VRIJE UNIVERSITEIT BRUSSEL

Proef ingediend met het oog op het behalen
van de graad van Master in de Geneeskunde

THE IMPACT OF FERTILITY PRESERVATION ON THE TIME INTERVAL BETWEEN CANCER DIAGNOSIS AND INITIATION OF CHEMOTHERAPY IN WOMEN DIAGNOSED WITH BREAST CANCER OR HODGKIN'S LYMPHOMA

DE IMPACT VAN FERTILITEITSPRESERVATIE OP HET TIJDSINTERVAL TUSSEN KANKERDIAGNOSE EN START VAN CHEMOTHERAPIE BIJ VROUWEN MET BORSTKANKER OF HODGKIN LYMFOOM

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2017-2018

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ABSTRACT

Purpose: The aim of this study was to verify whether fertility preservation (FP) in adult women diagnosed with Hodgkin's lymphoma (HL) or breast cancer (BC) has an impact on the time frame between diagnosis and initiation of chemotherapy.

Methods: A retrospective cohort study encompassing patients diagnosed with cancer between January 2012 and December 2017, who underwent FP before chemotherapy, and matched control patients, who were not referred to a fertility centre for FP counselling, was performed in three study population groups, more specifically HL patients, BC patients undergoing neo-adjuvant chemotherapy (NAC) and BC patients undergoing adjuvant chemotherapy. Case patients were selected from the patient database of the Centre for Reproductive Medicine (CRG) at Universitair Ziekenhuis Brussel (UZ Brussel). Control patients were selected from the patient database of the haematological department or the Breast Cancer Clinic at UZ Brussel. Cases and controls were matched for tumour characteristics and type of oncological treatment. Time intervals between oncological diagnosis and initiation of chemotherapy were analysed.

Results: Eight case patients with HL were selected and matched to suitable control patients. Fifty-nine BC patients who underwent FP, of which 29 received NAC and 30 received adjuvant chemotherapy, were selected and matched to control patients. The average time to chemotherapy in HL patients was 26.7 days (33.2 (range: 9.0-70.0) days in cases and 20.1 (range: 7.0-33.0) days in controls, $p = 0.102$), in BC patients with NAC 28.5 days (27.3 (range: 14.0-44.0) days in cases and 29.6 (range: 14.0-62.0) days in controls, $p = 0.441$) and in BC patients with adjuvant chemotherapy 58.9 days (57.2 (range: 36.0-106.0) days in cases and 60.7 (range: 31.0-105.0) days in controls, $p = 0.145$). The FP program itself (from referral for FP counselling to termination of the FP treatment) took on average 12.2 days in HL patients, 11.9 days in BC patients with NAC and 23.5 days in BC patients with adjuvant chemotherapy.

Conclusion: The initiation of chemotherapy is not delayed when adult women diagnosed with Hodgkin's lymphoma or breast cancer are referred to an oncofertility team to undergo fertility preservation.

Keywords: Fertility preservation; breast neoplasms; Hodgkin disease; antineoplastic agents; Time-to-Treatment

ABBREVIATIONS AND CONCEPTS

ABVD = Adriamycin, Bleomycin, Vinblastine and Dacarbazine

Ann Arbor Staging for Hodgkin's lymphoma: A = no symptoms, B = additional symptoms present, S = splenic involvement, X = bulky disease

ART = Assisted Reproductive Technology

ASCO = American Society of Clinical Oncology

BC = breast cancer

BEACOPP = Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Oncovin, Procarbazine and Prednisolone

Cat. = category

COS = controlled ovarian stimulation

CRG = Centre for Reproductive Medicine

DCIS = ductal carcinoma in situ

EMD = electronic medical file

ER = oestrogen receptor

ESMO = European Society for Medical Oncology

FP = fertility preservation

GnRHa = gonadotrophin releasing hormone agonists

HER2 = Human Epidermal growth factor Receptor 2

HL = Hodgkin's lymphoma

HR = hormone receptor

IDA = invasive ductal adenocarcinoma

IFHL = interfollicular Hodgkin's lymphoma

IPS = International Prognostic Score (Hasenclever Index)

IVM = in vitro maturation

KCE = Belgian Health Care Knowledge Centre

Ki67 = a proliferation marker that acts as a prognostic parameter in breast cancer

LCIS = lobular carcinoma in situ

MCHL = mixed cellularity Hodgkin's lymphoma

NA = not applicable

NAC = neo-adjuvant chemotherapy

NSHL = nodular sclerosing Hodgkin's lymphoma

OPU = oocyte pick-up

OTCP = ovarian tissue cryopreservation

PR = progesterone receptor

QoL = Quality of Life

SPSS = Statistical Package for the Social Sciences

Statistical measurements: SD = standard deviation, IQR = interquartile range, CI = confidence interval, Q-Q plot = quantile - quantile plot

TNM-classification: T = tumour, N = nodes, M = metastasis, c = clinical, p = pathologic, yp = pathologic after neo-adjuvant treatment

vs. = versus

Oncofertility: “an interdisciplinary field bridging biomedical, social sciences and examining issues regarding an individual's fertility options, choice and goals in light of cancer diagnosis, treatment and survivorship.” (cited as introduced by Woodruff TK (1))

Referring physician: the physician in charge with the patient’s oncological treatment (in the control group) or who actually referred the patient to a fertility centre (in the case group).

INTRODUCTION

In 2013, 65 487 de novo malignancies were diagnosed in Belgium, of which 53% were male cancer patients and 47% were female cancer patients. Over the last years the overall incidence of cancer is stagnating, although there is a slight increase in the female population. An important increase in the five-year survival rate over the past decades has been observed, resulting in a five-year relative survival of 58.7% in male and 68.6% in female patients between 2009 and 2013 in Belgium.(2)

In 2013, 10 695 females were diagnosed with breast cancer (BC) in Belgium, accounting for 35% of all female cancer diagnoses and 20% of the cancer deaths in female patients. The incidence remains stable and the mortality is decreasing by two percent annually, with a five-year relative survival proportion in female breast cancer patients of 90% in the period between 2009 and 2013.(2)

In 2012, 325 patients were diagnosed with Hodgkin's lymphoma (HL) in Belgium, of which 43% were female patients, with a mean age of 41 years at diagnosis. The incidence of Hodgkin's lymphoma was 4.9/100 000 in women aged 15-29 years and 2.5/100 000 in women aged 30-44 years. In this age category HL accounted for almost 50% of all lymphoid malignancies, with a five-year relative survival of 85-95% that decreases after the age of 45 years.(3)

The decrease in mortality rate is an effect of scientific evolutions in diagnostic and therapeutic techniques, leading to earlier detection and oncological treatment in a lower cancer stage. In addition, the oncological treatment becomes more effective and personalised for each patient.
A commonly used treatment modality is the administration of systemic chemotherapy. Depending on the type and regimen, chemotherapy has a gonadotoxic effect on the gonadal function of cancer patients. Chemotherapeutic drugs can damage the granulosa cells in the ovaries leading to a decrease or elimination of the remaining oocyte pool.(4)

Due to the increasing survival rate there is raising concern about the quality of life (QoL) of cancer patients. For young adults in remission, a substantial part of their wellbeing consists of their reproductive possibilities, which has an important psychological impact.(5)

In this context the advice has been given to refer cancer patients at reproductive age to a fertility centre before the initiation of the gonadotoxic chemotherapy in order to discuss their expected reproductive outcome and their options with regard to fertility preservation.(6-10) Several Assisted Reproductive Technologies (ART) are currently available in order to preserve a patient's fertility before the start of the oncological treatment. For female patients cryopreservation of oocytes, embryos and ovarian tissue are possible FP interventions. In case of oocyte or embryo cryopreservation, mature oocytes are routinely obtained using a controlled ovarian stimulation (COS) protocol, although in vitro maturation (IVM) of immature oocytes, retrieved transvaginally or obtained during an ovarian tissue cryopreservation (OTCP) procedure, is another emerging albeit experimental source of mature oocytes (11-13). The use of concomitant gonadotrophin releasing hormone agonists (GnRHa) during the chemotherapeutical treatment, as an additional treatment to preserve patients' fertility, remains an experimental but promising technique.(14, 15)

At present, not every eligible patient at risk of infertility due to gonadotoxic oncological treatment is referred to a fertility centre.(16) Insufficient information and knowledge about fertility-related side-effects is a possible cause. Another potential factor is the concern of patients and their oncologists that FP may delay the start of oncological treatment and that it may reduce a patient's survival after cancer.(17) Nevertheless, there is currently no literature evidence to substantiate these concerns.

Only a limited number of published studies have analysed the time frame of FP before the initiation of chemotherapy. In addition, oncological as well as fertility preservation approaches have evolved considerably over the last years and continue to evolve, as science is always on the move.

In 2007 *Madrigrano et al.* postulated the possibility to perform FP by means of conventional-start COS, starting at basal hormone levels in the beginning of the menstrual cycle, within the accepted time frame to start adjuvant chemotherapy in female breast cancer patients. Therefore, the group described the time intervals between several phases of the oncological and FP program in a population of 23 female breast cancer patients receiving a fertility treatment. An average of 46.8 days from surgery to chemotherapy and 87 days from diagnosis to chemotherapy was reported.(18)

In 2009 *Baynosa et al.* compared the time interval between oncological diagnosis and the start of an adjuvant chemotherapy in a population of 19 female patients performing FP using a conventional-start ovarian stimulation and 63 women who were not referred to a fertility centre. In conclusion, no significant difference and consequently no delay to start the chemotherapy due to the FP was observed, reporting a median time from surgery to chemotherapy of 30 and 29 days ($p = 0.79$) and from initial diagnosis to chemotherapy of 71 and 67 days ($p = 0.27$) respectively.(19)

In 2010 *Lee et al.* demonstrated the benefit of an early referral to a fertility centre. In this study a group of 93 women with the need for adjuvant chemotherapy for breast cancer who underwent controlled ovarian stimulation, not specified whether it concerned conventional- or random-start COS, were investigated, dividing the study population in a group with referral before surgery and a population with referral after surgery. In both groups the FP was performed after the surgery, but the stimulation itself as well as the chemotherapy appeared to start significantly sooner after the initial diagnosis (start of COS: 42.6 vs. 71.9 days, $p < 0.001$ and start of chemotherapy: 83.9 vs. 107.8 days, $p = 0.045$) in the first group. In addition, the women in the first group were more likely to perform a second cycle of ovarian stimulation (25.7% vs. 1.7% of patients, $p < 0.001$) and consequently cryopreserving a higher number of oocytes and embryos resulting in a possible higher chance to achieve pregnancy in the future.(20)

In 2012 *Kim et al*. described in a retrospective study the essential factors in the decision-making process to pursue with a conventional-start COS after being informed, during a consultation in a fertility centre, on the fertility risks and FP options. Therefore, demographical and medical data of 185 female breast cancer patients were analysed: 108 women who underwent FP and 77 who decided not to preserve. The authors noticed that an oncological treatment with neo-adjuvant chemotherapy (NAC) has a strong negative association with performing a FP, likely as a result of time constraints, with only one out of 19 patients receiving NAC undergoing COS. However, the authors argued that ovarian stimulation before NAC would have been feasible if the referral to a fertility clinic for FP would have been done shortly after the cancer diagnosis.(21)

As a solution for this lack of time *Jenninga et al.* suggested in 2012 a less time-consuming technique: ovarian tissue cryopreservation (OTCP). Although this fertility preservation method

was still considered experimental, this approach was considered as a plausible solution for cancer patients in need of an urgent gonadotoxic treatment.(22)

The domain of oncology has evolved immensely over the past years resulting in the development of several new treatment modalities. For example, the administration of chemotherapy in a neo-adjuvant setting for breast cancer patients has been widely accepted as an essential oncological therapeutic option.(23) However, this implies that the time available for FP is even more limited.

The domain of reproductive medicine is a continuously developing discipline as well, resulting in an improvement of established techniques and the development of new techniques: cryopreservation of oocytes and ovarian tissue has been accepted as an established non-experimental technique (24) and an ovarian stimulation can be initiated at a random moment in the menstrual cycle (random-start COS) instead of waiting until basal hormonal values are observed in blood sample analyses, at the beginning of the menstrual cycle (conventional-start COS). (25, 26) Furthermore, the addition of in vitro maturation of immature oocytes, retrieved transvaginally or during an ovarian tissue cryopreservation procedure, could increase the success rate for future pregnancy.(11-13)

In August 2017 *Chien et al.* published the results of a retrospective study concerning the time interval between the cancer diagnosis and the initiation of neo-adjuvant chemotherapy in a population of 82 female breast cancer patients. In this population 34 women underwent FP using random-start COS, nine were counselled about fertility preservation but eventually decided not to undergo FP and 39 patients were never referred to a fertility centre. The group concluded that neither random-start COS nor a consultation at the fertility centre had an influence on the timing of NAC. However, the included patients were selected from the study population of the prospective ISPY2 trial, a study comparing two different types of chemotherapy in a neo-adjuvant setting in women with a stage II or III breast cancer. This could mean a possible selection bias since patients recruited for a clinical trial generally have a closer follow-up.(27)

Letourneau et al. recently published the results of a cross-sectional retrospective study performed in a population of 87 breast cancer patients who had a fertility consultation before the start of a NAC. In this population, 58 women decided to undergo random-start COS while 29 declined the

treatment. There was no significant difference regarding the time interval between diagnosis and initiation of NAC, although there was a large difference in time between diagnosis and referral to the fertility centre (9.4 vs. 17.9 days, $p < 0.001$). A long time interval between diagnosis and referral may have had an important influence on the decision-making process of whether or not to perform a fertility preservation.(28)

Furthermore, in 2016 *Srikanthan et al.* published an article regarding the role of a dedicated program for young breast cancer patients in the frequency of discussing the fertility risk inherent to a gonadotoxic treatment and the number of referrals to and consultations at a fertility centre, prior to that chemotherapy. This dedicated program includes a nurse navigator, who coordinates the patient's oncological and fertility-related program. Therefore, a cohort of patients in two hospitals, one with a dedicated program and one without, was selected. Both hospitals were examined regarding their medical records on a retrospective basis, which was correlated with the prospectively collected answers of a survey sent to the patients. This survey interrogated the patient's perception on the fertility-related discussion, the date of referral and some demographic data to verify whether the responding population was representative for the larger cohort of patients whose medical records were examined. This study clearly showed that having a dedicated program results in a higher frequency of discussing the fertility-related risks with the patient and implicates more referrals to a physician specialised in reproductive medicine. One of the major reasons reported by the patients that may influence their decision whether or not to perform any form of FP was the patient's unwillingness to delay the chemotherapy. The authors argued that this kind of anxiety was not legitimate, because there was no significant difference in the time to chemotherapy after diagnosis or surgery, depending on whether the patient received neo-adjuvant or adjuvant chemotherapy, between the populations who underwent FP and who did not (median of 50 vs. 47 days, $p = 0.22$).(29)

Not all reproductive aged women facing potential fertility loss due to a gonadotoxic oncological treatment are referred to a fertility centre to discuss FP and have the possibility to undergo FP.(16) One of the essential elements for this problem appears to be the fear to delay the oncological treatment and consequently reducing the oncological outcome, not only in the patients' but also in the oncologists' mind.(17) At present, only a few studies have investigated the validity of this concern; all published studies refuted this concern although the quality of published evidence is

low. The oldest studies were performed using a conventional-start ovarian stimulation, starting at basal hormonal levels in the beginning of the menstrual cycle, in breast cancer patients treated with adjuvant chemotherapy.(18-20) The most recent studies reviewed the use of random-start ovarian stimulation in breast cancer patients treated with neo-adjuvant chemotherapy.(27, 28)

Besides breast cancer patients, which is the largest group of cancer patients referred for FP, patients with other types of cancer could also benefit from a fertility preservation treatment, for example patients with Hodgkin's lymphoma. (7, 10, 30, 31)

To our knowledge, no studies have been performed regarding the optimal time interval between diagnosis and initiation of the chemotherapy in patients diagnosed with Hodgkin's lymphoma. Both physicians and patients are consequently eager to start chemotherapy as soon as possible. This leads to a limited time frame wherein FP could be performed. Nevertheless, the first line treatment in most low-grade HL patients consists of a combination of Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD) which is not overtly gonadotoxic, in contrast with the more aggressive protocol using Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Oncovin, Procarbazine and Prednisolone (BEACOPP) that contains alkylating agents.(15, 30-32)

In addition, the reproductive technologies that are currently available include other, more experimental FP approaches, than COS. In vitro maturation of immature oocytes and ovarian tissue cryopreservation are emerging techniques that can be performed at very short notice, which could be highly relevant and promising for emergency FP in cancer patients.(10, 24, 33, 34)

In this context, there appears to be a necessity to enhance the awareness of both patients and oncologists concerning the gonadotoxic risks correlated with a systemic chemotherapy and the options to prevent potential infertility as a consequence of this treatment.

Investigating the impact of a fertility preservation procedure on the time interval between oncological diagnosis and initiation of the chemotherapy, considering a larger variety of cancer patients and including all fertility preservation techniques, may have a significant value in this debate.

METHODS

Objectives

The principal aim of this study is to verify whether fertility preservation in adult reproductive aged women with a new diagnosis of a breast cancer or Hodgkin's lymphoma would have an impact on the timing of the start of chemotherapy. We also investigated the total time frame of fertility preservation in these patients in order to optimise the clinical practice of fertility preservation.

Study design

We conducted a retrospective cohort study on a population of female patients with a new diagnosis of breast cancer or Hodgkin's lymphoma and who underwent fertility preservation before the start of (potentially) gonadotoxic cancer treatment at the Centre for Reproductive Medicine (CRG) at Universitair Ziekenhuis Brussel (UZ Brussel) between January 2012 and December 2017 (case group). This case group was compared to a matched control group of female cancer patients treated at the Breast Cancer Clinic or at the haematological department at UZ Brussel in the same period, but who were not referred to a fertility centre for FP.

The study protocol was approved by the Ethical Committee of the UZ Brussel (B.U.N. 143201834864).

Study population

The entire study population consists of two different groups: a case group and a control group.

Cohort 1: case-group

The case group consists of female cancer patients who underwent a FP treatment in the context of oncofertility. Patients were selected from the oncofertility database of the CRG and needed to fulfil the following inclusion and exclusion criteria:

Inclusion criteria:

- Women aged ≥18 and ≤40 years at diagnosis
- De novo diagnosis of breast cancer or Hodgkin's lymphoma with the need for systemic chemotherapy
- FP at the CRG at UZ Brussel between January 2012 and December 2017 in the context of this diagnosis and finished before initiation of the chemotherapy.

Exclusion criteria:

- Men, children, women aged >40 years at diagnosis
- FP in a non-oncological context
- Oncological diagnosis different from breast cancer or Hodgkin's lymphoma
- Systemic chemotherapy for the current diagnosis initiated before finishing the FP
- No need for chemotherapy
- Insufficient data available regarding the date of diagnosis, referral and/or initiation of chemotherapy.

Cohort 2: control-group

The control group consists of cancer patients treated at the UZ Brussel who were not referred to a fertility centre for FP. This population was matched individually to the patients in the case group and was selected from the database of the Breast Cancer Clinic and the haematological department at UZ Brussel, after fulfilling the following criteria:

Inclusion criteria:

- Women aged ≥18 years at diagnosis
- De novo diagnosis of breast cancer or Hodgkin's lymphoma with the need for systemic chemotherapy.

Exclusion criteria:

- Men and children
- FP or consultation at a fertility centre performed in the context of this diagnosis
- Oncological diagnosis different from breast cancer or Hodgkin's lymphoma
- No need for chemotherapy
- Insufficient data available regarding the date of diagnosis and/or initiation of chemotherapy.

Matching principles

Patients were matched based on the tumour characteristics and biology and the oncological treatment modalities used since those elements were considered as the crucial factors to determine the emergency of the chemotherapy. In order to match for all these factors, categories have been composed to define the priority of each different variable to match with. This is shown in **Table 1** for the patients diagnosed with Hodgkin's lymphoma and **Table 2** for the breast cancer patients. Factors in the first category are completely matched for, factors in the second category needed to match as good as possible and factors in the third category were used to select the control patient if there were still a few possible matches left.

Table 1: Matching criteria for Hodgkin's lymphoma patients

Category 1: necessary	Category 2: recommended	Category 3: ideally
Prognostic factor cat. 1 (early vs. advanced stage)	Ann Arbor Stage	Year of incidence
Prognostic factor cat. 2 (favourable vs. unfavourable in early stages)	Morphology	Age at oncological diagnosis
Treatment 1: start with ABVD vs. BEACOPP	Prognostic factor cat. 2 (risk based on IPS-score in advanced stages)	
	Treatment 2: combination with radiotherapy	

Table 2: Matching criteria for breast cancer patients

Category 1: necessary	Category 2: recommended	Category 3: ideally
Chemotherapy in a neo-adjuvant or adjuvant setting	cN-stage	Radiotherapy necessity
cT-stage	pTN-stage (in case of adjuvant chemotherapy)	ypTN-stage (in case of neo-adjuvant chemotherapy)
Hormone therapy necessity	Degree of differentiation	Year of incidence, time periods: 2012-2014 or 2015-2017
	Morphology	Age at oncological diagnosis
	Targeted therapy necessity (Trastuzumab)	

Data collection

The selection of women in the case group was based on a list of patients who underwent a fertility preservation in the context of oncofertility at the Centre for Reproductive Medicine (CRG) at UZ Brussel. This includes patients who were referred by a treating oncologist associated to the UZ Brussel or associated to another hospital in Belgium or Europe. The list of patients did not contain

any medical or oncological information. Therefore, we collected the medical data from medical reports already available in the patient's electronic medical file (EMD) at the UZ Brussel or requested at the referring physician's hospital.

The selection of women in the control group was based on a list of patients available from the Breast Cancer Clinic and the haematological department at UZ Brussel. At first, the patients who met the inclusion and exclusion criteria were selected from this list. Secondly, the possible matches in this control group for each case patient were listed. Finally, the best match for each case patient was selected and the data collection was completed. Medical data were collected from the EMD at the UZ Brussel.

The collected information concerned the fertility-related consultations and treatment method, and the oncological consultations, staging and treatment, with special attention for the dates on which the different oncological and fertility-related procedures were performed.

In more detail, the following information was collected for statistical analysis:

- Patient characteristics: age at oncological diagnosis, referring physician (internal or external referral)
- Oncological data: initial and final diagnosis and staging (tumour biology included), staging process, applicated treatment modalities
- Oncological course: date of oncological intake, date of initiation of the staging, date of biopsy, date of surgery, date of initiation of the chemotherapy
- Fertility preservation data (case group): process of fertility diagnostics and treatment, applicated fertility preservation treatment modalities
- Fertility course (case group): date of referral, date of fertility-related intake, date of initiation of the fertility preservation treatment and date of the last necessary physical contact at the fertility centre.

Data processing and analysis was performed anonymously in accordance to the 'Act of 8 December 1992 on the protection of privacy in relation to the processing of personal data (Privacy Act)'.

Endpoints

Primary endpoints

The primary endpoint of this study concerns the time interval between oncological diagnosis and initiation of chemotherapy, expressed in days and compared between the two cohorts. The date of the first biopsy showing malignancy was used as the date of diagnosis. The date of the first hospital visit in order to receive chemotherapy was used as the date of initiation of the chemotherapy, even when the therapy could not start due to a non-fertility-related reason (e.g. complications with the port used for the chemotherapy).

Secondary endpoints

Within the case group time intervals, expressed in days, between the different steps in the fertility-related and oncological process will be calculated, in particular:

- Diagnosis to referral: time interval between oncological diagnosis and referral
- Referral to intake: time interval between referral and FP intake
- Intake to initiation FP: time interval between FP intake and initiation of the FP treatment
- Duration FP: time interval between initiation of the FP treatment and last necessary physical presence at the CRG
- End FP to chemotherapy: time interval between termination of the FP (last physical presence at the CRG) and initiation of the chemotherapy
- Scheduling of the surgery within this time frame, in case of breast cancer patients
- Combinations of the time intervals mentioned above.

Subgroup analyses

Regarding to the fertility preservation procedure performed in the patients in the case group, we performed some additional subgroup analyses to detect whether there is a difference in duration in any of the phases in this program. Such differences would be interesting to ameliorate and fasten the fertility treatment in order to enhance the fertility-related and oncological outcome.

First of all, we performed subgroup analyses based on whether the referring physician was associated to the UZ Brussel, since we hypothesise that a close collaboration within the

multidisciplinary approach, which is encouraged when physicians work together in the same team, could fasten the procedures.

For the patients with Hodgkin's lymphoma we performed additional subgroup analyses based on the regimen of chemotherapy the patients started their treatment with. An oncological treatment starting with the more aggressive BEACOPP-regimen is mostly recommended in the advanced stages of the disease, further assuming this to be a more urgent treatment to start compared to a treatment starting with ABVD. Moreover the need for fertility preservation in patients receiving ABVD is controversial since it only has a limited risk for fertility loss of 10% in patients aged younger than 30 years, although quickly emerging above the age of 30 years.(30)

Since the oncological treatment for breast cancer patients has evolved over the past years with the implementation of chemotherapy in a neo-adjuvant setting, as well as the fertility preservation modalities, we also divided the breast cancer patients into a group diagnosed in the period between 2012 and 2014 and between 2015 and 2017.
At last we performed subgroup analyses on the group of breast cancer patients receiving adjuvant chemotherapy based on whether the patient was referred to the fertility centre before or after the breast cancer surgery. The sequence of the multiple steps in the procedure can also influence the time course and the oncological and fertility-related outcome.

Statistics

Statistical analyses were performed using IBM SPSS Statistics, Version 23.0.

Three main groups were regarded separately: patients diagnosed with Hodgkin's lymphoma, diagnosed with breast cancer treated with neo-adjuvant chemotherapy or treated with adjuvant chemotherapy.

Demographical and tumour characteristics were reported for each study population using descriptive statistics.
In the context of the main objective of this study the time interval between diagnosis and start of the chemotherapy was compared between the two cohorts in each of the three groups described

above. In the group of breast cancer patients receiving adjuvant chemotherapy the scheduling of the surgery was reported as well. In the context of the supplemental objectives some subgroup analyses based on descriptive and analytic statistics were performed.

In case of categorical variables Fisher's Exact and Pearson Chi-Square tests were used. Continuous variables were checked for normality using a Q-Q plot. If normality was observed independent t-tests were used, otherwise a non-parametric Mann-Whitney U test (with exact significance) was performed. We chose not to perform paired t-tests for the main study question although the two cohorts were accurately matched, since there are always confounding factors that differ in the two matched patients that were not taken into account in this study.
All tests were evaluated two-sided and a difference was assumed to be significant if $p < 0,05$.

RESULTS

Study population

Hodgkin's lymphoma patients

There were 24 patients with a haematological malignancy who underwent a fertility preservation treatment at the CRG at UZ Brussel between January 2012 and December 2017. In total, 13 patients were excluded from this study for multiple reasons: eight women had a malignancy other than Hodgkin's lymphoma, insufficient data was available for three patients, one patient had already received chemotherapy in the context of the current diagnosis before referral and one patient started radiotherapy as the first treatment long before the start of the chemotherapy. In total, 11 women with Hodgkin's lymphoma were included in the case group.

A total of 20 adult women were treated for Hodgkin's lymphoma at the haematological department at UZ Brussel in the period between January 2012 and December 2017, of which seven patients underwent fertility preservation and were included in the case group. This was insufficient to match for each case patient. Therefore, the population had been expanded with six patients who were treated at UZ Brussel between January 2010 and December 2011, resulting in a population of 19 adult women diagnosed with Hodgkin's lymphoma fulfilling the inclusion and exclusion criteria for the control group.

Eight pairs were matched based on the criteria mentioned above. Three patients in the case group have not been matched since their oncological treatment started with a BEACOPP-regimen and none of the patients in the control group received BEACOPP as the initial treatment. These three patients were not included to investigate the time interval between cancer diagnosis and initiation of chemotherapy comparing the case and control group, but were included in the case group to investigate the time frame of the fertility preservation in the subgroup analyses.

Breast cancer patients

A total of 87 adult women who underwent a fertility preservation treatment in the context of a breast cancer diagnosis between January 2012 and December 2017 were screened for inclusion. Twenty-eight women were excluded for several reasons: two women were aged 41 years or older at diagnosis, one woman never had chemotherapy, one woman refused to participate in the study, one woman had an ongoing fertility treatment at the moment of diagnosis, two women started fertility preservation at a fertility centre in another country and insufficient data was available for 21 patients. The resulting 59 women were divided into two groups: a group of 29 women who received chemotherapy in a neo-adjuvant setting and a group of 30 women receiving chemotherapy in an adjuvant setting.

These 59 patients were matched with 59 women diagnosed with breast cancer between January 2012 and December 2016 who were never referred to a fertility centre, selected from the database of the Breast Cancer Clinic at UZ Brussel.

Group 1: Hodgkin's lymphoma patients

Population characteristics

A description of the demographical and oncological characteristics of the eight pairs are presented in **Table 3a**. The case group (n = 8) was found to be significantly younger than the control group (n = 8), with an average of 24.7 versus 42.9 years (p = 0.008) respectively. Other patient and tumour characteristics, of which the majority was used as a fundamental variable for the matching, were not significantly different. Inherent to the study design all control patients were treated at the haematological department at UZ Brussel, while not all case patients were treated in this hospital (50%). In general, 68.8% of the patients (n = 16) had a nodular sclerosing Hodgkin's lymphoma (NSHL), whereas 31.3% had a mixed cellularity Hodgkin's lymphoma (MCHL). Twelve patients (75%) still had an early stage disease, of which 83.3% had favourable and 16.7% had unfavourable prognostic factors, and four patients (25%) already had an advanced stage of disease at diagnosis. A total of seven patients (43.8%) was treated with a combination of chemotherapy and radiotherapy, whereas nine patients (56.3%) only received chemotherapy.

Table 3a: Demographical and oncological characteristics in Hodgkin's lymphoma patients, case versus control

	Total n=16	Case n=8	Control n=8	p-value (2-sided)
Age (y)				
Mean ± SD	33,8 ± 13,8	24,7 ± 4,1	42,9 ± 14,2	0,008[a]
Median ± IQR	27,8 ± 24,0	24,2 ± 7,7	44,5 ± 27,4	
Year of incidence				
2010-2014	8 (50,0%)	3 (37,5%)	5 (62,5%)	0,619[b]
2015-2017	8 (50,0%)	5 (62,5%)	3 (37,5%)	
Referring physician				
Intern	12 (75,0%)	4 (50,0%)	8 (100,0%)	0,077[b]
Extern	4 (25,0%)	4 (50,0%)	0 (0,0%)	
Morphology				
NSHL	11 (68,8%)	6 (75,0%)	5 (62,5%)	1,000[b]
MCHL (incl IFHL)	5 (31,3%)	2 (25,0%)	3 (37,5%)	
Ann Arbor Stage				
IA	1 (6,3%)	1 (12,5%)	0 (0,0%)	—[c]
IIA	8 (50,0%)	5 (62,5%)	3 (37,5%)	
IIAX	1 (6,3%)	0 (0,0%)	1 (12,5%)	
IIB	2 (12,5%)	0 (0,0%)	2 (25,0%)	
IIIBS	2 (12,5%)	1 (12,5%)	1 (12,5%)	
IVA	2 (12,5%)	1 (12,5%)	1 (12,5%)	
Prognostic factors				
Early	12 (75,0%)	6 (75,0%)	6 (75,0%)	1,000[b]
- Early favourable	*10 (83,3%)*	*5 (83,3%)*	*5 (83,3%)*	*1,000*[b]
- Early unfavourable	*2 (16,7%)*	*1 (16,7%)*	*1 (16,7%)*	
Advanced	4 (25,0%)	2 (25,0%)	2 (25,0%)	
- Advanced good risk	*1 (25,0%)*	*1 (50,0%)*	*0 (0,0%)*	*1,000*[b]
- Advanced fair risk	*3 (75,0%)*	*1 (50,0%)*	*2 (100,0%)*	
- Advanced poor risk	*0 (0,0%)*	*0 (0,0%)*	*0 (0,0%)*	
Oncological therapy				
Chemotherapy	9 (56,3%)	4 (50,0%)	5 (62,5%)	1,000[b]
Chemotherapy + Radiotherapy	7 (43,8%)	4 (50,0%)	3 (37,5%)	

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate.
[a]= Independent t-test; [b]= Fisher's Exact test; [c]= assumptions for statistical analysis not met.

Comparing the subgroups within the case group (n = 11), based on the referring physician (internal referral (n = 7) or external referral (n = 4)) and the type of initial chemotherapy (ABVD (n = 8) or BEACOPP (n = 3)), no significant differences in patient or tumour characteristics were detected (**Table 3b**).

Table 3b: Demographical and oncological characteristics in Hodgkin's lymphoma case patients

	Total n=11	Intern n=7	Extern n=4	p-value (2-sided)	ABVD n=8	BEACOPP n=3	p-value (2-sided)
Age (y)							
Mean ± SD	24,4 ± 3,4	23,5 ± 2,7	25,9 ± 4,5	0,315[a]	24,7 ± 4,1	23,5 ± 0,3	1,000[a]
Median ± IQR	23,3 ± 5,0	23,3 ± 2,1	26,1 ± 8,5		24,2 ± 7,7	23,3 ± 0,0	
Year of incidence							
2012-2014	3 (27,3%)	1 (14,3%)	2 (50,0%)	0,491[b]	3 (37,5%)	0 (0,0%)	0,491[b]
2015-2017	8 (72,7%)	6 (85,7%)	2 (50,0%)		5 (62,5%)	3 (100,0%)	
Referring physician							
Intern	7 (63,6%)	NA	NA	NA	4 (50,0%)	3 (100,0%)	0,236[b]
Extern	4 (36,4%)	NA	NA		4 (50,0%)	0 (0,0%)	
Morphology							
NSHL	8 (72,7%)	6 (85,7%)	2 (50,0%)	0,491[b]	6 (75,0%)	2 (66,7%)	1,000[b]
MCHL (incl IFHL)	3 (27,3%)	1 (14,3%)	2 (50,0%)		2 (25,0%)	1 (33,3%)	
Ann Arbor Stage							
IA	1 (9,1%)	1 (14,3%)	0 (0,0%)	—[c]	1 (12,5%)	0 (0,0%)	—[c]
IIA	5 (45,5%)	3 (42,9%)	2 (50,0%)		5 (62,5%)	0 (0,0%)	
IIAX	1 (9,1%)	1 (14,3%)	0 (0,0%)		0 (0,0%)	1 (33,3%)	
IIIBS	1 (9,1%)	0 (0,0%)	1 (25,0%)		1 (12,5%)	0 (0,0%)	
IVA	2 (18,2%)	1 (14,3%)	1 (25,0%)		1 (12,5%)	1 (33,3%)	
IVBE	1 (9,1%)	1 (14,3%)	0 (0,0%)		0 (0,0%)	1 (33,3%)	
Prognostic factors							
Early	7 (63,6%)	5 (71,4%)	2 (50,0%)	0,576[b]	6 (75,0%)	1 (33,3%)	0,491[b]
- Early favourable	*5 (71,4%)*	*3 (60,0%)*	*2 (100,0%)*	*1,000[b]*	*5 (83,3%)*	*0 (0,0%)*	*0,286[b]*
- Early unfavourable	*2 (28,6%)*	*2 (40,0%)*	*0 (0,0%)*		*1 (16,7%)*	*1 (100,0%)*	
Advanced	4 (36,4%)	2 (28,6%)	2 (50,0%)		2 (25,0%)	2 (66,7%)	
- Advanced good risk	*1 (25,0%)*	*0 (0,0%)*	*1 (50,0%)*	*1,000[b]*	*1 (50,0%)*	*0 (0,0%)*	*1,000[b]*
- Advanced fair risk	*3 (75,0%)*	*2 (100,0%)*	*1 (50,0%)*		*1 (50,0%)*	*2 (100,0%)*	
- Advanced poor risk	*0 (0,0%)*	*0 (0,0%)*	*0 (0,0%)*		*0 (0,0%)*	*0 (0,0%)*	
Oncological therapy							
Chemotherapy	7 (63,6%)	5 (71,4%)	2 (50,0%)	0,576[b]	4 (50,0%)	3 (100,0%)	0,236[b]
Chemotherapy + Radiotherapy	4 (36,4%)	2 (28,6%)	2 (50,0%)		4 (50,0%)	0 (0,0%)	
Type of chemotherapy							
ABVD	8 (72,7%)	4 (57,1%)	4 (100,0%)	0,236[b]	NA	NA	NA
BEACOPP	3 (27,3%)	3 (42,9%)	0 (0,0%)		NA	NA	

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate. NA = not applicable.

[a]= Mann-Whitney U test (exact significance); [b]= Fisher's Exact test; [c]= assumptions for statistical analysis not met.

Oncological time course

Table 4a presents the time interval between oncological diagnosis and initiation of chemotherapy in comparison between the case and control group, while **Table 4b** presents the same interval in the different subgroups. Statistically no significant difference has been verified between the case (33.2 ± 18.7 days) and control (20.1 ± 9.9 days) group (p = 0.102), the case groups with an internal (25.0 ± 11.1 days) or external (34.0 ± 27.4 days) referral (p = 0.450) and the case groups initially treated with ABVD (33.2 ± 18.7 days) or BEACOPP (15.0 ± 1.0 days) (p = 0.138).

Table 4a: Time to chemotherapy in Hodgkin's lymphoma patients, case versus control

	Total n=16	Case n=8	Control n=8	p-value (2-sided)
Diagnosis to chemotherapy (d)				
Mean ± SD	26,7 ± 16,0	33,2 ± 18,7	20,1 ± 9,9	0,102[a]
Median ± IQR	26,0 ± 20,0	31,5 ± 24,0	18,5 ± 20,0	
Minimum	7,0	9,0	7,0	
Maximum	70,0	70,0	33,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Independent t-test.

Table 4b: Time to chemotherapy and duration of FP in Hodgkin's lymphoma case patients

	Total n=11	Intern n=7	Extern n=4	p-value (2-sided)	ABVD n=8	BEACOPP n=3	p-value (2-sided)
Diagnosis to chemotherapy (d)							
Mean ± SD	28,3 ± 17,8	25,0 ± 11,1	34,0 ± 27,4	0,450[a]	33,2 ± 18,7	15,0 ± 1,0	0,138[a]
Median ± IQR	24,0 ± 25,0	24,0 ± 20,0	28,5 ± 52,0		31,5 ± 24,0	15,0 ± 0,0	
Minimum	9,0	14,0	9,0		9,0	14,0	
Maximum	70,0	43,0	70,0		70,0	16,0	
Referral to end FP (d)							
Mean ± SD	12,2 ± 8,4	12,1 ± 7,2	12,2 ± 11,5	0,927[b]	13,8 ± 9,3	8,0 ± 3,5	0,497[b]
Median ± IQR	10,0 ± 15,0	10,0 ± 14,0	11,0 ± 21,0		17,0 ± 19,0	10,0 ± 0,0	
Minimum	2,0	4,0	2,0		2,0	4,0	
Maximum	25,0	23,0	25,0		25,0	10,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Independent t-test; [b]= Mann-Whitney U test (exact significance).

Time frame of fertility preservation

In **Table 5** the FP modalities used in the case group are presented and compared between the different subgroups. Overall in the group of 11 case patients, three underwent a controlled ovarian stimulation (COS), three underwent an oocyte pick-up for in vitro maturation (IVM OPU), two underwent the combination of a COS and an IVM OPU and three patients underwent the combination of an IVM OPU and an OTCP. Ovarian tissue cryopreservation was only performed in the patients receiving BEACOPP as the first oncological treatment, in combination with IVM OPU. The patients referred by an internal physician (including the three patients receiving BEACOPP) used all three FP modalities, while patients referred by an external physician only used controlled ovarian stimulation and IVM OPU.

Table 5: Fertility preservation modalities in Hodgkin's lymphoma case patient

	Total n=11	Intern n=7	Extern n=4	p-value (2-sided)	ABVD n=8	BEACOPP n=3	p-value (2-sided)
COS	5 (45,5%)	3 (42,9%)	2 (50,0%)	1,000[a]	5 (62,5%)	0 (0,0%)	0,182[a]
OTCP	3 (27,3%)	3 (42,9%)	0 (0,0%)	0,236[a]	0 (0,0%)	3 (100,0%)	0,006[a]
IVM OPU	8 (72,7%)	5 (71,4%)	3 (75,0%)	1,000[a]	5 (62,5%)	3 (100,0%)	0,491[a]
Combination	5 (45,5%)	4 (57,1%)	1 (25,0%)	0,545[a]	2 (25,0%)	3 (100,0%)	0,061[a]

Results are expressed as N (%) when appropriate.
[a]= Fisher's Exact test.

The fertility preservation program, from referral for FP counselling to termination of the FP treatment, took on average 12.2 ± 8.4 days, with no significant difference between the groups with an internal (12.1 ± 7.2 days) or external (12.2 ± 11.5 days) referral (p = 0.927), and with a, not significant, trend towards a shorter interval in patients treated with BEACOPP (8.0 ± 3.5 vs. 13.8 ± 9.3 days, p = 0.497). (**Table 4b**)

An overview of the entire time frame of the fertility preservation procedure was presented in **Figure 1**, showing the timelines of the entire case group and the different subgroups composed based on the mean of each time interval. Although no significant difference was found concerning the interval between oncological diagnosis and initiation of chemotherapy, some intervals appear to deviate from others. The entire time frame appears to be shorter in the group with an internal referring physician and in the group treated with BEACOPP. Within this time frame the referral appears to occur sooner after diagnosis when done by an internal physician and when the use of BEACOPP was indicated. In these groups, there appears to be a longer period between the FP

intake and the start of FP, whereas the duration of the FP procedure is shorter. Furthermore, in the group treated with BEACOPP and in the group referred by an external physician the chemotherapy appeared to be initiated sooner after the FP treatment.

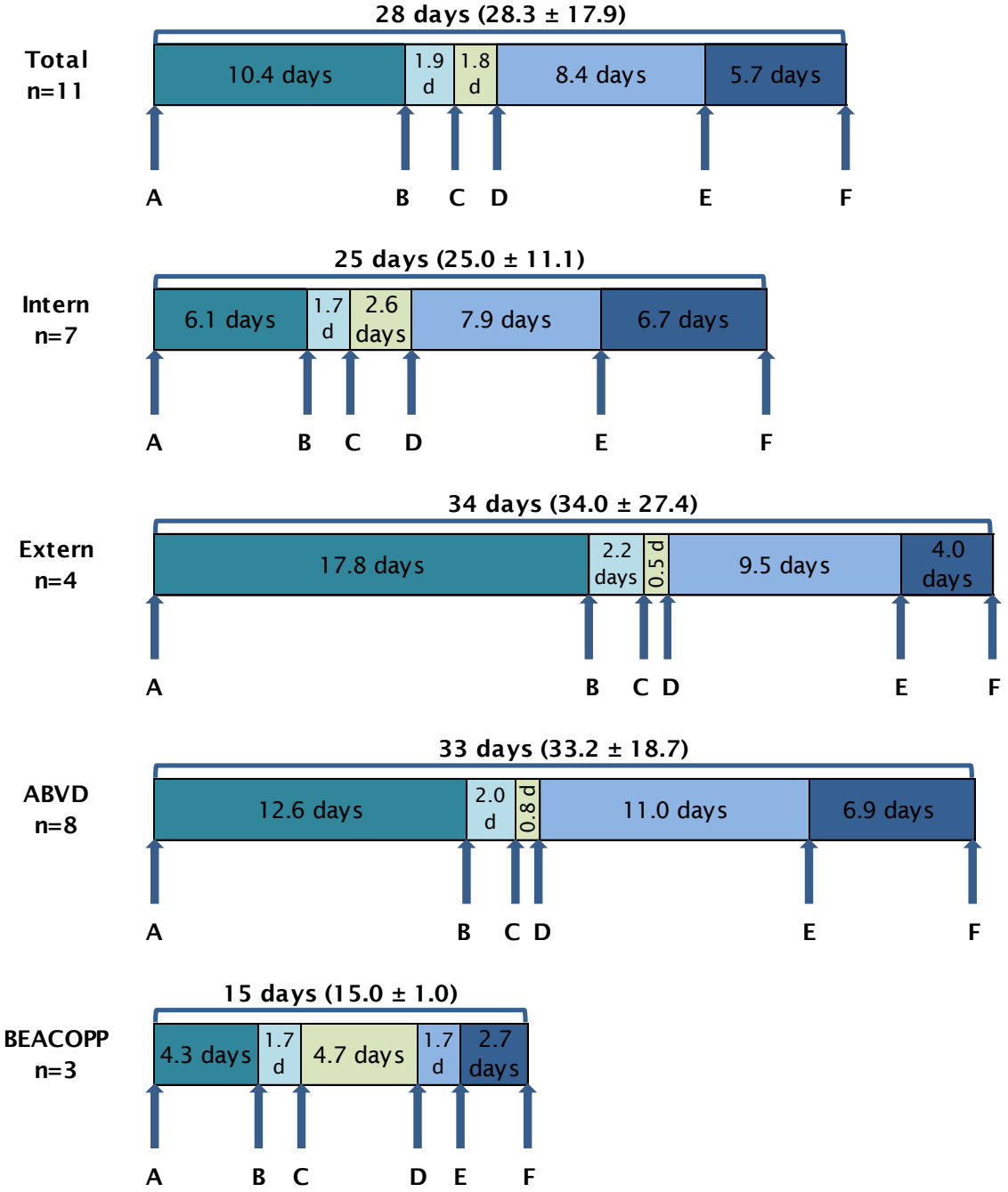
Additionally, some of the different steps in the FP procedure occurred on the same day (**Table 6**): only one patient had referral on the same day of diagnosis, two patients had an intake at the fertility centre on the same day as the referral and four patients were able to start their FP treatment on the day of intake.

Table 6: Steps in the fertility preservation procedure on the same day in Hodgkin's lymphoma case patients

		Total n=11	Intern n=7	Extern n=4	ABVD n=8	BEACOPP n=3
Diagnosis to referral	**Δ=0**	1 (9,1%)	1 (14,3%)	0 (0,0%)	1 (12,5%)	0 (0,0%)
Referral to intake	**Δ=0**	2 (18,2%)	2 (28,6%)	0 (0,0%)	1 (12,5%)	1 (33,3%)
Intake to initiation FP	**Δ=0**	4 (36,4%)	2 (28,6%)	2 (50,0%)	4 (50,0%)	0 (0,0%)
Duration FP	**Δ=0**	3 (27,3%)	1 (14,3%)	2 (50,0%)	2 (25,0%)	1 (33,3%)
End FP to chemotherapy	**Δ=0**	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)

Results are expressed as N (%).
Δ= Time interval.

Figure 1: Timelines of FP program in case patients with Hodgkin's lymphoma



Mean of each time interval is presented, mean ± SD is presented for the entire time frame. Values are expressed in days. A = oncological diagnosis; B = referral to fertility centre; C = intake at fertility centre; D = initiation of fertility preservation; E = termination of fertility preservation (last physical contact at the fertility centre); F = initiation of chemotherapy.

Group 2: Breast cancer patients with neo-adjuvant chemotherapy

Population characteristics

Demographical and oncological characteristics are presented in **Table 7a** in comparison between the case and control group, and in **Table 7b** in comparison between the different subgroups based on the referring physician and the year of incidence (two categories: 2012-2014 and 2015-2017). The case and control group only differed significantly regarding the age at diagnosis: patients who underwent a FP treatment were younger at diagnosis (31.1 years) compared to those who did not (54.6 years) ($p < 0,0001$). Most of the other variables were used for matching and did not differ significantly. Inherent to the study design all control patients were treated at the Breast Cancer Clinic at UZ Brussel, while not all case patients were (41.4%). Of all patients 82.8% had an invasive ductal adenocarcinoma (IDA), 15.5% had a combination of an IDA and a ductal or lobular carcinoma in situ (DCIS, LCIS) and 1.7% had another type of tumour morphology. Most of them were poorly differentiated (70.7%) and the majority was diagnosed at tumour stage II (65.5%) while 17.2% of the patients were diagnosed at stage I and 17.2% at stage III. A total of 55.2% of the patients had a hormone receptor (HR) positive status (oestrogen receptor (ER), progesterone receptor (PR) or both positive) and 21.1% had a Human Epidermal growth factor Receptor 2 (HER2) amplification.

Regarding the patient and tumour characteristics of the cases no significant difference was found in comparison between the subgroups.

Table 7a: Demographical and oncological characteristics in BC patients with NAC, case versus control

	Total n=58	Case n=29	Control n=29	p-value (2-sided)
Age (y)				
Mean ± SD	42,9 ± 14,6	31,1 ± 4,0	54,6 ± 11,5	<0,0001[a]
Median ± IQR	37,1 ± 23,3	30,2 ± 6,0	53,1 ± 15,7	
Year of incidence				
2012-2014	20 (34,5%)	12 (41,4%)	8 (27,6%)	0,408[b]
2015-2017	38 (65,5%)	17 (58,6%)	21 (72,4%)	
Referring physician				
Intern	41 (70,7%)	12 (41,4%)	29 (100,0%)	<0,0001[b]
Extern	17 (29,3%)	17 (58,6%)	0 (0,0%)	
Morphology				
IDA	48 (82,8%)	23 (79,3%)	25 (86,2%)	—[d]
IDA+DCIS/LCIS	9 (15,5%)	5 (17,2%)	4 (13,8%)	
Other	1 (1,7%)	1 (3,4%)	0 (0,0%)	
Differentiation				
Moderate	17 (29,3%)	8 (27,6%)	9 (31,0%)	1,000[b]
Poor	41 (70,7%)	21 (72,4%)	20 (69,0%)	
Clinical tumour stage				
I	10 (17,2%)	5 (17,2%)	5 (17,2%)	1,000[c]
II	38 (65,5%)	19 (65,5%)	19 (65,5%)	
III	10 (17,2%)	5 (17,2%)	5 (17,2%)	
ER positivity				
Negative	28 (48,3%)	13 (44,8%)	15 (51,7%)	—[d]
Weakly positive	7 (12,1%)	3 (10,3%)	4 (13,8%)	
Strongly positive	23 (39,7%)	13 (44,8%)	10 (34,5%)	
PR positivity				
Negative	34 (58,6%)	17 (58,6%)	17 (58,6%)	—[d]
Weakly positive	7 (12,1%)	3 (10,3%)	4 (13,8%)	
Strongly positive	17 (29,3%)	9 (31,0%)	8 (27,6%)	
HER2 amplification present	12 (21,1%)	6 (21,4%)[e]	6 (20,7%)	1,000[b]
Ki67 score				
0-15%	5 (10,0%)	2 (7,7%)[g]	3 (12,5%)[h]	—[d]
16-25%	8 (16,0%)	4 (15,4%)[g]	4 (16,7%)[h]	
26-35%	9 (18,0%)	3 (11,5%)[g]	6 (25,0%)[h]	
36-45%	4 (8,0%)	1 (3,8%)[g]	3 (12,5%)[h]	
>45%	24 (48,0%)	16 (61,5%)[g]	8 (33,3%)[h]	
Additional oncological therapy				
Radiotherapy	50 (89,3%)	24 (88,9%)[f]	26 (89,7%)	1,000[b]
Hormone therapy	32 (55,2%)	16 (55,2%)	16 (55,2%)	1,000[b]
Targeted therapy	12 (21,1%)	6 (21,4%)[e]	6 (20,7%)	1,000[b]

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate. [a]= Mann-Whitney U test (exact significance); [b]= Fisher's Exact test; [c]= Pearson Chi-Square; [d]=assumptions for statistical analysis not met; [e]= 1 value unknown, counts on 28; [f]= 2 values unknown, counts on 27; [g]= 3 values unknown, counts on 26; [h]= 5 values unknown, counts on 24.

Table 7b: Demographical and oncological characteristics in BC case patients with NAC

	Intern n=12	Extern n=17	p-value (2-sided)	2012-2014 n=12	2015-2017 n=17	p-value (2-sided)
Age (y)						
Mean ± SD	31,2 ± 3,8	31,0 ± 4,2	0,744[a]	31,5 ± 3,1	30,8 ± 4,6	0,616[a]
Median ± IQR	30,4 ± 6,8	29,2 ± 6,2		31,0 ± 6,4	29,2 ± 7,2	
Year of incidence						
2012-2014	5 (41,7%)	7 (41,2%)	1,000[b]	NA	NA	NA
2015-2017	7 (58,3%)	10 (58,8%)		NA	NA	
Referring physician						
Intern	NA	NA	NA	5 (41,7%)	7 (41,2%)	1,000[b]
Extern	NA	NA		7 (58,3%)	10 (58,8%)	
Morphology						
IDA	9 (75,0%)	14 (82,4%)	—[c]	9 (75,0%)	14 (82,4%)	—[c]
IDA+DCIS/LCIS	3 (25,0%)	2 (11,8%)		3 (25,0%)	2 (11,8%)	
Other	0 (0,0%)	1 (5,9%)		0 (0,0%)	1 (5,9%)	
Differentiation						
Moderate	4 (33,3%)	4 (23,5%)	0,683[b]	4 (33,3%)	4 (23,5%)	0,683[b]
Poor	8 (66,7%)	13 (76,5%)		8 (66,7%)	13 (76,5%)	
Clinical tumour stage						
I	2 (16,7%)	3 (17,6%)	—[c]	1 (8,3%)	4 (23,5%)	—[c]
II	7 (58,3%)	12 (70,6%)		8 (66,7%)	11 (64,7%)	
III	3 (25,0%)	2 (11,8%)		3 (25,0%)	2 (11,8%)	
ER positivity						
Negative	4 (33,3%)	9 (52,9%)	—[c]	3 (25,0%)	10 (58,8%)	—[c]
Weakly positive	2 (16,7%)	1 (5,9%)		2 (16,7%)	1 (5,9%)	
Strongly positive	6 (50,0%)	7 (41,2%)		7 (58,3%)	6 (35,3%)	
PR positivity						
Negative	6 (50,0%)	11 (64,7%)	—[c]	6 (50,0%)	11 (64,7%)	—[c]
Weakly positive	2 (16,7%)	1 (5,9%)		3 (25,0%)	0 (0,0%)	
Strongly positive	4 (33,3%)	5 (29,4%)		3 (25,0%)	6 (35,3%)	
HER2 amplification present	4 (33,3%)	2 (12,5%)[e]	0,354[b]	1 (8,3%)	5 (31,3%)[e]	0,196[b]
Ki67 score						
0-15%	1 (9,1%)[d]	1 (6,7%)[g]	—[c]	2 (20,0%)[f]	0 (0,0%)[e]	—[c]
16-25%	3 (27,3%)[d]	1 (6,7%)[g]		2 (20,0%)[f]	2 (12,5%)[e]	
26-35%	1 (9,1%)[d]	2 (13,3%)[g]		1 (10,0%)[f]	2 (12,5%)[e]	
36-45%	1 (9,1%)[d]	0 (0,0%)[g]		1 (10,0%)[f]	0 (0,0%)[e]	
>45%	5 (45,5%)[d]	11 (73,3%)[g]		4 (40,0%)[f]	12 (75,0%)[e]	
Additional oncological therapy						
Radiotherapy	10 (90,9%)[d]	14 (87,5%)[e]	1,000[b]	11 (91,7%)	13 (86,7%)[g]	1,000[b]
Hormone therapy	8 (66,7%)	8 (47,1%)	0,451[b]	9 (75,0%)	7 (41,2%)	0,130[b]
Targeted therapy	4 (33,3%)	2 (12,5%)[e]	0,354[b]	1 (8,3%)	5 (31,3%)[e]	0,196[b]

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate. NA= not applicable [a]= Mann-Whitney U test (exact significance); [b]= Fisher's Exact test; [c]=assumptions for statistical analysis not met; [d]= 1 value unknown, counts on 11; [e]= 1 value unknown, counts on 16; [f]= 2 values unknown, counts on 10; [g]= 2 values unknown, counts on 15.

Oncological time course

No significant difference was found regarding the time interval between oncological diagnosis and initiation of chemotherapy when comparing the case (27.3 ± 9.0 days) and control (29.6 ± 9.3 days) groups (p = 0.441) (**Table 8a**). Similar results apply to the subgroup analyses with an interval of 27.4 ± 8.8 days in patients with an internal and 27.3 ± 9.4 days in patients with an external referral (p = 1.000) and an interval of 25.0 ± 9.4 days in patients diagnosed in 2012-2014 and 29.0 ± 8.6 days in patients diagnosed in 2015-2017 (p = 0.245) (**Table 8b**).

Table 8a: Time to chemotherapy in BC patients with NAC, case versus control

	Total n=58	Case n=29	Control n=29	p-value (2-sided)
Diagnosis to chemotherapy (d)				
Mean ± SD	28,5 ± 9,2	27,3 ± 9,0	29,6 ± 9,3	0,441[a]
Median ± IQR	28,0 ± 11,0	27,0 ± 14,0	28,0 ± 10,0	
Minimum	14,0	14,0	14,0	
Maximum	62,0	44,0	62,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Mann-Whitney U test (exact significance).

Table 8b: Time to chemotherapy and duration of FP in BC case patients with NAC

	Total n=29	Intern n=12	Extern n=17	p-value (2-sided)	2012-2014 n=12	2015-2017 n=17	p-value (2-sided)
Diagnosis to chemotherapy (d)							
Mean ± SD	27,3 ± 9,0	27,4 ± 8,8	27,3 ± 9,4	1,000[a]	25,0 ± 9,4	29,0 ± 8,6	0,245[a]
Median ± IQR	27,0 ± 14,0	25,0 ± 14,0	28,0 ± 16,0		23,5 ± 17,0	28,0 ± 13,0	
Minimum	14,0	17,0	14,0		14,0	15,0	
Maximum	44,0	43,0	44,0		43,0	44,0	
Referral to end FP (d)							
Mean ± SD	11,9 ± 5,6	13,8 ± 5,1	10,5 ± 5,6	0,117[a]	11,2 ± 5,8	12,4 ± 5,5	0,679[a]
Median ± IQR	11,0 ± 10,0	13,0 ± 7,0	8,0 ± 10,0		9,5 ± 9,0	13,0 ± 11,0	
Minimum	3,0	6,0	3,0		3,0	5,0	
Maximum	22,0	22,0	21,0		21,0	22,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Mann-Whitney U test (exact significance).

Time frame of fertility preservation

Table 9 presents the fertility preservation modalities used in the case group in comparison between the different subgroups. A noticeable, albeit not significant trend is the fact that in the most recent years a controlled ovarian stimulation was more frequently applied (11/17 patients in 2015-2017 (64.7%) vs. 3/12 patients in 2012-2014 (25.0%), p = 0.060).

Table 9: Fertility preservation modalities in BC case patients treated with NAC

	Total n=29	Intern n=12	Extern n=17	p-value (2-sided)	2012-2014 n=12	2015-2017 n=17	p-value (2-sided)
COS	14 (48,3%)	6 (50,0%)	8 (47,1%)	1,000[a]	3 (25,0%)	11 (64,7%)	0,060[a]
OTCP	12 (41,4%)	5 (41,7%)	7 (41,2%)	1,000[a]	6 (50,0%)	6 (35,3%)	0,471[a]
IVM OPU	11 (37,9%)	5 (41,7%)	6 (35,3%)	1,000[a]	6 (50,0%)	5 (29,4%)	0,438[a]
Combination	8 (27,6%)	4 (33,3%)	4 (23,5%)	0,683[a]	3 (25,0%)	5 (29,4%)	1,000[a]

Results are expressed as N (%) when appropriate.
[a]= Fisher's Exact test.

Table 8b shows that the fertility preservation program, from referral for FP counselling to termination of the FP treatment, took on average 11.9 ± 5.6 days, with no significant difference between the groups treated in 2012-2014 and 2015-2017 (11.2 ± 5.8 vs. 12.4 ± 5.5 days, p = 0.679), and with a non-significant trend towards a shorter interval in patients with an external referral (10.5 ± 5.6 days) as compared to patients with an internal referral (13.8 ± 5.1 days) (p = 0.117).

Timelines regarding the time frame wherein the fertility preservation treatment was performed, are presented in **Figure 2** for the entire case group and the different subgroups. Although no significantly different time interval between oncological diagnosis and initiation of the chemotherapy was demonstrated, some trends can be noticed.

An internal referral and intake after the referral appears to occur sooner after diagnosis compared to patients with an external referral. However, the time interval between intake and initiation of the FP and the duration of the FP appears to be longer.

More recently it appears that the referral occurs later after the diagnosis, but the intake and initiation of the FP treatment are performed sooner. The duration of the FP appears to be prolonged in the last years. Overall, this results in an entire time frame that appears to be slightly longer in the most recent years, but no significance was found.

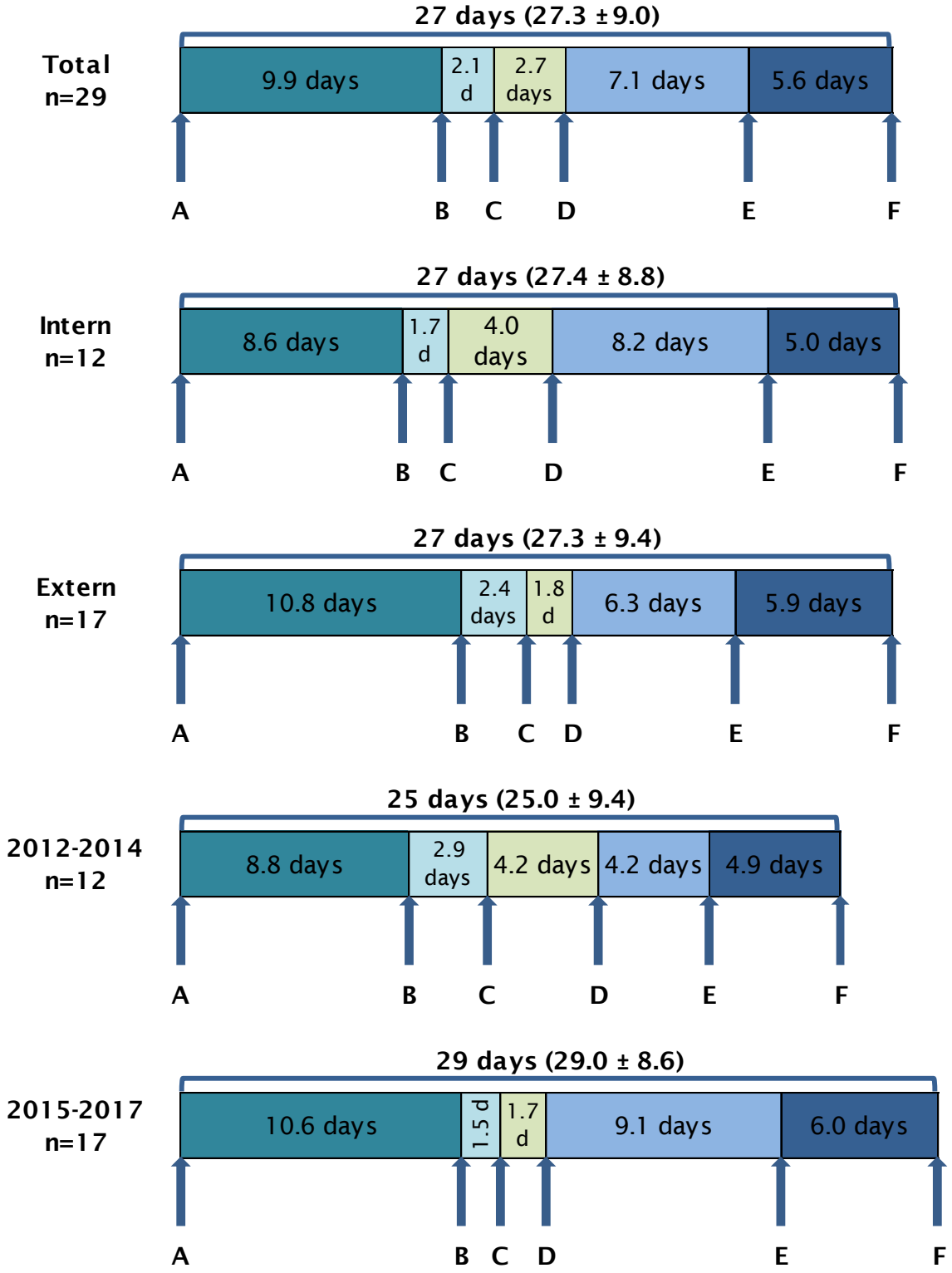
Furthermore, some of the different steps in the FP procedure appeared to occur on the same day (**Table 10**): one patient was referred on the day of diagnosis, five patients had an intake on the day of referral of which four had an internal referral, and 10 patients started their FP at the day of intake of which nine were treated between 2015 and 2017.

Table 10: Steps in the fertility preservation procedure on the same day in BC case patients with NAC

		Total n=29	Intern n=12	Extern n=17	2012-2014 n=12	2015-2017 n=17
Diagnosis to referral	**Δ=0**	1 (3,4%)	1 (8,3%)	0 (0,0%)	1 (8,3%)	0 (0,0%)
Referral to intake	**Δ=0**	5 (17,2%)	4 (33,3%)	1 (5,9%)	3 (25,0%)	2 (11,8%)
Intake to initiation of FP	**Δ=0**	10 (34,5%)	3 (25,0%)	7 (41,2%)	1 (8,3%)	9 (52,9%)
Duration FP	**Δ=0**	8 (27,5%)	2 (16,7%)	6 (35,3%)	5 (41,7%)	3 (17,6%)
End FP to chemo	**Δ=0**	1 (3,4%)	1 (8,3%)	0 (0,0%)	0 (0,0%)	1 (5,9%)

Results are expressed as N (%).
Δ= Time interval.

Figure 2: Timelines of FP program in BC case patients with NAC



Mean of each time interval is presented, mean ± SD is presented for the entire time frame. Values are expressed in days. A = oncological diagnosis; B = referral to fertility centre; C = intake at fertility centre; D = initiation of fertility preservation; E = termination of fertility preservation (last physical contact at the fertility centre); F = initiation of chemotherapy.

Group 3: Breast cancer patients with adjuvant chemotherapy

Population characteristics

Table 11a presents the comparative demographical and oncological characteristics of the group of breast cancer patients treated with adjuvant chemotherapy (cases vs. controls). Similar data are presented in **Table 11b** among different subgroups based on the year of incidence (two categories: 2012-2014 and 2015-2017) and based on whether the patient was referred before or after the breast cancer surgery.

The case and control groups differed significantly with regard to the age at diagnosis (31.6 vs. 55.4 years old, $p < 0.0001$), the year of incidence (16/30 case patients (53.3%) vs. 25/30 control patients (83.3%) diagnosed in 2012-2014, $p = 0.025$) and the referring physician (inherent to the study design). Other characteristics, of which most are used as a variable to match, were found to be similar. Of all patients 56.7% had an IDA, 30% had a combination of an IDA and a DCIS or LCIS and 13.3% had another type of tumour morphology. Most of the tumours were poorly differentiated (70.2%) and the majority was diagnosed at tumour stage II (65%) while 35% was diagnosed at tumour stage I. The tumour had a hormone receptor (HR) positive status (ER, PR or both positive) in 58.3% of the patients and an HER2 amplification in 35%.

In the multiple subgroups there were no significant differences in patient and tumour characteristics. Since only four patients (13.3%) had an internal referral, while 26 patients (86.7%) had an external referral, no subgroup analyses based on the referring physician were performed. The other subgroups appeared to be rather equal: 16/30 patients were diagnosed in 2012-2014 while 14/30 were diagnosed in 2015-2017 and 14/30 patients had surgery first while 16/30 had the referral for FP counselling before surgery. Of the 16 patients with referral before the surgery, 13 also had an intake at the fertility centre and three women even performed their fertility treatment before the surgery.

Table 11a: Demographical and oncological characteristics in BC patients with adjuvant chemotherapy, case versus control

	Total n=60	Case n=30	Control n=30	p-value (2-sided)
Age (y)				
Mean ± SD	43,5 ± 15,3	31,6 ± 4,8	55,4 ± 12,7	<0,0001[a]
Median ± IQR	38,6 ± 23,4	31,9 ± 6,7	54,7 ± 18,2	
Year of incidence				
2012-2014	41 (68,3%)	16 (53,3%)	25 (83,3%)	0,025[b]
2015-2017	19 (31,7%)	14 (46,7%)	5 (16,7%)	
Referring physician				
Intern	34 (56,7%)	4 (13,3%)	30 (100,0%)	<0,0001[b]
Extern	26 (43,3%)	26 (86,7%)	0 (0,0%)	
Morphology				
IDA	34 (56,7%)	13 (43,3%)	21 (70,0%)	—[c]
IDA+DCIS/LCIS	18 (30,0%)	10 (33,3%)	8 (26,7%)	
Other	8 (13,3%)	7 (23,3%)	1 (3,3%)	
Differentiation				
Well	2 (3,5%)	1 (3,7%)[d]	1 (3,3%)	—[c]
Moderate	15 (26,3%)	7 (25,9%)[d]	8 (26,7%)	
Poor	40 (70,2%)	19 (70,4%)[d]	21 (70,0%)	
Clinical tumour stage				
I	21 (35,0%)	10 (33,3%)	11 (36,7%)	1,000[b]
II	39 (65,0%)	20 (66,7%)	19 (63,3%)	
ER positivity				
Negative	27 (45,0%)	13 (43,3%)	14 (46,7%)	—[c]
Weakly positive	3 (5,0%)	2 (6,7%)	1 (3,3%)	
Strongly positive	30 (50,0%)	15 (50,0%)	15 (50,0%)	
PR positivity				
Negative	28 (46,7%)	15 (50,0%)	13 (43,3%)	—[c]
Weakly positive	7 (11,7%)	4 (13,3%)	3 (10,0%)	
Strongly positive	25 (41,7%)	11 (36,7%)	14 (46,7%)	
HER2 amplification present	21 (35,0%)	8 (26,7%)	13 (43,3%)	0,279[b]
Ki67 score				
0-15%	9 (21,4%)	4 (18,2%)[e]	5 (25,0%)[f]	—[c]
16-25%	9 (21,4%)	3 (13,6%)[e]	6 (30,0%)[f]	
26-35%	6 (14,3%)	4 (18,2%)[e]	2 (10,0%)[f]	
36-45%	7 (16,7%)	3 (13,6%)[e]	4 (20,0%)[f]	
>45%	11 (26,2%)	8 (36,4%)[e]	3 (15,0%)[f]	
Additional oncological therapy				
Radiotherapy	50 (83,3%)	23 (76,7%)	27 (90,0%)	0,299[b]
Hormone therapy	34 (56,7%)	17 (56,7%)	17 (56,7%)	1,000[b]
Targeted therapy	21 (35,0%)	8 (26,7%)	13 (43,3%)	0,279[b]

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate. [a]= Independent t-test; [b]= Fisher's Exact test; [c]= assumptions for statistical analysis not met; [d]= 3 values unknown, counts on 27; [e]= 8 values unknown, counts on 22; [f]= 10 values unknown, counts on 20.

Table 11b: Demographical and oncological characteristics in BC case patients with adjuvant chemotherapy

	2012-2014 n=16	2015-2017 n=14	p-value (2-sided)	Surgery first n=14	Referral before surgery n=16	p-value (2-sided)
Age (y)						
Mean ± SD	31,2 ± 5,4	32,0 ± 4,0	0,645[a]	31,6 ± 5,1	31,6 ± 4,6	0,992[a]
Median ± IQR	30,8 ± 8,5	32,3 ± 5,1		31,5 ± 6,3	32,0 ± 7,3	
Year of incidence						
2012-2014	NA	NA	NA	5 (35,7%)	11 (68,8%)	0,141[b]
2015-2017	NA	NA		9 (64,3%)	5 (31,3%)	
Referring physician						
Intern	4 (25,0%)	0 (0,0%)	0,103[b]	0 (0,0%)	4 (25,0%)	0,103[b]
Extern	12 (75,0%)	14 (100,0%)		14 (100,0%)	12 (75,0%)	
Morphology						
IDA	10 (62,5%)	3 (21,4%)	—[c]	4 (28,6%)	9 (56,3%)	—[c]
IDA+DCIS/LCIS	3 (18,8%)	7 (50,0%)		6 (42,9%)	4 (25,0%)	
Other	3 (18,8%)	4 (28,6%)		4 (28,6%)	3 (18,8%)	
Differentiation						
Well	0 (0,0%)[g]	1 (7,7%)[d]	—[c]	1 (8,3%)[f]	0 (0,0%)[e]	—[c]
Moderate	4 (28,6%)[g]	3 (23,1%)[d]		3 (25,0%)[f]	4 (26,7%)[e]	
Poor	10 (71,4%)[g]	9 (69,2%)[d]		8 (66,7%)[f]	11 (73,3%)[e]	
Clinical tumour stage						
I	4 (25,0%)	6 (42,9%)	0,442[b]	4 (28,6%)	6 (37,5%)	0,709[b]
II	12 (75,0%)	8 (57,1%)		10 (71,4%)	10 (62,5%)	
ER positivity						
Negative	9 (56,3%)	4 (28,6%)	—[c]	7 (50,0%)	6 (37,5%)	—[c]
Weakly positive	2 (12,5%)	0 (0,0%)		0 (0,0%)	2 (12,5%)	
Strongly positive	5 (31,3%)	10 (71,4%)		7 (50,0%)	8 (50,0%)	
PR positivity						
Negative	10 (62,5%)	5 (35,7%)	—[c]	7 (50,0%)	8 (50,0%)	—[c]
Weakly positive	2 (12,5%)	2 (14,3%)		2 (14,3%)	2 (12,5%)	
Strongly positive	4 (25,0%)	7 (50,0%)		5 (35,7%)	6 (37,5%)	
HER2 amplification present	4 (25,0%)	4 (28,6%)	1,000[b]	3 (21,4%)	5 (31,3%)	0,689[b]
Ki67 score						
0-15%	2 (20,0%)[h]	2 (16,7%)[f]	—[c]	3 (23,1%)[d]	1 (11,1%)[i]	—[c]
16-25%	1 (10,0%)[h]	2 (16,7%)[f]		2 (15,4%)[d]	1 (11,1%)[i]	
26-35%	2 (20,0%)[h]	2 (16,7%)[f]		3 (23,1%)[d]	1 (11,1%)[i]	
36-45%	1 (10,0%)[h]	2 (16,7%)[f]		1 (7,7%)[d]	2 (22,2%)[i]	
>45%	4 (40,0%)[h]	4 (33,3%)[f]		4 (30,8%)[d]	4 (44,4%)[i]	
Additional oncological therapy						
Radiotherapy	13 (81,3%)	10 (71,4%)	0,675[b]	11 (78,6%)	12 (75,0%)	1,000[b]
Hormone therapy	8 (50,0%)	9 (64,3%)	0,484[b]	7 (50,0%)	10 (62,5%)	0,713[b]
Targeted therapy	4 (25,0%)	4 (28,6%)	1,000[b]	3 (21,4%)	5 (31,3%)	0,689[b]

Results are expressed as mean ± SD, median ± IQR or N (%) when appropriate. NA= not applicable. [a]= Independent t-test; [b]= Fisher's Exact test; [c]= assumptions for statistical analysis not met; [d]= 1 value unknown, counts on 13; [e]= 1 value unknown, counts on 15; [f]= 2 values unknown, counts on 12; [g]= 2 values unknown, counts on 14; [h]= 6 values unknown, counts on 10; [i]= 7 values unknown, counts on 9.

Oncological time course

Surgery was performed significantly sooner after diagnosis in the case group (18.4 ± 10.8 days) compared to the control group (23.6 ± 8.1 days) (p = 0.020), but the overall time interval between diagnosis and initiation of chemotherapy did not differ significantly (57.2 ± 17.3 vs. 60.7 ± 15.3 days, p = 0.145) (**Table 12a**).

Table 12a: Oncological time intervals in BC patients with adjuvant chemotherapy, case versus control

	Total n=60	Case n=30	Control n=30	p-value (2-sided)
Diagnosis to surgery (d)				
Mean ± SD	21,0 ± 9,8	18,4 ± 10,8	23,6 ± 8,1	0,020[a]
Median ± IQR	21,0 ± 12,0	16,5 ± 14,0	23,0 ± 8,0	
Minimum	0,0	0,0	4,0	
Maximum	47,0	42,0	47,0	
Surgery to chemotherapy (d)				
Mean ± SD	38,0 ± 14,1	38,8 ± 16,6	37,1 ± 11,4	0,917[a]
Median ± IQR	34,0 ± 15,0	34,5 ± 18,0	33,5 ± 12,0	
Minimum	20,0	20,0	27,0	
Maximum	106,0	106,0	80,0	
Diagnosis to chemotherapy (d)				
Mean ± SD	58,9 ± 16,3	57,2 ± 17,3	60,7 ± 15,3	0,145[a]
Median ± IQR	56,5 ± 17,0	54,5 ± 19,0	59,5 ± 16,0	
Minimum	31,0	36,0	31,0	
Maximum	106,0	106,0	105,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Mann-Whitney U test (exact significance).

When comparing the groups who had surgery before or after referral, the first group had surgery sooner after diagnosis (13.1 ± 10.7 vs. 23.0 ± 8.8 days, p = 0.005) but the time interval between surgery and initiation of chemotherapy was longer (45.8 ± 19.6 vs. 32.6 ± 10.6 days, p = 0.012), resulting in an overall time frame that did not differ significantly (58.9 ± 19.6 vs. 55.6 ± 15.4 days, p = 0.608) (**Table 12b**). Furthermore, the table shows that in comparison between the other subgroups no significant difference was found, with an overall time frame for the group diagnosed

in 2012-2014 of 57.8 ± 19.2 days and for the group diagnosed in 2015-2017 of 56.4 ± 15.6 days (p = 0.951).

Table 12b: Oncological time intervals and duration of FP in BC case patients with adjuvant chemotherapy

	Total n=30	2012-2014 n=16	2015-2017 n=14	p-value (2-sided)	Surgery first n=14	Referral before surgery n=16	p-value (2-sided)
Diagnosis to surgery (d)							
Mean ± SD	18,4 ± 10,8	18,6 ± 9,4	18,2 ± 12,6	0,728[a]	13,1 ± 10,7	23,0 ± 8,8	0,005[a]
Median ± IQR	16,5 ± 14,0	17,5 ± 13,0	15,5 ± 16,0		12,0 ± 14,0	24,5 ± 12,0	
Minimum	0,0	0,0	0,0		0,0	9,0	
Maximum	42,0	36,0	42,0		39,0	42,0	
Surgery to chemotherapy (d)							
Mean ± SD	38,8 ± 16,6	39,2 ± 20,6	38,2 ± 10,9	0,637[a]	45,8 ± 19,6	32,6 ± 10,6	0,012[a]
Median ± IQR	34,5 ± 18,0	33,0 ± 15,0	39,5 ± 23,0		43,5 ± 13,0	30,5 ± 9,0	
Minimum	20,0	20,0	20,0		20,0	20,0	
Maximum	106,0	106,0	53,0		106,0	60,0	
Diagnosis to chemotherapy (d)							
Mean ± SD	57,2 ± 17,3	57,8 ± 19,2	56,4 ± 15,6	0,951[a]	58,9 ± 19,6	55,6 ± 15,4	0,608[a]
Median ± IQR	54,5 ± 19,0	53,5 ± 19,0	55,5 ± 22,0		57,5 ± 22,0	53,0 ± 19,0	
Minimum	36,0	38,0	36,0		36,0	38,0	
Maximum	106,0	106,0	91,0		106,0	96,0	
Referral to end FP (d)							
Mean ± SD	23,5 ± 11,5	26,4 ± 12,6	20,1 ± 9,5	0,110[a]	17,9 ± 9,0	28,4 ± 11,6	0,012[a]
Median ± IQR	22,0 ± 12,0	23,5 ± 12,0	19,0 ± 13,0		18,5 ± 14,0	25,5 ± 15,0	
Minimum	3,0	8,0	3,0		3,0	14,0	
Maximum	61,0	61,0	38,0		34,0	61,0	

Results are expressed as mean ± SD or median ± IQR when appropriate. Values are expressed in days.
[a]= Mann-Whitney U test (exact significance).

Time frame of fertility preservation

Table 13 presents the fertility preservation modalities used in the case group treated with adjuvant chemotherapy in the different subgroups. There were no significant differences with regard to the FP approach in the different subgroups, but there was a trend towards a more limited use of “experimental” approaches (OTCP) and combination of techniques in recent years, although the numbers are too small to draw conclusions.

Table 13: Fertility preservation modalities in BC case patients treated with adjuvant chemotherapy

	Total n=30	2012-2014 n=16	2015-2017 n=14	p-value (2-sided)	Surgery first n=14	Referral before surgery n=16	p-value (2-sided)
COS	25 (83,3%)	13 (81,3%)	12 (85,7%)	1,000[a]	11 (78,6%)	14 (87,5%)	0,642[a]
OTCP	7 (23,3%)	6 (37,5%)	1 (7,1%)	0,086[a]	3 (21,4%)	4 (25,0%)	1,000[a]
IVM OPU	4 (13,3%)	2 (12,5%)	2 (14,3%)	1,000[a]	3 (21,4%)	1 (6,3%)	0,315[a]
Combination	6 (20,0%)	5 (31,3%)	1 (7,1%)	0,175[a]	3 (21,4%)	3 (18,8%)	1,000[a]

Results are expressed as N (%) when appropriate.
[a]= Fisher's Exact test.

The fertility preservation program, from referral for FP counselling to termination of the FP treatment, took on average 23.5 ± 11.5 days, with a significant difference between the groups who had breast cancer surgery before (17.9 ± 9.0 days) and after (28.4 ± 11.6 days) referral (p = 0.012). A non-significant trend towards a shorter interval in the group treated in 2015-2017 (20.1 ± 9.5 vs. 26.4 ± 12.6 days, p = 0.110) was observed. (**Table 12b**)

Timelines regarding the time frame within which the fertility preservation treatment was performed are presented in **Figure 3** for the entire case group receiving adjuvant chemotherapy and completed with the different subgroups. Although no significant difference was found in the overall time interval when comparing the different subgroups, a slightly longer time interval has been noticed in the group of patients receiving surgery before the referral.

The group starting with surgery before the referral appears to have an interval between diagnosis and referral that is much longer when compared to the patients referred before the surgery. Nevertheless, in the first group the interval between intake and initiation of the FP treatment and between termination of the FP treatment and initiation of the chemotherapy appears to be shorter. In average, when patients were referred before surgery the FP treatment could start closely after the surgery, while in patients referred after the surgery more time was needed.

The group treated in 2012-2014 appeared to start their FP treatment with a larger time interval after the intake than the group treated in 2015-2017. However, their fertility treatment appeared to be less time-consuming and the chemotherapy appeared to begin sooner after the FP treatment. Additionally, it appeared that there was a larger time interval between surgery and referral in the period 2015-2017.

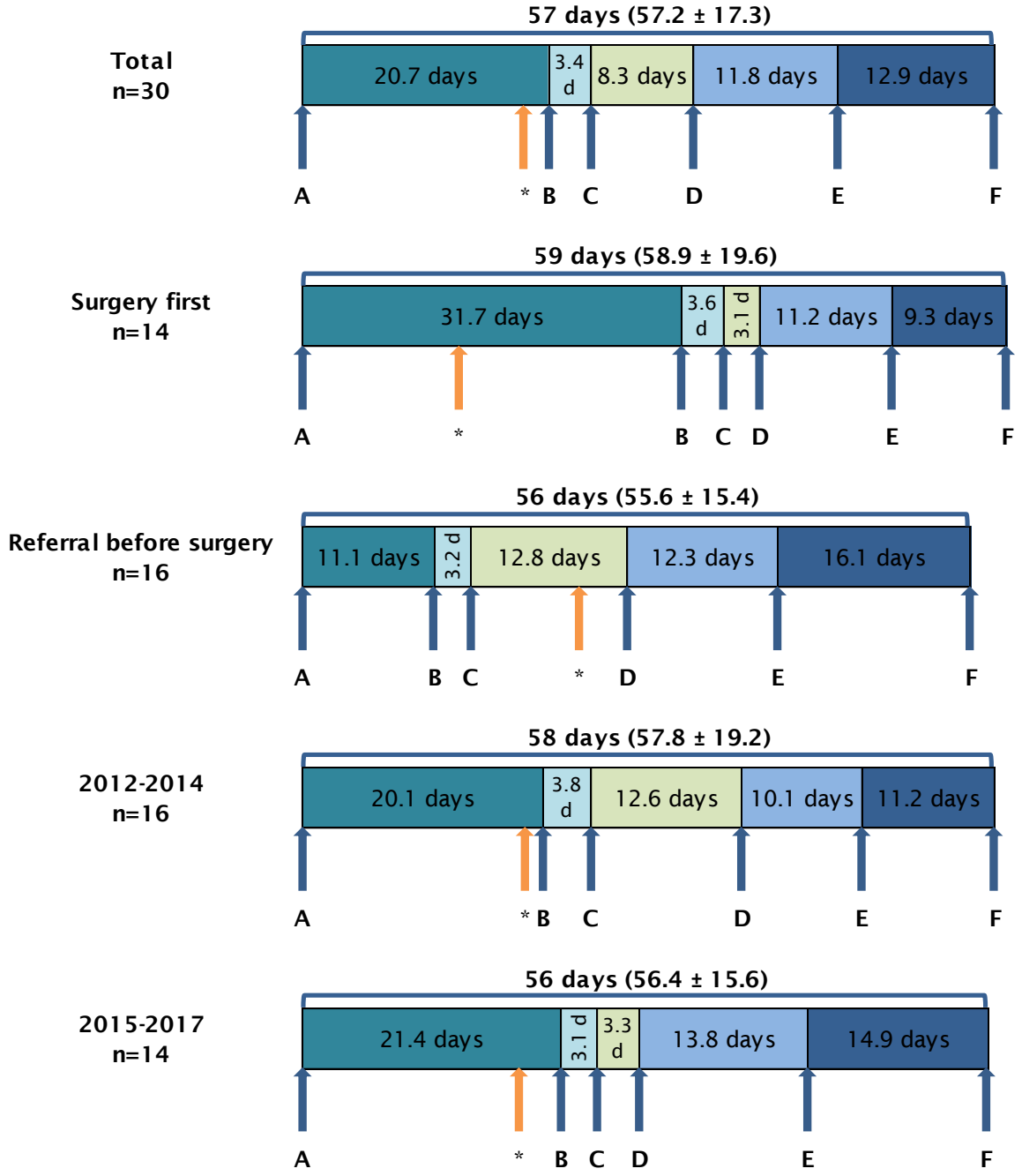
In the FP procedure some of the different steps occurred on the same day (**Table 14**): two patients referred before the surgery had referral and intake on the same day, five patients treated between 2015 and 2017 could start their FP on the day of intake and two patients referred before the surgery even started their chemotherapy on the day the fertility preservation treatment was completed.

Table 14: Steps in the fertility preservation procedure on the same day in BC case patients with adjuvant chemotherapy

		Total n=30	2012-2014 n=16	2015-2017 n=14	Surgery first n=14	Referral before surgery n=16
Diagnosis to referral	**Δ=0**	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
Referral to intake	**Δ=0**	2 (6,7%)	1 (6,2%)	1 (7,1%)	0 (0,0%)	2 (12,5%)
Intake to initiation of FP	**Δ=0**	5 (16,7%)	0 (0,0%)	5 (35,7%)	3 (21,4%)	2 (12,5%)
Duration FP	**Δ=0**	3 (10,0%)	2 (12,5%)	1 (7,1%)	2 (14,3%)	1 (6,2%)
End FP to chemo	**Δ=0**	2 (6,7%)	1 (6,2%)	1 (7,1%)	0 (0,0%)	2 (12,5%)

Results are expressed as N (%).
Δ= Time interval.

Figure 3: Timelines of FP program in BC case patients with adjuvant chemotherapy



Mean of each time interval is presented, mean ± SD is presented for the entire time frame. Values are expressed in days. A = oncological diagnosis; B = referral to fertility centre; C = intake at fertility centre; D = initiation of fertility preservation; E = termination of fertility preservation (last physical contact at the fertility centre); F = initiation of chemotherapy; *= surgery.

DISCUSSION

To our knowledge, this is the first study analysing the time from cancer diagnosis to cancer treatment in adult women diagnosed with Hodgkin's lymphoma, who underwent FP treatment before gonadotoxic treatment, compared to patients who were not referred for FP. Furthermore, the studies previously published in breast cancer patients only included patients undergoing FP based on controlled ovarian stimulation, without inclusion of other, more experimental, FP techniques.(18-20, 27, 28) Since the establishment of a multidisciplinary oncofertility team at UZ Brussel, the decision-making process for fertility preservation has been based on a patient-tailored approach and a shared decision of the patient, fertility specialists and the referring oncologist or haematologist. The choice of a specific FP approach or a combination of different FP approaches is based on multiple factors including the patient's medical condition, age and ovarian reserve, the oncological staging and the urgency to start the oncological treatment.(6, 10, 33) Additionally, this is the first study investigating the impact of fertility preservation on the pathway of oncological health care in breast cancer patients who were scheduled either for neo-adjuvant or adjuvant chemotherapy. Finally, this is the first study using meticulous matching of cases and controls, to diminish the factors potentially influencing the time interval. Nevertheless, it was impossible to match for age, which can be explained by the fact that the overwhelming majority of the reproductive aged women at UZ Brussel diagnosed with a malignancy requiring chemotherapy are referred to the fertility centre at UZ Brussel; this has indirectly resulted in over-representation of post-menopausal women in the control group.

In general

In none of the subgroups in this study there were significant differences with regard to the time interval between oncological diagnosis and initiation of chemotherapy. However, in the population of patients with Hodgkin's lymphoma our data demonstrated a longer interval between the time of diagnosis and treatment initiation in the case group, with a mean interval of 33.2 vs. 20.1 days in the control group ($p = 0.102$), although the number of patients with Hodgkin's lymphoma at UZ Brussel diagnosed between January 2010 and December 2017 was too small to reach a statistically significant difference. To our knowledge, no studies have been performed in patients with Hodgkin's lymphoma regarding the optimal time to treatment initiation to prevent

suboptimal oncological outcome. In this context we are not able to conclude whether the increased time interval in the study group of HL patients who received FP has any clinical relevance, nor can we point toward any causal link between FP and the increased time interval.

The average time to treatment in the population of breast cancer patients receiving chemotherapy in a neo-adjuvant setting is 28.5 days, without being significantly different between the case and control group (27.3 vs. 29.6 days, p = 0.441). In the control group 72.4% of the patients were diagnosed in 2015-2017 while only 58.6% case patients were treated in that period, which is surprising since we would expect that over time, the time to treatment would decrease as an indicator of good clinical practice. The most recent studies published by *Chien et al.* and *Letourneau et al.* describe an average time to treatment of respectively 39.8 and 38.1 days in the case group and 40.9 and 39.4 days in the control group.(27, 28) It is remarkable that patients in our study population could start their chemotherapy a lot faster. Moreover, the population investigated in these studies was selected from a study population included in a prospective trial where patients generally have a closer follow-up and more accurately organised therapeutic approach.

Only a few studies have been performed determining the optimal time to treatment in breast cancer patients receiving chemotherapy in a neo-adjuvant setting. *Smith et al.* concluded that a time interval from diagnosis to treatment longer than six weeks had a worse oncological outcome, in terms of five-year survival (78%), compared to a time to treatment shorter than two weeks (84%) or between two and four weeks (83%) (p = 0.005). This conclusion did not differ depending on whether the first treatment consisted of chemotherapy or surgery (with or without adjuvant chemotherapy).(35) At the Annual Meeting of the American Society of Clinical Oncology (ASCO) in 2016 *Sanford et al.* presented the results of a study regarding the impact of a delay in the admission of a systemic neo-adjuvant chemotherapy on the survival outcomes in breast cancer patients. The group reported a limited decrease in five-year overall survival in patients treated more than 56 days after diagnosis (81% vs. 86%, p = 0.011). However, selected patients with an amplification of HER2 in the tumour had an increased risk of death (Hazard Ratio = 1.67; 95% CI 1.06-2.64), suggesting that a delay in treatment in this population has a larger impact and should be avoided.(36)

Since the patients in our study population started their chemotherapy within an average interval of “only” 28.5 days, the question arises whether an additional round of ovarian stimulation and

fertility preservation would be acceptable. More studies are necessary to establish an optimal balance between safety from an oncological point of view and FP potential, although studies of this kind may be difficult to conduct for ethical reasons.

Comparable results are seen in the group treated with adjuvant chemotherapy, with an average time from diagnosis to initiation of chemotherapy of 58.9 days, without a significant difference between the case (57.2 days) and control group (60.7 days) ($p = 0.145$). However, the time from diagnosis to surgery appeared to be significantly shorter in the case group (18.4 vs. 23.6 days, $p = 0.020$) with an average of 21.0 days in the entire study population. The Belgian Health Care Knowledge Centre (KCE) recommends the initiation of adjuvant therapy in breast cancer patients within a period of eight weeks after surgery, since it would be less efficient when started after a period of 12 weeks.(37) The European Society for Medical Oncology (ESMO) also concluded chemotherapy administered 12 or more weeks after the surgery to have a decrease in efficacy, but recommends starting the adjuvant therapy within a period of two to six weeks after the surgery.(38) In addition, *Gagliato et al.* concluded a decrease in survival in patients with a triple negative or a HER2 positive breast cancer treated with chemotherapy more than 60 days after surgery (39) and *Chavez-McGregor et al.* found a decrease in overall and breast specific survival when chemotherapy was administered more than 91 days after surgery.(40) In our study population the average time from surgery to adjuvant chemotherapy was 38 days, rendering this interval below the recommended thresholds. Similarly, the question arises whether this short time interval results in improved oncological outcome (compared to longer intervals) and whether additional rounds of ovarian stimulation and fertility preservation would be acceptable.

Subgroup analyses

Internal versus external referral

In the cases diagnosed with Hodgkin's lymphoma and diagnosed with breast cancer treated with neo-adjuvant chemotherapy a subgroup analysis based on the referring physician was performed. In the population treated with adjuvant chemotherapy this was not performed since only four out of thirty case patients had an internal referral, which is an unbalanced distribution to draw conclusions. We see that inside our hospital there was an evolution towards a treatment with

neo-adjuvant chemotherapy over the past years resulting in a limited population that is still treated with adjuvant chemotherapy.

No significant time frame differences were found, although the overall interval appeared to be shorter in the HL patients referred internally compared to those referred by extra muros oncological health providers (25.0 vs. 34.0 days, p = 0.450). Nevertheless, the numbers of HL patients are small and the observations may be skewed by the three cases initially treated with BEACOPP (see section on 'ABVD versus BEACOPP') who were all internally referred. In the breast cancer group, the interval is almost the same in both groups (27.4 vs. 27.3 days, p = 1.000).

However, regarding to the FP program itself, some differences in the time intervals between the different steps were observed, although there were no significant differences in the modalities used (except for the three HL patients treated with BEACOPP who all underwent OTCP and IVM OPU, while no other patients with Hodgkin's lymphoma underwent an OTCP).

The shorter interval between diagnosis and referral and between referral and intake in the internally referred group is exactly what we would expect and why we performed this subgroup analysis. Inside the hospital there is a close collaboration between health care workers in a multidisciplinary team and referring a patient of reproductive age or younger to a fertility specialist has become a standard of care in the approach of a cancer patient facing an (urgent) potentially gonadotoxic treatment. In that context it seems logical that the oncologist or surgeon who is in charge of the patients' oncological treatment, refers patients sooner after diagnosis, starting from the moment when the need for systemic chemotherapy is suspected. Since patients are performing their oncological staging inside the hospital, the intake for FP counselling can be accurately organised within the oncological program, resulting in an intake sooner after the referral.

On the other hand, we cannot explain the longer time interval between FP intake and initiation of FP in the group of internally referred patients; we hypothesise that in patients who had an early referral after cancer diagnosis, it was preferred to complete the staging and have a final diagnosis before the initiation of the FP treatment. Furthermore, in the group with Hodgkin's lymphoma this can be due to the patients receiving BEACOPP who performed an OTCP with IVM OPU, an intervention that needs to be organised and scheduled with all partners involved (operating room, physician performing the intervention, anaesthesiologist, the laboratory team receiving the

tissue, …) and consequently cannot be performed on the day of intake. In contrast the FP itself takes less time since it consists only of one day when the intervention is performed, if necessary preceded by a few days of hormonal priming, resulting in a shorter duration of the FP treatment itself. The longer interval between termination of the FP and initiation of the chemotherapy in HL patients internally referred remains unexplained, but still results in an overall time to treatment that appears shorter than in patients externally referred. In the breast cancer patients, we observed an unexplained longer duration of the FP itself in the group with an internal referral, but the interval between termination of the FP and initiation of the chemotherapy was equal.

2012-2014 versus 2015-2017

The subgroup analysis based on the year of incidence demonstrates an evolution in the fertility preservation modalities used at the CRG at UZ Brussel with the application of more ovarian stimulation and less OTCP and IVM OPU, which can be explained by the gradual adoption of a random-start ovarian stimulation protocol with equal outcome (number of oocytes) compared to the outcome of a conventional-start ovarian stimulation but without the need to wait for basal hormonal levels at the beginning of the menstrual cycle to start the stimulation.(25, 26) This possibility reduces the need for OTCP and IVM OPU in an urgent FP program, since the fertility-related outcome of an ovarian stimulation is still better comparing to the promising but still experimental OTCP and in vitro maturation techniques.(10, 24, 33, 34, 41) Nevertheless, a random-start ovarian stimulation still takes more time than an OTCP and IVM OPU which only requires one day at the hospital for the intervention, if necessary preceded by a few days of hormonal priming.

No significant difference in the time from diagnosis to chemotherapy was found in both populations, although it appears that in the population treated with neo-adjuvant chemotherapy the time interval was longer in the period between 2015 and 2017 (29.0 vs. 25.0 days, p = 0.245) but remained below the recommended threshold. This could be explained by a trend in the use of more COS (64.7% vs. 25%, p = 0.060) and less OTCP (35.3% vs. 50%, p = 0.471) and IVM OPU (29.4% vs. 50%, p = 0.438) in this period.

Intriguingly, we noticed a trend towards a longer interval between cancer diagnosis and referral for FP in recent years, which can be explained by an increased time necessary to perform the

growing number of molecular analyses, on the tumour biopsy, that are essential for the staging and used as prognostic factors to determine patient's eligibility for neo-adjuvant chemotherapy, such as HR positivity (ER and PR), HER2 amplification and Ki67-score. However, the FP intake follows more closely after referral, reflecting a more efficient organisation, and the FP itself could start sooner after intake but takes more time on average, consistent with the more frequent use of controlled ovarian stimulation. In addition, there is a slightly larger interval between termination of the fertility preservation treatment and initiation of the chemotherapy in the latest years. An explaining hypothesis could be that the patient still needs an intervention to implant a port for the chemotherapy after the oocyte retrieval or that the necessary length of stimulation is not the same in every woman resulting in a moment of oocyte retrieval that cannot be predicted accurately in contrast to the intervention for an OTCP. Maybe there is still another plausible explanation for this trend, such as the patient's need to recover after a FP treatment or other oncological or personal reasons influencing the overall time to treatment, confirmed by the fact that there is no difference in time to treatment in the case group compared to the control group. Furthermore, we noticed that in the group treated with adjuvant chemotherapy the referral was more frequently performed before the surgery in the period 2012-2014 (see section on 'Breast cancer surgery before or after referral').

Specific subgroup analyses

ABVD versus BEACOPP

Although no significant difference in the time to treatment was found between the groups of case patients with Hodgkin's lymphoma treated with ABVD and BEACOPP due to the limited population size, it appears that the interval is shorter in the group treated with BEACOPP (15.0 vs. 33.2 days, $p = 0.138$). In the same group, all patients underwent FP treatment using an OTCP and IVM OPU while patients treated with ABVD underwent an ovarian stimulation, an IVM OPU or both. This could be explained by the administration of BEACOPP in more advanced disease stages, which is coherent with our data consisting of one patient diagnosed in an early unfavourable stage of disease and two patients in an advanced disease stage, rendering the perception that a treatment is more urgent than a treatment with ABVD.(7, 42, 43) Therefore, the fertility specialist should provide a fertility preservation modality that can be done in an even more limited period and still provides a good fertility-related outcome.

In this context the observations about the multiple time intervals seem logical: in the group treated with BEACOPP the referral occurs sooner after diagnosis, the initiation of the FP happens later after the intake since the OTCP needs time to be scheduled, the FP treatment itself takes less time and the chemotherapy starts sooner after termination of the fertility preservation.
Since ABVD is known to be less gonadotoxic compared to other types of chemotherapy such as BEACOPP (15, 30-32), it is possible that physicians and patients thoroughly consider whether they should go to a fertility centre and perform a fertility preservation treatment. This consideration can also have an impact on the entire time frame between diagnosis and chemotherapy.

Breast cancer surgery before or after referral

In the case group of breast cancer patients treated with adjuvant chemotherapy the scheduling of the surgery regarding the different steps in the fertility preservation program differed in several patients. We distinguished a group of 14 patients with surgery before the referral and a group of 16 patients with referral (and sometimes intake and FP treatment) before the surgery.
The group referred before the surgery consisted mostly of patients diagnosed in 2012-2014, including the four internally referred patients. Except the fact that most of the patients with adjuvant chemotherapy were externally referred, which could be a factor in the timing of the referral that was also observed in the other subgroup analyses, we have no plausible explanation for this trend towards referring patients after surgery in the last years. Additionally, we observe a trend towards the use of more ovarian stimulation and less IVM OPU in the group referred before surgery, although most of them were diagnosed in 2012-2014 when random-start ovarian stimulation was not as commonly used as compared to the period between 2015 and 2017, as observed in the other subgroup analyses.

This subgroup analysis revealed a significant difference between the groups with surgery first and referral before the surgery, regarding the interval between diagnosis and surgery (13.1 vs. 23.0 days, $p = 0.005$) and the interval between surgery and chemotherapy (45.8 vs. 32.6 days, $p = 0.012$), resulting in an overall time interval between diagnosis and chemotherapy that was not significantly different (58.9 vs. 55.6 days, $p = 0.608$). We conclude that the sequence of the surgery and the referral does not significantly influence the time from diagnosis to chemotherapy, although there is a slight benefit for the group with referral before the surgery.

In the group with surgery before referral the mean time needed for referral was 31.7 days, while the surgery already took place after 13.1 days, leaving a period of 18.6 days in between which could be valuable for both oncological and fertility-related treatment and outcome. In contrast, patients with a referral before surgery were referred after an average period of 11.1 days, while surgery took place after 23.0 days, generally in the interval between intake and start of the FP, which results in a longer interval between intake and initiation of the FP treatment. Finally, the time interval between termination of the FP and initiation of the chemotherapy is shorter in the group with surgery first, without a clear explanation.

Our findings are consistent with the findings of *Lee et al.* who concluded that in patients referred before the breast cancer surgery the ovarian stimulation and the chemotherapy could be initiated sooner after the surgery.(20)

Oncofertility organisation

At the CRG, UZ Brussel, a multidisciplinary team, with a midwife acting as a patient navigator, is dedicated to manage the fertility preservation program for patients who face an urgent, potentially gonadotoxic treatment in the context of a cancer diagnosis. Since the oncological treatment for cancer patients is initiated as soon as possible after the diagnosis of cancer, the time frame for fertility preservation is limited. To maximise efficiency of the FP time interval, the oncofertility team has established a fertility preservation program tailored to each individual patient, keeping in mind the available time, the multiple established and promising FP modalities, the patient's current medical and reproductive status and the patient's preferences.

When analysing the time frames in the different subgroups we noticed that it still takes a lot of time to refer a patient to a fertility centre, time that is crucial for a better reproductive outcome in the future or to start the oncological treatment earlier in order to influence the oncological outcome as less as possible. Therefore, referring a patient as soon as possible after suspecting the need for a gonadotoxic chemotherapy should be recommended.

Furthermore, the organisation of the oncofertility team is continuously improving. This results in shorter time intervals within the FP program, including the possibility of performing different steps on the same day. We noticed that overall two patients were referred by an internal physician on the day of diagnosis, nine patients mostly internally referred had an intake on the

day of referral, and 19 patients could start their fertility treatment on the day of intake, which were mostly patients treated between 2015 and 2017 using a (random-start) ovarian stimulation.

The time frame of the fertility preservation program, from referral to termination of the FP treatment, appears to be approximately 12 days in case patients with Hodgkin's lymphoma and breast cancer treated with neo-adjuvant chemotherapy and 23.5 days in breast cancer patients treated with adjuvant chemotherapy. There appears to be a trend towards a shorter interval in HL patients treated with BEACOPP, explained by the use of OTCP and IVM OPU in this group as compared to the more frequent use of COS in the group treated with ABVD, and towards a shorter interval in BC patients with NAC with an external referral, for which we have no plausible explanation. In the group of BC patients treated with adjuvant chemotherapy the interval is significantly longer in patients with referral before the breast cancer surgery (28.4 vs. 17.9 days, $p = 0.012$), explained by the performance of the surgery within this period. Since most of the patients in the period between 2015 and 2017 were referred after the surgery, the previous observation can also explain why the FP program took less time in 2015-2017 (20.1 vs. 26.4 days, $p = 0.110$). Furthermore, this can be explained partially by an improved organisation of the oncofertility team.

Since improvement is always pursued, one could search a method to diminish the time between referral and intake and the time necessary to organise an intervention at the operating room, such as an OTCP or IVM OPU. Furthermore, there is a need to continue developing promising fertility preservation techniques such as in vitro maturation of oocytes and ovarian tissue cryopreservation as well as techniques to protect the gonads during chemotherapy.

LIMITATIONS AND RECOMMENDATIONS

This study has a retrospective design, which implies difficulties to properly collect data and limits the possibility to explain some findings. Moreover, personal or medical factors that may influence the time to treatment, by the use of chemotherapy, were not explored. Therefore, a study with a prospective design will be required to confirm our findings, not only to include cancer patients referred to a fertility centre but also to include control patients who will not perform a fertility preservation. This offers the occasion to list all the steps in the fertility-related and oncological program accurately in chronological order, to note the reasons why a certain decision is made by a physician or a patient and to register whether the chemotherapy has to be delayed because of the fertility preservation treatment or because of another medical or personal reason as compared to the initially provided date to initiate the chemotherapy.

Furthermore, this was a single centred study in which all the case patients underwent a fertility preservation treatment at the CRG at UZ Brussel and all patients in the control group were treated at UZ Brussel, which may have caused a bias. It is unknown whether our results can be extrapolated to a larger population of cancer patients in Belgium or Europe. Additionally, the population size of this study was limited, certainly regarding the different subgroup analyses. Therefore, a study with a larger population size, preferentially multi-centred, is essential to provide some analyses representative for a larger population of cancer patients.

Finally, we did not investigate the fertility-related nor the oncological outcome of the patients in the case or control group. Composing an optimal fertility preservation program in different oncological settings based on several factors, including the outcome of and the required time for the different FP techniques and the time available before the start of an urgent potentially gonadotoxic chemotherapy, could be useful in the decision-making process concerning the best fertility-related approach for each individual patient. In addition, research about the optimal time to treatment in cancer patients to achieve an optimal oncological outcome that is feasible, could provide a time frame wherein the fertility preservation can be performed.

CONCLUSION

In adult women diagnosed with Hodgkin's lymphoma or breast cancer facing an (urgent) potentially gonadotoxic treatment with chemotherapy, conducting a fertility preservation procedure appears unlikely to significantly delay the initiation of the chemotherapy. The chemotherapy was applied as a monotherapy or combined with radiotherapy in HL patients or in a neo-adjuvant or adjuvant setting in breast cancer patients. In patients with Hodgkin's lymphoma this result needs to be carefully interpreted due to the limited sample size.

Subgroup analyses suggest that the time interval of referral (between cancer diagnosis and intake at the FP clinic) is shorter for intra muros UZ patients than patients referred from another hospital. Over time ovarian stimulation has become more commonly used in an oncofertility setting, due to the development of a random-start controlled ovarian stimulation protocol, which has resulted in a more timely start of the FP intervention. In breast cancer patients treated with adjuvant chemotherapy who were referred after the breast cancer surgery, a large interval between surgery and referral was observed. Moreover, patients referred before the surgery were offered more frequently the possibility to perform a controlled ovarian stimulation, which remains the most established FP technique. Therefore, a referral as soon as possible after the cancer diagnosis is recommended.

More research is required to determine the optimal and feasible time to treatment in different oncological settings and in order to optimise the decision-making process to choose the best fertility-related approach for each individual patient.

ACKNOWLEDGEMENTS

First, I would like to thank Prof. Dr. Michel De Vos, my promotor, who gave me the opportunity to perform my first scientific study, under supervision, but with a large autonomy. His door was always open to give some advice regarding the study design, difficulties in the entire process and to answer fertility-related questions. Secondly, I would like to express my gratitude towards Dr. Marian Vanhoeij, acting as a co-promotor, who was always available and willing to give advice regarding the oncological subfield of this study, even at 7.30 am. Moreover, without the database of the Breast Cancer Clinic at UZ Brussel, the breast cancer patients could not have been matched, remaining the main study question without an answer. Thirdly, I'm grateful to Prof. Dr. Rik Schots and his haematological department for the collaboration and advice regarding the patients diagnosed with Hodgkin's lymphoma. Additionally, I would like to address some special thanks towards Hanne Massa, one of my colleagues. I really appreciate you providing me your own database to select control patients from and I enjoyed our discussions about Hodgkin's lymphoma.

Furthermore, this study would not have been possible without the support of the oncofertility team and the research unit, consisting of study nurses, at the Centre for Reproductive Medicine (CRG) at UZ Brussel. They were always available for some advice regarding the data collection and to create a lovely atmosphere to work in. I hope we will work together again in the future.

Finally, I would like to thank my family and friends for their continuous and unconditional support through this stressful period and my entire career so far. In particular, I would like to express some special thanks to my parents for giving me the chance to chase my dreams by allowing me to study medicine.

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