SUPPLEMENTARY ONLINE MATERIAL

Methods

Patients

The study was part of a national multicenter cross-sectional study performed in 2012-2014.[1] The survivors were identified through treatment records and registries at each participating center. Lymphoma- and treatment-related data were obtained from patients' charts.

Of 399 eligible survivors, 311 (78%) consented and completed the questionnaire (supplementary figure S1). Of these, 270 also attended the clinical examination. The participants were slightly older than non-participants (mean 55 vs. 52 years, p=.03), but there was no statistically significant difference regarding age at HDT-ASCT, sex, observation time, lymphoma type or HDT regimen. There were no statistically significant differences between survivors consenting to complete both the questionnaire and the clinical examination and those completing the questionnaire only for any of these variables.

Treatment

Treatment of lymphomas in Norway, including HDT-ASCT, has followed international and national guidelines.[2] In the period 1987-1995 the conditioning regimen consisted of total body irradiation (TBI) and high-dose cyclophosphamide, and from 1996 chemotherapy only (BEAM: carmustine, etoposide, cytarabine and melphalan). The survivors were grouped based on time of HDT-ASCT: 1987-1995, 1996-2002 and 2003-2008 and primary diagnosis: Hodgkin lymphoma (HL), aggressive non-Hodgkin lymphoma (NHL) (diffuse large B-cell lymphoma, T-cell lymphomas, mantle cell lymphoma, Burkitt's lymphoma and lymphoblastic lymphoma) and indolent NHL (mostly follicular lymphoma).

Questionnaires

Chronic fatigue (CF) was assessed by the Fatigue Questionnaire (FQ).[3] The FQ contains 11 items concerning physical (7 items) and mental (4 items) fatigue during the last month, compared with when the respondent last felt well. Each item has four response alternatives (rated from 0 to 3). Two additional items cover duration and extent of fatigue. Responses were dichotomized (0 and 1 scored as 0, and 2 and 3 scored as 1), with CF defined as a sum score of ≥4 of the dichotomized responses with duration of ≥6 months.[3] Internal consistency (Cronbach's alpha) was 0.92 for total fatigue, 0.93 for physical fatigue and 0.80 for mental fatigue, respectively.

Paired relationship was defined as married or cohabiting. Level of education was dichotomized into low (<13 years) and high (≥13 years). According to the World Health Organization (WHO) guidelines, physical activity of ≥150 minutes/week of moderate intensity or ≥75 minutes/week of vigorous intensity was categorized as "meeting guidelines", any lower level as "not meeting guidelines".[4]

Neuroticism was assessed by an abbreviated version of the Eysenck Personality Questionnaire (EPQ) with six items,[5] with higher score implying more neuroticism. Internal consistency of EPQ was 0.79.

A 15-item version of the Impact of event scale (IES)[6] was used to measure post-traumatic symptoms related to HDT-ASCT, consisting of seven items on intrusion and eight on avoidance, with responses on a six-point frequency scale for each item. Internal consistency was 0.91 for both intrusion and avoidance.

Mental distress was measured by the Hospital Anxiety and Depression Scale (HADS).[7, 8] Each item has four response alternatives, scored from 0 (not present) to 3 (highly

present). Internal consistencies were 0.82 and 0.87 for the depression and anxiety subscales, respectively.

Clinical assessments and blood samples

Standardized medical consultations were performed by experienced physicians, including documentation of comorbidities and medications. Blood pressure was measured using the mean of the three most consistent measures. Body mass index (BMI) was calculated from measured height and weight, and obesity defined as BMI ≥30 kg/m2. Blood samples were drawn at 8 am after an overnight fast. Normal thyroid function was defined as normal levels of free thyroid hormone (fT4) (9.0-21.0 pmol/l) and thyroid stimulating hormone (TSH) (0.5-3.5 mU/l) and latent hypothyroidism was defined as TSH ≥3.6 mU/l and fT4 >9.0 pmol/l. Patients on "thyroid substitution" was defined as such. No participant had primary overt hypothyroidism (TSH ≥3.6 mU/l and fT4 <9.0 pmol/l), or secondary hypothyroidism (TSH <0.5 mU/l and fT4 <9.0 pmol/l). Morning serum cortisol was analyzed as a continuous variable. Anemia was defined as hemoglobin <11.7 g/dl and <13.4 g/dl for females and males, respectively.[9] Estimated glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease (MDRD) formula.[10]

Echocardiography

The echocardiographic examination with definitions of left ventricular ejection fraction (LVEF), left ventricular systolic dysfunction (LVSD) and symptomatic heart failure has been described elsewhere.[1] Valvular dysfunction was defined as either of regurgitations more than mild, any stenosis or prior valve replacement.[11]

Exercise capacity and pulmonary function

Symptom-limited exercise testing, measuring respiratory gas-exchange, was performed using an electrically braked ergometer bicycle and pulmonary function tests included dynamic spirometry, determination of static lung volumes and gas diffusion capacity (n=218), all described previously.[12] All calculations were based on recommended reference values.[13, 14]

Cytokines

Cytokine measurements were performed using the BioPlex XMap technology (Austin, Texas, USA) with a Luminex IS100 instrument (BIO-RAD, Hercules, California, USA), powered with the Bio-Plex manager Software version 6.0.1. After written informed consent, age and gender matched controls (40 female and 60 males) were recruited from healthy blood donors (Oslo University Hospital) with an age-distribution similar to patient samples.

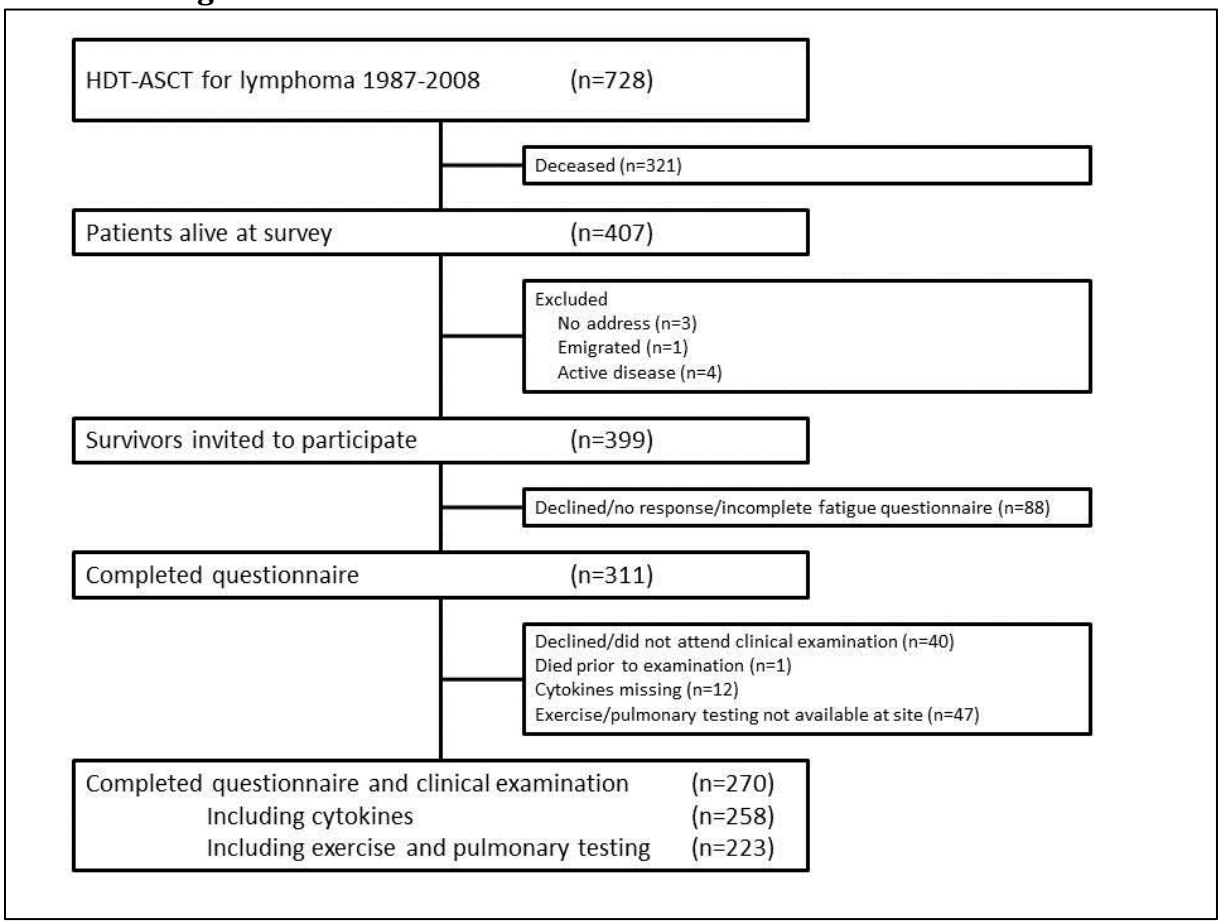
Serum samples were collected in serum tubes without gel, left to coagulate in room temperature for 30-60 minutes and centrifuged at 1000 g for 15 minutes at 4oC. Immediately after centrifugation samples were aliquoted and frozen at -70oC. Prior to analysis of interleukin-1 receptor antagonist (IL-RA), IL-1β, IL-6 and TNFα (tumor necrosis factor-α), serum aliquots were thawed on ice, centrifuged at 10.000 g for 10 minutes at 4oC and diluted 1:4. All samples were run in duplicate, and healthy control samples were evenly distributed and assayed on all 96 well plates (n=10) together with the patient samples. The assay was performed according to the manufacturer's instructions. Longitudinal controls were used to assess the inter-assay variation and the coefficient of variation for controls ranged from 6% to 21%.

Cytokines IL-1β, IL-6 and TNF-α were analytically undetectable in <50% of samples in at least one group, and therefore dichotomized as detectable vs. undetectable.

Statistics

Descriptive statistics and comparison of groups by t-tests, Mann-Whitney U tests, Chi-square test and Fischer's exact tests were performed as appropriate. Logistic regression analyses were performed with CF as the dependent variable. Independent variables with p-value <0.10 in univariate analyses were included in the multivariable model. The subscales of the HADS were excluded due to their high correlation with neuroticism. Similarly, workload and physical activity were excluded because of their high correlation to peak oxygen consumption (VO_{2peak}) and IL-1RA because of its correlation to IL-6. The multivariable model was adjusted for age and sex, since previous studies have shown an association between these factors and fatigue.[15-17] In the multivariable model, current smoking was not significant, thus left out of the final model to avoid too many explanatory variables. The significance level was set to 0.05, and all tests were two-sided. Analyses were performed using IBM SPSS statistics version 21.

Tables and Figures



Supplementary figure S1: Flowchart of recruitment of eligible lymphoma survivors after high dose therapy with autologous stem-cell transplantation (HDT-ASCT) in Norway.

Supplementary Table S1: Patient characteristics according to chronic fatigue

	No chronic fatigue (n = 214)		Chronic fatigue (n = 97)		p-value
	n	(%)	n	(%)	
Sex					.18
Males (n=187)	134	72 %	53	28 %	
Females (n=124)	80	65 %	44	35 %	
Age at diagnosis (years)					.31
Median (range)	43 (10-65)		40 (17-64)		
Age at survey (years)					.22
Median (range)	57 (25-76)		55 (24-77)		
Time diagnosis-survey (years)					.70
Median (range)	12.9 (4-34)		12.3 (4-31)		
Time diagnosis-HDT-ASCT (years)					.29
Median (range)	1.4 (0.2-21.4)		1.1 (0.2-22.7)		
Lymphoma type					.37
Hodgkin lymphoma (n=71)	44	62 %	27	38 %	
Aggressive NHL (n=209)	148	71 %	61	29 %	
Indolent NHL (n=31)	22	71 %	9	29 %	
Treatment period					.88
1987-1995 (n=45)	32	71 %	13	29 %	
1996-2002 (n=91)	61	67 %	30	33 %	
2003-2008 (n=175)	121	69 %	54	31 %	
Ann Arbor stage at diagnosis (missing n=1)					.53
I (n=25)	18	72 %	7	28 %	
II (n=71)	44	62 %	27	38 %	
III (n=66)	45	68 %	21	32 %	
IV (n=148)	106	72 %	42	28 %	
B-symptoms (missing n=4)					.62
No (n=194)	139	72 %	55	28 %	
Yes (n=113)	72	64 %	41	36 %	
High dose regimen					.62
TBI + Cyclophosphamide (n=43)	31	72 %	12	28 %	
BEAM (n=268)	183	68 %	85	32 %	
Treatment lines before HDT-ASCT					.97
1 (n=92)	64	70 %	28	30 %	
2 (n=176)	121	69 %	55	31 %	
≥3 (n=43)	29	67 %	14	33 %	
Mediastinal radiotherapy					**.03**
No (n=202)	148	73 %	54	27 %	
Yes (n=109)	66	61 %	43	39 %	
Rituximab					.46
No (n=178)	119	67 %	59	33 %	
Yes (n=133)	95	71 %	38	29 %	
Relapse after HDT-ASCT					.22
No (n=241)	170	71 %	71	29 %	
Yes (n=70)	44	63 %	26	37 %	
Second cancer after HDT-ASCT[a]					.33
No (n=283)	197	70 %	86	30 %	
Yes (n=28)	17	61 %	11	39 %	
Allogeneic SCT after HDT-ASCT					.23
No (n=290)	202	70 %	88	30 %	
Yes (n=21)	12	57 %	9	43 %	
Relationship status (missing n=1)					.24
In paired relationship (n=226)	151	67 %	75	33 %	
Not in paired relationship (n=84)	62	74 %	22	26 %	
Level of education (missing n=2)					.58
< 13 years (n=168)	113	67 %	55	33 %	
≥ 13 years (n=141)	99	70 %	42	30 %	

WHO physical activity recommendation met[b]					**<.001**
No (n=172)	102	59 %	70	41 %	
Yes (n=139)	112	81 %	27	19 %	
Current smoker (missing n=2)					.09
No (n=253)	179	79 %	74	29 %	
Yes (n=56)	33	59 %	23	41 %	
Neuroticism					
Score, median (range)	0 (0-6)		3 (0-6)		**<.001**
Impact of event scale (IES)					
Intrusion, median (range)	2 (0-27)		5 (0-29)		**<.001**
Avoidance, median (range)	4 (0-35)		9 (0-38)		**<.001**
Total score, median (range)	5 (0-60)		14 (0-60)		**<.001**
PTSD (missing n=2)					**<.001**
No[c] (n=259)	190	73 %	69	27 %	
Partial PTSD[d] (n=25)	11	44 %	14	56 %	
Full PTSD[e] (n=25)	11	44 %	14	56 %	
HADS-A					
Score, median (range)	3 (0-13)		5 (0-19)		**<.001**
HADS-D					
Score, median (range)	1 (0-12)		5 (0-15)		**<.001**

Patient- and disease characteristics and patients reported outcomes according to chronic fatigue. p-values obtained by X^2-test for categorical variables and independent T-test or Mann-Whitney (skewed data) for continuous variables. Statistically significant p-values are indicated in bold.

[a] Second cancer was defined as any new malignancy other than relapse of the primary lymphoma after HDT-ASCT (non-melanoma skin cancers excluded). [b] 150 min/week of moderate intensity or 75 min/week of vigorous intensity, [c] total IES score <26, [d] total IES score 26-34, [e] total IES score ≥35.

SD=standard deviation, HDT-ASCT=high dose therapy with autologous stem cell transplantation, NHL=non-Hodgkin lymphoma. TBI=total body irradiation, BEAM=carmustine, etoposide, cytarabine and melphalan, SCT=stem cell transplantation, WHO=world health organization, IES=impact of event scale, PTSD=post-traumatic stress disorder, HADS=hospital anxiety and depression scale (A=anxiety subscale, D=depression subscale).

Supplementary Table S2: Findings on clinical examination according to CF (n=270)

	No chronic fatigue (n = 184) n (%)	Chronic fatigue (n = 86) n (%)	p-value
Body mass index (kg/m^2)			
Mean (SD)	25.9 (3.4)	26.8 (5.6)	.19
<30 (not obese)[a] (n=230)	165 72 %	65 28 %	**.001**
≥30 (obese) (n=40)	19 48 %	21 52 %	
Blood samples			
Free T4 (pmol/l)			
Mean (SD)	15.3 (2.6)	15.7 (2.3)	.23
Thyroid hormone status (missing n=5)			.73
Normal (n=132)	93 70 %	39 30 %	
Subclinical hypothyroidism (n=79)	53 67 %	26 33 %	
On substitution (n=54)	35 65 %	19 35 %	
Morning cortisol (nmol/l)			
Mean (SD)	510 (165)	522 (168)	.60
Anemia (missing n=2)			
No (n=240)	164 68 %	76 32 %	.56
Yes (n=28)	19 68 %	9 32 %	
Estimated GFR (missing=1)			.21
≥60 (n=238)	165 69 %	73 31 %	
<60 (n=31)	18 58 %	13 42 %	
CRP (mg/L)			
Median (range)	1.7 (0.0-59.0)	5.6 (0.0-154.0)	.55
Echocardiography (n=270)			
LVEF			
Mean (SD)	55 (6)	54 (6)	.47
LVSD as LVEF <50% (missing n=1)			.56
No (n=228)	156 68 %	72 32 %	
Yes (n=41)	28 68 %	13 32 %	
Symptomatic heart failure			.24
No (n=241)	167 69 %	74 31 %	
Yes (n=29)	17 59 %	12 41 %	
Valvular dysfunction			.70
No (n=211)	145 69 %	66 31 %	
Yes (n=59)	39 66 %	20 34 %	
Pulmonary function (n=223)			
Restrictive impairment			.63
No (n=208)	140 67 %	68 33 %	
Yes (n=15)	11 73 %	4 27 %	
Obstructive impairment			.30
No (n=199)	137 69 %	62 31 %	
Yes (n=24)	14 58 %	10 42 %	
Gas transfer impairment			.32
No (n=116)	82 71 %	34 19 %	
Yes (n=107)	69 65 %	38 36 %	
Exercise capacity (n=218)			
VO_2 peak (ml/kg/min)			**.003**
Mean (SD)	28.0 (7.5)	24.9 (6.5)	
VO_2 peak % predicted (Hansen Wasserman)			**.005**
Mean (SD)	105 (21)	96 (19)	
Work load max (Watt)			**.002**
Mean (SD)	176 (58)	151 (50)	
RER max (VCO_2/VO_2)			.72
Mean (SD)	1.18 (0.07)	1.17 (0.08)	
Perceived exertion (Borg)			.84
Mean (SD)	17 (1.0)	17 (1.0)	

Findings on clinical examination according to chronic fatigue. Normal thyroid function was defined as normal levels of free thyroid hormone (fT4) (9.0-21.0 pmol/l) and thyroid stimulating hormone (TSH) (0.5-3.5 mU/l) and latent hypothyroidism was defined as TSH ≥3.6 mU/l and fT4 >9.0 pmol/l. Patients on "thyroid substitution" was defined as such. No participant had primary overt hypothyroidism (TSH ≥3.6 mU/l and fT4 <9.0 pmol/l), or secondary hypothyroidism (TSH <0.5 mU/l and fT4 <9.0 pmol/l). Morning serum cortisol was analyzed as a continuous variable. Anemia was defined as hemoglobin <11.7 g/dl and <13.4 g/dl for females and males, respectively. Estimated glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease (MDRD) formula.
p-values obtained by X^2-test for categorical variables and independent T-test or Mann-Whitney (skewed data) for continuous variables. Statistically significant p-values are indicated in bold.
[a] All four participants with BMI<18.5 kg/m^2 (underweight) had CF.
SD=standard deviation, LVEF=left ventricular ejection fraction, LVSD=left ventricular systolic dysfunction, VO_2=volume oxygen, VCO_2=volume carbon dioxide, RER=respiratory exchange ratio.

Supplementary table S3: Cytokine levels according to CF and versus controls

	Cases			Controls (n=100)		
	No chronic fatigue (n=170)	Chronic fatigue (n=79)	p[a]		p[b]	p[c]
Median (range) (pg/ml)						
IL-1RA	32.2 (0-608)	37.2 (12.3-598)	**0.04**	25.2 (6-6853)	**0.01**	**<.001**
Cytokines dichotomized (n (%) detectable of total)						
IL-1β	20 (11.8 %)	14 (17.7 %)	0.20	24 (24 %)	**0.01**	.27
IL-6	86 (50.6 %)	56 (70.9 %)	**0.003**	25 (25 %)	**<0.001**	**<.001**
TNF-α	38 (22.4 %)	20 (25.3 %)	0.60	19 (19 %)	0.48	.28

Cytokines according to chronic fatigue (CF) and versus age- and gender matched controls from the general population. Participants currently taking cyclosporine (n=2), anakinra (n=1) or prednisolone (n=6) were excluded. p-values obtained by X^2-test for categorical variables and Mann-Whitney for continuous variables (skewed data). Statistically significant p-values are indicated in bold. [a] p-value for comparison between non-CF and CF cases. [b] p-value for comparison between non-CF cases and controls. [c] p-value for comparison between CF cases and controls.

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