertility investigation and treatment. We also gathered information regarding relationship status.

The EQ-5D-3L questionnaire was used to evaluate quality of life, which concerns an individual's self-assessment of their health state and well-being. This questionnaire contains two parts: a descriptive system and a visual analogue scale (VAS). The descriptive system explores five dimensions including mobility, hygiene/self-care, usual activities, pain/discomfort, and anxiety/depression. For each dimension, there are three answers: 1=no problems, 2=some problems, and 3=extreme problems. In total, this gives 243 possible health states, e.g., the health state 11111 revealing no problems at all. These health states are converted into an index value, presenting the individual's health state in comparison to the preferences of the general population in a country or region (i.e., a value set). Value sets have been extracted for many countries, including Sweden, by applying the time trade-off (TTO) valuation technique or the VAS valuation technique. The second part of this questionnaire contains a VAS with a range of 0–100, reflecting the subject's health on the present day. On this scale, 0 and 100 represent the worst and the best imaginable health, respectively. This questionnaire provides a quantitative assessment of health at a group level [152]. In our *study II*, the health state is

presented as mean TTO values and well-being as mean EQ-VAS. CCSs with hypothalamic-pituitary-ovarian insufficiency were excluded due to the well-known influence of hypothalamic injuries on quality of life. Subjects who did not complete the EQ-5D-3L questionnaire or who had missing data were also excluded. Moreover, CCSs with POI were compared with CCSs without POI as well as with controls.

We retrieved comprehensive information concerning cancer diagnoses and treatments from medical records, the Childhood Cancer Registry, and BORISS (Paediatric Oncology Registry in South Sweden) [153]. For each CCS, the cumulative doses of chemotherapeutic agents and radiotherapy administered were collected.

In *study III*, CCSs were allocated into four infertility risk groups based on the Swedish guidelines for infertility risk assessment (Table 3) [130]. Recently, the cumulative doses for cyclophosphamide have been lowered from <9 to <6 g/m^2 for the moderate-risk group and from >9 to >6 g/m^2 for the high-risk group. CCSs that were incompatible with the risk groups in Table 3 were distributed to the no risk, surgery only, or unilateral oophorectomy groups. CCSs were also categorised according to the PanCareLIFE infertility risk groups, i.e., groups 1–4 (Table 2) [129]. The PanCareLIFE guidelines utilise CED for their infertility risk assessment, wherefore the following equation was applied: CED (mg/m^2) = 1.0 (cumulative cyclophosphamide dose [mg/m^2]) + 0.244 (cumulative ifosfamide dose [mg/m^2]) + 0.857 (cumulative procarbazine dose [mg/m^2]) + 14.286 (cumulative chlorambucil dose [mg/m^2]) + 15.0 (cumulative BCNU dose [mg/m^2]) + 16.0 (cumulative CCNU dose [mg/m^2]) + 40 (cumulative melphalan dose [mg/m^2]) + 50 (cumulative Thio-TEPA dose [mg/m^2]) + 100 (cumulative nitrogen mustard dose [mg/m^2]) + 8.823 (cumulative busulfan dose [mg/m^2]) (Table 1) [112]. As far as we know, no studies have compared similar cyclophosphamide doses administered intravenously and orally. We therefore chose not to include oral doses of cyclophosphamide in the Swedish infertility risk assessment or in the CED calculation. Furthermore, the definition of alkylating agents provided by van Dorp et al. was used. In this definition, carboplatin, cisplatin, mustine, and dacarbazine are included, but these agents are not incorporated into the calculation of CED [85].

For the final *study IV*, CCSs were divided into two groups based on the age at diagnosis, i.e., ≤8.4 and >8.4 years when diagnosed. This cut-off was derived from the median age when diagnosed.

Ethical approval

Before inclusion, all subjects gave their written informed consent. *Studies I–IV* were approved by the Regional Ethics Committee, Medical Faculty, Lund University, Sweden (approval no. 523/2009).

Ultrasound

All included subjects underwent clinical examination at the Reproductive Medicine Centre (RMC) at Skåne University Hospital, Malmö, Sweden, during the period October 2010–January 2015. To estimate AFC (i.e., the sum of follicles measuring 2–10 mm in diameter in both ovaries) and OV (i.e., the total volume of both ovaries), six different clinicians performed transvaginal ultrasound with BK Medical Flex Focus 500 and BK Medical Pro Focus scanners. Only females with an assessment of the total AFC and OV of both ovaries were included. The limit for diminished ovarian reserve was set at AFC below 10 [154].

Hormonal assays

Blood samples for AMH, inhibin B, FSH, and 17β-oestradiol (E2) were obtained during fasting on days 2–5 of the menstrual cycle (i.e., early follicular phase) for females with regular cycles and on a random day for those with irregular cycles, amenorrhea, or the use of HRT or OC. For the analyses of FSH and E2, females with ongoing use of HRT were excluded. Likewise, subjects who were taking OC as well as progesterone in the form of a pill, subcutaneous implant, or depot injection were also excluded. Since an intrauterine device, whether progesterone or copper, does not appear to affect the levels of FSH and E2, subjects with these devices were included in the analyses [155]. All blood samples were centrifuged and kept at -20°C prior to the analysis.

Serum AMH and inhibin B levels were analysed at the Laboratory of Reproductive Biology, Copenhagen, Denmark. The ultrasensitive enzyme-linked immunosorbent assay (ELISA) was used for AMH analysis, with a detection threshold of 0.023 ng/ml. Values below this limit were stated as 0. We measured inhibin B using MCA1312KZZ (Oxford Bio-Innovation Ltd., Oxfordshire, UK) with a detection limit of 20 pg/ml (i.e., values <20 pg/ml were specified as 0).

FSH levels (Roche) were analysed at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden. During *study I*, the FSH method was updated, with values for 56 CCSs analysed using the previous assay with a higher

detection limit (i.e., 0.2 IU/L). Other remaining CCSs, including controls, had FSH levels measured with the latest assay (Roche) (i.e., detection limit of 0.1 IU/L). The FSH levels measured with the previous assay were therefore recalculated to the most recent assay using a conversion factor as outlined by Bobjer et al. [156]. FSH values below the detection limit (i.e., <0.1 IU/L) were specified as 0.

E2 levels (DELFIA PerkinElmer Inc.) were measured at the same department as FSH. The detection threshold was 10 pmol/L, and values below this threshold were stated as 0. The intra- and inter-assay variation coefficients were less than 10%.

POI diagnosis

The former introduced definition of POI was not applicable in our studies, because nearly all subjects with ovarian insufficiency were treated with HRT. Therefore, FSH and E2 levels were not reliable for POI diagnosis. Hence, we defined POI as amenorrhoea and ongoing or earlier HRT treatment, together with undetectable (i.e., <0.023 ng/ml) or very low (i.e., <0.1 ng/ml) AMH levels, in females aged <40 years. Hypothalamic-pituitary-ovarian insufficiency was not included in this definition.

Assessment of ovarian radiotherapy dose

Altogether, 21 CCSs were treated with craniospinal or flank radiotherapy. Estimations of the radiotherapy doses to the right and left ovaries were extracted from the subject's radiotherapy records, along with x-ray images and/or anatomical sketches or photographs of radiotherapy fields, by two radiation physicists and a radiation oncologist. For many subjects, the dose to each ovary could not be estimated precisely because the positioning of the ovaries in relation to the radiation field was inconclusive, and these doses were therefore estimated as intervals. In case of a discrepancy between the dose to the ovaries, the subject was grouped into the infertility risk classifications based on the lowest dose or interval.

Statistical analyses

Study I

For evaluation of data distribution, histograms were applied. We presented descriptive data as mean (range), count, and percentage. Independent sample t-test and Fisher's exact test were used to analyse continuous and categorical variables, respectively. Scatter plots illustrated correlations, which were calculated with Pearson correlation. For continuous variables, linear regression was performed to compare CCSs and controls. As FSH data was not normally distributed, additional analyses were added such as Mann-Whitney U test and Spearman correlation. ROC curves were performed to evaluate variables as predictors of several outcomes. $P<0.05$ was regarded as statistically significant, and the confidence interval (CI) was set at 95%. The IBM Statistical Package for the Social Science (SPSS) version 24 was used for statistical analyses.

Study II

Univariate analysis of variance and independent sample t-test were used to compare continuous variables between the groups. Chi-squared test was performed to compare categorical variables among the groups. TTO values and EQ-VAS were analysed for normal distribution within the groups using the Shapiro-Wilk test. For pairwise comparisons between more than two groups, a relevant post hoc test was performed. Linear regression analysis was used with adjustment for variables such as age at examination, having a child (yes or no), current desire for a child, desire for future children, and partnership status. We chose these variables as they might impact TTO values and EQ-VAS. The same analyses were conducted to compare the group who had the number of children they wanted with the group who did not, but only adjusted for the variables age at examination and partnership status. The CI and statistically significant *p*-value were set at 95% and <0.05, respectively. All statical analyses were conducted in SPSS version 25.

Study III

Continuous variables were analysed with Mann-Whitney U test, Pearson correlation, partial correlation, and linear regression. Correlations between variables are presented with a scatter plot. Histograms were performed to evaluate data distribution. For comparisons of more than two groups, the Kruskal-Wallis test and the Bonferroni correction were used. The Fisher's exact test and chi-squared test were applied for categorical variable analyses. The statistical analyses were two-

sided. We showed data as median, range, count, and percentage. The significance level and CI were the same as mentioned above. SPSS version 26 was used for the statistical analyses.

Study IV

Version 29 of SPSS was used for data analysis. Data is presented as count, percentage, median, and range. Histograms evaluated data distribution. Mann-Whitney U test and linear regression were used to analyse continuous variables. A scatter plot presents correlation, calculated with partial correlation. Categorical variables were analysed with the Fisher's exact test. For analysis of a binary variable, we performed logistic regression. Statistical analyses were conducted as two-sided. The CI and significance level were as previously mentioned.

Alternative methods

Our methodology is quantitative research with a cross-sectional study design. The advantages of cross-sectional studies are that they are often less expensive and time-consuming to conduct. They are carried out during a specific point in time (i.e., without prospective or retrospective follow-up), and are therefore the best approach for measuring prevalence. Furthermore, participants are not treated or exposed to interventions as they are in clinical trials, making it easier to obtain an ethical approval. However, cross-sectional studies also have limitations, such as an inability to determine incidence and causality. Moreover, they are also prone to biases, including selection bias (e.g., nonresponse bias) and information bias (e.g., recall bias) [157]. For example, in our study population, CCSs with morbidities or known reproductive issues may have been more likely to participate. On the other hand, there is also a risk of including more healthy individuals in the study population, the so-called healthy volunteer bias [158]. Hence, in both cases, the characteristics of participants and nonparticipants may differ. To reduce the risk of selection bias, we performed a drop-out analysis on offspring data for both nonparticipants and participants since ovarian function was studied, as well as compared the distribution of cancer diagnoses among CCSs with the female population of the same age diagnosed with cancer in Sweden during the period 1984–2010. In addition, there is always a risk of confounding in cross-sectional studies. To minimise this risk, we conducted matching for several factors as previously mentioned. However, controls were not matched for the use of HRT and OC, and we therefore analysed data for groups and not paired data. We also adjusted several of our analyses by using multivariable regression analysis, which is another method to control for confounding [157]. The strengths and limitations of our studies are further outlined under *Discussion*.

An alternative observational study design that could have been applied is a cohort study (i.e., a prospective longitudinal study). This type of study with long follow-up could evaluate the change rate of ovarian markers and thus estimate the timing of POI onset as well as the incidence. Moreover, this study design could also estimate the prognostic potential of ovarian markers in predicting fertility outcomes (i.e., pregnancies and live births) in CCSs. However, the disadvantage is certainly the time interval between childhood cancer treatment (i.e., exposure) and POI occurrence (i.e., outcome), which sometimes spans several decades and could potentially contribute to a substantial loss to follow-up [159]. In addition, this type of study would be challenging to carry out during a PhD study period.

For our *study II*, instead of using questionnaires to assess quality of life, a qualitative method could have been used. Data could have been collected through face-to-face in-depth interviews or focus group discussions. The most suitable approach to analyse data would be using content analysis, providing a narrative summary of the findings. However, downsides to this method are that they are more time-consuming than questionnaires and can also be more expensive to undertake [160].

Results

Study I

Participants

Using data from the Swedish Cancer Registry, we identified 575 females from the southern region of Sweden who met the inclusion criteria. Besides the previously mentioned exclusion criteria, an additional 244 subjects were excluded because of gynaecological cell atypia or cancer in situ. Two hundred and two females agreed to participate, among them, four with severe disabilities, three pregnant, and 28 who withdrew due to time constraints were excluded. The final study population consisted of 167 CCSs at a mean age of 34.3 years (19.3–57.8). Initially, 167 female controls were enrolled, however, three dropped out after clinical examination, leaving 164 controls included at a mean age of 35.0 years (19.3–58.0). Background data for CCSs and controls are presented in Table 4.

Table 4. The main characteristics for CCSs and controls.

	CCSs n=167	Controls n=164
Age at examination (years)	34.3 (19.3–57.8)	35.0 (19.3–58.0)
Age at diagnosis (years)	8.9 (0.1–17.9)	n.a.
Years since diagnosis	25.4 (11.6–41.3)	n.a.
Height (cm)	164.3 (143.0–181.5)**	168.5 (150.0–186.4)
Weight (kg)	67.7 (41.0–125.0)	66.8 (46.6–107.2)
Body mass index (kg/m^2)	25.1 (16–44)**	23.5 (18–35)
Age at menarche (years)	12.8 (9–17)	13.0 (9–19)
HRT	n=20 (12%)**	n=0
OC (p-ring and systemic gestagens/progestins included)	n=48 (29%)	n=58 (34%)
POI (primary)	n=22 (13%)**	n=0
Hypothalamic-pituitary-ovarian insufficiency	n=5 (3%)	n=0

Data is shown as mean, range, count, and percent. Three CCSs administered OC as HRT, and are therefore included in both groups. CCSs: childhood cancer survivors; n.a.: not applicable; HRT: hormone replacement therapy; OC: oral contraceptives; POI: premature ovarian insufficiency. ** $p<0.01$ calculated with independent sample t-test and Fisher's exact test.

We collected data from the Swedish Multi-Generation Registry, Statistics Sweden, on offspring for both participating and nonparticipating CCSs, to conduct a drop-out analysis since ovarian function was studied. CCSs in our study were representative of female CCSs in Sweden, with similar distribution figures concerning number of children (0, 1, 2, or >2): 51, 14, 23, and 11% among included CCSs; 50, 26, 18, and 6% among nonrespondents; and 53, 13, 24, and 10% among those who declined, respectively. The distribution of cancer diagnoses for females <19 years of age in Sweden was derived from the Swedish Childhood Cancer Foundation, and was comparable with childhood cancer diagnoses in our study (Table 5).

Table 5. The childhood cancer diagnoses for CCSs as well as for females <19 years in Sweden in the period 1984–2010.

Diagnosis	CCSs n=167 (%)	Females <19 years in Sweden %
Leukaemia	51 (31)	29
Brain tumour	39 (23)	28
Lymphoma	21 (13)	9
Wilms tumour	19 (11)	6
Sarcoma	18 (11)	9
Ovarian tumour	11 (7)	5
Other	8 (5)	14

CCSs: childhood cancer survivors.

Ovarian markers

AMH and inhibin B levels were available for 166 CCSs and 163 controls. FSH was accessible for all participants, however, those on HRT or OC were excluded, with 102 CCSs and 106 controls included for analysis. E2 was also available for every participant, but those taking HRT or OC as well as those where a non-sensitive method was used for analysis (i.e., two CCSs and three controls) were excluded, resulting in 100 CCSs and 105 controls. AFC was estimated for 135 CCSs and 157 controls, and OV for 129 CCSs and 155 controls. Females were excluded from analysis of AFC and OV in the case of virginity, ovarian cysts, unilateral or bilateral oophorectomy, or non-imaging. Moreover, AFC was assessed in some subjects despite non-measurable OV due to follicular cysts.

Serum markers compared with ultrasound markers

We compared ovarian serum markers (i.e., AMH, inhibin B, FSH, and E2) with ultrasound markers (i.e., AFC and OV). Mean AMH levels were 2.9 and 3.1 ng/ml for CCSs and controls (p=0.486), respectively. AMH showed the strongest correlation with AFC among both CCSs and controls, with no significant difference between the groups. We also noted a significant correlation between AMH and OV, without any difference between CCSs and controls (Table 6).

Table 6. Correlations between ovarian serum markers and ultrasound markers among CCSs and controls.

	CCSs n=167 r	**Controls n=164 r**	**Linear regression analysis comparing CCSs and controls *p***
AMH-AFC	0.667** (n=134)	0.630** (n=156)	0.136
AMH-OV	0.433** (n=128)	0.529** (n=154)	0.096
Inhibin B-AFC	0.403** (n=134)	0.364** (n=156)	0.073
Inhibin B-OV	0.523** (n=128)	0.469** (n=154)	0.333
FSH-AFC	-0.444**, *-0.394*** (n=86)	-0.393**, *-0.520*** (n=101)	0.819
FSH-OV	-0.419**, *-0.291*** (n=81)	-0.288**, *-0.442*** (n=99)	0.870
E2-AFC	-0.022 (n=84)	-0.041 (n=99)	0.206
E2-OV	0.037 (n=79)	0.015 (n=97)	0.487
AFC-OV	0.635** (n=128)	0.528** (n=153)	0.051

Pearson and Spearman correlations as well as linear regression were used. CCSs: childhood cancer survivors; r: Pearson's correlation coefficient, in italics Spearman's correlation coefficient; AMH: anti-Müllerian hormone; AFC: antral follicle count; E2: 17β-oestradiol; FSH: follicle-stimulating hormone. ***p*<0.01.

There was a significant difference for mean inhibin B between the groups: 37.7 and 53.2 pg/ml for CCSs and controls ($p<0.01$), respectively. Inhibin B correlated significantly with both AFC and OV among CCSs and controls. No differences were noted between the groups (Table 6).

FSH mean levels were 17.8 vs. 11.6 IU/L, and median levels were 7.0 vs. 6.6 IU/L among CCSs and controls ($p=0.049$ and $p=0.47$), respectively. The FSH levels correlated negatively with AFC and OV in both groups. We observed no difference among CCSs and controls (Table 6).

E2 mean levels were 185.9 pmol/L for CCSs and 169.6 pmol/L for controls ($p=0.48$), with no correlation observed with AFC or OV for the groups. We noted no difference when comparing the groups (Table 6).

We observed no difference in mean AFC: 12.6 for CCSs and 14.2 for controls ($p=0.12$). Mean OV was significantly lower for CCSs compared with controls, 7.7 and 9.4 cm^3 ($p=0.01$), respectively. There was a significant correlation between AFC and OV among both groups, with no difference noted between them (Table 6).

POI

We found POI in 22/167 (13%) CCSs. Of these, 15 were on HRT, with an additional five CCSs taking HRT due to hypothalamic-pituitary-ovarian insufficiency. Among the 15 CCSs receiving therapy, 11 out of 12 were below the age of 40 years, three out of seven were aged between 40 and 50, and one out of three was over 50 years of age. None of the controls had primary or secondary amenorrhea <40 years or were treated with HRT.

ROC curve analysis for ovarian markers

ROC curve analysis was applied to test the accuracy of ovarian markers as predictors of POI and diminished ovarian reserve (i.e., AFC <10). Ovarian markers included in the analyses were AMH, inhibin B, AFC, and OV. FSH and E2 could not be assessed due to the limited number of participants included after exclusion for HRT and OC. ROC curves with area under the curve (AUC) values are presented in Figure 10 and Table 7.

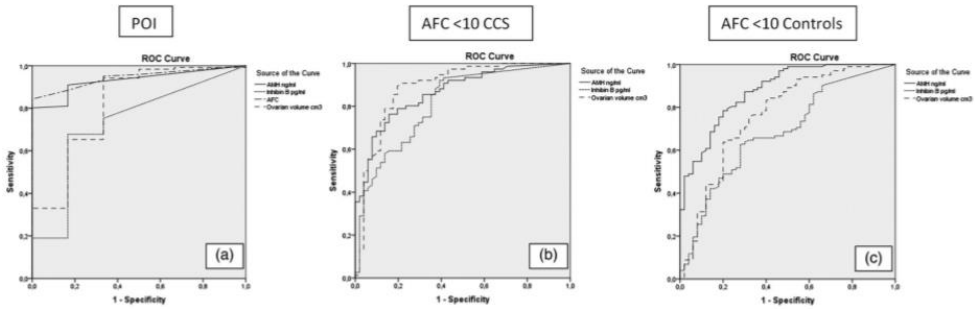

Figure 10. Receiving Operating Characteristic (ROC) curves with area under the curve (AUC). (a) Premature ovarian insufficiency (POI) in childhood cancer survivors (CCSs); (b) antral follicle count (AFC) <10 among CCSs; (c) AFC <10 among controls. AUC values are presented in Table 7.

Table 7. AUC values for ovarian markers for POI among CCSs and for AFC <10 among both CCSs and controls.

	CCSs POI[a] n=167	CCSs AFC <10[a] n=167	Controls AFC <10[a] n=164
AMH	0.930 (0.870–0.989)	0.866 (0.804–0.928)	0.878 (0.821–0.934)
Inhibin B	0.729 (0.516–0.941)	0.814 (0.738–0.891)	0.684 (0.595–0.774)
OV	0.815 (0.614–1.000)	0.890 (0.826–0.953)	0.765 (0.680–0.851)
AFC	0.944 (0.896–0.992)		

[a]AUC (95% CI). AUC: area under the curve; POI: premature ovarian insufficiency; CCSs: childhood cancer survivors; AFC: antral follicle count; AMH: anti-Müllerian hormone; OV: ovarian volume; CI: confidence interval.

Study II

Participants

The study population was the same as described in *study I*. Altogether, five CCSs had POI due to hypothalamic-pituitary-ovarian insufficiency and were therefore excluded. Two controls did not answer the EQ-5D-3L questionnaire and were also excluded from the analyses. In total, 22 CCSs with POI, 140 CCSs without POI, and 162 controls were included.

Age at examination differed between the three groups: CCSs with POI, CCSs without POI, and controls (p=0.031) (Table 8). This difference was no longer observable when all CCSs were compared with controls (p=0.454). Among the three groups, we also noted a significant difference regarding BMI, FSH, and HRT (p<0.000 for all comparisons). CCSs with POI had a reduced mean number of biological children compared with CCSs without POI and controls, 0.19, 1.05, and

1.10 (p=0.005), respectively. We noted that adoption and the use of ART were more pronounced among those with POI (p<0.000). In addition, no overall difference was noted for mean E2 levels and for partnership status (Table 8).

Table 8. Clinical parameters for CCSs with and without POI and controls.

	CCSs with POI n=22	**CCSs without POI n=140**	**Controls n=162**	***P*-value**
Age at examination (years)	38.9 (22–56)	33.6 (19–58)	35.0 (19–58)	**0.031**
BMI (kg/m^2)	23.4 (17.7–34.1)	25.1 (16.2–43.8)	19.8 (14.5–29.6)	**<0.000**
FSH (IU/L)	41.4 (0.6–131.0)	10.5 (0.0–124.0)	9.1 (0.2–109.0)	**<0.000**
E2 (pmol/L)	206.0 (25–1020)	178.8 (17–1342)	162.9 (15–1232)	0.511
HRT	n=15 (68%)	0	0	**<0.000**
Biological children	0.19 (0–2)	1.05 (0–5)	1.10 (0–5)	**0.005**
Adopted children	0.18 (0–1)	0 (0–0)	0.01 (0–1)	**<0.000**
ART	n=8 (36%)	n=9 (6%)	n=12 (7%)	**<0.000**
Married/domestic partnership	n=13 (59%)	n=80 (57%)	n=104 (64%)	0.450

Data is presented as count, mean, range, and percent. CCSs: childhood cancer survivors; POI: premature ovarian insufficiency; BMI: body mass index; FSH: follicle-stimulating hormone; E2: 17β-oestradiol; HRT: hormone replacement therapy; ART: assisted reproductive technology. *P*-value comparing the three groups, analysed with the univariate analysis of variance or chi-squared test.

When comparing CCSs with and without POI, those with POI were more often treated with radiotherapy, especially abdominal radiotherapy, total body irradiation (TBI), chemotherapy, particularly with alkylating agents, and HSCT (Table 9). None of the CCSs with POI were treated with surgery only.

Table 9. Treatments for CCSs with/without POI.

Type of treatment	CCSs with POI n=22 (%)	CCSs without POI n=140 (%)
All radiotherapy	17 (77)	65 (46)
Cranial radiotherapy	7 (23)	41 (29)
Abdominal radiotherapy	16 (73)	17 (12)
Cranial and abdominal radiotherapy	7 (32)	9 (6)
TBI	5 (23)	2 (1)
All chemotherapy	20 (91)	104 (74)
Alkylating agents	14 (64)	65 (46)
HSCT	7 (23)	4 (3)
Only surgery	0	19 (14)

Five CCSs with hypothalamic-pituitary-ovarian insufficiency were excluded. Data is shown as count and percent. CCSs: childhood cancer survivors; POI: premature ovarian insufficiency; TBI: total body irradiation; HSCT: hematopoietic stem cell transplantation.

TTO values

In total, 22 CCSs with POI, 138 CCSs without POI, and 159 controls were included for analyses of health state, described as mean TTO values. Participants with missing data were excluded. TTO values differed significantly across the three groups: 0.8892, 0.8913, and 0.9230 among CCSs with POI, CCSs without POI, and controls (unadjusted p=0.010, adjusted p=0.027), respectively (Table 10). When a post hoc test was performed for the unadjusted analysis, there was only a significant difference in mean TTO values between CCSs without POI and controls. All CCSs compared with controls had significantly lower mean TTO values: 0.8910 vs. 0.9230 (unadjusted p=0.002, adjusted p=0.007), respectively. We adjusted for the following factors: age at examination, having a child (yes or no), current desire for a child, desire for future children, and partnership status (i.e., marital status/domestic partnership).

Table 10. Health state presented as mean TTO values and well-being as mean EQ-VAS for CCSs with POI, CCSs without POI, and controls.

	CCSs with POI n=22	CCSs without POI n=138[a]/140[b]	Controls n=159[a]/161[b]	Unadjusted overall *p*-value (with post hoc test)	Adjusted overall *p*-value
TTO value	0.8892	0.8913	0.9230	**0.010**	**0.027**
EQ-VAS	74.5	79.0	83.4	**0.037[c], 0.024[d]**	**0.017**

[a]n in TTO analysis, [b]n in EQ-VAS analysis, [c]CCSs without POI compared with controls, [d]CCSs with POI compared with controls. TTO: time trade-off; VAS: visual analogue scale; CCSs: childhood cancer survivors; POI: premature ovarian insufficiency. Overall *p*-value analysed with the univariate analysis of variance.

EQ-VAS

For well-being presented as mean EQ-VAS, we included 22 CCSs with POI, 140 CCSs without POI, and 161 controls. Participants with missing data were not included. An overall difference between the three groups was observed (adjusted p=0.017) (Table 10). The entire group of CCSs had significantly reduced mean EQ-VAS compared with controls, 78.4 and 83.4 (unadjusted p=0.003, adjusted p=0.012), respectively. Mean EQ-VAS was 74.5 among CCSs with POI compared with 83.4 among controls (unadjusted p=0.024). CCSs without POI also had significantly lower mean EQ-VAS compared with controls, 79.0 and 83.4 (unadjusted p=0.037), respectively (Table 10). We adjusted for the same variables as mentioned under *TTO values*.

TTO values and EQ-VAS for current desire for a child

For these analyses, CCSs and controls were combined and thereafter divided into two groups, i.e., one group having the number of children they desired and one group not having their wishes fulfilled regarding number of children. The latter group had answered yes to one of the following: planning biological children in the future, unable to have biological children (i.e., due to ovarian insufficiency or uterine incapacity for pregnancy), partner unable to have children, partner not interested in having children, attempting pregnancy for less or more than two years, or I consider myself too old or I am too old (if not, I would wish for a child). For 39 participants, the desire to have children has never been a consideration and they were therefore excluded from the analyses. The group with the number of children they wanted consisted of 118 females, and the other group consisted of 167 females. The mean TTO values and EQ-VAS did not differ among the group with the number of children they wanted and the group that did not have the number of children they

wanted: 0.91 vs. 0.91 (unadjusted p=0.524, adjusted p=0.551) and 81.1 vs. 80.7 (unadjusted p=0.705, adjusted p=0.227), respectively. We adjusted for age at examination and partnership status.

Study III

Participants

The included study population was the same as described in previous studies, i.e., 167 CCSs and 164 controls. In this study, CCSs were divided into infertility risk groups according to the PanCareLIFE and Swedish guidelines, based on cancer treatments given (Tables 2 and 3, respectively). Cancer treatments given for all CCSs are presented in Table 11. Among the 167 CCSs, eight were excluded from the Swedish infertility risk grouping (i.e., six and two due to inconclusive data and bilateral oophorectomy, respectively). For the PanCareLIFE infertility risk groups, two CCSs were excluded because of bilateral oophorectomy, leaving 165 CCSs. As AMH levels decline with advanced age, we also performed analyses for females aged <40 years, including 120 CCSs and 113 controls for the PanCareLIFE infertility risk groups. For the Swedish infertility risk groups aged <40 years, two CCSs were excluded as ovarian radiotherapy doses could not be determined.

Table 11. Cancer treatments for all CCSs.

Treatment	All CCSs n=167 n (%)
Chemotherapy	127 (76)
Alkylating agents	81 (49)
Radiotherapy	87 (52)
Radiotherapy, abdominal and cranial	16 (10)
Radiotherapy, abdominal	34 (20)
Radiotherapy, cranial	53 (32)
TBI	7 (4)
HSCT	11 (7)
Surgery only	19 (11)

CCSs: childhood cancer survivors; TBI: total body irradiation; HSCT: hematopoietic stem cell transplantation.

AMH, AFC, and OV

In this study, only AMH is presented as an ovarian serum marker, as it was the most accurate serum marker for evaluating ovarian reserve in CCSs according to *study I*. We also included AFC and OV as ovarian markers for this study. The number of included participants for each of the ovarian reserve markers has previously been reported in *study I.*

Median AMH and AFC levels did not differ between CCSs and controls: 1.9 vs. 2.1 ng/ml and 12.0 vs. 13.0 (p=0.065 and p=0.096), respectively. However, median OV levels were significantly reduced in CCSs 6.8 cm^3 compared with controls 8.0 cm^3 (p=0.021). Adjustments for the use of HRT and OC were not performed in our analyses, and median AMH levels were therefore compared among CCSs (n=65) and controls (n=57) on HRT or OC: 1.8 and 2.4 ng/ml (p=0.077), respectively.

For females <40 years, we noted no difference regarding median AMH levels among CCSs (n=120) and controls (n=112), 3.3 and 3.2 ng/ml (p=0.108), respectively. Both median AFC and OV were significantly lower among CCSs compared with controls: 13.0 (n=102) vs. 15.0 (n=109) and 7.6 (n=98) vs. 9.1 cm^3 (n=108) (p=0.026 and p=0.009), respectively.

There was a significant negative correlation between AMH and age at examination for both CCSs (r=-0.435, p<0.001, n=166) and controls (r=-0.497, p<0.001, n=163). Linear regression analysis showed that CCSs had somewhat reduced levels of AMH at all ages compared with controls. However, the difference was neither significant (p=0.224) nor more prominent with advancing age (Figure 11).

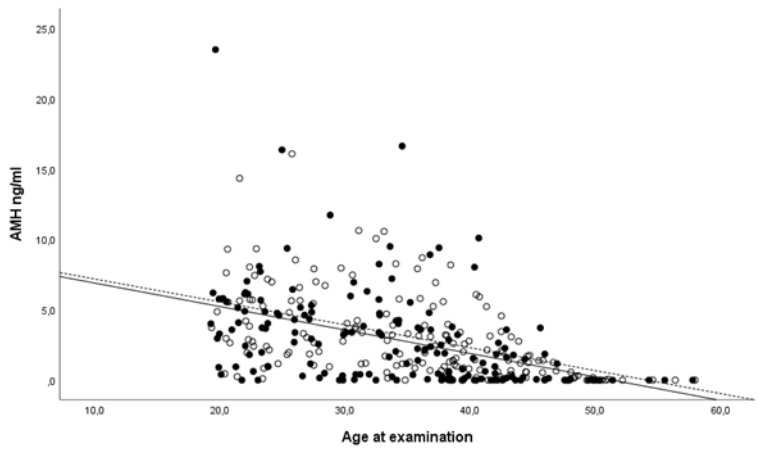

Figure 11. Scatter plot illustrating the negative correlation between AMH levels and age at examination among both CCSs (●+filled line, r=-0.435, p<0.001, n=166) and controls (○+dashed line, r=-0.497, p<0.001, n=163). AMH: anti-Müllerian hormone; CCSs: childhood cancer survivors.

Swedish infertility risk groups, <40 years

CCSs were allocated to seven different groups based on treatments. In addition to the four infertility risk groups outlined in the Swedish guidelines (Table 3), we added three more groups for the CCSs who were outside the treatment scope: no risk (i.e., received none of the therapies in Table 3), surgery only, and unilateral oophorectomy. Altogether, 118 CCSs and 113 controls <40 years of age were included for the analyses (i.e., two CCSs with inconclusive ovarian radiotherapy doses were excluded). We distributed 12 CCSs to the no risk group, 26 to the low-risk group, 24 to the moderate-risk group, 28 to the high-risk group, 10 to the very high-risk group, 11 to the only surgery group, and seven to the unilateral oophorectomy group (Table 12).

There was an overall difference for both age at examination and age at diagnosis between the groups (p=0.049 and p=0.007, respectively), but no differences were observed with pairwise comparisons. Furthermore, AMH and AFC varied significantly among the groups (p<0.001 for both analyses) (Table 12). Pairwise comparisons showed significantly reduced median AMH levels for the very high-risk group 0.0 ng/ml and the high-risk group 1.1 ng/ml, compared with controls 3.2 ng/ml (p<0.001 and p=0.034, respectively) (Figure 12a). Median AFC was also significantly lower in the very high-risk group and the high-risk group compared with controls: 3.0 and 9.0 vs. 15.0 (p=0.003 and p<0.001), respectively (Figure 12b). OV did not differ between the groups (p=0.054) (Table 12 and Figure 12c).

We performed linear regression analysis for AMH to adjust for age at examination, with the very high-risk group and the high-risk group having 3.5 and 1.7 ng/ml decreased levels of AMH compared with the controls (p=0.001, 95% CI -5.6 – -1.4 and p=0.012, 95% CI -3.0 – -0.4, adjusted for age at examination), respectively. The unadjusted analysis demonstrated comparable results (not presented).

Table 12. Ovarian markers in CCSs (n=120) and controls (n=113), <40 years.

	No risk	**Low-risk**	**Moderate-risk**	**High-risk**	**Very high-risk**	**Only surgery**	**Unilateral oophorectomy**	**All CCSs <40 years**	**Controls <40 years**	***p*-value All CCSs vs. controls <40 years**	**Overall *p*-value**
N	12	26	24	28	10	11	7	120	113	n.a.	n.a.
Age at examination (years)	30.3 (19.6–37.4)	28.5 (19.8–37.3)	25.3 (19.5–38.6)	33.7 (19.3–39.5)	30.3 (21.8–39.8)	26.4 (22.2–38.3)	38.3 (25.0–39.4)	30.6 (19.3–39.8)	30.8 (19.3–39.9)	0.599	**0.049**
Age at diagnosis (years)	10.2 (1.3–15.6)	3.5 (0.6–12.0)	3.4 (0.1–17.0)	8.1 (0.4–17.2)	9.7 (1.8–17.2)	6.7 (0.1–15.1)	12.5 (4.6–15.0)	6.4 (0.1–17.2)	n.a.	n.a.	**0.007**
AMH (ng/ml)	3.3 (0.5–23.5)	4.3 (0.4–8.9)	4.0 (0.2–16.6)	1.1 (0.0–7.2)	0.0 (0.0–4.9)	3.7 (0.1–9.4)	0.1 (0.0–16.4)	3.3 (0.0–23.5)	3.2 (0.0–16.1)	0.108	**<0.001**
AFC	13.5 (3–47)	14.0 (4–28)	19.0 (3–38)	9.0 (0–23)	3.0 (0–16)	21.0 (10–26)	n.a.	13.0 (0–47)	15.0 (5–40)	**0.026**	**<0.001**
OV (cm^3)	6.8 (1.5–15.4)	6.2 (1.7–21.4)	8.1 (2.2–20.2)	6.0 (0.7–20.5)	5.5 (0.5–21.0)	10.6 (3.2–15.5)	n.a.	7.6 (0.25–21.4)	9.1 (1.6–36.7)	**0.009**	0.054

CCSs were divided into Swedish infertility risk groups based on cancer treatments outlined in Table 3. Risk grouping was unavailable for two CCSs. Data is shown as count, median, and range. CCSs: childhood cancer survivors; AMH: anti-Müllerian hormone; AFC: antral follicle count; OV: ovarian volume; n.a.: not applicable. *P*-value for all CCSs vs. controls analysed with Mann-Whitney U test. Overall *p*-value analysed with Kruskal-Wallis test.

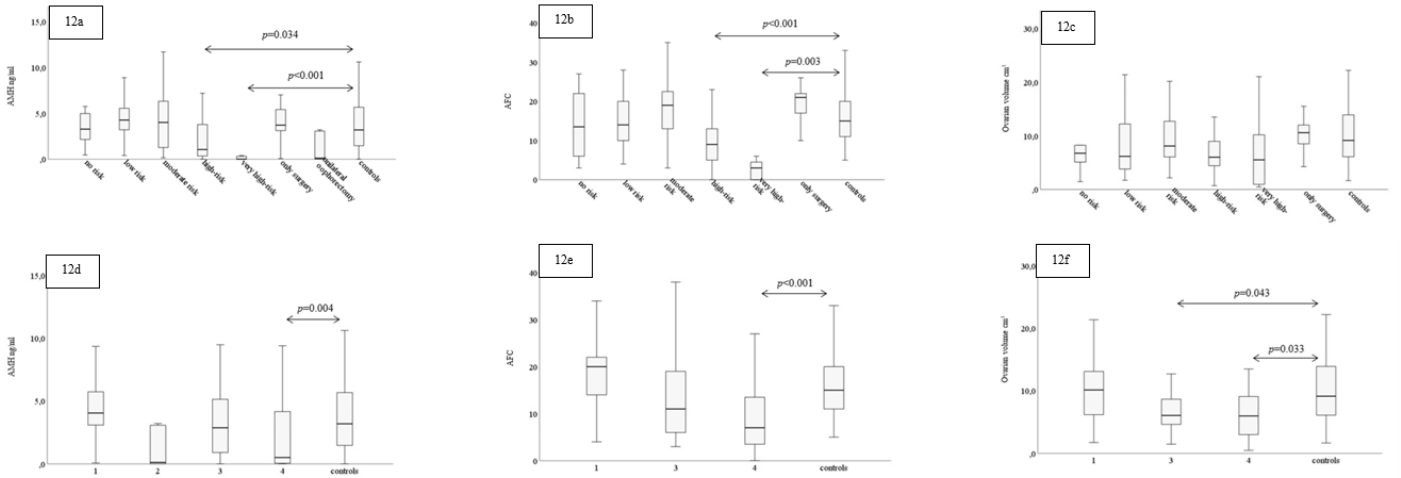

Figure 12. Pairwise comparisons presented for the Swedish infertility risk groups (12a–c) and the PanCareLIFE infertility risk groups (12d–f) in comparison with controls for AMH, AFC, and OV. 120 CCSs and 113 controls below 40 years of age were included. Two CCSs were excluded from the Swedish infertility risk groups because of inconclusive ovarian radiotherapy doses. Outliers are not visualised. AMH: anti-Müllerian hormone; AFC: antral follicle count; OV: ovarian volume; CCSs: childhood cancer survivors. The Kruskal-Wallis test and the Bonferroni correction were applied.

PanCareLIFE infertility risk groups, <40 years

In total, 120 CCSs and 113 controls below 40 years of age were included in the PanCareLIFE infertility risk groups based on Table 2. The cancer treatments for group 4 are considered the most gonadotoxic (i.e., CED ≥6 g/m^2, ovarian radiotherapy irrespective of dose, or HSCT). Group 3 underwent treatments including CED <6 g/m^2 or cranial radiotherapy. Those who were treated with unilateral oophorectomy were categorised into group 2, regardless of other cancer treatments. All other CCSs with treatment not consistent with those of groups 2–4 were assigned to group 1. We categorised 40 CCSs to group 1, seven to group 2, 38 to group 3, and 35 to group 4 (Table 13).

There was an overall difference in age at examination between the groups (p=0.005). Pairwise comparisons showed that age at examination was significantly lower in group 1 25.7 years compared with controls 30.8 years (p=0.048). Age at diagnosis also differed across the groups (p=0.010), however, no differences were observed with pairwise comparisons (Table 13).

AMH, AFC, and OV differed significantly between the groups (p<0.001, p<0.001, and p=0.002, respectively) (Table 13). Median AMH levels and AFC were significantly lower for group 4 in comparison with controls: 0.5 vs. 3.2 ng/ml and 7.0 vs. 15.0 (p=0.004 and p<0.001, respectively) (Figures 12d and e). In addition, OV was significantly decreased in group 3 6.1 cm^3, and in group 4 6.0 cm^3 compared with controls 9.1 cm^3 (p=0.043 and p=0.033, respectively) (Figure 12f).

Table 13. Ovarian markers in CCSs (n=120) and controls (n=113), <40 years.

Group	1	2	3	4	All CCSs <40 years	Controls <40 years	*p*-value All CCSs vs. controls <40 years	Overall *p*-value
N	40	7	38	35	120	113	n.a.	n.a.
Age at examination (years)	25.7 (19.6–38.3)	38.3 (25.0–39.4)	33.3 (19.9–39.4)	30.7 (19.3–39.8)	30.6 (19.3–39.8)	30.8 (19.3–39.9)	0.599	**0.005**
Age at diagnosis (years)	4.9 (0.1–15.1)	12.5 (4.6–15.0)	7.2 (0.3–17.2)	7.9 (0.4–17.2)	6.4 (0.1–17.2)	n.a.	n.a.	**0.010**
AMH (ng/ml)	4.0 (0.1–23.5)	0.1 (0.0–16.4)	2.9 (0.0–11.7)	0.5 (0.0–9.4)	3.3 (0.0–23.5)	3.2 (0.0–16.1)	0.108	**<0.001**
AFC	20.0 (4–47)	n.a.	11.0 (3–38)	7.0 (0–27)	13.0 (0–47)	15.0 (5–40)	**0.026**	**<0.001**
OV (cm^3)	10.1 (1.7–21.4)	n.a.	6.1 (1.5–20.8)	6.0 (0.5–21.0)	7.6 (0.25–21.4)	9.1 (1.6–36.7)	**0.009**	**0.002**

CCSs were divided into PanCareLIFE infertility risk groups based on cancer treatments outlined in Table 2. Data is shown as count, median, and range. CCSs: childhood cancer survivors; AMH: anti-Müllerian hormone; AFC: antral follicle count; OV: ovarian volume; n.a.: not applicable. *P*-value for all CCSs vs. controls analysed with Mann-Whitney U test. Overall *p*-value analysed with Kruskal-Wallis test.

Fertility outcomes for all ages

When comparing all CCSs with controls, we noted a tendency of decreased fertility in terms of conceiving as well as the number of children born. We also noted that fertility investigations and treatments were more often utilised by CCSs than by controls. Fertility treatments included IVF in eight CCSs and eight controls, ovarian stimulation in seven CCSs and four controls, and egg donation in six CCSs. Among CCSs who underwent fertility treatment, 11/18 (61%) successfully gave birth. In addition, the median age for the first childbirth was significantly lower among CCSs (n=72) compared with controls (n=87), 26.5 and 29.0 years (p=0.013), respectively. Despite the trend towards fertility impairment, no significant association was noted regarding future desire for a child between all CCSs and controls (p=0.883).

We observed an overall significant difference concerning number of pregnancies and number of children born for the Swedish infertility risk groups (p=0.005 and p=0.006, respectively). However, no differences were observed with pairwise comparisons. Only 23% of the females in the very high-risk group were able to conceive, in comparison with 59% of controls. Regarding the number of females who had given birth, we noted that 15% and 54% of the females in the very high-risk group and controls had given birth, respectively. Furthermore, utilisation of fertility investigation and treatment were more pronounced among those in the very high-risk group and in the unilateral oophorectomy group (Table 14).

For the PanCareLIFE infertility risk groups, no significant differences were observed regarding either the number of pregnancies or the number of children born between the groups (p=0.071 and p=0.279, respectively). Nevertheless, a trend towards reduced median number of pregnancies was apparent for groups 3 and 4 vs. controls: 0 vs. 1, respectively. The median number of children born was also lower among groups 1, 3, and 4 compared with controls. Moreover, females in groups 2 and 4 underwent fertility investigation and treatment more often than the controls (Table 15).

Table 14. Fertility outcomes among CCSs (n=167) and controls (n=164) based on the Swedish infertility risk grouping (see Table 3).

	No risk	Low-risk	Moderate-risk	High-risk	Very high-risk	Only surgery	Unilateral oophorectomy	All CCSs	Controls	*p*-value All CCSs vs. controls	Overall *p*-value
N	17	31	29	37	13	19	13	167	164	n.a.	n.a.
Age at examination (years)	32.9 (19.6–49.8)	31.8 (19.8–48.0)	28.8 (19.5–47.9)	36.6 (19.3–57.8)	30.8 (21.8–51.4)	38.1 (22.2–49.9)	39.4 (25.0–54.3)	34.6 (19.3–57.8)	35.8 (19.3–58.0)	0.385	**0.015**
Age at diagnosis (years)	11.1 (1.3–17.9)	5.0 (0.6–16.0)	6.1 (0.1–17.2)	7.9 (0.4–17.6)	12.3 (1.8–17.9)	8.8 (0.1–17.2)	12.6 (4.6–17.0)	8.4 (0.1–17.9)	n.a.	n.a.	**0.003**
POI, n	0	1 (3%)	0	5 (14%)	9 (69%)	0	4 (31%)	22 (13%)	0	**<0.001**	n.a.
Females pregnant, n	6 (35%)	13 (42%)	11 (38%)	22 (59%)	3 (23%)	14 (74%)	9 (69%)	84 (50%)	97 (59%)	0.122	**0.010**
Pregnancies, total n	10	37	34	49	5	43	32	224	263	n.a.	n.a.
Pregnancies/female, n	0 (0–3)	0 (0–6)	0 (0–6)	1 (0–5)	0 (0–3)	2 (0–11)	2 (0–7)	1 (0–11)	1 (0–7)	0.066	**0.005**
Females given birth, n	5 (29%)	11 (35%)	9 (31%)	21 (57%)	2 (15%)	13 (68%)	7 (54%)	74 (44%)	88 (54%)	0.100	**0.006**
Children born, total n	8	23	18	40	3	29	19	152	180	n.a.	n.a.
Children born/female, n	0 (0–3)	0 (0–3)	0 (0–4)	1 (0–4)	0 (0–2)	1 (0–5)	2 (0–4)	0 (0–5)	1 (0–5)	0.117	**0.006**
Children born/pregnancies	80%	62%	53%	82%	60%	67%	59%	68%	68%	n.a.	n.a.
Fertility investigation, n	3 (18%)	0	2 (7%)	5 (14%)	4 (31%)	4 (21%)	3 (23%)	25 (15%)	15 (9%)	0.129	n.a.
Fertility treatment, n	1 (6%)	0	2 (7%)	3 (8%)	2 (15%)	3 (16%)	3 (23%)	18 (11%)	12 (7%)	0.339	n.a.
Adoption	0	0	0	0	2 (15%)	0	1 (8%)	4 (2%)	1 (1%)	0.372	n.a.

Eight CCSs were excluded from risk grouping, six due to inconclusive data and two who underwent bilateral oophorectomy. Data is shown as count, median, range, and percentage. CCSs: childhood cancer survivors; POI: premature ovarian insufficiency; n.a.: not applicable. *P*-value among all CCSs vs. controls analysed with Mann-Whitney U test and Fisher's exact test. Overall *p*-value analysed with Kruskal-Wallis test and chi-squared test.

Table 15. Fertility outcomes among CCSs (n=167) and controls (n=164) based on the PanCareLIFE infertility risk grouping (see Table 2).

Group	1	2	3	4	All CCSs	Controls	*p*-value All CCSs vs. controls	Overall *p*-value
N	54	13	48	50	167	164	n.a.	n.a.
Age at examination (years)	30.6 (19.6–49.9)	39.4 (25.0–54.3)	35.1 (19.9–49.8)	37.2 (19.3–57.8)	34.6 (19.3–57.8)	35.8 (19.3–58.0)	0.385	0.053
Age at diagnosis (years)	6.2 (0.1–17.9)	12.6 (4.6–17.0)	7.7 (0.3–17.3)	8.5 (0.4–17.9)	8.4 (0.1–17.9)	n.a.	n.a.	**0.011**
POI, n	1 (2%)	4 (31%)	0	15 (30%)	22 (13%)	0	**<0.001**	**<0.001**
Females pregnant, n	28 (52%)	9 (69%)	22 (46%)	24 (48%)	84 (50%)	97 (59%)	0.122	0.285
Pregnancies, total n	81	32	54	56	224	263	n.a.	n.a.
Pregnancies/female, n	1 (0–11)	2 (0–7)	0 (0–6)	0 (0–5)	1 (0–11)	1 (0–7)	0.066	0.071
Females given birth, n	25 (46%)	7 (54%)	19 (40%)	22 (44%)	74 (44%)	88 (54%)	0.100	0.419
Children born, total n	53	19	35	44	152	180	n.a.	n.a.
Children born/female, n	0 (0–5)	2 (0–4)	0 (0–4)	0 (0–4)	0 (0–5)	1 (0–5)	0.117	0.279
Children born/pregnancies	65%	59%	65%	79%	68%	68%	n.a.	n.a.
Fertility investigation, n	4 (7%)	3 (23%)	6 (13%)	10 (20%)	25 (15%)	15 (9%)	0.129	n.a.
Fertility treatment, n	3 (6%)	3 (23%)	3 (6%)	7 (14%)	18 (11%)	12 (7%)	0.339	n.a.
Adoption	0	1 (8%)	0	2 (4%)	4 (2%)	1 (1%)	0.372	n.a.

Two CCSs who underwent bilateral oophorectomy were excluded. Data is shown as count, median, range, and percentage. CCSs: childhood cancer survivors; POI: premature ovarian insufficiency; n.a.: not applicable. *P*-value among all CCSs vs. controls analysed with Mann-Whitney U test and Fisher's exact test. Overall *p*-value analysed with Kruskal-Wallis test and chi-squared test.

POI

As previously reported, the prevalence of POI was 13% among CCSs. POI prevalence was notably higher for CCSs in the very high-risk group (69%), the high-risk group (14%), and the unilateral oophorectomy group (31%) according to the Swedish infertility risk assessment (Table 14). We also noted that POI was considerably more prominent in PanCareLIFE group 4 (30%) and group 2 (31%) (Table 15). None of the controls were diagnosed with POI.

Study IV

Participants

As previously reported, we included 167 CCSs and 164 controls at median ages of 34.6 (19.3–57.8) and 35.8 (19.3–58.0) years, respectively. The median age at diagnosis among CCSs was 8.4 years (0.1–17.9), with a median time since diagnosis of 25.4 years (11.6–41.3). Based on the median age at diagnosis, CCSs were divided into two groups: ≤8.4 and >8.4 years of age when diagnosed. We noticed for those aged >8.4 years at diagnosis that cancer diagnoses including brain tumour, lymphoma, sarcoma, and ovarian tumour were more pronounced. Among those aged ≤8.4 years when diagnosed, leukaemia, Wilms tumour, and other diagnoses were observed more frequently (Table 16).

Table 16. CCSs with various diagnoses and further grouped by age at diagnosis.

Age at diagnosis	≤8.4 years n=83 (%)	>8.4 years n=84 (%)	All CCSs n=167 (%)
Diagnoses			
Leukaemia	35 (42)	16 (19)	51 (31)
Brain tumour	15 (18)	24 (29)	39 (23)
Lymphoma	2 (2)	19 (23)	21 (13)
Sarcoma	7 (8)	11 (13)	18 (11)
Wilms tumour	17 (20)	2 (2)	19 (11)
Ovarian tumour	0	11 (13)	11 (7)
Other	7 (8)	1 (1)	8 (5)

CCSs: childhood cancer survivors.

When comparing treatments, the use of alkylating agents was significantly higher among those who were older when diagnosed (58%) compared with those who were younger at diagnosis (39%) (p=0.013). Moreover, with the cut-off for CED of ≥6000 mg/m^2, no significant difference was observed between the two groups (p=0.417) (Table 17). However, those >8.4 years of age at diagnosis were treated with higher median CED in comparison with those aged ≤8.4 years when diagnosed, 613 (0–31210) mg/m^2 and 0 (0–23300) mg/m^2 (p<0.001), respectively. We observed no differences between the groups in terms of abdominal radiotherapy, cranial radiotherapy, TBI, or HSCT (Table 17).

Table 17. CCSs with various treatments and further grouped by age at diagnosis.

Age at diagnosis	≤8.4 years n=83 (%)	>8.4 years n=84 (%)	All CCSs n=167 (%)	*p*-value
Treatments				
Chemotherapy	67 (81)	60 (71)	127 (76)	0.204
Alkylating agents	32 (39)	49 (58)	81 (49)	**0.013**
CED ≥6000 mg/m^2	11 (13)	21 (25)	32 (19)	0.417
Radiotherapy, abdominal	17 (20)	17 (20)	34 (20)	1.000
Radiotherapy, cranial	32 (39)	21 (25)	53 (32)	0.069
TBI	3 (4)	4 (5)	7 (4)	1.000
HSCT	4 (5)	7 (8)	11 (7)	0.535
Surgery only	8 (10)	11 (13)	19 (11)	0.627

CCSs: childhood cancer survivors; CED: Cyclophosphamide Equivalent Dose; TBI: total body irradiation; HSCT: hematopoietic stem cell transplantation. *P*-value ≤8.4 years vs. >8.4 years at diagnosis, analysed with Fisher's exact test.

Ovarian markers

CCSs aged >8.4 years at diagnosis were significantly older when examined, with a median age of 38.3 years (21.3–57.8), compared with those aged ≤8.4 years when diagnosed who were 29.9 years (19.3–46.9) at examination (p<0.001) (Table 18). Median follow-up time did not differ among the groups, 26.3 years (12.3–41.3) for CCSs aged ≤8.4 years when diagnosed and 24.7 years (11.6–40.7) for those aged >8.4 years at diagnosis (p=0.081).

For those aged >8.4 years at diagnosis, median AMH levels were significantly reduced when comparing with those aged ≤8.4 years when diagnosed: 0.3 and 3.7 ng/ml (p<0.001), respectively (Table 18). Linear regression analysis with adjustment for age at examination showed that those aged >8.4 years at diagnosis continued to have reduced AMH levels by 1.3 ng/ml in comparison with CCSs aged

≤8.4 years when diagnosed (p=0.017, 95% CI -2.3 – -0.2). In addition, median AFC was significantly lower in CCSs who were older when diagnosed 8.0 compared with those who were younger at diagnosis 14.5 (p<0.001) (Table 18). Nevertheless, this difference was no longer apparent when adjusting for age at examination (p=0.165, 95% CI -5.0 – 0.9). Furthermore, median OV did not differ among the groups (p=0.416) (Table 18).

Table 18. Ovarian markers among CCSs according to age when diagnosed.

Age at diagnosis	**≤8.4 years**	**>8.4 years**	***p*-value**
N	83	84	n.a.
Age at examination (years)	29.9 (19.3–46.9)	38.3 (21.3–57.8)	**<0.001**
Time since diagnosis (years)	26.3 (12.3–41.3)	24.7 (11.6–40.7)	0.081
AMH (ng/ml)	3.7 (0.0–23.5) (n=82)	0.3 (0.0–16.6) (n=84)	**<0.001**
AFC	14.5 (0–47) (n=74)	8.0 (0–38) (n=61)	**<0.001**
OV (cm^3)	7.1 (0.7–21.4) (n=72)	6.8 (0.3–21.0) (n=57)	0.416

Data is shown as count, median, and range. CCSs: childhood cancer survivors; AMH: anti-Müllerian hormone; AFC: antral follicle count; OV: ovarian volume. *P*-value analysed with Mann-Whitney U test.

We noticed that ovarian tumour as a diagnosis was restricted to the group >8.4 years at diagnosis. Additional analysis was therefore performed with exclusion of this diagnosis, demonstrating that those who were older at diagnosis still had significantly lower median AMH levels compared with those who were younger at diagnosis: 0.4 (0.0–16.6, n=73) vs. 3.7 (0.0–23.5, n=82) ng/ml, respectively (p<0.001). Furthermore, those who were older at diagnosis also presented with a 3.5-fold increased risk of unmeasurable AMH (i.e., <0.023 ng/ml) compared with those who were younger when diagnosed (p=0.024, 95% CI 1.2 – 10.4, adjusted for age at examination). In addition, there was a negative correlation between AMH levels and age at diagnosis (r=-0.234, p=0.002, adjusted for age at examination, n=166) (Figure 13). Moreover, those who were younger at diagnosis presented with longer follow-up time, i.e., time since diagnosis correlated negatively with age at diagnosis (r=-1.000, p<0.001, adjusted for age at examination, n=167).

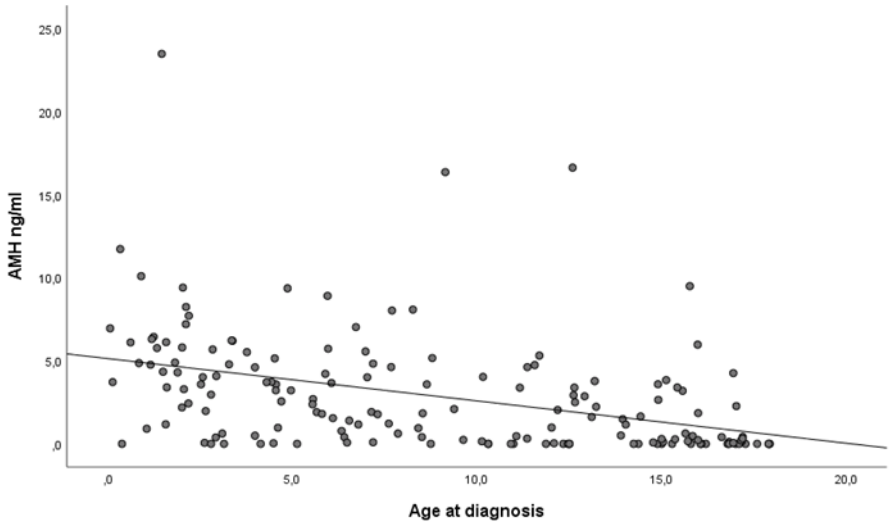

Figure 13. Correlation between AMH levels and age at diagnosis among CCSs. Scatter plot illustrating the negative correlation between AMH levels and age at diagnosis (r=-0.234, *p*=0.002, n=166, adjusted for age at examination). AMH: anti-Müllerian hormone; CCSs: childhood cancer survivors.

POI

Among 22 CCSs with POI, 15 presented with secondary amenorrhoea at 15–39 years of age and six with primary amenorrhoea. All CCSs with POI had either very low or undetectable AMH (i.e., <0.1 or <0.023 ng/ml, respectively), except for one CCS with an AMH level of 0.403 ng/ml along with primary amenorrhoea and ongoing use of HRT. For those with POI, the median age at examination was 39.4 years (21.8–55.5), with a median age at diagnosis of 11.7 years (0.4–17.9) and median time since diagnosis of 30.1 years (12.1–39.4). Cancer diagnoses identified in the POI group were leukaemia (n=8), ovarian tumour (n=5), lymphoma (n=5), Wilms tumour (n=2), brain tumour (n=1), and sarcoma (n=1). When dividing up CCSs according to age when diagnosed, those who were older at diagnosis had higher POI prevalence (19%) compared with those who were younger at diagnosis (7%) (*p*=0.038).

Discussion

Major improvements in childhood cancer survival and therapeutic strategies over time have resulted in a growing population of survivors, with many years left to live [3-5, 19, 20]. Concerns about the late complications of childhood cancer treatment have led to a greater focus on survivorship research, a field that is central to our work. In our studies, we have evaluated the detrimental effects of cancer treatment on ovarian function, quality of life, and fertility among adult female CCSs.

The findings of our first study indicated that serum markers including AMH, inhibin B, and FSH correlated well with the ultrasound markers AFC and OV among both CCSs and controls. As far as we know, this study was the first to evaluate the correlation between these markers in an adult population of CCSs. Furthermore, E2 correlated to neither AFC nor OV within the groups. Among ovarian serum markers, we found AMH to be the strongest predictor for detecting primary POI among CCSs and for diminished ovarian reserve (i.e., AFC <10) in both CCSs and controls. FSH and E2 were not included in the analyses due to the limited sample size after exclusion for the use of HRT or OC.

AFC, which is considered to be the gold standard method for evaluation of the ovarian reserve, also showed high accuracy in predicting primary POI [64]. However, this method, with its use of transvaginal ultrasound, is not suitable for screening in young females due to virginity or the discomfort it may cause. In comparison to other ovarian serum markers, AMH is not part of the negative feedback system, and thus has the advantage of being assessable at any time during the menstrual cycle [67, 68].

POI was identified in 13% (22/167) of CCSs in our study, compared with a previously reported prevalence of 8–11% [93, 96]. However, our CCSs were somewhat older when examined with longer follow-up time, which could account for the higher prevalence observed. Currently, HRT is advised up to the age of 50–51 years, when menopause naturally occurs [58, 83]. However, 14/19 of our CCSs ≤50 years of age had ongoing HRT use, which calls for improvements in offering treatment to reduce the morbidities associated with oestrogen deficiency.

All CCSs treated with alkylating agents and/or ovarian radiotherapy should be offered counselling and information about the risk of POI, as stated in the current recommendations. For POI screening among CCSs, AMH as a primary test is not suggested for those at risk. Evaluation of FSH and E2 levels is recommended in the

case of delayed puberty, disturbances in puberty progression, or if menstrual cycle irregularities arise. However, it is outlined that AMH might be used as an additional test for those aged ≥25 years presenting with menstrual cycle disturbances, or upon request for future fertility assessment [85]. We found limited support to recommend E2 testing for POI surveillance. In addition, previous studies have reported that FSH rise is a late indicator of diminished ovarian reserve, with AMH decline preceding the rise [74, 75]. George et al. found that almost 20% of CCSs had normal FSH but low AMH, indicating that diminished ovarian reserve would not have been detected in a substantial proportion of survivors with current surveillance recommendations [125]. Another study reported low AMH in 30% of CCSs with regular cycles [124]. Therefore, POI surveillance with observation of menstrual disturbances and testing with FSH and E2 is not optimal for detecting decreased ovarian reserve and impending POI. Our study suggests AMH as the most valuable serum marker for POI screening.

The psychological burden of POI includes lower quality of life, anxiety, and depression [90, 95]. Among somatic complications, impaired fertility has been reported as a major concern, with a negative impact on well-being and intimate relationships [95, 98]. Our second study investigated health state and well-being for all CCSs and for those CCSs with POI compared with controls. We found that both health state and well-being were significantly reduced for all CCSs in comparison with controls. When comparing only CCSs with POI with controls, no difference was found regarding health state, which could be due to the limited number included in the POI group. However, CCSs with POI demonstrated the lowest well-being compared with controls. To ensure that the controls in our study were representative of the general population, we compared EQ-VAS figures with those from a large Swedish study (n=30,431), which presented comparable scores [161]. Our findings in the present study align with those of previous studies [95, 98]. However, conflicting results also exist with an American study reporting better well-being among long-term cancer survivors compared with the nationally representative cohort [162]. Another study found no difference in quality of life measured by EQ-5D-3L questionnaire among breast cancer survivors five years after surgery when comparing with age-matched controls. However, for the dimensions of pain/discomfort and anxiety/depression, breast cancer survivors reported poorer outcomes than controls [163]. These somewhat unexpected findings might reflect long-term cancer survivors valuing their health more than those who have never been diagnosed with cancer. Even though we observed a significant difference in health state between CCSs as one group and the controls, the actual figures did not differ much. One could therefore argue that CCSs have a good quality of life in general, but that it is still lower than in females without a history of childhood cancer. It should be emphasised, however, that CCSs with POI still score notably worse on well-being, which reflects the current status in contrast to the health state rather revealing quality of life based on prior events. Importantly, CCSs with POI

should be identified promptly to ensure they receive appropriate support and treatment.

We observed that CCSs with POI had a lower number of biological children, which was anticipated given their low chance of conceiving spontaneously [87]. Our hypothesis that females who did not have the desired number of children would score worse on health state and well-being than those who did have their wishes fulfilled was rejected. This was unexpected, given the fact that childlessness is associated with emotional distress [147, 164, 165]. However, the group without the desired number of children included those who were planning to have children and those who had been trying to conceive for less than two years. In other words, this group did not only consist of infertile females, which might explain the finding. It could also reflect acceptance within this group of not being able to have children of their own. CCSs with POI utilised ART more often than those without POI and controls. This is a positive aspect, as it shows that they are recognised by healthcare professionals and offered treatment.

To further examine ovarian markers and fertility outcomes, we classified CCSs into infertility risk groups based on the Swedish and PanCareLIFE guidelines in our third study [129, 130]. For the Swedish infertility risk groups of females below 40 years, we found that both AMH and AFC were significantly reduced in the very high-risk group and the high-risk group in comparison with controls. Similarly, AMH and AFC were significantly lower when comparing PanCareLIFE group 4 with controls. In addition, 69%, 31%, and 14% of CCSs at all ages were diagnosed with POI in the very high-risk group, the unilateral oophorectomy group, and the high-risk group, respectively. POI prevalence was also notably higher in PanCareLIFE groups 4 (30%) and 2 (31%). A study conducted in Sweden on CCSs aged 19–40 years reported a POI prevalence of 9%, which increased to 35% for those treated with highly gonadotoxic therapies [166]. These figures are considerably higher than the spontaneous POI prevalence of 1.7% found by a Swedish register study [97]. Our results are in line with those of previous studies, and thus affirm treatments such as high-doses of alkylating agents, ovarian radiotherapy, and HSCT being the most gonadotoxic [109, 111, 167]. In the PanCareLIFE guidelines, the CED cut-off has been specified as a range, i.e., <6–8 g/m² for group 3 and ≥6–8 g/m² for group 4 [129]. We chose the lowest dose in this range, with our findings supporting CED of ≥6 g/m^2 as the preferable cut-off for recommending fertility preservation.

Regarding fertility outcomes, there was a trend towards decreased fertility in terms of conceiving and giving birth for all CCSs as well as for those at all ages in the very high-risk group, group 3, and group 4 compared with controls. Fertility investigation and treatment were more frequently utilised among CCSs and especially in those categorised as very high-risk of infertility and group 4. It was also more pronounced in CCSs who were treated with unilateral oophorectomy. Impaired fertility among CCSs has also been observed in earlier studies [107, 168]. Another study detected that CED of >7 g/m^2 and any radiotherapy dose to the lower

abdomen constitute an increased risk of compromised fertility in CCSs, which aligns with the results observed in group 4 [118]. One surprising result was that the high-risk group presented with no manifestation of fertility impairment concerning females being pregnant and children born. However, AMH and AFC were significantly reduced in this group for those aged <40 years compared with controls. A previous study's results correspond to this somewhat unexpected finding, reporting similar pregnancy rates among young cancer survivors despite reduced ovarian markers in comparison with age-matched controls [169]. One could therefore propose that ovarian markers mirror the quantity of remaining follicles, but to a lesser extent their quality. This theory is supported by a recent study reporting AMH as a limited prognostic factor of fertility [79]. Moreover, during follow-up, healthcare providers encourage CCSs to bring forward their childbearing plans, which is reflected in the fact that the CCSs in our study were younger than controls when they had their first child, with median ages of 26.5 and 29.0 years, respectively. Earlier childbearing among CCSs could be an additional explanation for the above-mentioned observation. In addition, we noted no difference between CCSs and controls concerning desire for future children. This may be viewed as either having the intended number of children or accepting the infertility situation. Given the trend of postponing childbearing in Europe, it may be valuable to monitor AMH in CCSs treated with highly gonadotoxic treatments to avoid missing the window of opportunity for family planning or fertility treatment.

CCSs who underwent treatment with unilateral oophorectomy are not originally stated as a separate group in the Swedish infertility risk classification. However, in the PanCareLIFE guidelines, those who underwent this treatment are classified as one group. It is reasoned in the PanCareLIFE guidelines that even though these females may be at risk of decreased fertility, they still have one healthy ovary left and are therefore not considered for fertility preservation [129]. This seems appropriate, as females who underwent unilateral oophorectomy enter menopause slightly earlier at a mean age of 49.6 years [170]. Nevertheless, in the case of cancer relapse and use of gonadotoxic treatments, the guidelines state that oocyte or embryo cryopreservation might be valuable prior to starting treatment. CCSs categorised to our unilateral oophorectomy group have received other cancer treatments in addition to removing one ovary, and thus constitute a heterogeneous group regarding therapy. Besides the diverse treatments adding complexity when evaluating the results, this group had a limited number of CCSs included. Median levels of AMH for females aged <40 years in this group were not significantly reduced in comparison with controls: 0.1 vs. 3.2 ng/ml, respectively. POI prevalence was 31% in this group, but with no sign of reduced fertility when assessing the numbers of pregnancies and children born. This could be explained by the observation of the greatest utilisation of fertility treatment (23%) among CCSs who underwent unilateral oophorectomy. Future studies including larger number of participants should investigate fertility outcomes further and evaluate whether a different fertility preservation approach is needed for this group.

One other major difference between the Swedish and PanCareLIFE guidelines is the inclusion of cranial radiotherapy for infertility risk assessment in the latter. Group 3 consisted of CCSs treated with cranial radiotherapy and/or CED <6 g/m^2, and could be comparable to the Swedish moderate-risk group except for cranial radiotherapy. As mentioned earlier, group 3 did not have reduced AMH and AFC compared with controls, but a tendency for impaired fertility was apparent. On the contrary, the moderate-risk group presented no reduction in ovarian markers and preserved fertility. Based on this, one could infer that the fertility impairment trend observed in group 3 is more likely attributable to hypogonadotropic hypogonadism rather than ovarian damage. In the event of injury to the HPG axis, ovarian stimulation can be performed by using hormonal treatment (i.e., FSH and human chorionic gonadotrophin) at the time when family planning is sought [129].

A positive implication in the overall context of cancer treatment is that the ovarian insult appears to happen in relation to the treatment without further follicular loss later in life [171, 172]. Our finding of a negative correlation between AMH and age at examination for all CCSs and controls showed that AMH levels were only slightly lower among CCSs, supporting the theory of no accelerated follicular loss over the years. However, future prospective studies with extended follow-up time beyond three years are necessary to confirm this statement.

In our final study, we investigated the impact of age at diagnosis on ovarian markers. CCSs aged ≤8.4 years at diagnosis had significantly higher median levels of AMH compared with those aged >8.4 years when diagnosed. We observed a significant difference among these groups regarding age at study enrolment. When adjusting for age at examination, the significant reduction in AMH levels remained for those who were older at diagnosis. This finding is comparable with those of previous studies, which report better preserved ovarian function among pre-pubescent girls [124-126]. Furthermore, those who were older at diagnosis were 3.5 times as likely to have unmeasurable AMH (i.e., <0.023 ng/ml) compared with CCSs who were younger at diagnosis. CCSs who were younger at diagnosis did indeed have longer follow-up time, which could not therefore account for these observations.

POI was more common among those aged >8.4 years at diagnosis (19%) compared with those aged ≤8.4 years when diagnosed (7%). Another study by Sklar et al., however, found no association between POI and age at diagnosis [93]. As mentioned, CCSs aged >8.4 years at diagnosis were older at study enrolment, which might contribute to the higher observed prevalence of POI. Nevertheless, it has been reported by a multicentre study that CCSs diagnosed between the ages of 13 and 19 years had a 2.3 times higher risk of self-reported menopause compared with sibling controls [127, 128]. In this multicentre study, menopause was reported as cessation of menstruation by the participants and was not verified by biochemical or ultrasound ovarian markers. Another study reported that 8% of CCSs experienced self-reported non-surgical premature menopause, compared with 0.8% of siblings, with a higher age at diagnosis identified as a risk factor [93].

Our study observed limited evidence regarding the impact of different cancer treatments on reduced AMH levels. We found that CCSs who were older when diagnosed underwent treatment with alkylating agents to a higher extent than those who were younger at diagnosis. Although CCSs aged >8.4 years at diagnosis were treated with significantly higher CED, the median was rather low (i.e., 613 mg/m^2), which recent guidelines classify as a low risk for POI [129]. In addition, no differences were observed regarding treatment including ovarian radiotherapy.

These findings potentially point to the ovaries being more susceptible to cancer treatments at an older age, and to growing follicles being more vulnerable. One could reason that the loss of growing follicles leads to an increased follicle turnover, consequently depleting the primordial follicle pool. Younger girls (i.e., those considered pre-pubescent) could therefore be less affected since their ovaries are in a dormant stage. Moreover, they also have a larger primordial follicle pool to start with compared with older girls. It has been reported that histological examination of cryopreserved ovarian tissue shows higher follicular density among younger paediatric patients [173], together with an acute AMH reduction following the initiation of cancer treatment [174], supporting this reasoning. It is also known that the number of primordial follicles differs at birth [51], and individuals might thus present with varying sensitivity to gonadotoxic treatments.

From the observations that pre-pubescent girls are less sensitive to ovarian damage, it has been hypothesised that the use of GnRHa could potentially offer protection to the ovaries during cancer treatment. To date, randomised trials evaluating GnRHa therapy are scarce with limited numbers of childhood cancer patients included [175]. Meta-analyses including pre-menopausal females with breast cancer reported that concurrent use of chemotherapy and GnRHa improves the restoration of regular menstrual cycles and pregnancy rates, as well as lowers the risk of POI [176, 177]. GnRHa use is currently considered experimental for childhood cancer patients, and future prospective randomised studies need to investigate the potential benefit for post-pubescent girls [129, 178]. Nevertheless, if GnRHa therapy will be implemented eventually, it should not serve as a substitute for proven fertility preservation methods, i.e., oocyte or embryo cryopreservation.

Limitations of our studies

We collected data during a period of five years, leading to a difference of zero to three years between CCSs and controls in the timing of blood sampling. Assessments of AFC and OV were more challenging among CCSs than controls, and were not measured in 19% and 4% and in 23% and 5%, respectively. Examination with transvaginal ultrasound was conducted by six different doctors, which could potentially contribute to interindividual differences. The definition of

POI was subtly changed, as we had no information on FSH and oestradiol levels at the time of diagnosis. Data was analysed based on groups instead of paired data, as matching was not performed for the use of HRT and OC. Moreover, studies report diverse results regarding the effect of oral hormonal therapy on lowering AMH levels [69-71]. Hence, a potential limitation is that analyses were not adjusted for the use of oral hormonal therapy, even though the numbers of CCSs and controls using HRT or OC were similar in our study. In addition, the number of CCSs was small in the infertility risk groups. Due to the cross-sectional design, we were unable to predict pregnancies and the timing of POI using ovarian reserve markers. In many cases, ovarian radiotherapy dose could not be exactly estimated and was therefore specified as an interval. Because of the limited study population and numerous cancer treatments, we could not determine the gonadotoxic threshold dose of alkylating agents and ovarian radiotherapy. Finally, we used a cut-off of 8.4 years of age when diagnosed, since data on pubescent status at the time of diagnosis was unavailable.

Strengths of our studies

To evaluate ovarian function, we collected detailed data on cancer treatments and performed physical examinations, including assessment of serum and ultrasound ovarian markers. We also gathered comprehensive data regarding quality of life from questionnaires. Our study population consisted of adult CCSs who were treated before fertility preservation methods could be offered, thus not affecting measurement of ovarian markers as in the case of removing one ovary for cryopreservation. Furthermore, CCSs were matched and compared with healthy controls. At last, our final study group of CCSs was representative concerning both the distribution of cancer diagnoses and offspring.

Conclusions

- POI prevalence was 13% among CCSs, which is somewhat higher than previously reported. AMH, inhibin B, and FSH correlated significantly with AFC and OV, both in CCSs and controls, with no difference observed between the groups. AMH was the strongest serum predictor for detection of POI as well as low AFC after childhood cancer treatment. Thus, we suggest AMH as a reliable ovarian reserve marker in follow-up programmes for POI surveillance in CCSs.
- Self-reported health state and well-being were significantly reduced among all CCSs compared with controls. When comparing health state, the actual scores were not very different between the groups. However, CCSs with POI reported the lowest well-being. Therefore, early identification of female CCSs with POI is important to guarantee that they receive the necessary treatment and support.
- Both the Swedish and PanCareLIFE infertility risk classifications serve as effective tools for detecting CCSs at risk of low AMH, fertility impairment, and POI. It is therefore evident that treatments such as CED $\geq$6 g/m^2, ovarian radiotherapy, and HSCT are most harmful to the ovaries. All childhood cancer patients and their parents should receive information on the expected future infertility risk, irrespective of planned treatment. In addition, those considered to be at a substantial risk of compromised fertility must be offered fertility preservation prior to starting treatment, along with follow-up during early reproductive years.
- CCSs who were older at diagnosis presented with reduced AMH levels and a higher prevalence of POI compared with those who were younger when diagnosed. It seems that the ovaries become more vulnerable to gonadotoxic treatments with increased age, as cancer treatments did not differ much between the groups. However, those who were older at diagnosis were more often treated with alkylating agents and with slightly higher CED. Age at diagnosis and CED can therefore aid in identifying those eligible for fertility preservation and monitoring throughout young reproductive age.

Future perspectives

- Further research is needed among CCSs aged <25 years to assess whether AMH is a valuable ovarian marker in follow-up programmes for POI surveillance in this age group. There is also a need to obtain age-specific ranges from healthy females below 20 years of age.
- Longitudinal follow-up is required to evaluate the decline in ovarian markers across time and to estimate the timing of POI onset. In addition, the prognostic value of ovarian markers to predict the likelihood of conceiving among CCSs needs to be further investigated by future prospective studies.
- Although our data has been analysed based on cancer treatments rather than cancer diagnoses, large amounts of data refer to CCSs treated several decades ago with therapeutic approaches that are no longer in use (e.g., prophylactic cranial radiotherapy for ALL patients) or might have been changed. Therefore, the findings of our studies may not be fully pertinent to childhood cancer patients undergoing therapy more recently. This calls for additional research to evaluate POI prevalence, ovarian markers, and fertility outcomes in patients treated according to more current treatment protocols.
- Efforts must continue to optimise treatment protocols, with the aim of minimising toxicity and late complications while maintaining survival outcomes.
- Interindividual sensitivity to gonadotoxic treatments need further investigation with the identification of genetic variants associated with susceptibility for decreased ovarian function in order to give more patient-tailored recommendations for fertility preservation.
- Improvements in ovarian tissue reimplantation technique are desired to limit ischemia causing extensive follicular loss in the graft. There is also a need to further investigate the developmental potential of immature oocytes in the graft harvested from pre-pubescent girls.
- Future studies including larger study populations with evaluations of cancer treatment factors will have to reproduce the protective effects of being diagnosed at a younger age on ovarian function.

- The potentially beneficial use of GnRHa treatment during chemotherapy in post-pubescent girls needs further investigation through larger prospective randomised trials.
- In vitro maturation of oocytes from cryopreserved ovarian tissue is currently considered experimental. Advances in this method, with potential future clinical use, could be particularly beneficial for females at risk of malignant cells in the cryopreserved tissue.

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