

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-018120
Article Type:	Protocol
Date Submitted by the Author:	07-Jun-2017
Complete List of Authors:	Krul, Inge; The Netherlands Cancer Institute, Epidemiology and Biostatistics Opstal-van Winden, Annemieke; the Netherlands Cancer Institute, Epidemiology and Biostatistics Zijlstra, Josée ; VU University Medical Centre, Haemato-oncology Appelman, Yolande; VU University Medical Center, Cardiology Schagen, SB; The Netherlands Cancer Institute , Epidemiology and Biostatistics Meijboom, Lilian; VU University Medical Center, Radiology Serné, Erik; VU University Medical Centre, Vascluar medicine Lambalk, Cornelis; VU University Medical Centre, Obstetrics and Gynecology Lips, Paul; VU University Medical Center, Internal medicine, Endocrine Section van Dulmen - den Broeder, Eline; VU University Medical Center, Pediatric Oncology and Hematology Hauptmann, M; The Netherlands Cancer Institute, Department of Epidemiology and Biostatistics Daniëls, Laurien; Leids Universitair Medisch Centrum, Radiotherapy Aleman, Berthe; The Netherlands Cancer Institute , Radiotherapy van Leeuwen, Flora; The Netherlands Cancer Insitute , Epidemiology and Biostatistics
Keywords:	Hodgkin lymphoma, Cancer survivor, Primary ovarian insufficiency, Bone mineral density, Cardiovascular disease, Neurocognitve function

SCHOLARONE™
Manuscripts

1 **Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of**
2
3 **Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)**
4

5 Inge M Krul, Msc¹, Annemieke WJ Opstal-van Winden, PhD¹, Josée M Zijlstra, MD, PhD², Yolande Appelman,
6 MD, PhD³, Sanne B. Schagen, PhD¹, Lillian J. Meijboom, MD, PhD⁴, Erik Serné, MD, PhD⁵, Cornelis B Lambalk,
7 MD, PhD⁶, Paul Lips, MD, PhD⁷, Eline van Dulmen-den Broeder, MD, PhD⁸, Michael Hauptmann, PhD¹, Laurien A
8 Daniëls, MD, PhD⁹, Berthe MP Aleman, MD, PhD¹⁰, Flora E van Leeuwen, PhD¹
9
10
11
12
13
14

- 15 1. Department of Epidemiology and Biostatistics, The Netherlands Cancer Institute, Amsterdam, the
16 Netherlands
- 17 2. Department of Haemato-oncology, VU University Medical Center, Amsterdam, the Netherlands
- 18 3. Department of Cardiology, VU University Medical Center, Amsterdam, the Netherlands
- 19 4. Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands
- 20 5. Department of Vascular Medicine, VU University Medical Center, Amsterdam, the Netherlands
- 21 6. Department of Obstetrics and Gynecology, VU University Medical after Center, Amsterdam, the
22 Netherlands
- 23 7. Department of Internal medicine, Endocrine Section, VU University Medical Center, Amsterdam,
24 the Netherlands
- 25 8. Department of Pediatric Oncology and Hematology, VU University Medical Center, Amsterdam, the
26 Netherlands
- 27 9. Department of Radiotherapy, Leiden University Medical Center, Leiden, the Netherlands
- 28 10. Department of Radiation Oncology ,The Netherlands Cancer Institute, Amsterdam, the Netherlands
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43

44 **Corresponding author**

45 Flora E. van Leeuwen, PhD

46 Department of Epidemiology and Biostatistics, The Netherlands Cancer institute

47 Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

48 Email: f.v.leeuwen@nki.nl

49 Phone: +31205122480 / Fax: +3120512 2232

50
51
52
53
54
55
56
57 **Word count:** 3,945
58
59
60

ABSTRACT

Introduction: Hodgkin lymphoma (HL) has become the prototype of a curable disease. However, many young survivors suffer from late adverse effects of treatment. Both chemotherapy (CT) and radiotherapy (RT) may induce primary ovarian insufficiency (POI), which has been associated with reduced bone mineral density (BMD), neurocognitive dysfunction and possibly cardiovascular disease (CVD). While the general assumption is that POI increases CVD risk, other hypotheses postulate reverse causality, suggesting that cardiovascular risk factors determine menopausal age or that biological aging underlies both POI and CVD risk. None of these hypotheses are supported by convincing evidence. Furthermore, most studies on POI-associated conditions have been conducted in women with early natural or surgery-induced menopause with short follow-up times. In this study we will examine the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status, neurocognitive function and quality of life (QoL) in female HL survivors.

Methods and analysis: This study will be performed within an existing Dutch cohort of HL survivors. Eligible women were treated for HL at ages 15-39 years in three large hospitals since 1965 and survived for ≥ 8 years after their diagnosis. Women will be invited to visit a survivorship care outpatient clinic (SSC) for a neurocognitive, cardiovascular and BMD assessment, and asked to complete several questionnaires and to provide a blood sample. Using multivariable regression analyses we will compare the outcomes of HL survivors who developed POI with those who did not. Cardiovascular status will also be compared with women with natural POI.

Ethics and dissemination: This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL44714.031.13). Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL survivors.

STRENGTHS AND LIMITATIONS

- This study is the first to examine to the long-term effects of chemotherapy- and radiotherapy -induced primary ovarian insufficiency in female Hodgkin lymphoma survivors
- Furthermore, this study is embedded in an infrastructure of several multidisciplinary survivorship care clinics, enabling a broad scope of medical tests and extensive follow-up care

- Results of this study will help to identify and timely refer those Hodgkin lymphoma survivors who are at increased risk for osteoporosis, neurocognitive dysfunction and possibly cardiovascular disease due to treatment-induced primary ovarian insufficiency for interventions in order to reduce morbidity and enhance quality of life
- Moreover, this study sheds light on different hypotheses regarding the association between primary ovarian insufficiency and cardiovascular disease risk
- Data collection is dependent on routine care procedures of the participating survivorship care clinics and of visiting patients

KEY WORDS

Hodgkin lymphoma, cancer survivor, primary ovarian insufficiency, bone mineral density, cardiovascular disease, neurocognitive function

BACKGROUND

Primary ovarian insufficiency in Hodgkin lymphoma survivors

Due to the improvements in treatment since 1960, Hodgkin lymphoma (HL) has become the prototype of a curable malignancy. Nowadays, overall 10-year survival rates exceed 80%.[1-3] However, survivors are faced with several late adverse effects of treatment, such as second malignancies and cardiovascular disease (CVD) [4-10]. Moreover, 30-40% of female HL survivors treated before 1985 developed primary ovarian insufficiency (POI) (menopause before the age of 40 years) [11-15], compared with 1% of women in the general population [16]. Women treated with more recent, less gonadotoxic treatment regimens are likely to have a lower risk, but the long-term risk has not yet been quantified sufficiently.

The risk of POI strongly depends on type of HL treatment, with the highest cumulative risks reported following pelvic RT (81%) and alkylating CT (42-60%).[11-15 17] Older age at treatment (up to 40 years) does not appear to increase risk. Although women treated for HL at older age will develop POI sooner after treatment compared with those treated at younger ages, the cumulative incidence at age 40 is nearly equal in both groups.[11 13 14] In our earlier study among female 5-year HL survivors treated between 1965 and 1995, women developed POI at a median age of 33 years.[11] POI occurring this early potentially has a large impact on quality of life (QoL) as it results in infertility, and menopausal symptoms including hot flushes, vaginal dryness and mood swings that are more severe than after menopause at later ages.[18-20] In addition, POI has been associated with reduced bone mineral density (BMD), and increased risk of CVD and neurocognitive dysfunction.

Bone mineral density

Women who reach menopause early (before age 45 years), have a lower BMD and higher incidence of osteoporosis than women who enter menopause at ages ≥ 50 years.[21-23] Moreover, an early menopause has been associated with a 1.5-3-fold increased fracture risk.[21 22 24] The most common osteoporotic fractures in postmenopausal women occur in the hip, wrist and spine.[24 25] However, it is unclear whether the association between early menopause and BMD and fracture risk persists over time. Some studies have shown that the association becomes much weaker with increasing age (mainly above age 70) [26-28], while others reported a lifetime increased fracture risk.[22 29] Possibly, not only oestrogens but also aging mechanisms are of importance in BMD status.[30]

So far, many studies have been conducted in women with an early natural or surgery-induced menopause, while studies evaluating the long-term effects of CT- and/or RT-induced POI on BMD and fracture risk are limited. Two small studies among HL survivors reported a significantly reduced BMD after treatment-induced

1 POI [31 32], while another study found no association.[33] More research is needed to identify the extent of
2
3 reduced BMD and prevalence of osteoporotic fractures among female HL survivors who developed POI.
4
5

6 **Cardiovascular disease**

7
8 In the general population, early menopause has been associated with an increased incidence of CVD [34-36]. A
9
10 recent meta-analysis of CVD risk among women with POI showed a pooled HR of 1.6 for total CVD and 1.7 for
11
12 ischemic heart disease when compared with menopause at ages ≥ 50 years.[37] Moreover, epidemiological data
13
14 show a 2% decrease in cardiovascular mortality for each year menopause is delayed.[38] Low levels of
15
16 testosterone in women have also been associated with increased intima media thickness (IMT) of the carotid
17
18 artery.[39-41]

19
20 An intriguing hypothesis postulates that reverse causality may operate, i.e., that CVD risk factors such as
21
22 weight, cholesterol and blood pressure determine menopausal age. This is in contrast with the general
23
24 assumption that endocrine changes due to early menopause are responsible for CVD development. Indeed, in
25
26 the Framingham Heart Study cohort, a 1% higher premenopausal cardiovascular risk score was associated with
27
28 a subsequent decrease in menopausal age of 1.8 years.[42] However, it has also been suggested that several
29
30 risk factors are associated with both CVD and early menopause. A meta-analysis of 22 genome-wide association
31
32 studies (GWAS) on natural early menopause revealed predominantly genes that are involved in general repair
33
34 mechanisms [43], arguing for a role of generalized aging rather than ovarian dysfunction in early menopause.

35
36 To date, the important question whether accelerated biological aging underlies both early menopause and an
37
38 increased CVD risk is unresolved and no recent evidence has been provided to support the reverse causality
39
40 hypothesis. A direct comparison of CVD risk between HL survivors and women with natural POI might provide
41
42 new insights, as POI among HL survivors is induced by treatment (exogenous factors) instead of natural early
43
44 depletion of the primordial follicle pool (endogenous factors). If POI would increase CVD risk, this should be
45
46 considered in the light of an established increased risk of CVD due to mediastinal RT and anthracycline-
47
48 containing CT.[9]

49 **Neurocognitive function**

50
51 Although in-vitro studies suggest a neuroprotective effect of oestrogen in the brain, the influence of decreased
52
53 estrogen levels on cognitive performance is still unclear. Some in-vivo studies have shown an increased risk of
54
55 neurocognitive impairment or dementia after a surgery-induced early menopause (approximately 1.5-fold),
56
57 while others found no association.[44-48] These contradicting findings may be due to differences in the
58
59

1 cognitive domains that were evaluated, as not all cognitive functions are equally influenced by oestrogens.
2
3 Hormonal influences seem to mainly concern aspects of memory, information processing speed and executive
4
5 functioning.[49 50]

6
7 Up to now, the long-term effects of POI on cognition are largely unknown, as most studies had short follow-up
8
9 times and included only women with menopausal ages above 40 years and/or women who used hormone
10
11 replacement therapy (HRT). Moreover, the majority of studies looked at the effects after oophorectomy,
12
13 characterized by an abrupt drop of oestrogen levels, while oestrogen levels may decrease gradually in CT-
14
15 induced POI occurring many years after treatment.[20 51]

18 **Hormone replacement therapy**

19
20 Much debate surrounds the use of HRT since the Women's Health Initiative (WHI) study reported increased
21
22 risks of breast cancer, CVD and cognitive impairment after oestrogen and progestin supplementation.[52] More
23
24 recent studies suggest that the benefit of oestrogen supplementation strongly depends on starting age and timing
25
26 with respect to menopause.[53-56] Among HL survivors in the Netherlands, HRT has been mainly prescribed to
27
28 relieve menopausal symptoms and to prevent osteoporosis.[57] However, in several HL treatment centres, HL
29
30 survivors have been advised to refrain from using HRT against menopausal symptoms because of a potential
31
32 increase in breast cancer risk.[58] This provides the unique opportunity to examine the effects of HRT use in
33
34 this population as the long-term effects of HRT on BMD, CVD and neurocognitive function in HL survivors with
35
36 POI have not been examined yet.

39 **Aim**

40
41 This article describes the design and methods of a Study On Primary ovarian insufficiency in female survivors of
42
43 Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA). The study aims to examine the long-term
44
45 effects of treatment-induced POI on BMD, cardiovascular status, neurocognitive function and QoL. We
46
47 hypothesize that women with treatment-induced POI will have an increased risk of osteoporosis and
48
49 neurocognitive dysfunction and a lower QoL than HL survivors without POI. However, based on the hypotheses
50
51 on reverse causality and biological aging, we hypothesize that CVD risk may not be increased in female HL
52
53 survivors with POI compared to HL survivors without POI.

54
55 Furthermore, we will examine whether long-term effects differ between women with CT- and RT-induced POI,
56
57 and between acute and more gradually developed POI. We will also investigate the effects of type and timing
58
59 of HRT on all outcomes if there is sufficient power. Finally, we will compare cardiovascular status and the

possible influence of HRT between HL survivors with treatment-induced POI and women from the general population with natural (non-treatment-induced) POI.

METHODS

Design and study population

Hodgkin lymphoma survivors

The SOPHIA-study is an observational cross-sectional study in which participants will be invited to complete several questionnaires and to visit an outpatient clinic for a neurocognitive, cardiovascular and BMD assessment. Participants will be recruited from a large previously described cohort of 5-year HL survivors treated in the Netherlands between 1965 and 2000 [4 59], which has been extended with more recently treated patients. This study is a collaboration of three large Dutch Medical Centres: The Netherlands Cancer Institute (NKI), VU University Medical Centre (VUmc) and Leiden University Medical Centre (LUMC). Eligible women were treated for HL at the age of 15-39 years at the adult Haematology-Oncology departments of the three medical centres, and survived ≥ 8 years after HL diagnosis. Exclusion criteria are: current age of ≥ 75 years, current treatment for a second malignancy, insufficient understanding of the Dutch language or any psychological, familial, sociological or geographical condition that potentially hampers study participation. The study has been approved by the Institutional Review Board of The Netherlands Cancer Institute. Recruitment started in 2014 (VUmc). We will follow the study population longitudinally to examine changes in risk factor and outcomes over time for which additional funding will be acquired.

External control group

To enable the comparison of cardiovascular status between HL survivors and women with natural (non-treatment-induced) POI, we will use data from an ongoing nationwide multicentre study on hypergonadotropic oligomenorrhoea/amenorrhoea. This study is a collaboration of three University Medical Centres and includes women aged ≥ 40 years with a diagnosis of polycystic ovarian syndrome (PCOS) or POI between 1992 and 2012. Data collection consists of a cardiovascular risk assessment at the outpatient clinic.[60 61]

Study parameters and data collection

Main outcome measures and other relevant study parameters are briefly described below by method of data collection (see also Table 1). The main exposure POI is defined as amenorrhoea for ≥ 4 months with two serum

1 follicle-stimulating hormone (FSH) levels in the menopausal range (obtained at least 1 month apart), or
2
3 amenorrhea for ≥ 12 months before the age of 40 years.
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Overview of outcome measures and corresponding data collection methods

Primary exposure and outcomes	Data collection methods		Outcome variables	Justification of methods
Primary ovarian insufficiency	Blood sample	Hormone level	If indicated for routine care: level of follicle-stimulating hormone (FSH) in mIU/mL	Routine care – diagnostic value
	Questionnaire		Date of last menstruation, menopausal age	
	Medical record		Date of last menstruation, menopausal age	
Bone mineral density	Medical tests	DEXA-scan of lumbar spine and hip by means of Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	BMD values in g/cm ² T and Z scores Presence of osteopenia (defined as T-scores of -1 to -2.5) Presence of osteoporosis (defined as T-scores of \geq -2.5)	Routine care – diagnostic value The DEXA-scan is most widely used in clinical practice to screen for osteoporosis and regarded as the 'Golden standard'
		Instant vertebral assessment (IVA) by Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	Vertebral height reduction in % Presence of clinical and non-clinical vertebral fractures	There is a strong additive value of IVA compared with DEXA alone [62-64]
		Anthropomorphic measurements	Height in cm and weight in kg	
	Blood sample	Bone turnover markers	Bone formation by P1NP - mean value in ng/ml Bone resorption by β -CTX - mean value in pg/ml	These markers have been used in previous studies and are recommended for research purposes [65]
		Vitamin D	Level of 25-hydroxyvitamine D in serum in nmol/l	Vitamin D has been associated with bone turnover markers, BMD, fracture risk and risk of falling [66-68]
	Questionnaire	Food frequency questionnaire (FFQ)	Mean score of calcium intake	The FFQ is a validated questionnaire [69] Reference values are available [70]
		General questionnaire	Previous fractures, use of calcium and vitamin D supplements use of glucocorticoids, family history of osteoporosis	
Medical record		Earlier DEXA-scans (yes,no), if applicable treatment plan for osteoporosis such as vitamin D supplementation, recommendations for lifestyle changes		
Cardiovascular status	Medical tests	Echocardiogram If contra-indicated: cardiac magnetic resonance imaging (MRI)	Abnormalities in heart structure Left ventricular function (LVF) by E/A ratio, deceleration time, isovolumic relaxation time (IVRT), left ventricular ejection fraction (LVEF), diastolic and systolic diameter and volume, E/e' ratio Right ventricular function: tricuspid annular plane systolic excursion (TAPSE) Presence of mitral, aortic or tricuspid valve defects i.e. insufficiencies or stenoses Wall motion score index	Routine care – diagnostic value
		Electrocardiogram (ECG)	Sinus rhythm, QRS complex, ST morphology (elevation or depression), PQ interval and left ventricle hypertrophy	Routine care – diagnostic value
		Coronary computed tomography angiography (CCTA) by a 320-detector	Coronary artery calcium (CAC) score according to Agatston Presence of luminal narrowing and if applicable: type of	High sensitivity and specificity [71] Most valid alternative method for detecting

		row volumetric scanner (Aquilion ONE) (LUMC) and 256 Scanner Philips (VUmc)	narrowing and number of plaques for the left main coronary artery (LMCA), left anterior descending (LAD), circumflex artery (CRX) and right coronary artery (RCA)	significant coronary disease (golden standard is invasive coronary angiography) [72]
		Vascular measurements	Presence of atherosclerosis by carotid intima media thickness (cIMT) and femoral IMT in mm Arterial stiffness (VUmc only)	Predictors of future cardiovascular events [73-75]
		Blood pressure	Mean of three consecutive measurements in mm/Hg	
		Anthropomorphic measurements	Height in cm, weight in kg, body mass index (BMI) in kg/cm ² , hip circumference in cm, waist circumference in cm, hip-waist ratio	
	Blood sample	Biomarkers	Left ventricular function and presence of ischemia and infarction by NT-pro-BNP in pmol/l Chronic inflammation (associated with atherosclerosis) by CRP in mg/L	In general population: strong predictor of coronary heart disease [76 77]
		Lipid spectrum	Total cholesterol, HDL, LDL, triglycerides	Established risk factors for CVD
		Glucose	Fasting blood glucose	Established risk factor for diabetes
		Kidney function	Creatinine, estimated glomerular filtration rate (eGFR)	Routine care before CCTA
	Questionnaire	'General questionnaire'	(Family) history of CVD and risk factors for CVD and if applicable date of diagnosis and treatment	
	Medical record		Cardiovascular risk score based on SCORE chart and Framingham chart, adjusted for age Cardiovascular history, contra-indications for echocardiogram	
Neurocognitive function	Neurocognitive tests	15 Words test	Verbal memory in total number of words	These tests were selected based on their reliability, validity and availability of reference norms. The domains examined are potentially sensitive for the effect of oestrogens [48,62-67]
		Trail making test A&B	Information processing speed) in seconds to complete	
		COWA verbal fluency test	Verbal fluency in total number of words	
		Letter-number sequencing	Working memory in total correct trials	
		WAIS III Digit span	Measures concentration in total number of items/ lists correctly repeated) can be converted to a scaled score, which is an age-based, norm-referenced score for each subject	
		Dutch Adult Reading Test (NART)	Verbal intelligence in mean IQ estimate	
Quality of life	Questionnaire	SF-12	General health	Shortened version of the validated questionnaire SF-36, which has been previously used in Dutch studies [78]
		MOS cognitive functioning scale	Cognitive functioning	Frequently used questionnaire [79]
		Hospital Anxiety and Depression Scale (HADS)	Anxiety and depression	Valid and reliable Dutch reference values are available [80]
		Sexual Activity Questionnaire (SAQ)	Sexual functioning	The SAQ is a valid, reliable and acceptable measure for describing the sexual functioning of women in terms of activity, pleasure and discomfort. It is quick and easy to administer and has good face validity discriminating

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

				between the sexual functioning of pre- and post-menopausal women [81]
		Shortened fatigue questionnaire (VW)	Fatigue	Reliable and validated questionnaire [82]

For peer review only

Medical records

Data on HL diagnosis (date, pathology), primary and recurrence treatment (including date, RT fields, chemotherapeutic regimens and doses) and follow-up data have been previously collected from medical records [4]. Since treatment for HL has changed considerably over time, a variety of treatment regimens was used. Primary treatment was usually given according to treatment protocols of the European Organization of Research and Treatment of Cancer (EORTC) and German Hodgkin lymphoma Study Group (GHSG), while treatment for recurrences was generally not standardized. Furthermore, data on reproductive factors (e.g., menopausal age) will be obtained.

Patient questionnaire data

Four questionnaires will be used to ascertain data on women's general characteristics, QoL, calcium intake and menopause related topics. The 'General characteristics questionnaire' will obtain information on the following items: reproductive history (e.g., age at menarche and menopause, parity, hormone use), general cardiovascular history, bone health status (e.g., previous fractures, use of medication), and lifestyle factors (e.g. current and previous smoking habits, alcohol use and physical activity). The 'QoL questionnaire' consists of five short validated and/or frequently used questionnaires regarding health, cognition, sexual activity, depression and fatigue (Table 1). A validated food frequency questionnaire (FFQ) will be used to assess calcium intake [69]. Reference values will be obtained from the report on dietary intake by the Health Council of the Netherlands [70]. The 'Menopause questionnaire' is specifically aimed at postmenopausal women and will collect information on climacteric symptoms (i.e., severity and frequency) and changes in lifestyle factors after the onset of menopause. Data regarding infertility issues will be ascertained for women who experienced POI.

Neurocognitive, cardiovascular and fracture risk assessments

For the neurocognitive assessment, we have chosen tests that measure cognitive domains potentially sensitive for effects of oestrogens.[49 83-88] Tests were selected based on their reliability, validity and availability of Dutch reference norms. The cardiovascular assessment includes an echocardiogram, electrocardiogram (ECG), coronary computed tomography angiography (CCTA), and measurement of the carotid and femoral IMT, blood pressure and hip/waist circumference. The BMD assessment consists of a DEXA-scan with instant vertebral assessment (IVA). These medical tests were chosen based on their availability and use in clinical practice and their evidence-based diagnostic or predictive value (Table 1).

Blood samples

A blood sample will be drawn to examine bone turnover (i.e., β -CTX for bone resorption and P1NP for bone formation) and cardiac (e.g. NT-pro-BNP, CRP, lipid spectrum) markers. Since new techniques in this research field develop rapidly, we will collect an additional blood sample for future analyses on new biomarkers, such as biomarkers predictive for late effects. Moreover, this sample will be used for future DNA extraction and analyses (e.g. to examine modifying effects of genetic factors, such as single nucleotide polymorphisms (SNP's) associated with POI). These blood samples will be frozen and stored at -80 °C.

Study procedures

Recruitment

Women will be recruited through the Survivorship Care outpatient Clinic (SCC) for HL survivors, established by the Dutch nationwide BETER consortium (Better care after Hodgkin lymphoma, Evaluation of long-Term Treatment Effects and screening Recommendations). This consortium consists of haematologists and radiation oncologists of >20 hospitals and has developed evidence-based guidelines on follow-up care, including recommendations on cardiovascular risk assessment in order to reduce morbidity and mortality [89]. The three medical centres participating in the SOPHIA-study all have an active SCC where HL survivors can be screened. Approximately 30% of the 5-year HL survivors already receive routine follow-up care on a yearly basis in their original HL treatment centre. All 5-year HL survivors who are currently not under surveillance will be invited for screening (if treated at the age of 15-60 years and currently aged <75 years) by the BETER consortium in the upcoming years.

If a woman, eligible for the SOPHIA-study, visits the SCC of NKI, LUMC or VUmc (either during an intake or follow-up care visit), she will be invited by her treating physician. As mentioned above, follow-up care is provided according to the BETER guidelines and depends on the specific treatments a patient received (e.g., chest or pelvic RT, anthracycline-containing CT). As some of the medical tests in the SOPHIA-study are incorporated in the BETER guidelines, these tests may be part of routine care. Therefore, the physician will determine for each patient which medical tests will be performed for routine care and which will be additionally performed for research purposes. Tests that are considered for routine care may have been recently performed in a participant. If there is no clinical reason to repeat the test, the result of the previous test will be abstracted from the medical record. More details on the distinction between routine care and research tests, and the study procedures are described in Figure 1.

1 We aim to integrate study participation as much as possible with the routine care provided at the SCC. Whether
2
3 or not a patient is willing to participate in the study will not have any influence on the routine care she receives
4
5 during follow-up.
6
7

8 *Study implementation*

9
10 If the patient is interested to participate in the SOPHIA-study, the treating physician will hand out an invitation
11
12 for the SOPHIA-study, together with a patient information letter and an informed consent form. After one or
13
14 two weeks, the treating physician or research nurse will contact the patient by telephone to answer any
15
16 remaining questions. If the woman agrees to participate, she will be asked to return the signed informed
17
18 consent form, and subsequently the 'General questionnaire' and 'Menopause questionnaire' (if applicable) will
19
20 be sent to her home. Patients will be asked to bring their completed questionnaires with them to a follow-visit
21
22 at the SSC or to return the questionnaires by mail.
23

24 The neurocognitive, cardiovascular and BMD assessments will be performed during a follow-up visit at the SCC.
25
26 Ideally, all medical tests will be performed during two to three follow-up visits, depending on availability and
27
28 timing of other routine medical care tests (e.g. breast cancer screening). The planned tests with allocated time
29
30 are shown in Figure 1.

31 Patients will be tested for renal failure before undergoing the CCTA. Women with severe renal insufficiency,
32
33 defined as a e-GFR value of <60 ml per minute per 1.73m^2 , will undergo a computed tomography coronary
34
35 calcium score without contrast fluid.
36

37 Blood will be drawn at two time points. The first blood sample will be taken at the SCC during a routine care
38
39 blood withdrawal. The second blood sample will be drawn in fasting state before the CCTA.

40 Patients are offered the possibility to perform the neurocognitive tests at home. In that case, a separate
41
42 appointment will be made. To ensure sufficient time between two memory tests, patients will be asked to
43
44 complete the FFQ during this appointment.
45
46
47

48 **Statistical issues**

49 *Power calculation*

50
51 Approximately 500 women are eligible for participation in the three selected hospitals. Based on our previous
52
53 studies we expect that 60% of the eligible women will participate in the current study (N=300). Power
54
55 calculations were performed separately for the outcomes BMD, cardiovascular status, neurocognitive function
56
57
58
59

1 and QoL. When comparing BMD of women who developed POI (expected N=60 (20%)) with those who did not
2 (expected N=240), there is over 80% power to detect a difference of 0.05 g/cm² in BMD (1.00 (SD 0.1) vs 1.05
3 (SD 0.1)).

4
5
6 The power calculation for cardiovascular status is based on the IMT measurement. There is over 80% power to
7 detect a difference of 0.1 mm in mean IMT between women with POI and women with normal menopausal
8 ages (0.6 (SD 0.2) versus 0.5 (SD 0.2)). An increase of 0.1 mm in IMT has been associated with an increase in risk
9 of 12% for myocardial infarction.[74]

10
11 Previous retrospective neuropsychological studies, in which effects of systemic cancer treatments and POI on
12 cognitive functioning were examined, have yielded significant findings with somewhat smaller group sizes of 39
13 and 53 patients.[90-92]

14 For the outcome QoL, we followed the calculations of Cohen.[93] The proposed study has over 80% power to
15 detect moderate difference between women who developed POI and those who did not. All power calculations
16 used a 5% chance for a Type-1 error and a minimal effect size of 0.5.

27 *Statistical analyses*

28
29 Outcomes of female HL survivors who developed POI will be compared with those of female HL survivors who
30 did not by using chi square tests or Fisher's exact tests (categorical variables) and two tailed t-tests (continuous
31 variables) after appropriate transformation, if necessary. Multivariate regression analyses will be used to
32 examine the effects of POI, menopausal age and HRT use on the primary outcome variables (BMD,
33 cardiovascular status and neurocognitive function) and to assess the effect of these primary outcome variables
34 on QoL. All analyses will be adjusted for confounders (HL treatment regimen, lifestyle factors, reproductive
35 factors, climacteric symptoms, medication) where applicable. Effect modification and mediation will be tested
36 using interaction terms. We will also perform subgroup analyses to evaluate the difference between a CT- and
37 RT-induced POI in more detail and we will compare cardiovascular status between HL survivors with POI and
38 other women with natural POI. P values <0.05 will be considered statistically significant.

49 **DISCUSSION**

50
51 In the current study, the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status,
52 neurocognitive function and QoL will be examined by measurements within a cohort of female HL survivors.
53
54 Approximately 30-40% of the female survivors in our HL cohort treated between 1965 and 1985 experienced

1 treatment-induced POI. The majority of these women have now been postmenopausal for over 20 to 30 years
2
3 which enables us to examine the very late effects of POI.

4 Treatment-induced POI might put female HL survivors at high risk for developing adverse POI-associated
5
6 conditions such as osteoporosis, neurocognitive dysfunction and CVD, while they already have an increased risk
7
8 for late adverse effects due to the HL treatment itself.[4 9 21 37 46] This may lead to problems in daily
9
10 functioning and can have a large impact on their QoL, in particular because the conditions may occur at very
11
12 young ages. Results of this study will help to identify those HL survivors who are at increased risk for
13
14 osteoporosis, neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely
15
16 referral of high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL.
17
18 Moreover, by identifying these long-term risks, physicians can better inform women with POI in the future.
19
20 Findings of this study will also be relevant for other female cancer patients who received gonadotoxic
21
22 treatment at premenopausal ages. CT is a major contributor to the development of POI and its use has
23
24 intensified considerably over the years in many malignancies.[94 95] Therefore, it is expected that the
25
26 occurrence of POI-associated adverse effects will increase in female cancer survivors in the near future. Since
27
28 HRT has become subject of much debate in recent years, investigating the effects of HRT on POI-associated
29
30 conditions will produce valuable knowledge with regard to the HRT-suppletion policy for female cancer
31
32 survivors in the Netherlands.

33 Finally, this study provides a unique possibility to challenge the conventional view that reproductive hormone
34
35 deprivation in females is of key importance in CVD development. This is relevant in the light of other
36
37 hypotheses that general biological aging mechanisms underlie a combination of POI and CVD, or that CVD risk
38
39 factors determine age at menopause (reverse causality hypothesis).[42] Comparison of cardiovascular status
40
41 between women with POI after HL treatment and women with natural POI will allow examination of a cause-
42
43 effect relationship between early menopause and CVD. We will be able to adjust for the potential effects of HL
44
45 treatment on CVD risk, as extensive data on HL treatment regimens are available within our cohort.

46 This study has several notable strengths and limitations. First, this study is embedded in an infrastructure of
47
48 several multidisciplinary SCCs, enabling a broad scope of medical tests and extensive follow-up care. Detailed
49
50 treatment and reproductive data will be available from medical records and patient questionnaires. Second,
51
52 women received a variety of treatments and have long-term follow-up, rendering it possible to examine the
53
54 effects of different HL treatments, POI and menopausal age on several outcomes. A limitation of the study
55
56 includes patient selection. Due to the high risk of late adverse effects in HL survivors, some women are already
57
58
59

1 deceased or not able to participate in the SOPHIA-study. Moreover, some women are under surveillance in a
2 local hospital rather than their original HL treatment centre, while women who are (feeling) healthy may not
3 visit the SCC because of medical costs and/or other obligations (e.g., work, family). We will account for this by
4 obtaining medical data for women under surveillance in others hospitals, and we have near complete data on
5 important competing late adverse effects such as CVD, second malignancies and vital status, as this study is
6 nested within an existing cohort.
7

8 Another limitation is that this study is dependent on routine care procedures of the participating SSCs.
9 Therefore, differences between SSCs may occur regarding available equipment and registration of results from
10 medical tests. In addition, the time interval between two medical tests may be variable between patients due
11 to planning issues (e.g., long waiting lists, the aim to plan multiple medical tests in one day), or because one
12 medical test has already been performed in the past year and will not be repeated. We made a standardized
13 abstraction form to ensure all relevant data are gathered and differences between timing of medical tests and
14 hospitals will be accounted for in the analyses.
15

16 In conclusion, this article describes the study protocol of the SOPHIA-study which aims to increase knowledge
17 about BMD, cardiovascular status and neurocognitive function in long-term female HL survivors with and
18 without treatment-induced POI, and the potential influence of these long-term effects on QoL. Results of this
19 study will lead to the identification of those HL survivors who are at increased risk for osteoporosis,
20 neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely referral of
21 high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL.
22 Furthermore, results will shed light on existing hypotheses regarding the association between POI and CVD risk.
23 Moreover, HL survivors or other cancer survivors who will experience treatment-induced POI in the future can
24 be better informed about potential long-term effects.
25

26 **AUTHORS' CONTRIBUTIONS**

27 The study protocol has been written by IK, AOvW, BA, EvD-dB and FvL. All authors contributed to study design.
28 JZ, LD, and BA are responsible for patient accrual and inclusion, and JZ, YA, SB, LM, ES, PL, and LD and BA are
29 responsible for the assessment of the outcome variables.
30

31 FvL is the principal investigator and responsible for the funding of the study.
32

33 All authors revised the manuscript critically for intellectual content and have approved the final manuscript.
34
35
36
37
38
39
40
41
42
43
44

FUNDING

This study is financially supported by the Dutch Cancer Society (grant number NKI 2010-4720).

COMPETING INTERESTS

JZ declares she has conducted a research project funded by Roche in the past two years (unrelated to the current study). CBL's department of Reproductive Medicine has received educational and research grants from Merck Serono, Ferring and Auxogyn, and he received speakers fees from MSD, Merck Serono, Ferring and Auxogyn. He is also a consultant for Ferring. PL provided advice to Friesland Campina. All other authors declare no competing interests.

ETHICS AND DISSEMINATION

This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL44714.031.13). Reporting of serious adverse events was exempted for this study, as the burden and risks associated with participation are very low and in accordance with routine medical care. Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL survivors.

REFERENCES

1. Ferme C, Eghbali H, Meerwaldt JH, et al. Chemotherapy plus involved-field radiation in early-stage Hodgkin's disease. *N Engl J Med* 2007;**357**(19):1916-27 doi: 10.1056/NEJMoa064601[published Online First: Epub Date] .
2. Raemaekers J, Burgers M, Henry-Amar M, et al. Patients with stage III/IV Hodgkin's disease in partial remission after MOPP/ABV chemotherapy have excellent prognosis after additional involved-field radiotherapy: interim results from the ongoing EORTC-LCG and GPMC phase III trial. The EORTC Lymphoma Cooperative Group and Groupe Pierre-et-Marie-Curie. *Ann Oncol* 1997;**8 Suppl 1**:111-4
3. Klimm B, Goergen H, Fuchs M, et al. Impact of risk factors on outcomes in early-stage Hodgkin's lymphoma: an analysis of international staging definitions. *Ann Oncol* 2013;**24**(12):3070-6 doi: 10.1093/annonc/mdt413[published Online First: Epub Date] .

- 1 4. Schaapveld M, Aleman BM, van Eggermond AM, et al. Second Cancer Risk Up to 40 Years after
2 Treatment for Hodgkin's Lymphoma. *N Engl J Med* 2015;**373**(26):2499-511 doi:
3 10.1056/NEJMoa1505949[published Online First: Epub Date]].
- 4 5. Bhatia S, Yasui Y, Robison LL, et al. High risk of subsequent neoplasms continues with extended follow-
5 up of childhood Hodgkin's disease: report from the Late Effects Study Group. *J Clin Oncol*
6 2003;**21**(23):4386-94
- 7 6. Hodgson DC, Gilbert ES, Dores GM, et al. Long-term solid cancer risk among 5-year survivors of
8 Hodgkin's lymphoma. *J Clin Oncol* 2007;**25**(12):1489-97
- 9 7. Punnett A, Tsang RW, Hodgson DC. Hodgkin lymphoma across the age spectrum: epidemiology,
10 therapy, and late effects. *Semin Radiat Oncol* 2010;**20**(1):30-44
- 11 8. Aleman BM, AW vdB-D, de Bruin ML, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma.
12 *Blood* 2007;**109**(5):1878-86
- 13 9. van Nimwegen FA, Schaapveld M, Janus CP, et al. Cardiovascular disease after Hodgkin lymphoma
14 treatment: 40-year disease risk. *JAMA Intern Med* 2015;**175**(6):1007-17 doi:
15 10.1001/jamainternmed.2015.1180[published Online First: Epub Date]].
- 16 10. de Bruin ML, Dorresteijn LD, van't Veer MB, et al. Increased risk of stroke and transient ischemic attack
17 in 5-year survivors of Hodgkin lymphoma. *J Natl Cancer Inst* 2009;**101**(13):928-37
- 18 11. de Bruin ML, Huisbrink J, Hauptmann M, et al. Treatment-related risk factors for premature
19 menopause following Hodgkin lymphoma. *Blood* 2008;**111**(1):101-08
- 20 12. Swerdlow AJ, Cooke R, Bates A, et al. Risk of premature menopause after treatment for Hodgkin's
21 lymphoma. *J Natl Cancer Inst* 2014;**106**(9) doi: 10.1093/jnci/dju207[published Online First: Epub
22 Date]].
- 23 13. Haukvik UK, Dieset I, Bjoro T, et al. Treatment-related premature ovarian failure as a long-term
24 complication after Hodgkin's lymphoma. *Ann Oncol* 2006;**17**(9):1428-33
- 25 14. van der Kaaij MA, Heutte N, Meijnders P, et al. Premature ovarian failure and fertility in long-term
26 survivors of Hodgkin's lymphoma: a European Organisation for Research and Treatment of Cancer
27 Lymphoma Group and Groupe d'Etude des Lymphomes de l'Adulte Cohort Study. *J Clin Oncol*
28 2012;**30**(3):291-99
- 29 15. Behringer K, Breuer K, Reineke T, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced
30 by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives

- 1 during therapy: a report from the German Hodgkin's Lymphoma Study Group. *J Clin Oncol*
2
3 2005;**23**(30):7555-64
- 4
5 16. Luborsky JL, Meyer P, Sowers MF, et al. Premature menopause in a multi-ethnic population study of
6
7 the menopause transition. *Hum Reprod* 2003;**18**(1):199-206
- 8
9 17. Meiorow D. Reproduction post-chemotherapy in young cancer patients. *Mol Cell Endocrinol*
10
11 2000;**169**(1-2):123-31
- 12
13 18. Nelson HD. Menopause. *Lancet* 2008;**371**(9614):760-70
- 14
15 19. Kenemans P. Menopause, HRT and menopausal symptoms. *J Epidemiol Biostat* 1999;**4**(3):141-46
- 16
17 20. Hendrix SL. Bilateral oophorectomy and premature menopause. *Am J Med* 2005;**118** Suppl 12B:131-5
18
19 doi: 10.1016/j.amjmed.2005.09.056[published Online First: Epub Date] .
- 20
21 21. Gallagher JC. Effect of early menopause on bone mineral density and fractures. *Menopause* 2007;**14**(3
22
23 Pt 2):567-71
- 24
25 22. Der Voort DJ, Der Weijer PH, Barentsen R. Early menopause: increased fracture risk at older age.
26
27 *Osteoporos Int* 2003;**14**(6):525-30
- 28
29 23. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, et al. Bone density patterns after normal and premature
30
31 menopause. *Maturitas* 2003;**44**(4):279-86
- 32
33 24. van der Klift M, de Laet CE, McCloskey EV, et al. Risk factors for incident vertebral fractures in men and
34
35 women: the Rotterdam Study. *J Bone Miner Res* 2004;**19**(7):1172-80
- 36
37 25. Schuit SC, van der Klift M, Weel AE, et al. Fracture incidence and association with bone mineral
38
39 density in elderly men and women: the Rotterdam Study. *Bone* 2004;**34**(1):195-202
- 40
41 26. Kritz-Silverstein D, von Muhlen DG, Barrett-Connor E. Hysterectomy and oophorectomy are unrelated
42
43 to bone loss in older women. *Maturitas* 2004;**47**(1):61-69
- 44
45 27. Ahlborg HG, Johnell O, Nilsson BE, et al. Bone loss in relation to menopause: a prospective study
46
47 during 16 years. *Bone* 2001;**28**(3):327-31
- 48
49 28. Gerdhem P, Obrant KJ. Bone mineral density in old age: the influence of age at menarche and
50
51 menopause. *J Bone Miner Metab* 2004;**22**(4):372-75
- 52
53 29. Svejme O, Ahlborg HG, Nilsson JA, et al. Early menopause and risk of osteoporosis, fracture and
54
55 mortality: a 34-year prospective observational study in 390 women. *BJOG* 2012;**119**(7):810-16
- 56
57 30. Khosla S, Melton LJ, III, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of
58
59 osteoporosis: is a revision needed? *J Bone Miner Res* 2011;**26**(3):441-51
- 60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
31. Kreuser ED, Felsenberg D, Behles C, et al. Long-term gonadal dysfunction and its impact on bone mineralization in patients following COPP/ABVD chemotherapy for Hodgkin's disease. *Ann Oncol* 1992;**3 Suppl 4**:105-10
32. Redman JR, Bajorunas DR, Wong G, et al. Bone mineralization in women following successful treatment of Hodgkin's disease. *Am J Med* 1988;**85**(1):65-72
33. Howell SJ, Berger G, Adams JE, et al. Bone mineral density in women with cytotoxic-induced ovarian failure. *Clin Endocrinol (Oxf)* 1998;**49**(3):397-402
34. Atsma F, Bartelink ML, Grobbee DE, et al. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. *Menopause* 2006;**13**(2):265-79
35. Hu FB, Grodstein F, Hennekens CH, et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med* 1999;**159**(10):1061-66
36. Lobo RA. Surgical menopause and cardiovascular risks. *Menopause* 2007;**14**(3 Pt 2):562-66
37. Roeters van Lennep JE, Heida KY, Bots ML, et al. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2016;**23**(2):178-86 doi: 10.1177/2047487314556004[published Online First: Epub Date]].
38. van der Schouw YT, van der Graaf Y, Steyerberg EW, et al. Age at menopause as a risk factor for cardiovascular mortality. *Lancet* 1996;**347**(9003):714-18
39. Montalcini T, Gorgone G, Gazzaruso C, et al. Role of endogenous androgens on carotid atherosclerosis in non-obese postmenopausal women. *Nutr Metab Cardiovasc Dis* 2007;**17**(10):705-11
40. Golden SH, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid atherosclerosis: a case-control study of the atherosclerosis risk in communities cohort. *Am J Epidemiol* 2002;**155**(5):437-45
41. Bernini GP, Sgro' M, Moretti A, et al. Endogenous androgens and carotid intimal-medial thickness in women. *J Clin Endocrinol Metab* 1999;**84**(6):2008-12
42. Kok HS, van Asselt KM, van der Schouw YT, et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol* 2006;**47**(10):1976-83
43. Stolk L, Perry JR, Chasman DI, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* 2012;**44**(3):260-68
44. Farrag AK, Khedr EM, Abdel-Aleem H, et al. Effect of surgical menopause on cognitive functions. *Dement Geriatr Cogn Disord* 2002;**13**(3):193-98

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
45. McLay RN, Maki PM, Lyketsos CG. Nulliparity and late menopause are associated with decreased cognitive decline. *J Neuropsychiatry Clin Neurosci* 2003;**15**(2):161-67
46. Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 2007;**69**(11):1074-83
47. Henderson VW, Sherwin BB. Surgical versus natural menopause: cognitive issues. *Menopause* 2007;**14**(3 Pt 2):572-79
48. Vearncombe KJ, Pachana NA. Is cognitive functioning detrimentally affected after early, induced menopause? *Menopause* 2009;**16**(1):188-98
49. Lethaby A, Hogervorst E, Richards M, et al. Hormone replacement therapy for cognitive function in postmenopausal women. *Cochrane Database Syst Rev* 2008(1):CD003122
50. Zwart W, Terra H, Linn SC, et al. Cognitive effects of endocrine therapy for breast cancer: keep calm and carry on? *Nat Rev Clin Oncol* 2015;**12**(10):597-606 doi: 10.1038/nrclinonc.2015.124[published Online First: Epub Date]].
51. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. *J Clin Oncol* 1996;**14**(5):1718-29 doi: 10.1200/JCO.1996.14.5.1718[published Online First: Epub Date]].
52. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;**288**(3):321-33
53. Rosano GM, Vitale C, Fini M. Hormone replacement therapy and cardioprotection: what is good and what is bad for the cardiovascular system? *Ann N Y Acad Sci* 2006;**1092**:341-48
54. Maki PM, Henderson VW. Hormone therapy, dementia, and cognition: the Women's Health Initiative 10 years on. *Climacteric* 2012;**15**(3):256-62
55. Maki PM, Dennerstein L, Clark M, et al. Perimenopausal use of hormone therapy is associated with enhanced memory and hippocampal function later in life. *Brain Res* 2011;**1379**:232-43
56. King J, Wynne CH, Assersohn L, et al. Hormone replacement therapy and women with premature menopause--a cancer survivorship issue. *Eur J Cancer* 2011;**47**(11):1623-32
57. Marjoribanks J, Farquhar C, Roberts H, et al. Long term hormone therapy for perimenopausal and postmenopausal women. *Cochrane Database Syst Rev* 2012(7):CD004143 doi: 10.1002/14651858.CD004143.pub4[published Online First: Epub Date]].

- 1 58. Lagro-Janssen A, Knufing MW, Schreurs L, et al. Significant fall in hormone replacement therapy
2 prescription in general practice. *Fam Pract* 2010;**27**(4):424-9 doi: 10.1093/fampra/cmz018[published
3 Online First: Epub Date]].
- 4
5
6 59. van Leeuwen FE, Klokman WJ, Hagenbeek A, et al. Second cancer risk following Hodgkin's disease: a
7 20-year follow-up study. *J Clin Oncol* 1994;**12**(2):312-25
- 8
9
10 60. Daan NM, Muka T, Koster MP, et al. Cardiovascular Risk in Women With Premature Ovarian
11 Insufficiency Compared to Premenopausal Women at Middle Age. *J Clin Endocrinol Metab*
12 2016;**101**(9):3306-15 doi: 10.1210/jc.2016-1141[published Online First: Epub Date]].
- 13
14
15 61. Janse F, Knauff EA, Niermeijer MF, et al. Similar phenotype characteristics comparing familial and
16 sporadic premature ovarian failure. *Menopause* 2010;**17**(4):758-65 doi:
17 10.1097/gme.0b013e3181cf8521[published Online First: Epub Date]].
- 18
19
20 62. Olenginski TP, Newman ED, Hummel JL, et al. Development and evaluation of a vertebral fracture
21 assessment program using IVA and its integration with mobile DXA. *J Clin Densitom* 2006;**9**(1):72-7 doi:
22 10.1016/j.jocd.2005.08.002[published Online First: Epub Date]].
- 23
24
25 63. Greenspan SL, von Stetten E, Emond SK, et al. Instant vertebral assessment: a noninvasive dual X-ray
26 absorptiometry technique to avoid misclassification and clinical mismanagement of osteoporosis. *J*
27 *Clin Densitom* 2001;**4**(4):373-80
- 28
29
30 64. Netelenbos JC, Lems WF, Geusens PP, et al. Spine radiographs to improve the identification of women
31 at high risk for fractures. *Osteoporos Int* 2009;**20**(8):1347-52 doi: 10.1007/s00198-008-0801-
32 1[published Online First: Epub Date]].
- 33
34
35 65. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and
36 monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*
37 2011;**22**(2):391-420 doi: 10.1007/s00198-010-1501-1[published Online First: Epub Date]].
- 38
39
40 66. van Schoor NM, Visser M, Pluijm SM, et al. Vitamin D deficiency as a risk factor for osteoporotic
41 fractures. *Bone* 2008;**42**(2):260-6 doi: 10.1016/j.bone.2007.11.002[published Online First: Epub
42 Date]].
- 43
44
45 67. Kuchuk NO, Pluijm SM, van Schoor NM, et al. Relationships of serum 25-hydroxyvitamin D to bone
46 mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin*
47 *Endocrinol Metab* 2009;**94**(4):1244-50 doi: 10.1210/jc.2008-1832[published Online First: Epub Date]].
- 48
49
50
51
52
53
54
55
56
57
58
59

- 1 68. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and
2 active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ* 2009;**339**:b3692 doi:
3 10.1136/bmj.b3692[published Online First: Epub Date]].
4
5
6
7 69. Angus RM, Sambrook PN, Pocock NA, et al. A simple method for assessing calcium intake in Caucasian
8 women. *J Am Diet Assoc* 1989;**89**(2):209-14
9
10
11 70. Netherlands. THHcot. Dietary reference values: calcium, vitamin D, thiamin, riboflavin, niacin,
12 panthothenic acid, and biotin, 2000.
13
14
15 71. Schussler JM, Grayburn PA. Non-invasive coronary angiography using multislice computed
16 tomography. *Heart* 2007;**93**(3):290-7 doi: 10.1136/hrt.2005.069195[published Online First: Epub
17 Date]].
18
19
20 72. Schuetz GM, Zacharopoulou NM, Schlattmann P, et al. Meta-analysis: noninvasive coronary
21 angiography using computed tomography versus magnetic resonance imaging. *Ann Intern Med*
22 2010;**152**(3):167-77 doi: 10.7326/0003-4819-152-3-201002020-00008[published Online First: Epub
23 Date]].
24
25
26
27 73. Nair SB, Malik R, Khattar RS. Carotid intima-media thickness: ultrasound measurement, prognostic
28 value and role in clinical practice. *Postgrad Med J* 2012;**88**(1046):694-9 doi: 10.1136/postgradmedj-
29 2011-130214[published Online First: Epub Date]].
30
31
32
33 74. Den Ruijter HM, Peters SA, Anderson TJ, et al. Common carotid intima-media thickness measurements
34 in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012;**308**(8):796-803
35
36
37
38 75. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause
39 mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol*
40 2010;**55**(13):1318-27 doi: 10.1016/j.jacc.2009.10.061[published Online First: Epub Date]].
41
42
43
44 76. Buckley DI, Fu R, Freeman M, et al. C-reactive protein as a risk factor for coronary heart disease: a
45 systematic review and meta-analyses for the U.S. Preventive Services Task Force. *Ann Intern Med*
46 2009;**151**(7):483-95
47
48
49 77. Kavousi M, Elias-Smale S, Rutten JH, et al. Evaluation of newer risk markers for coronary heart disease
50 risk classification: a cohort study. *Ann Intern Med* 2012;**156**(6):438-44 doi: 10.7326/0003-4819-156-6-
51 201203200-00006[published Online First: Epub Date]].
52
53
54
55
56
57
58
59

- 1 78. Brennehan SK, Barrett-Connor E, Sajjan S, et al. Impact of recent fracture on health-related quality of
2 life in postmenopausal women. *J Bone Miner Res* 2006;**21**(6):809-16 doi:
3 10.1359/jbmr.060301[published Online First: Epub Date]].
4
5
6
7 79. Stewart AL WJ, Sherbourne CD, et al. Psychological distress/well-being and cognitive functioning
8 measures. *Measuring Functioning and Well-Being: The Medical Outcomes Study Approach*. Durham,
9 NC: Duke University, 1992. p.102–142.
10
11
12 80. Spinhoven P OJ, Sloekers PP, Kempen GI, Speckens AE, Van Hemert AM (1997) A validation study of
13 the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychol Med*
14 27:363-70.
15
16
17
18 81. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual
19 functioning. *Qual Life Res* 1996;**5**(1):81-90
20
21
22 82. Alberts M SE, Vercoulen JHMM, Garssen B, Bleijenberg G. Verkorte Vermoeidheidsvragenlijst: een
23 praktisch hulpmiddel bij het scoren van vermoeidheid. *Nederlands Tijdschrift voor Geneeskunde* 1997;
24 141:1526-1530.
25
26
27
28 83. Sherwin BB. Estrogen therapy: is time of initiation critical for neuroprotection? *Nat Rev Endocrinol*
29 2009;**5**(11):620-7 doi: 10.1038/nrendo.2009.193[published Online First: Epub Date]].
30
31
32 84. Schilder CM, Eggens PC, Seynaeve C, et al. Neuropsychological functioning in postmenopausal breast
33 cancer patients treated with tamoxifen or exemestane after AC-chemotherapy: cross-sectional
34 findings from the neuropsychological TEAM-side study. *Acta Oncol* 2009;**48**(1):76-85 doi:
35 10.1080/02841860802314738[published Online First: Epub Date]].
36
37
38
39 85. van Dam FS, Schagen SB, Muller MJ, et al. Impairment of cognitive function in women receiving
40 adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. *J Natl*
41 *Cancer Inst* 1998;**90**(3):210-8
42
43
44
45 86. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, et al. Normative data for the Animal, Profession and
46 Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education,
47 and sex. *J Int Neuropsychol Soc* 2006;**12**(1):80-9 doi: 10.1017/S1355617706060115[published Online
48 First: Epub Date]].
49
50
51
52 87. Mulder J DR, Dekker P. *Verbale Leer & Geheugen Test*. Lisse: Swets & Zeitlinger; 1996.
53
54
55 88. Reitan R. Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and*
56 *Motor Skills*. 1958;**8**:271-6.
57
58
59

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
89. Dekker N, van 't Veer MB, Aleman BM, et al. [The BETER survivorship care initiative for Hodgkin lymphoma; tailored survivorship care for late effects of treatment]. *Ned Tijdschr Geneeskd* 2015;**159**:A9269
90. Castellon SA, Ganz PA, Bower JE, et al. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. *J Clin Exp Neuropsychol* 2004;**26**(7):955-69
91. Collins B, Mackenzie J, Stewart A, et al. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. *Psychooncology* 2009;**18**(2):134-43
92. Schagen SB, van Dam FS, Muller MJ, et al. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. *Cancer* 1999;**85**(3):640-50
93. Cohen J. A power primer. *Psychol Bull* 1992;**112**(1):155-59
94. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**(9472):1687-717 doi: 10.1016/S0140-6736(05)66544-0[published Online First: Epub Date]].
95. Rodriguez-Wallberg KA, Oktay K. Fertility preservation in women with breast cancer. *Clin Obstet Gynecol* 2010;**53**(4):753-62 doi: 10.1097/GRF.0b013e3181f96e00[published Online First: Epub Date]].

33 FIGURE LEGENDS

34 **Figure 1. Study procedures and patient burden, stratified by medical tests for routine care and research**

35 Abbreviations: RT = radiotherapy; CT = chemotherapy; BETTER = Better care after Hodgkin lymphoma,
36 Evaluation of long-Term Treatment Effects and screening Recommendations; CRP = C-reactive protein; BMD =
37 bone mineral density; DEXA = Dual Energy X-ray Absorptiometry

38 ^a Expected for > 90% of women ^b Expected for 15-40% of women

39 In case criteria for care are not fulfilled, tests will be performed for research purpose

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

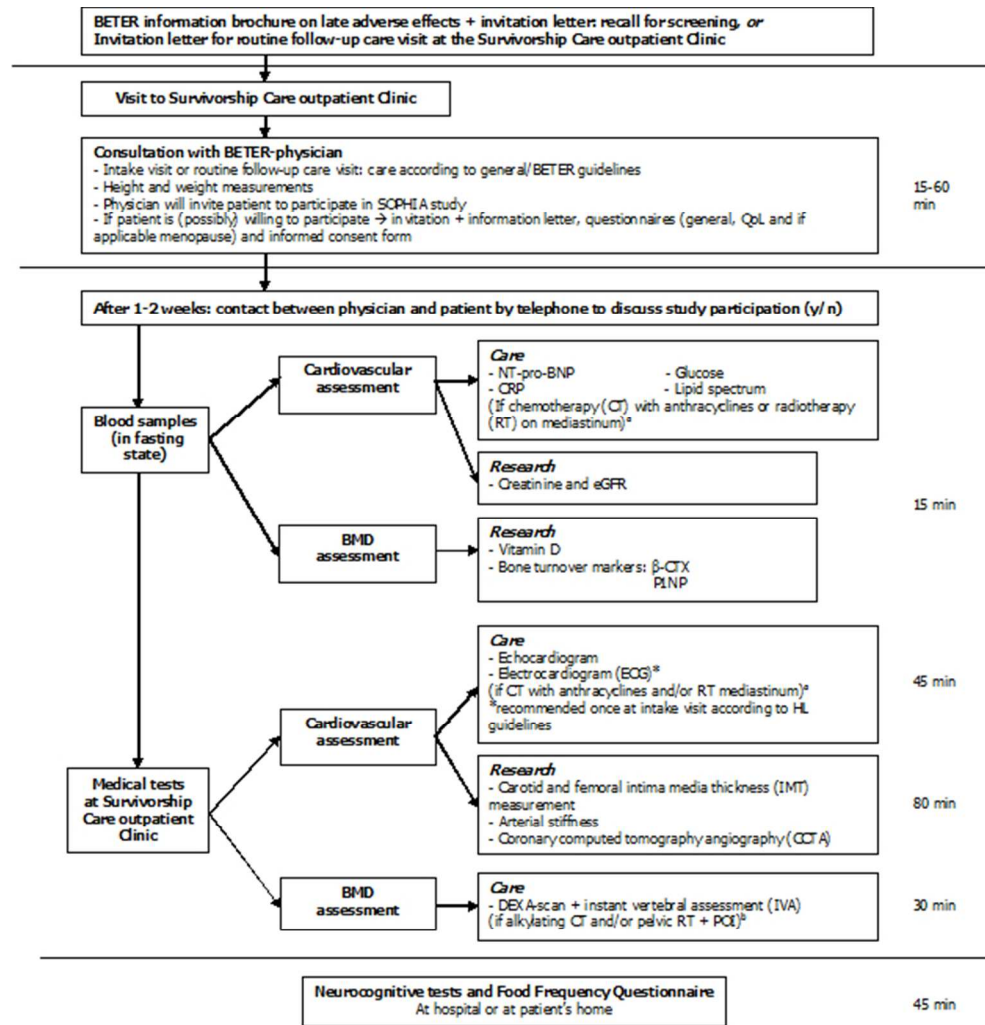


Figure 1. Study procedures and patient burden, stratified by medical tests for routine care and research

169x172mm (96 x 96 DPI)

BMJ Open

Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-018120.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Nov-2017
Complete List of Authors:	Krul, Inge; The Netherlands Cancer Institute, Epidemiology and Biostatistics Opstal-van Winden, Annemieke; the Netherlands Cancer Institute, Epidemiology and Biostatistics Zijlstra, Josée ; VU University Medical Centre, Haemato-oncology Appelman, Yolande; VU University Medical Center, Cardiology Schagen, SB; The Netherlands Cancer Institute , Epidemiology and Biostatistics Meijboom, Lilian; VU University Medical Center, Radiology Serné, Erik; VU University Medical Centre, Vascluar medicine Lambalk, Cornelis; VU University Medical Centre, Obstetrics and Gynecology Lips, Paul; VU University Medical Center, Internal medicine, Endocrine Section van Dulmen - den Broeder, Eline; VU University Medical Center, Pediatric Oncology and Hematology Hauptmann, M; The Netherlands Cancer Institute, Department of Epidemiology and Biostatistics Daniëls, Laurien; Leids Universitair Medisch Centrum, Radiotherapy Aleman, Berthe; The Netherlands Cancer Institute , Radiotherapy van Leeuwen, Flora; The Netherlands Cancer Insitute , Epidemiology and Biostatistics
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Cardiovascular medicine, Epidemiology, Oncology
Keywords:	Hodgkin lymphoma, Cancer survivor, Primary ovarian insufficiency, Bone mineral density, Cardiovascular disease, Neurocognitve function

SCHOLARONE™
Manuscripts

1 **Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of**
2
3 **Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)**
4

5 Inge M Krul, Msc¹, Annemieke WJ Opstal-van Winden, PhD¹, Josée M Zijlstra, MD, PhD², Yolande Appelman,
6 MD, PhD³, Sanne B. Schagen, PhD¹, Lillian J. Meijboom, MD, PhD⁴, Erik Serné, MD, PhD⁵, Cornelis B Lambalk,
7 MD, PhD⁶, Paul Lips, MD, PhD⁷, Eline van Dulmen-den Broeder, MD, PhD⁸, Michael Hauptmann, PhD¹, Laurien A
8 Daniëls, MD, PhD⁹, Berthe MP Aleman, MD, PhD¹⁰, Flora E van Leeuwen, PhD¹
9
10
11
12
13
14

- 15 1. Department of Epidemiology and Biostatistics, The Netherlands Cancer Institute, Amsterdam, the
16 Netherlands
- 17 2. Department of Haemato-oncology, VU University Medical Center, Amsterdam, the Netherlands
- 18 3. Department of Cardiology, VU University Medical Center, Amsterdam, the Netherlands
- 19 4. Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands
- 20 5. Department of Vascular Medicine, VU University Medical Center, Amsterdam, the Netherlands
- 21 6. Department of Obstetrics and Gynecology, VU University Medical after Center, Amsterdam, the
22 Netherlands
- 23 7. Department of Internal medicine, Endocrine Section, VU University Medical Center, Amsterdam,
24 the Netherlands
- 25 8. Department of Pediatric Oncology and Hematology, VU University Medical Center, Amsterdam, the
26 Netherlands
- 27 9. Department of Radiotherapy, Leiden University Medical Center, Leiden, the Netherlands
- 28 10. Department of Radiation Oncology ,The Netherlands Cancer Institute, Amsterdam, the Netherlands
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43

44 **Corresponding author**

45 Flora E. van Leeuwen, PhD

46 Department of Epidemiology and Biostatistics, The Netherlands Cancer institute

47 Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

48 Email: f.v.leeuwen@nki.nl

49 Phone: +31205122480 / Fax: +3120512 2232

50
51
52
53
54
55
56
57 **Word count:** 4,338
58
59
60

ABSTRACT

Introduction: Hodgkin lymphoma (HL) has become the prototype of a curable disease. However, many young survivors suffer from late adverse effects of treatment. Both chemotherapy (CT) and radiotherapy (RT) may induce primary ovarian insufficiency (POI), which has been associated with reduced bone mineral density (BMD), neurocognitive dysfunction and possibly cardiovascular disease (CVD). While the general assumption is that POI increases CVD risk, other hypotheses postulate reverse causality, suggesting that cardiovascular risk factors determine menopausal age or that biological aging underlies both POI and CVD risk. None of these hypotheses are supported by convincing evidence. Furthermore, most studies on POI-associated conditions have been conducted in women with early natural or surgery-induced menopause with short follow-up times. In this study we will examine the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status, neurocognitive function and quality of life (QoL) in female HL survivors.

Methods and analysis: This study will be performed within an existing Dutch cohort of HL survivors. Eligible women were treated for HL at ages 15-39 years in three large hospitals since 1965 and survived for ≥ 8 years after their diagnosis. Women visiting a survivorship care outpatient clinic (SSC) will be invited for a neurocognitive, cardiovascular and BMD assessment, and asked to complete several questionnaires and to provide a blood sample. Using multivariable regression analyses we will compare the outcomes of HL survivors who developed POI with those who did not. Cardiovascular status will also be compared with women with natural POI.

Ethics and dissemination: This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL44714.031.13). Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL survivors.

STRENGTHS AND LIMITATIONS

- This study is the first to examine the long-term effects of chemotherapy- and radiotherapy -induced primary ovarian insufficiency in female Hodgkin lymphoma survivors
- Furthermore, this study is embedded in an infrastructure of several multidisciplinary survivorship care clinics, enabling a broad scope of medical tests and extensive follow-up care

- Results of this study may help to identify and timely refer those Hodgkin lymphoma survivors who are at increased risk for osteoporosis, neurocognitive dysfunction and possibly cardiovascular disease due to treatment-induced primary ovarian insufficiency for interventions in order to reduce morbidity and enhance quality of life
- Moreover, this study sheds light on different hypotheses regarding the association between primary ovarian insufficiency and cardiovascular disease risk
- Data collection is dependent on routine care procedures of the participating survivorship care clinics and of visiting patients

KEY WORDS

Hodgkin lymphoma, cancer survivor, primary ovarian insufficiency, bone mineral density, cardiovascular disease, neurocognitive function

BACKGROUND

Primary ovarian insufficiency in Hodgkin lymphoma survivors

Due to the improvements in treatment since 1960, Hodgkin lymphoma (HL) has become the prototype of a curable malignancy. Nowadays, overall 10-year survival rates exceed 80%.[1-3] However, survivors are faced with several late adverse effects of treatment, such as second malignancies and cardiovascular disease (CVD) [4-10]. Moreover, 30-40% of female HL survivors treated before 1985 developed primary ovarian insufficiency (POI) (menopause before the age of 40 years) [11-15], compared with 1% of women in the general population [16]. Women treated with more recent, less gonadotoxic treatment regimens are likely to have a lower risk, but the long-term risk has not yet been quantified sufficiently.

The risk of POI strongly depends on type of HL treatment, with the highest cumulative risks reported following pelvic RT (81%) and alkylating CT (42-60%).[11-15 17] Older age at treatment (up to 40 years) does not appear to increase risk. Although women treated for HL at older age will develop POI sooner after treatment compared with those treated at younger ages, the cumulative incidence at age 40 is nearly equal in both groups.[11 13 14] In our earlier study among female 5-year HL survivors treated between 1965 and 1995, women developed POI at a median age of 33 years (range 19-39 years).[11] POI occurring this early potentially has a large impact on quality of life (QoL) as it results in infertility, and menopausal symptoms including hot flushes, vaginal dryness and mood swings that are more severe than after menopause at later ages.[18-20] In addition, POI has been associated with reduced bone mineral density (BMD), and increased risk of CVD and neurocognitive dysfunction.

Bone mineral density

Women who reach menopause early (before age 45 years), have a lower BMD and higher incidence of osteoporosis than women who enter menopause at ages ≥ 50 years.[21-23] Moreover, an early menopause has been associated with a 1.5-3-fold increased fracture risk.[21 22 24] The most common osteoporotic fractures in postmenopausal women occur in the hip, wrist and spine.[24 25] However, it is unclear whether the association between early menopause and BMD and fracture risk persists over time. Some studies have shown that the association becomes much weaker with increasing age (mainly above age 70) [26-28], while others reported a lifetime increased fracture risk.[22 29] Possibly, not only oestrogens but also aging mechanisms are of importance in BMD status.[30]

So far, many studies have been conducted among breast cancer survivors or in women with an early natural or surgery-induced menopause, while studies evaluating the long-term effects of CT- and/or RT-induced POI on

1 BMD and fracture risk are limited. Two small studies among HL survivors reported a significantly reduced BMD
2 after treatment-induced POI [31 32], while another study found no association.[33] Since HL survivors develop
3 POI at a younger age than breast cancer survivors or the general population, more research is needed to
4 identify the extent of reduced BMD and prevalence of osteoporotic fractures among female HL survivors who
5 developed POI.
6
7
8
9

10 11 12 **Cardiovascular disease**

13
14 In the general population, early menopause has been associated with an increased incidence of CVD [34-36]. A
15 recent meta-analysis of CVD risk among women with POI showed a pooled HR of 1.6 for total CVD and 1.7 for
16 ischemic heart disease when compared with menopause at ages ≥ 50 years.[37] Moreover, epidemiological data
17 show a 2% decrease in cardiovascular mortality for each year menopause is delayed.[38] Low levels of
18 testosterone in women have also been associated with increased intima media thickness (IMT) of the carotid
19 artery.[39-41]
20

21
22 An intriguing hypothesis postulates that reverse causality may operate, i.e., that CVD risk factors such as
23 weight, cholesterol and blood pressure determine menopausal age. This is in contrast with the general
24 assumption that endocrine changes due to early menopause are responsible for CVD development. Indeed, in
25 the Framingham Heart Study cohort, a 1% higher premenopausal cardiovascular risk score was associated with
26 a subsequent decrease in menopausal age of 1.8 years.[42] However, it has also been suggested that several
27 risk factors are associated with both CVD and early menopause. A meta-analysis of 22 genome-wide association
28 studies (GWAS) on natural early menopause revealed predominantly genes that are involved in general repair
29 mechanisms [43], arguing for a role of generalized aging rather than ovarian dysfunction in early menopause.
30

31
32 To date, the important question whether accelerated biological aging underlies both early menopause and an
33 increased CVD risk is unresolved and no recent evidence has been provided to support the reverse causality
34 hypothesis. A direct comparison of CVD risk between HL survivors and women with natural POI might provide
35 new insights, as POI among HL survivors is induced by treatment (exogenous factors) instead of natural early
36 depletion of the primordial follicle pool (endogenous factors). If POI would increase CVD risk, this should be
37 considered in the light of an established increased risk of CVD due to mediastinal RT and anthracycline-
38 containing CT.[9]
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Neurocognitive function

Although in-vitro studies suggest a neuroprotective effect of oestrogen in the brain, the influence of decreased estrogen levels on cognitive performance is still unclear. Some in-vivo studies have shown an increased risk of neurocognitive impairment or dementia after a surgery-induced early menopause (approximately 1.5-fold), while others found no association.[44-48] These contradicting findings may be due to differences in the cognitive domains that were evaluated, as not all cognitive functions are equally influenced by oestrogens. Hormonal influences seem to mainly concern aspects of memory, information processing speed and executive functioning.[49 50]

Up to now, the long-term effects of POI on cognition are largely unknown, as most studies had short follow-up times and included only women with menopausal ages above 40 years and/or women who used hormone replacement therapy (HRT). Moreover, the majority of studies looked at the effects after oophorectomy, characterized by an abrupt drop of oestrogen levels, while oestrogen levels may decrease gradually in CT-induced POI occurring many years after treatment.[20 51] Preliminary data on POI in HL survivors within our cohort show that women who developed POI had a median duration of ovarian function after HL treatment of 4 years (interquartile range 1-10 years).

Hormone replacement therapy

Much debate surrounds the use of HRT since the Women's Health Initiative (WHI) study reported increased risks of breast cancer, CVD and cognitive impairment after oestrogen and progestin supplementation.[52] More recent studies suggest that the benefit of oestrogen supplementation strongly depends on starting age and timing with respect to menopause.[53-56] Among HL survivors in the Netherlands, HRT has been mainly prescribed to relieve menopausal symptoms and to prevent osteoporosis.[57] However, in several HL treatment centres, HL survivors have been advised to refrain from using HRT against menopausal symptoms because of a potential increase in breast cancer risk.[58] This provides the unique opportunity to examine the effects of HRT use in this population as the long-term effects of HRT on BMD, CVD and neurocognitive function in HL survivors with POI have not been examined yet.

Aim

This article describes the design and methods of a Study On Primary ovarian insufficiency in female survivors of Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA). The primary aim of this study is to examine the long-term effects of treatment-induced POI on BMD, cardiovascular status, neurocognitive

1 function and QoL. We hypothesize that women with treatment-induced POI will have an increased risk of
2 osteoporosis and neurocognitive dysfunction and a lower QoL than HL survivors without POI. However, based
3 on the hypotheses on reverse causality and biological aging, we hypothesize that CVD risk may not be increased
4 in female HL survivors with POI compared to HL survivors without POI.
5
6

7 The secondary aims of this study are:
8
9

- 10 1. To examine whether long-term effects differ between women with CT- and RT-induced POI
- 11 2. To examine whether long-term effects differ between acute (<1 year after HL treatment) and more
12 gradually (≥ 1 year after HL treatment) developed POI if there is sufficient power.
- 13 3. To investigate the effects of type and timing of HRT on all outcomes if there is sufficient power.
- 14 4. To compare cardiovascular status and the possible influence of HRT between HL survivors with
15 treatment-induced POI and women from the general population with natural (non-treatment-
16 induced) POI.
17
18
19
20
21
22
23
24
25

26 METHODS

27 Design and study population

28 *Hodgkin lymphoma survivors*

29 The SOPHIA-study is an observational cross-sectional study among female HL survivors who are being followed
30 in an outpatient survivorship care clinic. Participants will be invited for a neurocognitive, cardiovascular and
31 BMD assessment and asked to complete several questionnaires and to provide a blood sample. Participants will
32 be recruited from a large previously described cohort of 5-year HL survivors treated in the Netherlands
33 between 1965 and 2000 [4 59], which has been extended with more recently treated patients. Registry data on
34 HL patients treated before 1965 are not available.
35
36
37
38
39
40
41
42

43 This study is a collaboration of three large Dutch Medical Centres: The Netherlands Cancer Institute (NKI), VU
44 University Medical Centre (VUmc) and Leiden University Medical Centre (LUMC). Eligible women were treated
45 for HL at the age of 15-39 years at the adult Haematology-Oncology departments of the three medical centres,
46 and survived ≥ 8 years after HL diagnosis. The latter criterion was chosen because we are interested in the long-
47 term effects of POI. Exclusion criteria are: current age of ≥ 75 years, current treatment for a second malignancy,
48 insufficient understanding of the Dutch language or any psychological, familial, sociological or geographical
49 condition that potentially hampers study participation. General patient characteristics, HL treatment data and
50 follow-up data on vital status, second malignancies and CVD are already available for all 8-year HL-survivors in
51 these three hospitals, enabling us to monitor possible differences between patients who participate and those
52
53
54
55
56
57
58
59

1 who decline. Also, we will be able to examine whether eligible 8-year survivors who died before study invitation
2 died due to one of our outcomes of interest. Due to the high risk of late adverse effects in HL survivors, some
3 women are already deceased. If it would turn out that a relatively large proportion of patients in the POI group
4 (compared to the comparison group) has died of CVD, we will be able to report this, which is a big advantage in
5 a cross-sectional study.
6
7
8
9

10 The study has been approved by the Institutional Review Board of The Netherlands Cancer Institute
11 Recruitment started in 2014 (VUmc). We will follow the study population longitudinally to examine changes in
12 risk factor and outcomes over time for which additional funding will be acquired. Moreover, eligible women will
13 be followed through clinical care, where permission is asked to store future blood samples as well.
14
15
16
17
18
19

20 *External control group*

21 To enable the comparison of cardiovascular status between HL survivors and women with natural (non-
22 treatment-induced) POI, we will use data from an ongoing nationwide multicentre study on hypergonadotropic
23 oligomenorrhoea/amenorrhoea. This study is a collaboration of three University Medical Centres and includes
24 women aged ≥ 40 years with a diagnosis of polycystic ovarian syndrome (PCOS) or POI between 1992 and 2012.
25 Data collection consists of a cardiovascular risk assessment at the outpatient clinic.[60 61]
26
27
28
29
30
31
32
33

34 **Study parameters and data collection**

35 Main outcome measures and other relevant study parameters are briefly described below by method of data
36 collection (see also Table 1). The main exposure POI is defined as amenorrhoea for ≥ 4 months with two serum
37 follicle-stimulating hormone (FSH) levels in the menopausal range (obtained at least 1 month apart), or
38 amenorrhoea for ≥ 12 months before the age of 40 years. In case a woman has already been postmenopausal for
39 many years at study enrolment, POI is defined as the date of or age at last menstruation. Because we
40 performed earlier studies on POI, for the majority of women we already know their menopausal status and age,
41 either from the medical records or from questionnaires sent in the 1990s-2000s. For the remaining women
42 these data will be abstracted from the medical records and/or obtained through the patient questionnaire.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Overview of outcome measures and corresponding data collection methods

Primary exposure and outcomes	Data collection methods		Outcome variables	Justification of methods
Primary ovarian insufficiency	Blood sample	Hormone level	If indicated for routine care: level of follicle-stimulating hormone (FSH) in mIU/mL	Routine care – diagnostic value
	Questionnaire		Date of last menstruation, menopausal age	
	Medical record		Date of last menstruation, menopausal age	
Bone mineral density	Medical tests	DEXA-scan of lumbar spine and hip by means of Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	BMD values in g/cm ² T and Z scores Presence of osteopenia (defined as T-scores of -1 to -2.5) Presence of osteoporosis (defined as T-scores of \geq -2.5)	Routine care – diagnostic value The DEXA-scan is most widely used in clinical practice to screen for osteoporosis and regarded as the 'Golden standard'
		Instant vertebral assessment (IVA) by Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	Vertebral height reduction in % Presence of clinical and non-clinical vertebral fractures	There is a strong additive value of IVA compared with DEXA alone [62-64]
		Anthropomorphic measurements	Height in cm and weight in kg	
	Blood sample	Bone turnover markers	Bone formation by P1NP - mean value in ng/ml Bone resorption by β -CTX - mean value in pg/ml	These markers have been used in previous studies and are recommended for research purposes [65]
		Vitamin D	Level of 25-hydroxyvitamine D in serum in nmol/l	Vitamin D has been associated with bone turnover markers, BMD, fracture risk and risk of falling [66-68]
	Questionnaire	Food frequency questionnaire (FFQ)	Mean score of calcium intake	The FFQ is a validated questionnaire [69] Reference values are available [70]
		General questionnaire	Previous fractures, use of calcium and vitamin D supplements use of glucocorticoids, family history of osteoporosis	
Medical record		Earlier DEXA-scans (yes,no), if applicable treatment plan for osteoporosis such as vitamin D supplementation, recommendations for lifestyle changes		
Cardiovascular status	Medical tests	Echocardiogram If contra-indicated: cardiac magnetic resonance imaging (MRI)	Abnormalities in heart structure Left ventricular function (LVF) by E/A ratio, deceleration time, isovolumic relaxation time (IVRT), left ventricular ejection fraction (LVEF), diastolic and systolic diameter and volume, E/e' ratio Right ventricular function: tricuspid annular plane systolic excursion (TAPSE) Presence of mitral, aortic or tricuspid valve defects i.e. insufficiencies or stenoses Wall motion score index	Routine care – diagnostic value
		Electrocardiogram (ECG)	Sinus rhythm, QRS complex, ST morphology (elevation or depression), PQ interval and left ventricle hypertrophy	Routine care – diagnostic value
		Coronary computed tomography angiography (CCTA) by a 320-detector	Coronary artery calcium (CAC) score according to Agatston Presence of luminal narrowing and if applicable: type of	High sensitivity and specificity [71] Most valid alternative method for detecting

		row volumetric scanner (Aquilion ONE) (LUMC) and 256 Scanner Philips (VUmc)	narrowing and number of plaques for the left main coronary artery (LMCA), left anterior descending (LAD), circumflex artery (CRX) and right coronary artery (RCA)	significant coronary disease (golden standard is invasive coronary angiography) [72]
		Vascular measurements	Presence of atherosclerosis by carotid intima media thickness (cIMT) and femoral IMT in mm Arterial stiffness (VUmc only)	Predictors of future cardiovascular events [73-75]
		Blood pressure	Mean of three consecutive measurements in mm/Hg	
		Anthropomorphic measurements	Height in cm, weight in kg, body mass index (BMI) in kg/cm ² , hip circumference in cm, waist circumference in cm, hip-waist ratio	
	Blood sample	Biomarkers	Left ventricular function and presence of ischemia and infarction by NT-pro-BNP in pmol/l Chronic inflammation (associated with atherosclerosis) by CRP in mg/L	In general population: strong predictor of coronary heart disease [76 77]
		Lipid spectrum	Total cholesterol, HDL, LDL, triglycerides	Established risk factors for CVD
		Glucose	Fasting blood glucose	Established risk factor for diabetes
		Kidney function	Creatinine, estimated glomerular filtration rate (eGFR)	Routine care before CCTA
	Questionnaire	'General questionnaire'	(Family) history of CVD and risk factors for CVD and if applicable date of diagnosis and treatment	
	Medical record		Cardiovascular risk score based on SCORE chart and Framingham chart, adjusted for age Cardiovascular history, contra-indications for echocardiogram	
Neurocognitive function	Neurocognitive tests	15 Words test	Verbal memory in total number of words	These tests were selected based on their reliability, validity and availability of reference norms. The domains examined are potentially sensitive for the effect of oestrogens [48,62-67]
		Trail making test A&B	Information processing speed) in seconds to complete	
		COWA verbal fluency test	Verbal fluency in total number of words	
		Letter-number sequencing	Working memory in total correct trials	
		WAIS III Digit span	Measures concentration in total number of items/ lists correctly repeated) can be converted to a scaled score, which is an age-based, norm-referenced score for each subject	
		Dutch Adult Reading Test (NART)	Verbal intelligence in mean IQ estimate	
Quality of life	Questionnaire	SF-12	General health	Shortened version of the validated questionnaire SF-36, which has been previously used in Dutch studies [78]
		MOS cognitive functioning scale	Cognitive functioning	Frequently used questionnaire [79]
		Hospital Anxiety and Depression Scale (HADS)	Anxiety and depression	Valid and reliable Dutch reference values are available [80]
		Sexual Activity Questionnaire (SAQ)	Sexual functioning	The SAQ is a valid, reliable and acceptable measure for describing the sexual functioning of women in terms of activity, pleasure and discomfort. It is quick and easy to administer and has good face validity discriminating

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

				between the sexual functioning of pre- and post-menopausal women [81]
		Shortened fatigue questionnaire (VW)	Fatigue	Reliable and validated questionnaire [82]

For peer review only

Medical records

Data on HL diagnosis (date, pathology), primary and recurrence treatment (including date, RT fields, chemotherapeutic regimens and doses) and follow-up data have been previously collected from medical records [4]. Since treatment for HL has changed considerably over time, a variety of treatment regimens was used. Primary treatment was usually given according to treatment protocols of the European Organization of Research and Treatment of Cancer (EORTC) and German Hodgkin lymphoma Study Group (GHSG), while treatment for recurrences was generally not standardized. Furthermore, data on reproductive factors (e.g., menopausal age) will be obtained.

Patient questionnaire data

Four questionnaires will be used to ascertain data on women's general characteristics, QoL, calcium intake and menopause related topics. The 'General characteristics questionnaire' will obtain information on the following items: reproductive history (e.g., age at menarche and menopause, parity, hormone use), general cardiovascular history, bone health status (e.g., previous fractures, use of medication), and lifestyle factors (e.g. current and previous smoking habits, alcohol use and physical activity). The 'QoL questionnaire' consists of five short validated and/or frequently used questionnaires regarding health, cognition, sexual activity, depression and fatigue (Table 1). A validated food frequency questionnaire (FFQ) will be used to assess calcium intake [69]. Reference values will be obtained from the report on dietary intake by the Health Council of the Netherlands [70]. The 'Menopause questionnaire' is specifically aimed at postmenopausal women and will collect information on climacteric symptoms (i.e., severity and frequency) and changes in lifestyle factors after the onset of menopause. Data regarding infertility issues will be ascertained for women who experienced POI.

Neurocognitive, cardiovascular and fracture risk assessments

For the neurocognitive assessment, we have chosen tests that measure cognitive domains potentially sensitive for effects of oestrogens.[49 83-88] Tests were selected based on their reliability, validity and availability of Dutch reference norms. The cardiovascular assessment includes an echocardiogram, electrocardiogram (ECG), coronary computed tomography angiography (CCTA), and measurement of the carotid and femoral IMT, blood pressure and hip/waist circumference. The BMD assessment consists of a DEXA-scan with instant vertebral assessment (IVA). These medical tests were chosen based on their availability and use in clinical practice and their evidence-based diagnostic or predictive value (Table 1).

Blood samples

A blood sample will be drawn to examine bone turnover (i.e., β -CTX for bone resorption and P1NP for bone formation) and cardiac (e.g. NT-pro-BNP, CRP, lipid spectrum) markers. Since new techniques in this research field develop rapidly, we will collect an additional blood sample for future analyses on new biomarkers, such as biomarkers predictive for late effects. Moreover, this sample will be used for future DNA extraction and analyses (e.g. to examine modifying effects of genetic factors, such as single nucleotide polymorphisms (SNP's) associated with POI). These blood samples will be frozen and stored at -80 °C.

Study procedures

Recruitment

Women will be recruited through the Survivorship Care outpatient Clinic (SCC) for HL survivors, established by the Dutch nationwide BETER consortium (Better care after Hodgkin lymphoma, Evaluation of long-Term Treatment Effects and screening Recommendations). This consortium consists of haematologists and radiation oncologists of >20 hospitals and has developed evidence-based guidelines on follow-up care, including recommendations on cardiovascular risk assessment in order to reduce morbidity and mortality [89]. The three medical centres participating in the SOPHIA-study all have an active SCC where HL survivors can be screened. Approximately 30% of the 5-year HL survivors already receive routine follow-up care on a yearly basis in their original HL treatment centre. All 5-year HL survivors who are currently not under surveillance will be invited for screening (if treated at the age of 15-60 years and currently aged <75 years) by the BETER consortium in the upcoming years.

If a woman, eligible for the SOPHIA-study, visits the SCC of NKI, LUMC or VUmc (either during an intake or follow-up care visit), she will be invited by her treating physician. As mentioned above, follow-up care is provided according to the BETER guidelines and depends on the specific treatments a patient received (e.g., chest or pelvic RT, anthracycline-containing CT). As some of the medical tests in the SOPHIA-study are incorporated in the BETER guidelines, these tests may be part of routine care. Therefore, the physician will determine for each patient which medical tests will be performed for routine care and which will be additionally performed for research purposes. Tests that are considered for routine care may have been recently performed in a participant. If there is no clinical reason to repeat the test, the result of the previous test will be abstracted from the medical record. More details on the distinction between routine care and research tests, and the study procedures are described in Figure 1.

1 We aim to integrate study participation as much as possible with the routine care provided at the SCC. Whether
2
3 or not a patient is willing to participate in the study will not have any influence on the routine care she receives
4
5 during follow-up.
6
7

8 *Study implementation*

9
10 If the patient is interested to participate in the SOPHIA-study, the treating physician will hand out an invitation
11
12 for the SOPHIA-study, together with a patient information letter and an informed consent form. After one or
13
14 two weeks, the treating physician or research nurse will contact the patient by telephone to answer any
15
16 remaining questions. If the woman agrees to participate, she will be asked to return the signed informed
17
18 consent form, and subsequently the 'General questionnaire' and 'Menopause questionnaire' (if applicable) will
19
20 be sent to her home. Patients will be asked to bring their completed questionnaires with them to a follow-visit
21
22 at the SSC or to return the questionnaires by mail.
23

24 The neurocognitive, cardiovascular and BMD assessments will be performed during a follow-up visit at the SCC.
25
26 Ideally, all medical tests will be performed during two to three follow-up visits, depending on availability and
27
28 timing of other routine medical care tests (e.g. breast cancer screening). The planned tests with allocated time
29
30 are shown in Figure 1.

31 Patients will be tested for renal failure before undergoing the CCTA. Women with severe renal insufficiency,
32
33 defined as a e-GFR value of <60 ml per minute per 1.73m², will undergo a computed tomography coronary
34
35 calcium score without contrast fluid.
36

37 Blood will be drawn at two time points. The first blood sample will be taken at the SCC during a routine care
38
39 blood withdrawal. The second blood sample will be drawn in fasting state before the CCTA.
40
41 Patients are offered the possibility to perform the neurocognitive tests at home. In that case, a separate
42
43 appointment will be made. To ensure sufficient time between two memory tests, patients will be asked to
44
45 complete the FFQ during this appointment.
46
47

48 **Statistical issues**

49 *Power calculation*

50
51
52 Approximately 500 women are eligible for participation in the three selected hospitals. Based on our previous
53
54 studies we expect that 60% of the eligible women will participate in the current study (N=300). Power
55
56 calculations were performed separately for the outcomes BMD, cardiovascular status, neurocognitive function
57
58
59

1 and QoL. When comparing BMD of women who developed POI (expected N=60 (20%)) with those who did not
2
3 (expected N=240), there is over 80% power to detect a difference of 0.05 g/cm² in BMD (1.00 (SD 0.1) vs 1.05
4
5 (SD 0.1)).

6 The power calculation for cardiovascular status is based on the IMT measurement. There is over 80% power to
7
8 detect a difference of 0.1 mm in mean IMT between women with POI and women with normal menopausal
9
10 ages (0.6 (SD 0.2) versus 0.5 (SD 0.2)). An increase of 0.1 mm in IMT has been associated with an increase in risk
11
12 of 12% for myocardial infarction.[74]

13
14 Previous retrospective neuropsychological studies, in which effects of systemic cancer treatments and POI on
15
16 cognitive functioning were examined, have yielded significant findings with somewhat smaller group sizes of 39
17
18 and 53 patients.[90-92]

19
20 For the outcome QoL, we followed the calculations of Cohen.[93] The proposed study has over 80% power to
21
22 detect moderate difference between women who developed POI and those who did not. All power calculations
23
24 used a 5% chance for a Type-1 error and a minimal effect size of 0.5.

25 26 27 28 *Statistical analyses*

29
30 Outcomes of female HL survivors who developed POI will be compared with those of female HL survivors who
31
32 did not by using chi square tests or Fisher's exact tests (categorical variables) and two tailed t-tests (continuous
33
34 variables) after appropriate transformation, if necessary. Multivariate regression analyses will be used to
35
36 examine the effects of POI, menopausal age and HRT use on the primary outcome variables (BMD,
37
38 cardiovascular status and neurocognitive function) and to assess the effect of these primary outcome variables
39
40 on QoL. Cox regression models with age as a time scale will be used to examine the independent effect of age
41
42 at HL treatment and age at developing POI. All analyses will be adjusted for confounders (HL treatment
43
44 regimen, lifestyle factors, reproductive factors, climacteric symptoms, medication) where applicable. Effect
45
46 modification and mediation will be tested using interaction terms. We will also perform subgroup analyses to
47
48 evaluate the difference between a CT- and RT-induced POI in more detail and we will compare cardiovascular
49
50 status between HL survivors with POI and other women with natural POI. P values <0.05 will be considered
51
52 statistically significant.

53 54 55 **DISCUSSION**

56
57 In the current study, the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status,
58
59

1 neurocognitive function and QoL will be examined by measurements within a cohort of female HL survivors.
2
3 Approximately 30-40% of the female survivors in our HL cohort treated between 1965 and 1985 experienced
4 treatment-induced POI. The majority of these women have now been postmenopausal for over 20 to 30 years
5 which enables us to examine the very late effects of POI.
6
7

8 Treatment-induced POI might put female HL survivors at high risk for developing adverse POI-associated
9 conditions such as osteoporosis, neurocognitive dysfunction and CVD, while they already have an increased risk
10 for late adverse effects due to the HL treatment itself.[4 9 21 37 46] This may lead to problems in daily
11 functioning and can have a large impact on their QoL, in particular because the conditions may occur at very
12 young ages. Results of this study will help to identify those HL survivors who are at increased risk for
13 osteoporosis, neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely
14 referral of high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL.
15
16 Moreover, by identifying these long-term risks, physicians can better inform women with POI in the future.
17 Findings of this study will also be relevant for other female cancer patients who received gonadotoxic
18 treatment at premenopausal ages. CT is a major contributor to the development of POI and its use has
19 intensified considerably over the years in many malignancies.[94 95] Therefore, it is expected that the
20 occurrence of POI-associated adverse effects will increase in female cancer survivors in the near future. Since
21 HRT has become subject of much debate in recent years, investigating the effects of HRT on POI-associated
22 conditions will produce valuable knowledge with regard to the HRT-suppletion policy for female cancer
23 survivors in the Netherlands.
24
25

26 Finally, this study provides a unique possibility to challenge the conventional view that reproductive hormone
27 deprivation in females is of key importance in CVD development. This is relevant in the light of other
28 hypotheses that general biological aging mechanisms underlie a combination of POI and CVD, or that CVD risk
29 factors determine age at menopause (reverse causality hypothesis).[42] Comparison of cardiovascular status
30 between women with POI after HL treatment and women with natural POI will allow examination of a cause-
31 effect relationship between early menopause and CVD. We will be able to adjust for the potential effects of HL
32 treatment on CVD risk, as extensive data on HL treatment regimens are available within our cohort.
33
34

35 This study has several notable strengths and limitations. First, this study is embedded in an infrastructure of
36 several multidisciplinary SCCs, enabling a broad scope of medical tests and extensive follow-up care. Detailed
37 treatment and reproductive data will be available from medical records and patient questionnaires. Second,
38 women received a variety of treatments and have long-term follow-up, rendering it possible to examine the
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 effects of different HL treatments, POI and menopausal age on several outcomes. A limitation of the study
2
3 includes patient selection. Due to the high risk of late adverse effects in HL survivors, some women are already
4
5 deceased or not able to participate in the SOPHIA-study. Moreover, some women are under surveillance in a
6
7 local hospital rather than their original HL treatment centre, while women who are (feeling) healthy may not
8
9 visit the SCC because of medical costs and/or other obligations (e.g., work, family). We will account for this by
10
11 obtaining medical data for women under surveillance in others hospitals, and we have near complete data on
12
13 important competing late adverse effects such as CVD, second malignancies and vital status, as this study is
14
15 nested within an existing cohort.

16 Another limitation is that this study is dependent on routine care procedures of the participating SSCs.
17
18 Therefore, differences between SSCs may occur regarding available equipment and registration of results from
19
20 medical tests. In addition, the time interval between two medical tests may be variable between patients due
21
22 to planning issues (e.g., long waiting lists, the aim to plan multiple medical tests in one day), or because one
23
24 medical test has already been performed in the past year and will not be repeated. We made a standardized
25
26 abstraction form to ensure all relevant data are gathered and differences between timing of medical tests and
27
28 hospitals will be accounted for in the analyses.

29 In conclusion, this article describes the study protocol of the SOPHIA-study which aims to increase knowledge
30
31 about BMD, cardiovascular status and neurocognitive function in long-term female HL survivors with and
32
33 without treatment-induced POI, and the potential influence of these long-term effects on QoL. Results of this
34
35 study will lead to the identification of those HL survivors who are at increased risk for osteoporosis,
36
37 neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely referral of
38
39 high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL.
40
41 Furthermore, results will shed light on existing hypotheses regarding the association between POI and CVD risk.
42
43 Moreover, HL survivors or other cancer survivors who will experience treatment-induced POI in the future can
44
45 be better informed about potential long-term effects. Finally, prospective follow-up of the study population will
46
47 provide insight into longitudinal changes in risk factors and study outcomes.
48
49

50 **AUTHORS' CONTRIBUTIONS**

51 The study protocol has been written by IK, AOvW, BA, EvD-dB and FvL. All authors contributed to study design.
52
53 MH provided statistical advice. JZ, LD, and BA are responsible for patient accrual and inclusion, and JZ, YA, SB,
54
55 LM, ES, CL, PL, and LD and BA are responsible for the assessment of the outcome variables.
56
57
58
59

1 FvL is the principal investigator and responsible for the funding of the study.

2
3 All authors revised the manuscript critically for intellectual content and have approved the final manuscript.

6 7 **FUNDING**

8 This study is financially supported by the Dutch Cancer Society (grant number NKI 2010-4720).

11 12 **COMPETING INTERESTS**

13
14 JZ declares she has conducted a research project funded by Roche in the past two years (unrelated to the
15 current study). CBL's department of Reproductive Medicine has received educational and research grants from
16 Merck Serono, Ferring and Auxogyn, and he received speakers fees from MSD, Merck Serono, Ferring and
17 Auxogyn. He is also a consultant for Ferring. PL provided advice to Friesland Campina. All other authors declare
18 no competing interests.
19
20
21
22
23
24
25

26 27 **ETHICS AND DISSEMINATION**

28 This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has
29 been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects
30 (file number NL44714.031.13). Reporting of serious adverse events was exempted for this study, as the burden
31 and risks associated with participation are very low and in accordance with routine medical care. Results will be
32 disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL
33 survivors.
34
35
36
37
38
39
40

41 42 **References**

- 43
44 1. Ferme C, Eghbali H, Meerwaldt JH, et al. Chemotherapy plus involved-field radiation in early-stage
45 Hodgkin's disease. *N Engl J Med* 2007;**357**(19):1916-27 doi: 10.1056/NEJMoa064601[published Online
46 First: Epub Date]].
- 47
48 2. Raemaekers J, Burgers M, Henry-Amar M, et al. Patients with stage III/IV Hodgkin's disease in partial
49 remission after MOPP/ABV chemotherapy have excellent prognosis after additional involved-field
50 radiotherapy: interim results from the ongoing EORTC-LCG and GPMC phase III trial. The EORTC
51 Lymphoma Cooperative Group and Groupe Pierre-et-Marie-Curie. *Ann Oncol* 1997;**8 Suppl 1**:111-4
52
53
54
55
56
57
58
59
60

- 1 3. Klimm B, Goergen H, Fuchs M, et al. Impact of risk factors on outcomes in early-stage Hodgkin's
2 lymphoma: an analysis of international staging definitions. *Ann Oncol* 2013;**24**(12):3070-6 doi:
3 10.1093/annonc/mdt413[published Online First: Epub Date]].
- 4 4. Schaapveld M, Aleman BM, van Eggermond AM, et al. Second Cancer Risk Up to 40 Years after
5 Treatment for Hodgkin's Lymphoma. *N Engl J Med* 2015;**373**(26):2499-511 doi:
6 10.1056/NEJMoa1505949[published Online First: Epub Date]].
- 7 5. Bhatia S, Yasui Y, Robison LL, et al. High risk of subsequent neoplasms continues with extended follow-
8 up of childhood Hodgkin's disease: report from the Late Effects Study Group. *J Clin Oncol*
9 2003;**21**(23):4386-94
- 10 6. Hodgson DC, Gilbert ES, Dores GM, et al. Long-term solid cancer risk among 5-year survivors of
11 Hodgkin's lymphoma. *J Clin Oncol* 2007;**25**(12):1489-97
- 12 7. Punnett A, Tsang RW, Hodgson DC. Hodgkin lymphoma across the age spectrum: epidemiology,
13 therapy, and late effects. *Semin Radiat Oncol* 2010;**20**(1):30-44
- 14 8. Aleman BM, AW vdB-D, de Bruin ML, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma.
15 *Blood* 2007;**109**(5):1878-86
- 16 9. van Nimwegen FA, Schaapveld M, Janus CP, et al. Cardiovascular disease after Hodgkin lymphoma
17 treatment: 40-year disease risk. *JAMA Intern Med* 2015;**175**(6):1007-17 doi:
18 10.1001/jamainternmed.2015.1180[published Online First: Epub Date]].
- 19 10. de Bruin ML, Dorresteijn LD, van't Veer MB, et al. Increased risk of stroke and transient ischemic attack
20 in 5-year survivors of Hodgkin lymphoma. *J Natl Cancer Inst* 2009;**101**(13):928-37
- 21 11. de Bruin ML, Huisbrink J, Hauptmann M, et al. Treatment-related risk factors for premature
22 menopause following Hodgkin lymphoma. *Blood* 2008;**111**(1):101-08
- 23 12. Swerdlow AJ, Cooke R, Bates A, et al. Risk of premature menopause after treatment for Hodgkin's
24 lymphoma. *J Natl Cancer Inst* 2014;**106**(9) doi: 10.1093/jnci/dju207[published Online First: Epub
25 Date]].
- 26 13. Haukvik UK, Dieset I, Bjoro T, et al. Treatment-related premature ovarian failure as a long-term
27 complication after Hodgkin's lymphoma. *Ann Oncol* 2006;**17**(9):1428-33
- 28 14. van der Kaaij MA, Heutte N, Meijnders P, et al. Premature ovarian failure and fertility in long-term
29 survivors of Hodgkin's lymphoma: a European Organisation for Research and Treatment of Cancer
30 Lymphoma Group and Groupe d'Etude des Lymphomes de l'Adulte Cohort Study. *J Clin Oncol*
31 2012;**30**(3):291-99
- 32 15. Behringer K, Breuer K, Reineke T, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced
33 by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives
34 during therapy: a report from the German Hodgkin's Lymphoma Study Group. *J Clin Oncol*
35 2005;**23**(30):7555-64
- 36 16. Luborsky JL, Meyer P, Sowers MF, et al. Premature menopause in a multi-ethnic population study of
37 the menopause transition. *Hum Reprod* 2003;**18**(1):199-206
- 38 17. Meiorow D. Reproduction post-chemotherapy in young cancer patients. *Mol Cell Endocrinol*
39 2000;**169**(1-2):123-31

18. Nelson HD. Menopause. *Lancet* 2008;**371**(9614):760-70
19. Kenemans P. Menopause, HRT and menopausal symptoms. *J Epidemiol Biostat* 1999;**4**(3):141-46
20. Hendrix SL. Bilateral oophorectomy and premature menopause. *Am J Med* 2005;**118 Suppl 12B**:131-5
doi: 10.1016/j.amjmed.2005.09.056[published Online First: Epub Date] .
21. Gallagher JC. Effect of early menopause on bone mineral density and fractures. *Menopause* 2007;**14**(3 Pt 2):567-71
22. Der Voort DJ, Der Weijer PH, Barentsen R. Early menopause: increased fracture risk at older age. *Osteoporos Int* 2003;**14**(6):525-30
23. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, et al. Bone density patterns after normal and premature menopause. *Maturitas* 2003;**44**(4):279-86
24. van der Klift M, de Laet CE, McCloskey EV, et al. Risk factors for incident vertebral fractures in men and women: the Rotterdam Study. *J Bone Miner Res* 2004;**19**(7):1172-80
25. Schuit SC, van der Klift M, Weel AE, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. *Bone* 2004;**34**(1):195-202
26. Kritz-Silverstein D, von Muhlen DG, Barrett-Connor E. Hysterectomy and oophorectomy are unrelated to bone loss in older women. *Maturitas* 2004;**47**(1):61-69
27. Ahlborg HG, Johnell O, Nilsson BE, et al. Bone loss in relation to menopause: a prospective study during 16 years. *Bone* 2001;**28**(3):327-31
28. Gerdhem P, Obrant KJ. Bone mineral density in old age: the influence of age at menarche and menopause. *J Bone Miner Metab* 2004;**22**(4):372-75
29. Svejme O, Ahlborg HG, Nilsson JA, et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *BJOG* 2012;**119**(7):810-16
30. Khosla S, Melton LJ, III, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? *J Bone Miner Res* 2011;**26**(3):441-51
31. Kreuser ED, Felsenberg D, Behles C, et al. Long-term gonadal dysfunction and its impact on bone mineralization in patients following COPP/ABVD chemotherapy for Hodgkin's disease. *Ann Oncol* 1992;**3 Suppl 4**:105-10
32. Redman JR, Bajorunas DR, Wong G, et al. Bone mineralization in women following successful treatment of Hodgkin's disease. *Am J Med* 1988;**85**(1):65-72
33. Howell SJ, Berger G, Adams JE, et al. Bone mineral density in women with cytotoxic-induced ovarian failure. *Clin Endocrinol (Oxf)* 1998;**49**(3):397-402
34. Atsma F, Bartelink ML, Grobbee DE, et al. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. *Menopause* 2006;**13**(2):265-79
35. Hu FB, Grodstein F, Hennekens CH, et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med* 1999;**159**(10):1061-66
36. Lobo RA. Surgical menopause and cardiovascular risks. *Menopause* 2007;**14**(3 Pt 2):562-66
37. Roeters van Lennep JE, Heida KY, Bots ML, et al. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2016;**23**(2):178-86 doi: 10.1177/2047487314556004[published Online First: Epub Date] .

- 1 38. van der Schouw YT, van der Graaf Y, Steyerberg EW, et al. Age at menopause as a risk factor for
2 cardiovascular mortality. *Lancet* 1996;**347**(9003):714-18
- 3
- 4 39. Montalcini T, Gorgone G, Gazzaruso C, et al. Role of endogenous androgens on carotid atherosclerosis
5 in non-obese postmenopausal women. *Nutr Metab Cardiovasc Dis* 2007;**17**(10):705-11
- 6
- 7 40. Golden SH, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid
8 atherosclerosis: a case-control study of the atherosclerosis risk in communities cohort. *Am J Epidemiol*
9 2002;**155**(5):437-45
- 10
- 11 41. Bernini GP, Sgro' M, Moretti A, et al. Endogenous androgens and carotid intimal-medial thickness in
12 women. *J Clin Endocrinol Metab* 1999;**84**(6):2008-12
- 13
- 14 42. Kok HS, van Asselt KM, van der Schouw YT, et al. Heart disease risk determines menopausal age rather
15 than the reverse. *J Am Coll Cardiol* 2006;**47**(10):1976-83
- 16
- 17 43. Stolk L, Perry JR, Chasman DI, et al. Meta-analyses identify 13 loci associated with age at menopause
18 and highlight DNA repair and immune pathways. *Nat Genet* 2012;**44**(3):260-68
- 19
- 20 44. Farrag AK, Khedr EM, Abdel-Aleem H, et al. Effect of surgical menopause on cognitive functions.
21 *Dement Geriatr Cogn Disord* 2002;**13**(3):193-98
- 22
- 23 45. McLay RN, Maki PM, Lyketsos CG. Nulliparity and late menopause are associated with decreased
24 cognitive decline. *J Neuropsychiatry Clin Neurosci* 2003;**15**(2):161-67
- 25
- 26 46. Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in
27 women who underwent oophorectomy before menopause. *Neurology* 2007;**69**(11):1074-83
- 28
- 29 47. Henderson VW, Sherwin BB. Surgical versus natural menopause: cognitive issues. *Menopause*
30 2007;**14**(3 Pt 2):572-79
- 31
- 32 48. Vearncombe KJ, Pachana NA. Is cognitive functioning detrimentally affected after early, induced
33 menopause? *Menopause* 2009;**16**(1):188-98
- 34
- 35 49. Lethaby A, Hogervorst E, Richards M, et al. Hormone replacement therapy for cognitive function in
36 postmenopausal women. *Cochrane Database Syst Rev* 2008(1):CD003122
- 37
- 38 50. Zwart W, Terra H, Linn SC, et al. Cognitive effects of endocrine therapy for breast cancer: keep calm
39 and carry on? *Nat Rev Clin Oncol* 2015;**12**(10):597-606 doi: 10.1038/nrclinonc.2015.124[published
40 Online First: Epub Date]].
- 41
- 42 51. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant
43 chemotherapy for breast cancer. *J Clin Oncol* 1996;**14**(5):1718-29 doi:
44 10.1200/JCO.1996.14.5.1718[published Online First: Epub Date]].
- 45
- 46 52. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy
47 postmenopausal women: principal results From the Women's Health Initiative randomized controlled
48 trial. *JAMA* 2002;**288**(3):321-33
- 49
- 50 53. Rosano GM, Vitale C, Fini M. Hormone replacement therapy and cardioprotection: what is good and
51 what is bad for the cardiovascular system? *Ann N Y Acad Sci* 2006;**1092**:341-48
- 52
- 53 54. Maki PM, Henderson VW. Hormone therapy, dementia, and cognition: the Women's Health Initiative
54 10 years on. *Climacteric* 2012;**15**(3):256-62
- 55
- 56
- 57
- 58
- 59
- 60

- 1 55. Maki PM, Dennerstein L, Clark M, et al. Perimenopausal use of hormone therapy is associated with
2 enhanced memory and hippocampal function later in life. *Brain Res* 2011;**1379**:232-43
3
- 4 56. King J, Wynne CH, Assersohn L, et al. Hormone replacement therapy and women with premature
5 menopause--a cancer survivorship issue. *Eur J Cancer* 2011;**47**(11):1623-32
6
- 7 57. Marjoribanks J, Farquhar C, Roberts H, et al. Long term hormone therapy for perimenopausal and
8 postmenopausal women. *Cochrane Database Syst Rev* 2012(7):CD004143 doi:
9 10.1002/14651858.CD004143.pub4[published Online First: Epub Date]].
10
- 11 58. Lagro-Janssen A, Knufing MW, Schreurs L, et al. Significant fall in hormone replacement therapy
12 prescription in general practice. *Fam Pract* 2010;**27**(4):424-9 doi: 10.1093/fampra/cm018[published
13 Online First: Epub Date]].
14
- 15 59. van Leeuwen FE, Klokman WJ, Hagenbeek A, et al. Second cancer risk following Hodgkin's disease: a
16 20-year follow-up study. *J Clin Oncol* 1994;**12**(2):312-25
17
- 18 60. Daan NM, Muka T, Koster MP, et al. Cardiovascular Risk in Women With Premature Ovarian
19 Insufficiency Compared to Premenopausal Women at Middle Age. *J Clin Endocrinol Metab*
20 2016;**101**(9):3306-15 doi: 10.1210/jc.2016-1141[published Online First: Epub Date]].
21
- 22 61. Janse F, Knauff EA, Niermeijer MF, et al. Similar phenotype characteristics comparing familial and
23 sporadic premature ovarian failure. *Menopause* 2010;**17**(4):758-65 doi:
24 10.1097/gme.0b013e3181cf8521[published Online First: Epub Date]].
25
- 26 62. Olinginski TP, Newman ED, Hummel JL, et al. Development and evaluation of a vertebral fracture
27 assessment program using IVA and its integration with mobile DXA. *J Clin Densitom* 2006;**9**(1):72-7 doi:
28 10.1016/j.jocd.2005.08.002[published Online First: Epub Date]].
29
- 30 63. Greenspan SL, von Stetten E, Emond SK, et al. Instant vertebral assessment: a noninvasive dual X-ray
31 absorptiometry technique to avoid misclassification and clinical mismanagement of osteoporosis. *J*
32 *Clin Densitom* 2001;**4**(4):373-80
33
- 34 64. Netelenbos JC, Lems WF, Geusens PP, et al. Spine radiographs to improve the identification of women
35 at high risk for fractures. *Osteoporos Int* 2009;**20**(8):1347-52 doi: 10.1007/s00198-008-0801-
36 1[published Online First: Epub Date]].
37
- 38 65. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and
39 monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*
40 2011;**22**(2):391-420 doi: 10.1007/s00198-010-1501-1[published Online First: Epub Date]].
41
- 42 66. van Schoor NM, Visser M, Pluijm SM, et al. Vitamin D deficiency as a risk factor for osteoporotic
43 fractures. *Bone* 2008;**42**(2):260-6 doi: 10.1016/j.bone.2007.11.002[published Online First: Epub
44 Date]].
45
- 46 67. Kuchuk NO, Pluijm SM, van Schoor NM, et al. Relationships of serum 25-hydroxyvitamin D to bone
47 mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin*
48 *Endocrinol Metab* 2009;**94**(4):1244-50 doi: 10.1210/jc.2008-1832[published Online First: Epub Date]].
49
- 50 68. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and
51 active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ* 2009;**339**:b3692 doi:
52 10.1136/bmj.b3692[published Online First: Epub Date]].
53
54
55
56
57
58
59

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
69. Angus RM, Sambrook PN, Pocock NA, et al. A simple method for assessing calcium intake in Caucasian women. *J Am Diet Assoc* 1989;**89**(2):209-14
70. The Hague: Health council of the Netherlands. Dietary reference values: calcium, vitamin D, thiamin, riboflavin, niacin, panthothenic acid, and biotin. 2000. Report No.: 90-5549-323-6.
71. Schussler JM, Grayburn PA. Non-invasive coronary angiography using multislice computed tomography. *Heart* 2007;**93**(3):290-7 doi: 10.1136/hrt.2005.069195[published Online First: Epub Date]].
72. Schuetz GM, Zacharopoulou NM, Schlattmann P, et al. Meta-analysis: noninvasive coronary angiography using computed tomography versus magnetic resonance imaging. *Ann Intern Med* 2010;**152**(3):167-77 doi: 10.7326/0003-4819-152-3-201002020-00008[published Online First: Epub Date]].
73. Nair SB, Malik R, Khattar RS. Carotid intima-media thickness: ultrasound measurement, prognostic value and role in clinical practice. *Postgrad Med J* 2012;**88**(1046):694-9 doi: 10.1136/postgradmedj-2011-130214[published Online First: Epub Date]].
74. Den Ruijter HM, Peters SA, Anderson TJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012;**308**(8):796-803
75. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;**55**(13):1318-27 doi: 10.1016/j.jacc.2009.10.061[published Online First: Epub Date]].
76. Buckley DI, Fu R, Freeman M, et al. C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive Services Task Force. *Ann Intern Med* 2009;**151**(7):483-95
77. Kavousi M, Elias-Smale S, Rutten JH, et al. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study. *Ann Intern Med* 2012;**156**(6):438-44 doi: 10.7326/0003-4819-156-6-201203200-00006[published Online First: Epub Date]].
78. Brennum SK, Barrett-Connor E, Sajjan S, et al. Impact of recent fracture on health-related quality of life in postmenopausal women. *J Bone Miner Res* 2006;**21**(6):809-16 doi: 10.1359/jbmr.060301[published Online First: Epub Date]].
79. Stewart AL WJ, Sherbourne CD, et al. Psychological distress/well-being and cognitive functioning measures. *Measuring Functioning and Well-Being: The Medical Outcomes Study Approach*. Durham, NC: Duke University, 1992. p.102–142.
80. Spinhoven P OJ, Sloekers PP, Kempen GI, Speckens AE, Van Hemert AM (1997) A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychol Med* 27:363-70.
81. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual functioning. *Qual Life Res* 1996;**5**(1):81-90
82. Alberts M SE, Vercoulen JHMM, Garssen B, Bleijenberg G. Verkorte Vermoeidheidsvragenlijst: een praktisch hulpmiddel bij het scoren van vermoeidheid. *Nederlands Tijdschrift voor Geneeskunde* 1997; 141:1526-1530.

- 1 83. Sherwin BB. Estrogen therapy: is time of initiation critical for neuroprotection? *Nat Rev Endocrinol* 2009;**5**(11):620-7 doi: 10.1038/nrendo.2009.193[published Online First: Epub Date]].
- 2
- 3
- 4 84. Schilder CM, Eggens PC, Seynaeve C, et al. Neuropsychological functioning in postmenopausal breast cancer patients treated with tamoxifen or exemestane after AC-chemotherapy: cross-sectional findings from the neuropsychological TEAM-side study. *Acta Oncol* 2009;**48**(1):76-85 doi: 10.1080/02841860802314738[published Online First: Epub Date]].
- 5
- 6
- 7
- 8
- 9
- 10 85. van Dam FS, Schagen SB, Muller MJ, et al. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. *J Natl Cancer Inst* 1998;**90**(3):210-8
- 11
- 12
- 13
- 14 86. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, et al. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. *J Int Neuropsychol Soc* 2006;**12**(1):80-9 doi: 10.1017/S1355617706060115[published Online First: Epub Date]].
- 15
- 16
- 17
- 18
- 19
- 20 87. Mulder J DR, Dekker P. *Verbale Leer & Geheugen Test*. Lisse: Swets & Zeitlinger; 1996.
- 21
- 22 88. Reitan R. Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and Motor Skills*. 1958;**8**:271-6
- 23
- 24 89. Dekker N, van 't Veer MB, Aleman BM, et al. [The BETER survivorship care initiative for Hodgkin lymphoma; tailored survivorship care for late effects of treatment]. *Ned Tijdschr Geneesk* 2015;**159**:A9269
- 25
- 26
- 27
- 28 90. Castellon SA, Ganz PA, Bower JE, et al. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. *J Clin Exp Neuropsychol* 2004;**26**(7):955-69
- 29
- 30
- 31 91. Collins B, Mackenzie J, Stewart A, et al. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. *Psychooncology* 2009;**18**(2):134-43
- 32
- 33
- 34 92. Schagen SB, van Dam FS, Muller MJ, et al. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. *Cancer* 1999;**85**(3):640-50
- 35
- 36
- 37 93. Cohen J. A power primer. *Psychol Bull* 1992;**112**(1):155-59
- 38
- 39 94. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**(9472):1687-717 doi: 10.1016/S0140-6736(05)66544-0[published Online First: Epub Date]].
- 40
- 41
- 42 95. Rodriguez-Wallberg KA, Oktay K. Fertility preservation in women with breast cancer. *Clin Obstet Gynecol* 2010;**53**(4):753-62 doi: 10.1097/GRF.0b013e3181f96e00[published Online First: Epub Date]].
- 43
- 44
- 45
- 46
- 47
- 48

FIGURE LEGENDS

Figure 1. Study procedures and patient burden, stratified by medical tests for routine care and research

Abbreviations: RT = radiotherapy; CT = chemotherapy; BETTER = Better care after Hodgkin lymphoma, Evaluation of long-Term Treatment Effects and screening Recommendations; CRP = C-reactive protein; BMD = bone mineral density; DEXA = Dual Energy X-ray Absorptiometry; POI = primary ovarian insufficiency

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

^a Expected for > 90% of women ^b Expected for 15-40% of women

In case criteria for care are not fulfilled, tests will be performed for research purpose

For peer review only

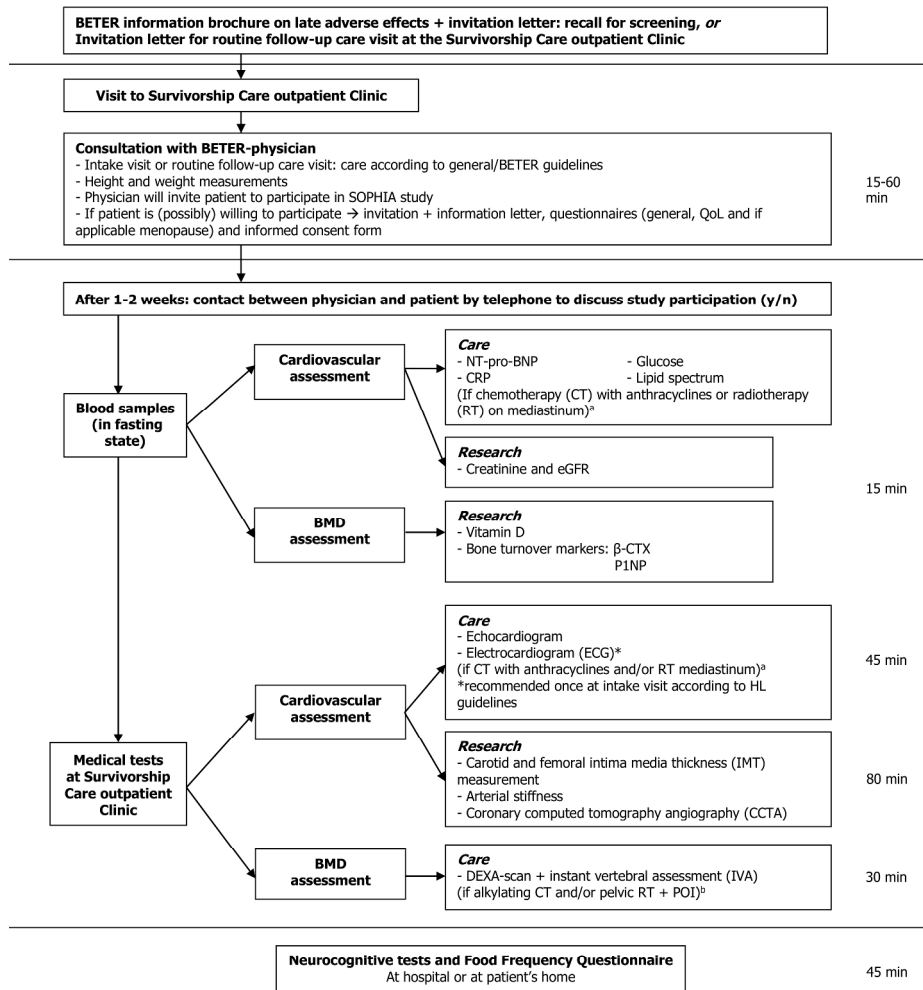


Figure 1. Overview of study procedures and patient burden, stratified by medical tests for routine care and research

279x361mm (300 x 300 DPI)

BMJ Open

Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-018120.R2
Article Type:	Protocol
Date Submitted by the Author:	14-Apr-2018
Complete List of Authors:	Krul, Inge; The Netherlands Cancer Institute, Epidemiology and Biostatistics Opstal-van Winden, Annemieke; the Netherlands Cancer Institute, Epidemiology and Biostatistics Zijlstra, Josée ; VU University Medical Centre, Haemato-oncology Appelman, Yolande; VU University Medical Center, Cardiology Schagen, SB; The Netherlands Cancer Institute , Epidemiology and Biostatistics Meijboom, Lilian; VU University Medical Center, Radiology Serné, Erik; VU University Medical Centre, Vascluar medicine Lambalk, Cornelis; VU University Medical Centre, Obstetrics and Gynecology Lips, Paul; VU University Medical Center, Internal medicine, Endocrine Section van Dulmen - den Broeder, Eline; VU University Medical Center, Pediatric Oncology and Hematology Hauptmann, M; The Netherlands Cancer Institute, Department of Epidemiology and Biostatistics Daniëls, Laurien; Leids Universitair Medisch Centrum, Radiotherapy Aleman, Berthe; The Netherlands Cancer Institute , Radiotherapy van Leeuwen, Flora; The Netherlands Cancer Insitute , Epidemiology and Biostatistics
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Cardiovascular medicine, Epidemiology, Oncology
Keywords:	Hodgkin lymphoma, Cancer survivor, Primary ovarian insufficiency, Bone mineral density, Cardiovascular disease, Neurocognitve function

SCHOLARONE™
Manuscripts

1 **Rationale and design of a cohort Study On Primary ovarian insufficiency in female survivors of**
2
3 **Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA)**
4

5 Inge M Krul, Msc¹, Annemieke WJ Opstal-van Winden, PhD¹, Josée M Zijlstra, MD, PhD², Yolande Appelman,
6 MD, PhD³, Sanne B. Schagen, PhD¹, Lillian J. Meijboom, MD, PhD⁴, Erik Serné, MD, PhD⁵, Cornelis B Lambalk,
7 MD, PhD⁶, Paul Lips, MD, PhD⁷, Eline van Dulmen-den Broeder, MD, PhD⁸, Michael Hauptmann, PhD¹, Laurien A
8 Daniëls, MD, PhD⁹, Berthe MP Aleman, MD, PhD¹⁰, Flora E van Leeuwen, PhD¹
9
10
11
12
13
14

- 15 1. Department of Epidemiology and Biostatistics, The Netherlands Cancer Institute, Amsterdam, the
16 Netherlands
- 17 2. Department of Haemato-oncology, VU University Medical Center, Amsterdam, the Netherlands
- 18 3. Department of Cardiology, VU University Medical Center, Amsterdam, the Netherlands
- 19 4. Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands
- 20 5. Department of Vascular Medicine, VU University Medical Center, Amsterdam, the Netherlands
- 21 6. Department of Obstetrics and Gynecology, VU University Medical after Center, Amsterdam, the
22 Netherlands
- 23 7. Department of Internal medicine, Endocrine Section, VU University Medical Center, Amsterdam,
24 the Netherlands
- 25 8. Department of Pediatric Oncology and Hematology, VU University Medical Center, Amsterdam, the
26 Netherlands
- 27 9. Department of Radiotherapy, Leiden University Medical Center, Leiden, the Netherlands
- 28 10. Department of Radiation Oncology ,The Netherlands Cancer Institute, Amsterdam, the Netherlands
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43

44 **Corresponding author**

45 Flora E. van Leeuwen, PhD

46 Department of Epidemiology and Biostatistics, The Netherlands Cancer institute

47 Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

48 Email: f.v.leeuwen@nki.nl

49 Phone: +31205122480 / Fax: +3120512 2232

50
51
52
53
54
55
56
57 **Word count:** 4,478
58
59
60

ABSTRACT

Introduction: Hodgkin lymphoma (HL) has become the prototype of a curable disease. However, many young survivors suffer from late adverse effects of treatment. Both chemotherapy (CT) and radiotherapy (RT) may induce primary ovarian insufficiency (POI), which has been associated with reduced bone mineral density (BMD), neurocognitive dysfunction and possibly cardiovascular disease (CVD). While the general assumption is that POI increases CVD risk, other hypotheses postulate reverse causality, suggesting that cardiovascular risk factors determine menopausal age or that biological aging underlies both POI and CVD risk. None of these hypotheses are supported by convincing evidence. Furthermore, most studies on POI-associated conditions have been conducted in women with early natural or surgery-induced menopause with short follow-up times. In this study we will examine the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status, neurocognitive function and quality of life (QoL) in female HL survivors.

Methods and analysis: This study will be performed within an existing Dutch cohort of HL survivors. Eligible women were treated for HL at ages 15-39 years in three large hospitals since 1965 and survived for ≥ 8 years after their diagnosis. Women visiting a survivorship care outpatient clinic (SSC) will be invited for a neurocognitive, cardiovascular and BMD assessment, and asked to complete several questionnaires and to provide a blood sample. Using multivariable regression analyses we will compare the outcomes of HL survivors who developed POI with those who did not. Cardiovascular status will also be compared with women with natural POI.

Ethics and dissemination: This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL44714.031.13). Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL survivors.

STRENGTHS AND LIMITATIONS

- This study is the first to examine to the long-term effects of chemotherapy- and radiotherapy -induced primary ovarian insufficiency in female Hodgkin lymphoma survivors
- Furthermore, this study is embedded in an infrastructure of several multidisciplinary survivorship care clinics, enabling a broad scope of medical tests and extensive follow-up care

- Results of this study may help to identify and timely refer those Hodgkin lymphoma survivors who are at increased risk for osteoporosis, neurocognitive dysfunction and possibly cardiovascular disease due to treatment-induced primary ovarian insufficiency for interventions in order to reduce morbidity and enhance quality of life
- Moreover, this study sheds light on different hypotheses regarding the association between primary ovarian insufficiency and cardiovascular disease risk
- Data collection is dependent on routine care procedures of the participating survivorship care clinics and of visiting patients

KEY WORDS

Hodgkin lymphoma, cancer survivor, primary ovarian insufficiency, bone mineral density, cardiovascular disease, neurocognitive function

BACKGROUND

Primary ovarian insufficiency in Hodgkin lymphoma survivors

Due to the improvements in treatment since 1960, Hodgkin lymphoma (HL) has become the prototype of a curable malignancy. Nowadays, overall 10-year survival rates exceed 80%.[1-3] However, survivors are faced with several late adverse effects of treatment, such as second malignancies and cardiovascular disease (CVD) [4-10]. Moreover, 30-40% of female HL survivors treated before 1985 developed primary ovarian insufficiency (POI) (menopause before the age of 40 years) [11-15], compared with 1% of women in the general population [16]. Women treated with more recent, less gonadotoxic treatment regimens are likely to have a lower risk, but the long-term risk has not yet been quantified sufficiently.

The risk of POI strongly depends on type of HL treatment, with the highest cumulative risks reported following pelvic RT (81%) and alkylating CT (42-60%).[11-15 17] Older age at treatment (up to 40 years) does not appear to increase risk. Although women treated for HL at older age will develop POI sooner after treatment compared with those treated at younger ages, the cumulative incidence at age 40 is nearly equal in both groups.[11 13 14] In our earlier study among female 5-year HL survivors treated between 1965 and 1995, women developed POI at a median age of 33 years (range 19-39 years).[11] POI occurring this early potentially has a large impact on quality of life (QoL) as it results in infertility, and menopausal symptoms including hot flushes, vaginal dryness and mood swings that are more severe than after menopause at later ages.[18-20] In addition, POI has been associated with reduced bone mineral density (BMD), and increased risk of CVD and neurocognitive dysfunction.

Bone mineral density

Women who reach menopause early (before age 45 years), have a lower BMD and higher incidence of osteoporosis than women who enter menopause at ages ≥ 50 years.[21-23] Moreover, an early menopause has been associated with a 1.5-3-fold increased fracture risk.[21 22 24] The most common osteoporotic fractures in postmenopausal women occur in the hip, wrist and spine.[24 25] However, it is unclear whether the association between early menopause and BMD and fracture risk persists over time. Some studies have shown that the association becomes much weaker with increasing age (mainly above age 70) [26-28], while others reported a lifetime increased fracture risk.[22 29] Possibly, not only oestrogens but also aging mechanisms are of importance in BMD status.[30]

So far, many studies have been conducted among breast cancer survivors or in women with an early natural or surgery-induced menopause, while studies evaluating the long-term effects of CT- and/or RT-induced POI on

1 BMD and fracture risk are limited. Two small studies among HL survivors reported a significantly reduced BMD
2 after treatment-induced POI [31 32], while another study found no association.[33] Since HL survivors develop
3 POI at a younger age than breast cancer survivors or the general population, more research is needed to
4 identify the extent of reduced BMD and prevalence of osteoporotic fractures among female HL survivors who
5 developed POI.
6
7
8
9

10 11 12 **Cardiovascular disease**

13
14 In the general population, early menopause has been associated with an increased incidence of CVD [34-36]. A
15 recent meta-analysis of CVD risk among women with POI showed a pooled HR of 1.6 for total CVD and 1.7 for
16 ischemic heart disease when compared with menopause at ages ≥ 50 years.[37] Moreover, epidemiological data
17 show a 2% decrease in cardiovascular mortality for each year menopause is delayed.[38] Low levels of
18 testosterone in women have also been associated with increased intima media thickness (IMT) of the carotid
19 artery.[39-41]
20
21
22
23
24

25
26 An intriguing hypothesis postulates that reverse causality may operate, i.e., that CVD risk factors such as
27 weight, cholesterol and blood pressure determine menopausal age. This is in contrast with the general
28 assumption that endocrine changes due to early menopause are responsible for CVD development. Indeed, in
29 the Framingham Heart Study cohort, a 1% higher premenopausal cardiovascular risk score was associated with
30 a subsequent decrease in menopausal age of 1.8 years.[42] However, it has also been suggested that several
31 risk factors are associated with both CVD and early menopause. A meta-analysis of 22 genome-wide association
32 studies (GWAS) on natural early menopause revealed predominantly genes that are involved in general repair
33 mechanisms [43], arguing for a role of generalized aging rather than ovarian dysfunction in early menopause.
34
35
36
37
38
39
40

41 To date, the important question whether accelerated biological aging underlies both early menopause and an
42 increased CVD risk is unresolved and no recent evidence has been provided to support the reverse causality
43 hypothesis. Since POI among HL survivors is induced by exogenous factors (i.e. HL treatment) rather than by
44 endogenous factors (i.e. natural early depletion of the primordial follicle pool) occurring in women with natural
45 POI, a direct comparison between HL survivors and women with natural POI might provide new insights into
46 the association between POI and CVD. If POI would increase CVD risk, this should be considered in the light of
47 an established increased risk of CVD due to mediastinal RT and anthracycline-containing CT.[9]
48
49
50
51
52
53
54
55
56
57
58
59
60

Neurocognitive function

Although in-vitro studies suggest a neuroprotective effect of oestrogen in the brain, the influence of decreased estrogen levels on cognitive performance is still unclear. Some in-vivo studies have shown an increased risk of neurocognitive impairment or dementia after a surgery-induced early menopause (approximately 1.5-fold), while others found no association.[44-48] These contradicting findings may be due to differences in the cognitive domains that were evaluated, as not all cognitive functions are equally influenced by oestrogens. Hormonal influences seem to mainly concern aspects of memory, information processing speed and executive functioning.[49 50]

Up to now, the long-term effects of POI on cognition are largely unknown, as most studies had short follow-up times and included only women with menopausal ages above 40 years and/or women who used hormone replacement therapy (HRT). Moreover, the majority of studies looked at the effects after oophorectomy, characterized by an abrupt drop of oestrogen levels, while oestrogen levels may decrease gradually in CT-induced POI occurring many years after treatment.[20 51] Preliminary data on POI in HL survivors within our cohort show that women who developed POI had a median duration of ovarian function after HL treatment of 4 years (interquartile range 1-10 years).

Hormone replacement therapy

Much debate surrounds the use of HRT since the Women's Health Initiative (WHI) study reported increased risks of breast cancer, CVD and cognitive impairment after oestrogen and progestin supplementation.[52] More recent studies suggest that the benefit of oestrogen supplementation strongly depends on starting age and timing with respect to menopause.[53-56] Among HL survivors in the Netherlands, HRT has been mainly prescribed to relieve menopausal symptoms and to prevent osteoporosis.[57] However, in several HL treatment centres, HL survivors have been advised to refrain from using HRT against menopausal symptoms because of a potential increase in breast cancer risk.[58] This provides the unique opportunity to examine the effects of HRT use in this population as the long-term effects of HRT on BMD, CVD and neurocognitive function in HL survivors with POI have not been examined yet.

Aim

This article describes the design and methods of a Study On Primary ovarian insufficiency in female survivors of Hodgkin lymphoma: Influence on long-term Adverse effects (SOPHIA). The primary aim of this study is to examine the long-term effects of treatment-induced POI on BMD, cardiovascular status, neurocognitive

1 function and QoL. We hypothesize that women with treatment-induced POI will have an increased risk of
2 osteoporosis and neurocognitive dysfunction and a lower QoL than HL survivors without POI. However, based
3 on the hypotheses on reverse causality and biological aging, we hypothesize that CVD risk may not be increased
4 in female HL survivors with POI compared to HL survivors without POI.
5
6

7
8 The secondary aims of this study are:
9

- 10 1. To examine whether long-term effects differ between women with CT- and RT-induced POI
- 11 2. To compare cardiovascular status and the possible influence of HRT between HL survivors with
12 treatment-induced POI and women from the general population with natural (non-treatment-
13 induced) POI.
14
15
16
17
18
19

20 In addition, we will perform exploratory analyses to examine potential differences between subgroups
21 regarding acute (<1 year after HL treatment) and more gradually (≥ 1 year after HL treatment) developed POI
22 and to explore the effects of type and timing of HRT on all outcomes.
23
24
25
26
27

28 **METHODS**

29 **Design and study population**

30 *Hodgkin lymphoma survivors*

31 The SOPHIA-study is an observational cross-sectional study among female HL survivors who are being followed
32 in an outpatient survivorship care clinic. Participants will be invited for a neurocognitive, cardiovascular and
33 BMD assessment and asked to complete several questionnaires and to provide a blood sample. Participants will
34 be recruited from a large previously described cohort of 5-year HL survivors treated in the Netherlands
35 between 1965 and 2000 [4 59], which has been extended with more recently treated patients. Registry data on
36 HL patients treated before 1965 are not available.
37
38
39
40
41
42
43
44

45 This study is a collaboration of three large Dutch Medical Centres: The Netherlands Cancer Institute (NKI), VU
46 University Medical Centre (VUmc) and Leiden University Medical Centre (LUMC). Eligible women were treated
47 for HL at the age of 15-39 years at the adult Haematology-Oncology departments of the three medical centres,
48 and survived ≥ 8 years after HL diagnosis. The latter criterion was chosen because we are interested in the long-
49 term effects of POI. Exclusion criteria are: current age of ≥ 75 years, current treatment for a second malignancy,
50 insufficient understanding of the Dutch language or any psychological, familial, sociological or geographical
51 condition that potentially hampers study participation. General patient characteristics, HL treatment data and
52 follow-up data on vital status, second malignancies and CVD are already available for all 8-year HL-survivors in
53
54
55
56
57
58
59

1 these three hospitals, enabling us to monitor possible differences between patients who participate and those
2 who decline. Also, we will be able to examine whether eligible 8-year survivors who died before study invitation
3 died due to one of our outcomes of interest. Due to the high risk of late adverse effects in HL survivors, some
4 women are already deceased. If it would turn out that a relatively large proportion of patients in the POI group
5 (compared to the comparison group) has died of CVD, we will be able to report this, which is a big advantage in
6 a cross-sectional study.
7

8
9
10
11
12 The study has been approved by the Institutional Review Board of The Netherlands Cancer Institute
13 Recruitment started in 2014 (VUmc). We will follow the study population longitudinally to examine changes in
14 risk factor and outcomes over time for which additional funding will be acquired. Moreover, eligible women will
15 be followed through clinical care, where permission is asked to store future blood samples as well.
16
17
18
19
20

21 22 *External control group*

23 To enable the comparison of cardiovascular status between HL survivors and women with natural (non-
24 treatment-induced) POI, we will use data from an ongoing nationwide multicentre study on hypergonadotropic
25 oligomenorrhoea/amenorrhoea. This study is a collaboration of three University Medical Centres and includes
26 women aged ≥ 40 years with a diagnosis of polycystic ovarian syndrome (PCOS) or POI between 1992 and 2012.
27
28
29
30
31 Data collection consists of a cardiovascular risk assessment at the outpatient clinic.[60 61]
32
33
34

35 **Study parameters and data collection**

36
37 Main outcome measures and other relevant study parameters are briefly described below by method of data
38 collection (see also Table 1). The main exposure POI is defined as amenorrhoea for ≥ 4 months with two serum
39 follicle-stimulating hormone (FSH) levels in the menopausal range (obtained at least 1 month apart), or
40 amenorrhoea for ≥ 12 months before the age of 40 years. In case a woman has already been postmenopausal for
41 many years at study enrolment, POI is defined as the date of or age at last menstruation. Because we
42 performed earlier studies on POI, for the majority of women we already know their menopausal status and age,
43 either from the medical records or from questionnaires sent in the 1990s-2000s. For the remaining women
44 these data will be abstracted from the medical records and/or obtained through the patient questionnaire.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Primary exposure and outcomes	Data collection methods		Outcome variables	Justification of methods
Primary ovarian insufficiency	Blood sample	Hormone level	If indicated for routine care: level of follicle-stimulating hormone (FSH) in mIU/mL	Routine care – diagnostic value
	Questionnaire		Date of last menstruation, menopausal age	
	Medical record		Date of last menstruation, menopausal age	
Bone mineral density	Medical tests	DEXA-scan of lumbar spine and hip by means of Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	BMD values in g/cm ² T and Z scores Presence of osteopenia (defined as T-scores of -1 to -2.5) Presence of osteoporosis (defined as T-scores of ≥-2.5)	Routine care – diagnostic value The DEXA-scan is most widely used in clinical practice to screen for osteoporosis and regarded as the 'Golden standard'
		Instant vertebral assessment (IVA) by Hologic Delphi densitometer (VUmc) or General Electric Scanner (LUMC)	Vertebral height reduction in % Presence of clinical and non-clinical vertebral fractures	There is a strong additive value of IVA compared with DEXA alone [62-64]
		Anthropomorphic measurements	Height in cm and weight in kg	
	Blood sample	Bone turnover markers	Bone formation by P1NP - mean value in ng/ml Bone resorption by β-CTX - mean value in pg/ml	These markers have been used in previous studies and are recommended for research purposes [65]
		Vitamin D	Level of 25-hydroxyvitamine D in serum in nmol/l	Vitamin D has been associated with bone turnover markers, BMD, fracture risk and risk of falling [66-68]
	Questionnaire	Food frequency questionnaire (FFQ)	Mean score of calcium intake	The FFQ is a validated questionnaire [69] Reference values are available [70]
		General questionnaire	Previous fractures, use of calcium and vitamin D supplements use of glucocorticoids, family history of osteoporosis	
Medical record		Earlier DEXA-scans (yes,no), if applicable treatment plan for osteoporosis such as vitamin D supplementation, recommendations for lifestyle changes		
Cardiovascular status	Medical tests	Echocardiogram If contra-indicated: cardiac magnetic resonance imaging (MRI)	Abnormalities in heart structure Left ventricular function (LVF) by E/A ratio, deceleration time, isovolumic relaxation time (IVRT), left ventricular ejection fraction (LVEF), diastolic and systolic diameter and volume, E/e' ratio Right ventricular function: tricuspid annular plane systolic excursion (TAPSE) Presence of mitral, aortic or tricuspid valve defects i.e. insufficiencies or stenoses Wall motion score index	Routine care – diagnostic value
		Electrocardiogram (ECG)	Sinus rhythm, QRS complex, ST morphology (elevation or depression), PQ interval and left ventricle hypertrophy	Routine care – diagnostic value
		Coronary computed tomography angiography (CCTA) by a 320-detector	Coronary artery calcium (CAC) score according to Agatston Presence of luminal narrowing and if applicable: type of	High sensitivity and specificity [71] Most valid alternative method for detecting

		row volumetric scanner (Aquilion ONE) (LUMC) and 256 Scanner Philips (VUmc)	narrowing and number of plaques for the left main coronary artery (LMCA), left anterior descending (LAD), circumflex artery (CRX) and right coronary artery (RCA)	significant coronary disease (golden standard is invasive coronary angiography) [72]
		Vascular measurements	Presence of atherosclerosis by carotid intima media thickness (cIMT) and femoral IMT in mm Arterial stiffness (VUmc only)	Predictors of future cardiovascular events [73-75]
		Blood pressure	Mean of three consecutive measurements in mm/Hg	
		Anthropomorphic measurements	Height in cm, weight in kg, body mass index (BMI) in kg/cm ² , hip circumference in cm, waist circumference in cm, hip-waist ratio	
	Blood sample	Biomarkers	Left ventricular function and presence of ischemia and infarction by NT-pro-BNP in pmol/l Chronic inflammation (associated with atherosclerosis) by CRP in mg/L	In general population: strong predictor of coronary heart disease [76 77]
		Lipid spectrum	Total cholesterol, HDL, LDL, triglycerides	Established risk factors for CVD
		Glucose	Fasting blood glucose	Established risk factor for diabetes
		Kidney function	Creatinine, estimated glomerular filtration rate (eGFR)	Routine care before CCTA
	Questionnaire	'General questionnaire'	(Family) history of CVD and risk factors for CVD and if applicable date of diagnosis and treatment	
	Medical record		Cardiovascular risk score based on SCORE chart and Framingham chart, adjusted for age Cardiovascular history, contra-indications for echocardiogram	
Neurocognitive function	Neurocognitive tests	15 Words test	Verbal memory in total number of words	These tests were selected based on their reliability, validity and availability of reference norms. The domains examined are potentially sensitive for the effect of oestrogens [48,62-67]
		Trail making test A&B	Information processing speed) in seconds to complete	
		COWA verbal fluency test	Verbal fluency in total number of words	
		Letter-number sequencing	Working memory in total correct trials	
		WAIS III Digit span	Measures concentration in total number of items/ lists correctly repeated) can be converted to a scaled score, which is an age-based, norm-referenced score for each subject	
		Dutch Adult Reading Test (NART)	Verbal intelligence in mean IQ estimate	
Quality of life	Questionnaire	SF-12	General health	Shortened version of the validated questionnaire SF-36, which has been previously used in Dutch studies [78]
		MOS cognitive functioning scale	Cognitive functioning	Frequently used questionnaire [79]
		Hospital Anxiety and Depression Scale (HADS)	Anxiety and depression	Valid and reliable Dutch reference values are available [80]
		Sexual Activity Questionnaire (SAQ)	Sexual functioning	The SAQ is a valid, reliable and acceptable measure for describing the sexual functioning of women in terms of activity, pleasure and discomfort. It is quick and easy to administer and has good face validity discriminating

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

				between the sexual functioning of pre- and post-menopausal women [81]
		Shortened fatigue questionnaire (VVV)	Fatigue	Reliable and validated questionnaire [82]

Table 1. Overview of outcome measures and corresponding data collection methods

For peer review only

Medical records

Data on HL diagnosis (date, pathology), primary and recurrence treatment (including date, RT fields, chemotherapeutic regimens and doses) and follow-up data have been previously collected from medical records [4]. Since treatment for HL has changed considerably over time, a variety of treatment regimens was used. Primary treatment was usually given according to treatment protocols of the European Organization of Research and Treatment of Cancer (EORTC) and German Hodgkin lymphoma Study Group (GHSG), while treatment for recurrences was generally not standardized. Furthermore, data on reproductive factors (e.g., menopausal age) will be obtained.

Patient questionnaire data

Four questionnaires will be used to ascertain data on women's general characteristics, QoL, calcium intake and menopause related topics. The 'General characteristics questionnaire' will obtain information on the following items: reproductive history (e.g., age at menarche and menopause, parity, hormone use), general cardiovascular history, bone health status (e.g., previous fractures, use of medication), and lifestyle factors (e.g. current and previous smoking habits, alcohol use and physical activity). The 'QoL questionnaire' consists of five short validated and/or frequently used questionnaires regarding health, cognition, sexual activity, depression and fatigue (Table 1). A validated food frequency questionnaire (FFQ) will be used to assess calcium intake [69]. Reference values will be obtained from the report on dietary intake by the Health Council of the Netherlands [70]. The 'Menopause questionnaire' is specifically aimed at postmenopausal women and will collect information on climacteric symptoms (i.e., severity and frequency) and changes in lifestyle factors after the onset of menopause. Data regarding infertility issues will be ascertained for women who experienced POI.

Neurocognitive, cardiovascular and fracture risk assessments

For the neurocognitive assessment, we have chosen tests that measure cognitive domains potentially sensitive for effects of oestrogens.[49 83-88] Tests were selected based on their reliability, validity and availability of Dutch reference norms. The cardiovascular assessment includes an echocardiogram, electrocardiogram (ECG), coronary computed tomography angiography (CCTA), and measurement of the carotid and femoral IMT, blood pressure and hip/waist circumference. The BMD assessment consists of a DEXA-scan with instant vertebral assessment (IVA). These medical tests were chosen based on their availability and use in clinical practice and their evidence-based diagnostic or predictive value (Table 1).

Blood samples

A blood sample will be drawn to examine bone turnover (i.e., β -CTX for bone resorption and P1NP for bone formation) and cardiac (e.g. NT-pro-BNP, CRP, lipid spectrum) markers. Since new techniques in this research field develop rapidly, we will collect an additional blood sample for future analyses on new biomarkers, such as biomarkers predictive for late effects. Moreover, this sample will be used for future DNA extraction and analyses (e.g. to examine modifying effects of genetic factors, such as single nucleotide polymorphisms (SNP's) associated with POI). These blood samples will be frozen and stored at -80 °C.

Study procedures

Recruitment

Women will be recruited through the Survivorship Care outpatient Clinic (SCC) for HL survivors, established by the Dutch nationwide BETER consortium (Better care after Hodgkin lymphoma, Evaluation of long-Term Treatment Effects and screening Recommendations). This consortium consists of haematologists and radiation oncologists of >20 hospitals and has developed evidence-based guidelines on follow-up care, including recommendations on cardiovascular risk assessment in order to reduce morbidity and mortality [89]. The three medical centres participating in the SOPHIA-study all have an active SCC where HL survivors can be screened. Approximately 30% of the 5-year HL survivors already receive routine follow-up care on a yearly basis in their original HL treatment centre. All 5-year HL survivors who are currently not under surveillance will be invited for screening (if treated at the age of 15-60 years and currently aged <75 years) by the BETER consortium in the upcoming years.

If a woman, eligible for the SOPHIA-study, visits the SCC of NKI, LUMC or VUmc (either during an intake or follow-up care visit), she will be invited by her treating physician. As mentioned above, follow-up care is provided according to the BETER guidelines and depends on the specific treatments a patient received (e.g., chest or pelvic RT, anthracycline-containing CT). As some of the medical tests in the SOPHIA-study are incorporated in the BETER guidelines, these tests may be part of routine care. Therefore, the physician will determine for each patient which medical tests will be performed for routine care and which will be additionally performed for research purposes. Tests that are considered for routine care may have been recently performed in a participant. If there is no clinical reason to repeat the test, the result of the previous test will be abstracted from the medical record. More details on the distinction between routine care and research tests, and the study procedures are described in Figure 1.

1 We aim to integrate study participation as much as possible with the routine care provided at the SCC. Whether
2
3 or not a patient is willing to participate in the study will not have any influence on the routine care she receives
4
5 during follow-up.
6
7

8 *Study implementation*

9
10 If the patient is interested to participate in the SOPHIA-study, the treating physician will hand out an invitation
11
12 for the SOPHIA-study, together with a patient information letter and an informed consent form. After one or
13
14 two weeks, the treating physician or research nurse will contact the patient by telephone to answer any
15
16 remaining questions. If the woman agrees to participate, she will be asked to return the signed informed
17
18 consent form, and subsequently the 'General questionnaire' and 'Menopause questionnaire' (if applicable) will
19
20 be sent to her home. Patients will be asked to bring their completed questionnaires with them to a follow-visit
21
22 at the SSC or to return the questionnaires by mail.
23

24 The neurocognitive, cardiovascular and BMD assessments will be performed during a follow-up visit at the SCC.
25
26 Ideally, all medical tests will be performed during two to three follow-up visits, depending on availability and
27
28 timing of other routine medical care tests (e.g. breast cancer screening). The planned tests with allocated time
29
30 are shown in Figure 1.

31 Patients will be tested for renal failure before undergoing the CCTA. Women with severe renal insufficiency,
32
33 defined as a e-GFR value of <60 ml per minute per 1.73m², will undergo a computed tomography coronary
34
35 calcium score without contrast fluid.
36

37 Blood will be drawn at two time points. The first blood sample will be taken at the SCC during a routine care
38
39 blood withdrawal. The second blood sample will be drawn in fasting state before the CCTA.
40
41 Patients are offered the possibility to perform the neurocognitive tests at home. In that case, a separate
42
43 appointment will be made. To ensure sufficient time between two memory tests, patients will be asked to
44
45 complete the FFQ during this appointment.
46
47

48 *Patient and public involvement*

49
50 This study was designed in collaboration with several physicians working at the Survivorship Care outpatient
51
52 Clinic (BETER). They provided relevant insights into so far unrecognized health and psychosocial issues
53
54 encountered by HL survivors, which were incorporated in both our research questions and our study
55
56 procedures.
57
58
59

Moreover, before the start of the study, we presented the study design and procedures at a Survivorship meeting for patients from the Dutch Lymphoma Association. We asked for feedback and five female HL survivors volunteered to review our questionnaires and study information letters. Several changes were made following their comments. These women were also involved in the decision-making regarding the name and logo of the study.

Results from the study will be disseminated through the Dutch website for HL survivors: www.beternahodkgin.nl and the Dutch Lymphoma Association.

Statistical issues

Power calculation

Approximately 500 women are eligible for participation in the three selected hospitals. Based on our previous studies we expect that 60% of the eligible women will participate in the current study (N=300). Power calculations were performed separately for the outcomes BMD, cardiovascular status, neurocognitive function and QoL. However, conclusions about associations between POI and the different outcomes will not be based on a single test but on how plausible a true association is given the results of the various analyses specified. Instead of formally adjusting p-values for multiple comparisons, we will consider the possibility of a type 1 error in our interpretation of the results for each outcome.

When comparing BMD of women who developed POI (expected N=60 (20%)) with those who did not (expected N=240), there is over 80% power to detect a difference of 0.05 g/cm² in BMD (1.00 (SD 0.1) vs 1.05 (SD 0.1)).

The power calculation for cardiovascular status is based on the IMT measurement. There is over 80% power to detect a difference of 0.1 mm in mean IMT between women with POI and women with normal menopausal ages (0.6 (SD 0.2) versus 0.5 (SD 0.2)). An increase of 0.1 mm in IMT has been associated with an increase in risk of 12% for myocardial infarction.[74]

Previous retrospective neuropsychological studies, in which effects of systemic cancer treatments and POI on cognitive functioning were examined, have yielded significant findings with somewhat smaller group sizes of 39 and 53 patients.[90-92]

For the outcome QoL, we followed the calculations of Cohen.[93] The proposed study has over 80% power to detect moderate difference between women who developed POI and those who did not. All power calculations used a 5% chance for a Type-1 error and a minimal effect size of 0.5.

Statistical analyses

Characteristics of female HL survivors who developed POI will be compared with those of female HL survivors who did not by using chi square tests or Fisher's exact tests (categorical variables) and two tailed t-tests (continuous variables) after appropriate transformation, if necessary. Multivariate regression analyses will be used to examine the effects of POI, menopausal age and HRT use on the primary outcome variables (BMD, cardiovascular status and neurocognitive function) and to assess the effect of these primary outcome variables on QoL. Cox regression models with age as a time scale will be used to examine the independent effect of age at HL treatment on age at developing POI. Potential confounders (HL treatment, lifestyle factors, reproductive factors, climacteric symptoms, and medications) will be added one by one to models with POI and the different main outcome variables to determine whether they are confounding the POI effect. Propensity score analyses will be used instead of adjustment if the number of confounders is large. Effect modification and mediation will be tested using interaction terms. We will also perform subgroup analyses to evaluate the difference between a CT- and RT-induced POI in more detail and we will compare cardiovascular status between HL survivors with POI and other women with natural POI. P values <0.05 will be considered statistically significant.

DISCUSSION

In the current study, the long-term effects of CT- and/or RT-induced POI on BMD, cardiovascular status, neurocognitive function and QoL will be examined by measurements within a cohort of female HL survivors. Approximately 30-40% of the female survivors in our HL cohort treated between 1965 and 1985 experienced treatment-induced POI. The majority of these women have now been postmenopausal for over 20 to 30 years which enables us to examine the very late effects of POI.

Treatment-induced POI might put female HL survivors at high risk for developing adverse POI-associated conditions such as osteoporosis, neurocognitive dysfunction and CVD, while they already have an increased risk for late adverse effects due to the HL treatment itself.[4 9 21 37 46] This may lead to problems in daily functioning and can have a large impact on their QoL, in particular because the conditions may occur at very young ages. Results of this study will help to identify those HL survivors who are at increased risk for osteoporosis, neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely referral of high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL. Moreover, by identifying these long-term risks, physicians can better inform women with POI in the future. Findings of this study will also be relevant for other female cancer patients who received gonadotoxic

1 treatment at premenopausal ages. CT is a major contributor to the development of POI and its use has
2 intensified considerably over the years in many malignancies.[94 95] Therefore, it is expected that the
3 occurrence of POI-associated adverse effects will increase in female cancer survivors in the near future. Since
4 HRT has become subject of much debate in recent years, investigating the effects of HRT on POI-associated
5 conditions will produce valuable knowledge with regard to the HRT-suppletion policy for female cancer
6 survivors in the Netherlands.
7

8
9
10
11
12 Finally, this study provides a unique possibility to challenge the conventional view that reproductive hormone
13 deprivation in females is of key importance in CVD development. This is relevant in the light of other
14 hypotheses that general biological aging mechanisms underlie a combination of POI and CVD, or that CVD risk
15 factors determine age at menopause (reverse causality hypothesis).[42] Comparison of cardiovascular status
16 between women with POI after HL treatment and women with natural POI will allow examination of a cause-
17 effect relationship between early menopause and CVD. We will be able to adjust for the potential effects of HL
18 treatment on CVD risk, as extensive data on HL treatment regimens are available within our cohort.
19

20
21
22
23
24
25
26 This study has several notable strengths and limitations. First, this study is embedded in an infrastructure of
27 several multidisciplinary SCCs, enabling a broad scope of medical tests and extensive follow-up care. Detailed
28 treatment and reproductive data will be available from medical records and patient questionnaires. Second,
29 women received a variety of treatments and have long-term follow-up, rendering it possible to examine the
30 effects of different HL treatments, POI and menopausal age on several outcomes. A limitation of the study
31 includes patient selection. Due to the high risk of late adverse effects in HL survivors, some women are already
32 deceased or not able to participate in the SOPHIA-study. Moreover, some women are under surveillance in a
33 local hospital rather than their original HL treatment centre, while women who are (feeling) healthy may not
34 visit the SCC because of medical costs and/or other obligations (e.g., work, family). We will account for this by
35 obtaining medical data for women under surveillance in others hospitals, and we have near complete data on
36 important competing late adverse effects such as CVD, second malignancies and vital status, as this study is
37 nested within an existing cohort. This also allows us to evaluate potential differences in disease risks between
38 participants and our entire cohort in order to quantify any survivorship bias and to adequately interpret the
39 strength of our results.
40

41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Another limitation is that this study is dependent on routine care procedures of the participating SCCs.
Therefore, differences between SSCs may occur regarding available equipment and registration of results from
medical tests. In addition, the time interval between two medical tests may be variable between patients due

1 to planning issues (e.g., long waiting lists, the aim to plan multiple medical tests in one day), or because one
2
3 medical test has already been performed in the past year and will not be repeated. We made a standardized
4
5 abstraction form to ensure all relevant data are gathered and differences between timing of medical tests and
6
7 hospitals will be accounted for in the analyses.

8
9 In conclusion, this article describes the study protocol of the SOPHIA-study which aims to increase knowledge
10
11 about BMD, cardiovascular status and neurocognitive function in long-term female HL survivors with and
12
13 without treatment-induced POI, and the potential influence of these long-term effects on QoL. Results of this
14
15 study will lead to the identification of those HL survivors who are at increased risk for osteoporosis,
16
17 neurocognitive dysfunction and possibly CVD due to treatment-induced POI. This enables timely referral of
18
19 high-risk women for interventions, thereby reducing (subclinical) adverse events and improving QoL.
20
21 Furthermore, results will shed light on existing hypotheses regarding the association between POI and CVD risk.
22
23 Moreover, HL survivors or other cancer survivors who will experience treatment-induced POI in the future can
24
25 be better informed about potential long-term effects. Finally, prospective follow-up of the study population will
26
27 provide insight into longitudinal changes in risk factors and study outcomes.

28 29 30 **AUTHORS' CONTRIBUTIONS**

31 The study protocol has been written by IK, AOvW, BA, EvD-dB and FvL. All authors contributed to study design.
32
33 MH is involved as statistician and performed the sample size calculations together with IK. JZ, LD, and BA are
34
35 responsible for patient accrual and inclusion, and JZ, YA, SB, LM, ES, CL, PL, and LD and BA are responsible for
36
37 the assessment of the outcome variables.

38
39 FvL is the principal investigator and responsible for the funding of the study.

40
41 All authors revised the manuscript critically for intellectual content and have approved the final manuscript.

42 43 44 **FUNDING**

45
46 This study is financially supported by the Dutch Cancer Society (grant number NKI 2010-4720).

47 48 49 **COMPETING INTERESTS**

50
51 JZ declares she has conducted a research project funded by Roche in the past two years (unrelated to the
52
53 current study). CBL's department of Reproductive Medicine has received educational and research grants from
54
55 Merck Serono, Ferring and Auxogyn, and he received speakers fees from MSD, Merck Serono, Ferring and
56
57

1 Auxogyn. He is also a consultant for Ferring. PL provided advice to Friesland Campina. All other authors declare
2
3 no competing interests.
4
5

6 **ETHICS AND DISSEMINATION**

7
8 This study has been approved by the Institutional Review Board of the Netherlands Cancer Institute and has
9
10 been registered at "Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects
11
12 (file number NL44714.031.13). Reporting of serious adverse events was exempted for this study, as the burden
13
14 and risks associated with participation are very low and in accordance with routine medical care. Results will be
15
16 disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines for HL
17
18 survivors.
19

20 **ACKNOWLEDGEMENTS**

21
22 Special thanks to the five female HL survivors who were willing to discuss our study procedures with us and to
23
24 review our questionnaires and information letters.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Ferme C, Eghbali H, Meerwaldt JH, et al. Chemotherapy plus involved-field radiation in early-stage Hodgkin's disease. *N Engl J Med* 2007;**357**(19):1916-27 doi: 10.1056/NEJMoa064601[published Online First: Epub Date]].
2. Raemaekers J, Burgers M, Henry-Amar M, et al. Patients with stage III/IV Hodgkin's disease in partial remission after MOPP/ABV chemotherapy have excellent prognosis after additional involved-field radiotherapy: interim results from the ongoing EORTC-LCG and GPMC phase III trial. The EORTC Lymphoma Cooperative Group and Groupe Pierre-et-Marie-Curie. *Ann Oncol* 1997;**8 Suppl 1**:111-4
3. Klimm B, Goergen H, Fuchs M, et al. Impact of risk factors on outcomes in early-stage Hodgkin's lymphoma: an analysis of international staging definitions. *Ann Oncol* 2013;**24**(12):3070-6 doi: 10.1093/annonc/mdt413[published Online First: Epub Date]].
4. Schaapveld M, Aleman BM, van Eggermond AM, et al. Second Cancer Risk Up to 40 Years after Treatment for Hodgkin's Lymphoma. *N Engl J Med* 2015;**373**(26):2499-511 doi: 10.1056/NEJMoa1505949[published Online First: Epub Date]].
5. Bhatia S, Yasui Y, Robison LL, et al. High risk of subsequent neoplasms continues with extended follow-up of childhood Hodgkin's disease: report from the Late Effects Study Group. *J Clin Oncol* 2003;**21**(23):4386-94
6. Hodgson DC, Gilbert ES, Dores GM, et al. Long-term solid cancer risk among 5-year survivors of Hodgkin's lymphoma. *J Clin Oncol* 2007;**25**(12):1489-97
7. Punnett A, Tsang RW, Hodgson DC. Hodgkin lymphoma across the age spectrum: epidemiology, therapy, and late effects. *Semin Radiat Oncol* 2010;**20**(1):30-44
8. Aleman BM, AW vdB-D, de Bruin ML, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma. *Blood* 2007;**109**(5):1878-86
9. van Nimwegen FA, Schaapveld M, Janus CP, et al. Cardiovascular disease after Hodgkin lymphoma treatment: 40-year disease risk. *JAMA Intern Med* 2015;**175**(6):1007-17 doi: 10.1001/jamainternmed.2015.1180[published Online First: Epub Date]].
10. de Bruin ML, Dorresteyn LD, van't Veer MB, et al. Increased risk of stroke and transient ischemic attack in 5-year survivors of Hodgkin lymphoma. *J Natl Cancer Inst* 2009;**101**(13):928-37
11. de Bruin ML, Huisbrink J, Hauptmann M, et al. Treatment-related risk factors for premature menopause following Hodgkin lymphoma. *Blood* 2008;**111**(1):101-08
12. Swerdlow AJ, Cooke R, Bates A, et al. Risk of premature menopause after treatment for Hodgkin's lymphoma. *J Natl Cancer Inst* 2014;**106**(9) doi: 10.1093/jnci/dju207[published Online First: Epub Date]].
13. Haukvik UK, Dieset I, Bjoro T, et al. Treatment-related premature ovarian failure as a long-term complication after Hodgkin's lymphoma. *Ann Oncol* 2006;**17**(9):1428-33
14. van der Kaaij MA, Heutte N, Meijnders P, et al. Premature ovarian failure and fertility in long-term survivors of Hodgkin's lymphoma: a European Organisation for Research and Treatment of Cancer Lymphoma Group and Groupe d'Etude des Lymphomes de l'Adulte Cohort Study. *J Clin Oncol* 2012;**30**(3):291-99
15. Behringer K, Breuer K, Reineke T, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's Lymphoma Study Group. *J Clin Oncol* 2005;**23**(30):7555-64
16. Luborsky JL, Meyer P, Sowers MF, et al. Premature menopause in a multi-ethnic population study of the menopause transition. *Hum Reprod* 2003;**18**(1):199-206
17. Meirou D. Reproduction post-chemotherapy in young cancer patients. *Mol Cell Endocrinol* 2000;**169**(1-2):123-31
18. Nelson HD. Menopause. *Lancet* 2008;**371**(9614):760-70
19. Kenemans P. Menopause, HRT and menopausal symptoms. *J Epidemiol Biostat* 1999;**4**(3):141-46
20. Hendrix SL. Bilateral oophorectomy and premature menopause. *Am J Med* 2005;**118 Suppl 12B**:131-5 doi: 10.1016/j.amjmed.2005.09.056[published Online First: Epub Date]].
21. Gallagher JC. Effect of early menopause on bone mineral density and fractures. *Menopause* 2007;**14**(3 Pt 2):567-71
22. Der Voort DJ, Der Weijer PH, Barentsen R. Early menopause: increased fracture risk at older age. *Osteoporos Int* 2003;**14**(6):525-30
23. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, et al. Bone density patterns after normal and premature menopause. *Maturitas* 2003;**44**(4):279-86

24. van der Klift M, de Laet CE, McCloskey EV, et al. Risk factors for incident vertebral fractures in men and women: the Rotterdam Study. *J Bone Miner Res* 2004;**19**(7):1172-80
25. Schuit SC, van der Klift M, Weel AE, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. *Bone* 2004;**34**(1):195-202
26. Kritz-Silverstein D, von Muhlen DG, Barrett-Connor E. Hysterectomy and oophorectomy are unrelated to bone loss in older women. *Maturitas* 2004;**47**(1):61-69
27. Ahlborg HG, Johnell O, Nilsson BE, et al. Bone loss in relation to menopause: a prospective study during 16 years. *Bone* 2001;**28**(3):327-31
28. Gerdhem P, Obrant KJ. Bone mineral density in old age: the influence of age at menarche and menopause. *J Bone Miner Metab* 2004;**22**(4):372-75
29. Svejme O, Ahlborg HG, Nilsson JA, et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *BJOG* 2012;**119**(7):810-16
30. Khosla S, Melton LJ, III, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? *J Bone Miner Res* 2011;**26**(3):441-51
31. Kreuser ED, Felsenberg D, Behles C, et al. Long-term gonadal dysfunction and its impact on bone mineralization in patients following COPP/ABVD chemotherapy for Hodgkin's disease. *Ann Oncol* 1992;**3 Suppl 4**:105-10
32. Redman JR, Bajorunas DR, Wong G, et al. Bone mineralization in women following successful treatment of Hodgkin's disease. *Am J Med* 1988;**85**(1):65-72
33. Howell SJ, Berger G, Adams JE, et al. Bone mineral density in women with cytotoxic-induced ovarian failure. *Clin Endocrinol (Oxf)* 1998;**49**(3):397-402
34. Atsma F, Bartelink ML, Grobbee DE, et al. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. *Menopause* 2006;**13**(2):265-79
35. Hu FB, Grodstein F, Hennekens CH, et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med* 1999;**159**(10):1061-66
36. Lobo RA. Surgical menopause and cardiovascular risks. *Menopause* 2007;**14**(3 Pt 2):562-66
37. Roeters van Lennep JE, Heida KY, Bots ML, et al. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2016;**23**(2):178-86 doi: 10.1177/2047487314556004[published Online First: Epub Date]].
38. van der Schouw YT, van der Graaf Y, Steyerberg EW, et al. Age at menopause as a risk factor for cardiovascular mortality. *Lancet* 1996;**347**(9003):714-18
39. Montalcini T, Gorgone G, Gazzaruso C, et al. Role of endogenous androgens on carotid atherosclerosis in non-obese postmenopausal women. *Nutr Metab Cardiovasc Dis* 2007;**17**(10):705-11
40. Golden SH, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid atherosclerosis: a case-control study of the atherosclerosis risk in communities cohort. *Am J Epidemiol* 2002;**155**(5):437-45
41. Bernini GP, Sgro' M, Moretti A, et al. Endogenous androgens and carotid intimal-medial thickness in women. *J Clin Endocrinol Metab* 1999;**84**(6):2008-12
42. Kok HS, van Asselt KM, van der Schouw YT, et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol* 2006;**47**(10):1976-83
43. Stolk L, Perry JR, Chasman DI, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* 2012;**44**(3):260-68
44. Farrag AK, Khedr EM, Abdel-Aleem H, et al. Effect of surgical menopause on cognitive functions. *Dement Geriatr Cogn Disord* 2002;**13**(3):193-98
45. McLay RN, Maki PM, Lyketsos CG. Nulliparity and late menopause are associated with decreased cognitive decline. *J Neuropsychiatry Clin Neurosci* 2003;**15**(2):161-67
46. Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 2007;**69**(11):1074-83
47. Henderson VW, Sherwin BB. Surgical versus natural menopause: cognitive issues. *Menopause* 2007;**14**(3 Pt 2):572-79
48. Vearncombe KJ, Pachana NA. Is cognitive functioning detrimentally affected after early, induced menopause? *Menopause* 2009;**16**(1):188-98
49. Lethaby A, Hogervorst E, Richards M, et al. Hormone replacement therapy for cognitive function in postmenopausal women. *Cochrane Database Syst Rev* 2008(1):CD003122
50. Zwart W, Terra H, Linn SC, et al. Cognitive effects of endocrine therapy for breast cancer: keep calm and carry on? *Nat Rev Clin Oncol* 2015;**12**(10):597-606 doi: 10.1038/nrclinonc.2015.124[published Online First: Epub Date]].

- 1 51. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant
2 chemotherapy for breast cancer. *J Clin Oncol* 1996;**14**(5):1718-29 doi:
3 10.1200/JCO.1996.14.5.1718[published Online First: Epub Date]].
- 4 52. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy
5 postmenopausal women: principal results From the Women's Health Initiative randomized controlled
6 trial. *JAMA* 2002;**288**(3):321-33
- 7 53. Rosano GM, Vitale C, Fini M. Hormone replacement therapy and cardioprotection: what is good and
8 what is bad for the cardiovascular system? *Ann N Y Acad Sci* 2006;**1092**:341-48
- 9 54. Maki PM, Henderson VW. Hormone therapy, dementia, and cognition: the Women's Health Initiative
10 10 years on. *Climacteric* 2012;**15**(3):256-62
- 11 55. Maki PM, Dennerstein L, Clark M, et al. Perimenopausal use of hormone therapy is associated with
12 enhanced memory and hippocampal function later in life. *Brain Res* 2011;**1379**:232-43
- 13 56. King J, Wynne CH, Assersohn L, et al. Hormone replacement therapy and women with premature
14 menopause—a cancer survivorship issue. *Eur J Cancer* 2011;**47**(11):1623-32
- 15 57. Marjoribanks J, Farquhar C, Roberts H, et al. Long term hormone therapy for perimenopausal and
16 postmenopausal women. *Cochrane Database Syst Rev* 2012(7):CD004143 doi:
17 10.1002/14651858.CD004143.pub4[published Online First: Epub Date]].
- 18 58. Lagro-Janssen A, Knufing MW, Schreurs L, et al. Significant fall in hormone replacement therapy
19 prescription in general practice. *Fam Pract* 2010;**27**(4):424-9 doi: 10.1093/fampra/cmq018[published
20 Online First: Epub Date]].
- 21 59. van Leeuwen FE, Klokman WJ, Hagenbeek A, et al. Second cancer risk following Hodgkin's disease: a
22 20-year follow-up study. *J Clin Oncol* 1994;**12**(2):312-25
- 23 60. Daan NM, Muka T, Koster MP, et al. Cardiovascular Risk in Women With Premature Ovarian
24 Insufficiency Compared to Premenopausal Women at Middle Age. *J Clin Endocrinol Metab*
25 2016;**101**(9):3306-15 doi: 10.1210/jc.2016-1141[published Online First: Epub Date]].
- 26 61. Janse F, Knauff EA, Niermeijer MF, et al. Similar phenotype characteristics comparing familial and
27 sporadic premature ovarian failure. *Menopause* 2010;**17**(4):758-65 doi:
28 10.1097/gme.0b013e3181cf8521[published Online First: Epub Date]].
- 29 62. Olenginski TP, Newman ED, Hummel JL, et al. Development and evaluation of a vertebral fracture
30 assessment program using IVA and its integration with mobile DXA. *J Clin Densitom* 2006;**9**(1):72-7 doi:
31 10.1016/j.jocd.2005.08.002[published Online First: Epub Date]].
- 32 63. Greenspan SL, von Stetten E, Emond SK, et al. Instant vertebral assessment: a noninvasive dual X-ray
33 absorptiometry technique to avoid misclassification and clinical mismanagement of osteoporosis. *J*
34 *Clin Densitom* 2001;**4**(4):373-80
- 35 64. Netelenbos JC, Lems WF, Geusens PP, et al. Spine radiographs to improve the identification of women
36 at high risk for fractures. *Osteoporos Int* 2009;**20**(8):1347-52 doi: 10.1007/s00198-008-0801-
37 1[published Online First: Epub Date]].
- 38 65. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and
39 monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*
40 2011;**22**(2):391-420 doi: 10.1007/s00198-010-1501-1[published Online First: Epub Date]].
- 41 66. van Schoor NM, Visser M, Pluijm SM, et al. Vitamin D deficiency as a risk factor for osteoporotic
42 fractures. *Bone* 2008;**42**(2):260-6 doi: 10.1016/j.bone.2007.11.002[published Online First: Epub
43 Date]].
- 44 67. Kuchuk NO, Pluijm SM, van Schoor NM, et al. Relationships of serum 25-hydroxyvitamin D to bone
45 mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin*
46 *Endocrinol Metab* 2009;**94**(4):1244-50 doi: 10.1210/jc.2008-1832[published Online First: Epub Date]].
- 47 68. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and
48 active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ* 2009;**339**:b3692 doi:
49 10.1136/bmj.b3692[published Online First: Epub Date]].
- 50 69. Angus RM, Sambrook PN, Pocock NA, et al. A simple method for assessing calcium intake in Caucasian
51 women. *J Am Diet Assoc* 1989;**89**(2):209-14
- 52 70. Netherlands. THHcot. Dietary reference values: calcium, vitamin D, thiamin, riboflavin, niacin,
53 panthothenic acid, and biotin, 2000.
- 54 71. Schussler JM, Grayburn PA. Non-invasive coronary angiography using multislice computed
55 tomography. *Heart* 2007;**93**(3):290-7 doi: 10.1136/hrt.2005.069195[published Online First: Epub
56 Date]].
- 57 72. Schuetz GM, Zacharopoulou NM, Schlattmann P, et al. Meta-analysis: noninvasive coronary
58 angiography using computed tomography versus magnetic resonance imaging. *Ann Intern Med*

- 2010;**152**(3):167-77 doi: 10.7326/0003-4819-152-3-201002020-00008[published Online First: Epub Date]].
73. Nair SB, Malik R, Khattar RS. Carotid intima-media thickness: ultrasound measurement, prognostic value and role in clinical practice. *Postgrad Med J* 2012;**88**(1046):694-9 doi: 10.1136/postgradmedj-2011-130214[published Online First: Epub Date]].
74. Den Ruijter HM, Peters SA, Anderson TJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012;**308**(8):796-803
75. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;**55**(13):1318-27 doi: 10.1016/j.jacc.2009.10.061[published Online First: Epub Date]].
76. Buckley DI, Fu R, Freeman M, et al. C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive Services Task Force. *Ann Intern Med* 2009;**151**(7):483-95
77. Kavousi M, Elias-Smale S, Rutten JH, et al. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study. *Ann Intern Med* 2012;**156**(6):438-44 doi: 10.7326/0003-4819-156-6-201203200-00006[published Online First: Epub Date]].
78. Brennehan SK, Barrett-Connor E, Sajjan S, et al. Impact of recent fracture on health-related quality of life in postmenopausal women. *J Bone Miner Res* 2006;**21**(6):809-16 doi: 10.1359/jbmr.060301[published Online First: Epub Date]].
79. Stewart AL WJ, Sherbourne CD, et al. Psychological distress/well-being and cognitive functioning measures. *Measuring Functioning and Well-Being: The Medical Outcomes Study Approach*. Durham, NC: Duke University, 1992. p.102–142.
80. Spinhoven P OJ, Sloekers PP, Kempen GI, Speckens AE, Van Hemert AM (1997) A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychol Med* 27:363-70.
81. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual functioning. *Qual Life Res* 1996;**5**(1):81-90
82. Alberts M SE, Vercoelen JHMM, Garssen B, Bleijenberg G. Verkorte Vermoeidheidsvragenlijst: een praktisch hulpmiddel bij het scoren van vermoeidheid. *Nederlands Tijdschrift voor Geneeskunde* 1997; 141:1526-1530.
83. Sherwin BB. Estrogen therapy: is time of initiation critical for neuroprotection? *Nat Rev Endocrinol* 2009;**5**(11):620-7 doi: 10.1038/nrendo.2009.193[published Online First: Epub Date]].
84. Schilder CM, Eggens PC, Seynaeve C, et al. Neuropsychological functioning in postmenopausal breast cancer patients treated with tamoxifen or exemestane after AC-chemotherapy: cross-sectional findings from the neuropsychological TEAM-side study. *Acta Oncol* 2009;**48**(1):76-85 doi: 10.1080/02841860802314738[published Online First: Epub Date]].
85. van Dam FS, Schagen SB, Muller MJ, et al. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. *J Natl Cancer Inst* 1998;**90**(3):210-8
86. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, et al. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. *J Int Neuropsychol Soc* 2006;**12**(1):80-9 doi: 10.1017/S1355617706060115[published Online First: Epub Date]].
87. Mulder J DR, Dekker P. *Verbale Leer & Geheugen Test*. Lisse: Swets & Zeitlinger; 1996.
88. Reitan R. Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and Motor Skills*. 1958;**8**:271-6
89. Dekker N, van 't Veer MB, Aleman BM, et al. [The BETER survivorship care initiative for Hodgkin lymphoma; tailored survivorship care for late effects of treatment]. *Ned Tijdschr Geneesk* 2015;**159**:A9269
90. Castellon SA, Ganz PA, Bower JE, et al. Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. *J Clin Exp Neuropsychol* 2004;**26**(7):955-69
91. Collins B, Mackenzie J, Stewart A, et al. Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. *Psychooncology* 2009;**18**(2):134-43
92. Schagen SB, van Dam FS, Muller MJ, et al. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. *Cancer* 1999;**85**(3):640-50
93. Cohen J. A power primer. *Psychol Bull* 1992;**112**(1):155-59
94. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**(9472):1687-717 doi: 10.1016/S0140-6736(05)66544-0[published Online First: Epub Date]].

- 1 95. Rodriguez-Wallberg KA, Oktay K. Fertility preservation in women with breast cancer. Clin Obstet
2 Gynecol 2010;**53**(4):753-62 doi: 10.1097/GRF.0b013e3181f96e00[published Online First: Epub Date]].
3
4

5 **FIGURE LEGENDS**

6 **Figure 1. Study procedures and patient burden, stratified by medical tests for routine care and research**

7
8 Abbreviations: RT = radiotherapy; CT = chemotherapy; BETTER = Better care after Hodgkin lymphoma,
9 Evaluation of long-Term Treatment Effects and screening Recommendations; CRP = C-reactive protein; BMD =
10 bone mineral density; DEXA = Dual Energy X-ray Absorptiometry
11
12

13
14 ^a Expected for > 90% of women ^b Expected for 15-40% of women
15

16 In case criteria for care are not fulfilled, tests will be performed for research purpose
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

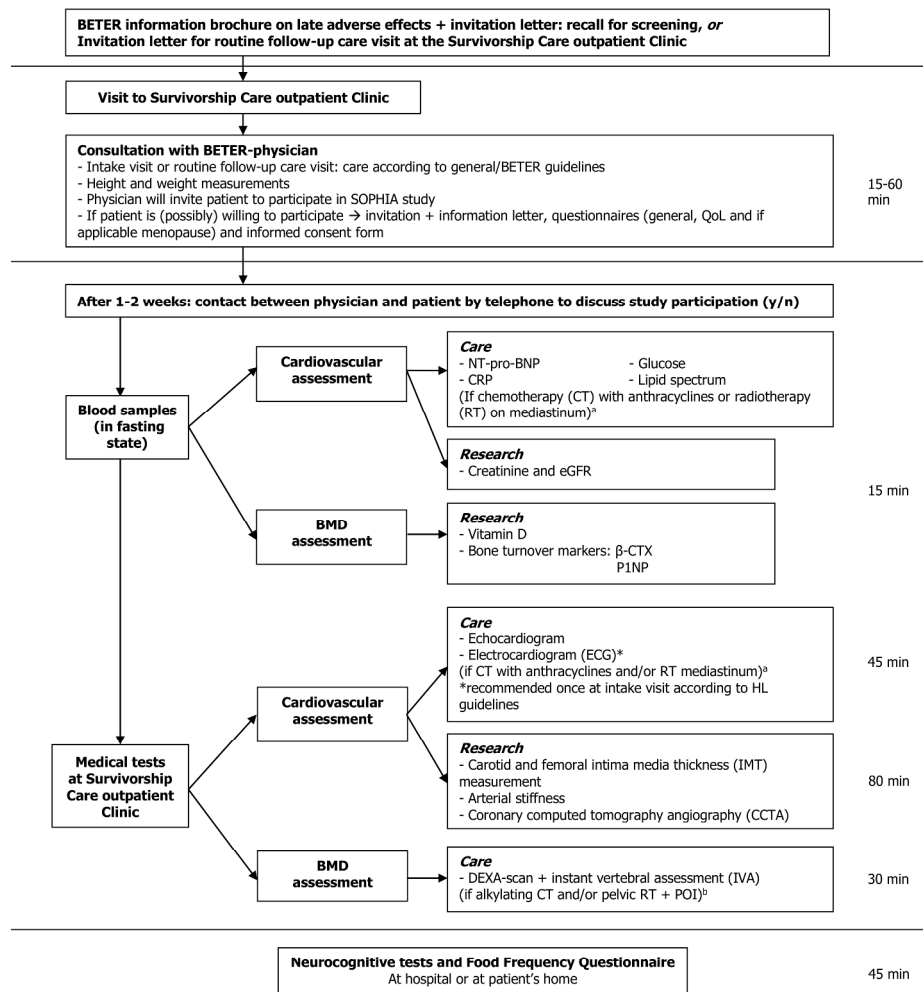


Figure 1. Overview of study procedures and patient burden, stratified by medical tests for routine care and research

279x361mm (300 x 300 DPI)