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Serum cytokines and chronic fatigue in adults surviving after childhood leukemia and lymphoma

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ABSTRACT

Introduction: Fatigue is a common and distressing symptom in all phases of the cancer trajectory. Chronic fatigue (CF) is defined as fatigue with duration ⩾6 months. The etiology of CF in cancer survivors is poorly understood, but a link to inflammatory activity has been suggested. In the present study we explored the relation between CF and the levels of 17 cytokines among a national representative sample of 232 adult survivors after childhood lymphoma and acute lymphoblastic leukemia (ALL).
Methods: Chalder's fatigue questionnaire assessed CF. The sera of the survivors were analyzed for 27 cytokines, where of 17 were detectable.
Results: Median age at survey and diagnosis was 29.7 years (range 18.6–54.5 years) and 9.6 years (range 0.3–18.0 years), respectively. Median follow-up time was 21.5 years (range 7.1–40.0 years). CF was not associated with increased levels of any of the 17 detectable cytokines when all three diagnostic groups were included in the analyses. In sub-analyses of the non-Hodgkin lymphoma survivors only, those with CF had significant higher levels of IL-9, FGF, PDGF and eotaxin compared to those without CF ($p < 0.05$). Gender, age, diagnosis, obesity, or reduced heart function did not impact upon the results. Differences in cytokine levels between the diagnostic groups were observed irrespective of the presence/absence of CF.
Conclusion: This study could not confirm a relation between levels of cytokines and CF in adults who survived childhood lymphoma and ALL, except for among NHL survivors. Despite the broad spectrum of cytokines and relatively large sample, small aberrances may not have been traced.

1. Introduction

Fatigue is a common symptom in all phases of the cancer trajectory and is defined as a subjective experience of tiredness, exhaustion and lack of energy that is not proportional to recent activities. It might negatively impact upon quality of life and daily activities including work ability (Mock et al., 2007; Spelten et al., 2003;Stone and Minton, 2008a).

Among cancer survivors, attention has been drawn to chronic fatigue (CF), which is defined as persistent or relapsing fatigue lasting for six months or longer (Fukuda et al., 1994). Studies among long-term tumor-free survivors after adult-onset cancer report prevalences of CF between 17% and 35% of the survivors (Ganz and Bower, 2007; Loge et al., 1999; Orre et al., 2008; Prue et al., 2006; Reinertsen et al., 2010). There is increasing evidence that CF is common also among childhood cancer survivors with prevalences ranging from 11% to 30% (Johannsdottir et al., 2012; Langeveld et al., 2003; Meeske et al., 2005; Mulrooney et al., 2008).

The etiology of cancer-related fatigue is relatively poorly understood, but is assumed to be multifactorial, involving physiological, biochemical, and psychological factors (Ryan et al., 2007). It has been hypothesized that inflammatory cytokines and enhanced immune activation play a role in fatigue experienced by cancer patients (Bower et al., 2002). Interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) might be released as a host response to the tumor or as a response to the treatment-related tissue damage (Ganz and Bower, 2007). In addition to acting systemically, these cytokines might also act centrally in the brain, inducing fatigue and "sickness behavior" (i.e. depressed mood, sleep disturbances, decreased activity) in different populations (Dantzer, 2009).

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The potential role of inflammation in CF among long-time cancer-free survivors is less obvious than in acute fatigue experienced during active disease or treatment. In general only a handful of inflammatory markers [e.g., IL-6, IL-1 receptor antagonist (IL-1ra), C-reactive protein (CRP)] have been explored in relation to fatigue among cancer survivors, most of them in relatively small patient samples (Bower et al., 2002; Orre et al., 2009). To our best knowledge, no previous studies have investigated a possible association between levels of pro-inflammatory cytokines and CF in long-term survivors after childhood cancer.

B-symptoms at diagnosis (night sweats, fever of unknown cause, weight loss), which has been reported to be associated with elevated levels of IL-6 and IL-10 (Gaiolla et al., 2011), have been found to be associated with higher prevalences of CF in two previous studies among disease-free long-term Hodgkin lymphoma (HL) survivors (Hjermstad et al., 2005; Loge et al., 1999; Ruffer et al., 2003). These findings point to a possible linkage between CF and the inflammatory system among these survivors. A possible relation between inflammation in CF among survivors after acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) has to our knowledge not been explored previously. In particular, if these mechanisms differ between ALL/NHL survivors treated for disease of T-cell compared to those treated for disease of B-cell origin is unknown.

Heart failure has been shown to impact on cytokine levels (Yndestad et al., 2007). Further, age, gender, obesity and smoking may impact on cytokine levels (Calder et al., 2011;Cioffi et al., 2002;Sher et al., 1999). Finally, the use of anti-inflammatory analgesics is expected to impact on cytokine production.

We have recently reported a relatively high prevalence of CF (28%) among adult survivors after childhood lymphoma and ALL with a median follow-up time of 20 years (Hamre et al., 2012). The aim of the present study was to explore the relation between CF and levels of multiple cytokines in this unselected group of survivors. Cytokines previously found to be associated with fatigue among other groups of cancer survivors were specifically examined for. Secondary, we aimed to assess associations between disease and treatment related variables, late effects and CF, and to explore if any of the variables associated with CF also were associated with differences in cytokine levels.

2. Patients and methods

2.1. Patients and study design

All Norwegian adult survivors after childhood lymphoma and ALL were identified by the Cancer Registry of Norway. The study's eligibility criteria were: Treatment for HL as first time cancer at any University Hospital in Norway or treatment at Oslo University Hospital (OUH) for acute lymphoblastic leukemia (ALL) or non-Hodgkin lymphoma (NHL), period of diagnosis 1970–2000 (ALL 1970–2002), age at diagnosis $\leqslant$18 years (ALL $\leqslant$16 years) and survival for at least five years since diagnosis, age at time of survey >18 years and alive as of June 2007. For HL the survey was national. For NHL and ALL, the survey was, due to resource limitations, restricted to all survivors from the South-Eastern Health Region of Norway, including approximately 50% of the Norwegian population.

In 2008–2011, eligible survivors were invited to participate in a cross-sectional study on late effects comprising completion of a mailed questionnaire and a 2-days out-patient examination at OUH which included echocardiography as previously reported (Hess et al., 2011). Reduced heart function (defined as the presence of aortic valve dysfunction and/or shortening fraction (the percent change in the internal diameter from diastole to systole) $\leqslant$28% and/or pro-brain natriuretic peptide (pro-BNP) $\geqslant$20 pmol/L) was observed in 70 patients (30%) but was not considered as a reason for exclusion because the majority of the survivors had asymptomatic (sub-clinical) findings of reduced heart function (Hamre et al., 2012). Major psychiatric disorders, other organ failures, chronic infections, rheumatic and inflammatory diseases, major neurological diseases, major endocrine diseases and primary sleep disorders were not observed among the survivors. Survivors with on-going pregnancy ($n = 6$) or living with the diagnosis of a second cancer ($n = 5$) were excluded.

The cytokine values of the survivors who did not display CF served as controls.

2.2. Disease characteristics and treatment

The initial extent of the lymphomas was classified according to the Ann Arbor classification (Lister et al., 1989). If possible, the NHL and ALL were sub-grouped in relation to B and T-cell origin of the disease. Information about the cancer treatment was retrieved from the hospitals' medical records. Radiation dose was registered in Grays (Gy) and doses of cumulative anthracycline were converted into doxorubicin-equivalents according to recommendations (Fulbright, 2011).

2.3. Chronic fatigue

Fatigue was assessed by the 11 items of the Fatigue Questionnaire (FQ) (Chalder et al., 1993). One additional item asks for the duration of fatigue. Dichotomized (0, 0, 1, 1) scores are used for the definition of CF where CF is defined by a sum score of $\geqslant$4 of all 11 dichotomized items and a duration of $\geqslant$6 months (Chalder et al., 1993; Fukuda et al., 1994). Missing scores were substituted by the respondent's mean score across the completed items, if the respondent had responded to 50% or more of the scale's items. Other symptoms applied in the definition of chronic fatigue syndrome (CFS) (impaired memory, sore throat, tender lymph nodes, muscle pain, multi-joint pain, new headaches, unrefreshing sleep, post-exertion malaise) were not evaluated in our cohort, since these symptoms are not associated with fatigue in cancer patients (Bennett et al., 2007).

2.4. Blood sampling and cytokine assays

Fasting blood samples were drawn at 8 o'clock Day 2 of the clinical examination. Blood was allowed to clot and the serum was immediately frozen to a temperature of −70 °C after centrifugation.

The serum levels of 27 cytokines were analyzed in one batch by a multiplex cytokine assay (BioPlex Human Cytokine 27-Plex Panel, Bio-Rad Laboratories Inc.) comprising 27 cytokines, of which the following 17 were detected in amounts above the lower detection limit of the assay: Il-1ra, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12, fibroblast growth factor (FGF), eotaxin/CCL11, interferon inducing protein-10 (IP-10)/CXCL10, monocyte chemoattractant protein (MCP)-1/CCL2, macrophage inflammatory peptide (MIP)-1 β/CCL4, regulated and normal T cell expressed and secreted (RANTES)/CCL5, platelet-derived growth factor (PDGF), TNF, vascular endothelial growth factor (VEGF) and interferon (IFN)-γ. The following 10 markers were not detectable or displayed values close to the lower detection limit by the current assay and are not reported: IL-1 β, IL-2, IL-4, IL-5, IL-13, IL-15, IL-17, granulocyte colony stimulating factor (CSF), granulocyte and macrophage CSF, and MIP-1 β/CCL4.

The intra- and inter-assay coefficients of variation were 8.3% (range 5–15) and 7.6% (range 5–11), respectively. Coefficients of variation for each cytokine are displayed in Supplementary Table 1.

2.5. Clinical findings relevant to cytokine status

Reduced cardiac function was evaluated with blood tests/echocardiography. Individuals who reported current regular smoking were classified as smokers. Any report of the use of analgesics >1 month during the last year was classified as recent use of analgesics. The term "analgesic" in the questionnaire did not discriminate between NSAIDs and other analgesic drugs. Any report of use of anti-asthmatic medicine was defined as active asthma.

2.6. Statistics

Standard descriptive analyses (mean, standard deviation, median, range) were applied. To explore the association between CF and cytokine levels non-parametric tests were performed (Mann–Whitney U test, Kruskal–Wallis test in analyses comparing >2 groups). Since our material consisted of survivors of three different diagnostic groups, each diagnostic group underwent separate sub-analyses on CF and cytokine levels.

Logistic regression analysis was performed, with CF as the dependent variable, in order to reveal variables which were significantly associated with CF. First, the impact of possible confounders were explored in univariate analyses, Variables which displayed odds ratio's (OR) with p-values $\leqslant 0.1$, were included in the final analysis (diagnosis, age, gender, BMI and reduced heart function). The covariates B-symptoms at diagnosis and T-cell-origin were not applied in the multivariate analysis due to the high number of unclassified cases ($n = 141$ and $n = 140$, respectively). P-values <0.05 were considered statistical significant, but due to multiple testing, particular attention should be drawn against p-values <0.01. One participant with extreme values in 12 of the 17 cytokines was excluded.

SPSS for PC version 18 was used.

2.7. Ethics

The study was approved by Regional Committee for Medical Research Ethics.

3. Results

The present study included 232 out of the total population of adult survivors after childhood lymphoma and ALL (Fig. 1). There were no significant differences concerning age at diagnosis, age at survey or follow-up time between participants and non-participants.

Among the participants median (range) age at survey and diagnosis was 29.7 (18.6–54.5) years and 9.6 (0.3–18.0) years, respectively (Table 1). Median follow-up time was 21.5 (7.1–40.0) years. Compared to the lymphoma survivors, the ALL survivors were younger at diagnosis ($p = 0.001$) (Table 2).

Most of the ALL (90%) and the majority of the NHL (57%) survivors had been treated with chemotherapy only. Among the HL survivors, the majority had received radiotherapy in addition to chemotherapy (63%). A total of 15 survivors had received radiotherapy to the central nervous system (CNS), 12 being ALL survivors. Among the 62 survivors who had undergone treatment with mediastinal irradiation 90% were HL survivors.

3.1. Prevalence of CF and relation with other variables

A total of 66 of the survivors (28%) had CF, with the highest prevalence of CF among the HL survivors ($n = 26$, 36%), followed by the NHL survivors ($n = 12$, 29%) and the ALL survivors ($n = 28$, 24%), but the differences did not reach statistical significance

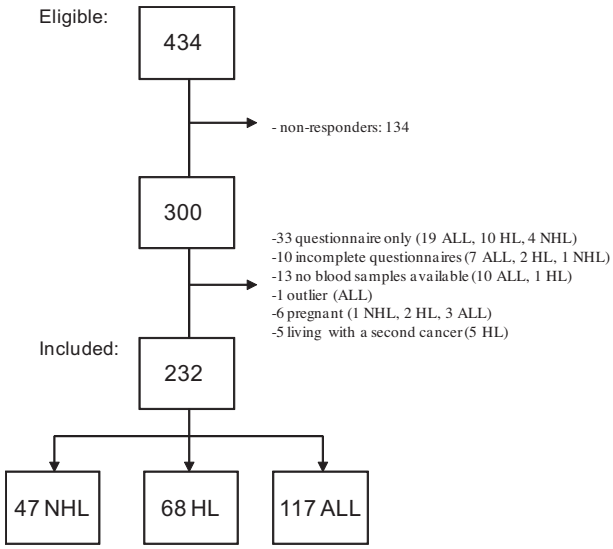

Fig. 1. Flowchart of eligible subjects, non-responders and excluded subjects.

($p = 0.08$) (Table 2). Older age at survey, lymphoma survivors with B-symptoms at diagnosis and NHL/ALL of T-cell origin displayed an increased risk of having CF (all $p \leqslant 0.05$, Table 3). Further, HL-survivors, survivors with elevated BMI and survivors with reduced heart function tended to have increased risk of CF, although not significantly ($p \leqslant 0.1$).

3.2. Levels of cytokines and relation with other variables

The levels of the 17 detectable cytokines varied between the three diagnostic groups, with particularly high levels in the ALL group (Table 4).

3.3. Cytokine levels and CF

When analyzing all three diagnostic groups together there were no significant differences in cytokine levels between survivors with CF compared to those without CF for any of the detectable cytokines (Table 5). However, when analyzing NHL survivors separately, survivors with CF had significantly raised serum levels of FGF, PDGF and eotaxin ($p < 0.05$) and in particular of IL-9 ($p = 0.008$) as compared with those without CF. In contrast, among the ALL and HL survivors there were no significant differences in cytokine levels between survivors with CF compared to those without CF.

No cytokine levels were associated to the presence of CF in a multivariate analysis when adjusting for diagnosis, age, gender, BMI and reduced heart function (Table 6).

4. Discussion

In this study of 232 unselected adult survivors after childhood lymphoma and ALL whereof 28% reported to have CF, no positive associations between CF and the levels of 17 cytokines were observed when including all the survivors in the analyses. This was also the case when analyzing the diagnostic subgroups separately, with an exception of a significantly raised concentrations of FGF, PDGF, eotaxin and in particular of IL-9 among the NHL survivors with CF. Collectively, these data for the first time demonstrate similar serum cytokine profiles in adult survivors after childhood lymphoma and ALL with and without CF, suggesting, though not

Table 1
Demographics, disease characteristics, treatment and physical health status (Hamre H: Serum cytokines and chronic fatigue).

	Total *N* = 232	NHL *N* = 42	HL *N* = 73	ALL *N* = 117
Demographics				
Age at survey, years[a]	29.7 (18.6–54.5)	30.0 (18.9–48.4)	35.0 (19.5–54.5)	28.4 (18.6–46.5)
Age at diagnosis, years[a]	9.6 (0.3–18.0)	12.2 (4.1–17.9)	14.7 (2.1–18.0)	5.0 (0.3–16.0)
Observation time, years[a]	21.5 (7.1–40.0)	19.0 (8.5–36.3)	20.7 (7.1–37.0)	22.5(7.4–40.0)
Female	116 (50%)	14 (33%)	39 (53%)	63 (54%)
Male	116 (50%)	28 (67%)	34 (47%)	54 (46%)
Disease characteristics				
T-cell subtype	12 (6%)	7 (17%)		5 (4%)
B-cell subtype	80 (34%)	19 (45%)		61 (52%)
Unknown	67 (29%)	16 (38%)		51 (44%)
Stage				
1–2	67 (29%)	21 (50%)	46 (68%)	
3–4	43 (19%)	21 (50%)	22 (32%)	
B-symptoms				
Yes	29 (13%)	11(26%)	18 (25%)	
No	62 (27%)	14 (33%)	48 (65%)	
Unknown	24 (10%)	17 (40%)	7 (10%)	
Treatment				
Radiotherapy only	14 (6%)	1 (2%)	13 (18%)	0
Chemotherapy only	143 (62%)	24 (57%)	14 (19%)	105 (90%)
Radiotherapy + chemo	75 (32%)	17 (41%)	46 (63%)	12 (10%)
Radiotherapy CNS	15 (6%)	3 (7%)	0	12 (11%)
Radiotherapy mediastinum	62 (27%)	6 (14%)	56 (77%)	0
Anthracycline, yes	173 (75%)	39 (93%)	45 (62%)	89 (76%)
Cumulative AC dose, mg/m^2[b,c]	120 (40–510)	150 (50–460)	160 (50–401)	120 (40–510)
Physical health status				
Smokers	71 (31%)	15 (36%)	20 (27%)	36 (31%)
BMI > 30 kg/m^2	25 (11%)	4 (10%)	4 (5%)	17 (15%)
Reduced heart function[d]	70 (30%)	5 (11%)	33 (45%)	32 (27%)
Analgetic use > 1 month last year	29 (13%)	10 (24%)	5 (7%)	14 (12%)

[a] Median, range.
[b] Pr patient who has received anthracycline (AC). Doxorubicine equvalents.
[c] Mean ± SD.
[d] Five patients missed heart examination.

Table 2
Number (%) of patients with chronic fatigue (CF), total and pr. diagnosis (Hamre H: Serum cytokines and chronic fatigue).

	Total *N* = 232	NHL *N* = 42	HL *N* = 73	ALL *N* = 117
CF (% of total)	66 (28)	12 (29)	26 (36)	28 (24)
Gender				
Men (% CF)	34/116 (29)	8/14 (29)	12/39 (35)	14/63 (26)
Women (% CF)	32/116 (28)	4/28 (29)	14/34 (36)	14/54 (22)
Lymphogeneic origin				
T-cell derived disease (% CF)	**8/12 (67)**[b]	4/7 (57)		**4/5 (80)**[b]
B-cell derived disease (% CF)	13/80 (16)	3/19 (16)		10/61 (16)
Unknown (% CF)	19/67 (28)	5/16 (31)		14/51 (28)
B-symptoms				
Yes (% CF)	**14/29 (48)**[a]	4/11 (36)	10 /18(56)	
No (% CF)	17/62 (27)	3/14 (21)	14/48 (29)	
Unknown (% CF)	7/24 (29)	5/17 (21)	2/7 (29)	
Cranial irradiation				
Yes (% CF)	4/15 (23)	1/3 (33)		3/12 (25)
No (% CF)	62/217 (29)	11/39 (28)	26/73 (36)	25/105 (24)

Significant associations given in bold letters
[a] $p < 0.05$
[b] $p \leqslant 0.01$, unknown not included in analyses

excluding, that cytokines are not major biologic players in CF among these survivors.

While the presence and clinical relevance of fatigue as a late effect after cancer has been confirmed during the last decades, the links between fatigue and inflammation remain vague. A meta-analysis from 2007 and a recent review concluded that selected cytokines (IL-1ra, IL-6, IL-1β and neopterin may be linked to cancer-related fatigue (Saligan and Kim, 2012;Schubert et al., 2007). However, among the few studies that have investigated the association between cytokine levels and fatigue among tumor free cancer survivors, only IL-1ra has been found to be associated with fatigue in more than one study, two including survivors after breast cancer and one after testicular cancer (mean age at study, 57, 54 and 47 years, respectively) (Bower et al., 2002;Collado-Hidalgo et al., 2006; Orre et al., 2009). On the other side, such association has not been confirmed in other studies by the same research groups

Table 3
Chronic fatigue and other variables. Logistic regression analysis. Odds ratio (OR) reported with 95% confidence interval (CI). $N = 232$, whereof $N_{CF} = 66$, $N_{non\text{-}CF} = 166$. Levels of cytokines applied as pg/mL:100 (Hamre H: Serum cytokines and chronic fatigue).

	OR (95% CI)	*p*-value
Age	1.04 (1.00–1.1)	0.03
Female gender	1.09 (0.6–1.9)	0.8
Diagnosis		
ALL	(ref)	
NHL	1.3 (0.6–2.8)	0.6
HL	1.8 (0.9–3.3)	0.08
Smoking	1.34(0.7–2.5)	0.3
BMI (kg/m^2)	1.1 (1.0–1.1)	0.1
Regular use of analgesics	1.6 (0.7–3.7)	0.2
Reduced heart function	1.8 (1.0–3.3)	0.06
T-cell origin		
No	(ref)	
Yes	10.3 (2.7–39.3)	0.01
Unknown	1.7 (0.7–3.9)	0.2
CNS-irradiation	0.9 (0.3–2.9)	0.9
B-symptoms at diagnosis		
No	(ref)	
Yes	2.5 (1.0–6.2)	0.05
Unknown	1.1 (0.4–3.1)	0.9

p-values < 0.1 given in bold characters

(Bower et al., 2011;Orre et al., 2011), both studies performed on breast cancer survivors, mean age at study 51 years and 55 years, respectively. In a recent longitudinal study of breast cancer patients, no associations could be found between the levels of eight cytokines (IL-1β, IL-1ra, IL-6, IL-10, IL-12, TNF, IFN-γ, neopterin) and fatigue at any time point from start of treatment until 6 months after treatment compared to controls (mean age at survey 52 years) (Cameron et al., 2012). In the only published study investigating the association between fatigue and levels of IL-1ra, IL-6 and neopterin among male and female survivors after hematological malignancies (adulthood NHL, HL and leukemia, mean age at study 51 years), no associations were observed three months after end of treatment (Dimeo et al., 2004). Finally, the present study examining a wide range of inflammatory markers in a fairly large and representative cohort of childhood lymphoma and ALL lend very limited or no support to the hypothesis that CF is associated with different levels of cytokines.

Most previous studies of cytokines and fatigue in cancer survivors have been performed in female survivors of breast cancer who were older at survey compared to the survivors in the present study. The recruitment to these studies has focused on thoroughly selected patients avoiding potentially disturbing comorbidities (Bower et al., 2002, 2011; Cameron et al., 2012; Collado-Hidalgo et al., 2006). We, in contrast, chose to encircle a larger and unselected population of fatigued cancer survivors. This approach increased the risk of confounding biological factors across the sample. However, even if confounding factors were adjusted for in a multivariate analysis we could still not show any association between cytokine levels and the presence of CF.

When analyzing the association between cytokine levels and CF by diagnoses, FGF, eotaxin and PDGF, and in particular IL-9 were significantly elevated in NHL survivors with CF compared to the NHL survivors without CF, but based on the multiple testing performed, only the IL-9 finding should attract some attention. This cytokine has been linked to activation of a particular T cell subset (Th9), which is associated with the promotion of allergic responses in the guts and the lungs, and in asthma in particular (Stassen et al., 2012). However, when revising the questionnaires, only one of the 42 NHL survivors reported active asthma (8 in the total material of 232). Further, IL-9 has been associated to increased autoimmune inflammation of the CNS in mice models (Li et al., 2011), but any link to sickness behavior would be speculative. Indeed, the numerical differences of IL-9 were modest, and at present the clinical implication is unclear. Further investigation in larger study populations should be performed. In contrast to NHL, these findings were not confirmed when analyzing cytokine levels among the survivors of ALL and HL with and without CF. The NHL group was the smallest group ($N = 42$). Further, while HL and ALL were considered to be the two most distinctive diseases, NHL shares disease and treatment characteristics with both ALL and HL. We were therefore surprised that the NHL group was the only group with some significant findings.

Our cohort displayed detectable but very low levels of IL-4 and IL-5, which stands in contrast to findings in non-cancer CFS populations (Fletcher et al., 2009).

The cytokine expression per se differed between the three diagnostic groups, with significantly higher levels in the ALL group compared to the HL group. The reasons for this are unclear, but differences in lymphogenic origins, in age at diagnosis and treatment modalities could be involved. Notably, these differences were not related to the presence or absence of CF.

Table 4
Cytokine levels, total and pr. diagnosis (Hamre H: Serum cytokines and chronic fatigue).

Cytokine	Total $N = 232$	NHL $N = 42$	HL $N = 73$	ALL $N = 117$
Il-1ra	99 (31–918)	94 (52–918)	95 (43–418)	105 (31–583)
IL-6	7 (2–341)	8 (2–193)	7 (2–341)	8 (3–185)
IL-7	8 (0–64)	8 (3–18)	10 (1–33)	**5 (0–64)**[a]
IL-8/CXCL8	13 (1–36)	13 (3–36)	15 (3–28)	**10 (1–27)**[a]
IL-9	27 (1–1630)	27 (1–1289)	25 (1–213)	**28 (7–1630)**[a]
IL-10	10 (0–360)	10 (2–28)	7 (0–31)	**11 (0–360)**[a]
IL-12	18 (0–420)	21 (2–73)	19 (2–58)	19 (0–420)
FGF	19 (0–95)	21 (0–95)	21 (0–52)	14 (0–75)
Eotaxin/CCL11	119 (0–3337)	138 (0–3337)	96 (0–2763)	**119 (9–420)**[a]
IP-10/CXCL10	1118 (273–11,110)	1202 (336–4091)	864 (273–11,110)	**1160 (49–7716)**[a]
MCP-1/CCL2	36 (10–123)	32 (13–92)	30 (10–123)	**39 (15–109)**[a]
MIP-1 β/CCL4	82 (23–290)	91 (29–223)	77 (28–290)	85 (23–254)
RANTES/CCL5	22,734 (1951–7 × 10^5)	17,253 (1951–7 × 10^5)	19,798 (2212–7 × 10^5)	**27,237 (4120–1 × 10^5)**[b]
PDGF	11,7684 (63–32,488)	10,438 (95–21,996)	10,753 (63–32,488)	**129,086 (3444–24,773)**[a]
TNF-α	44 (0–364)	38 (0–253)	43 (0–141)	**48 (1–364)**[a]
VEGF	66 (0–350)	65 (0–117)	52 (0–315)	79.0 (0–350)
IFN-γ	109 (26–1390)	84 (26–319)	85 (29–282)	**134 (29–1390)**[b]

Significant associations given in bold letters, all values given as pg/mL, median (range).
[a] Significantly higher compared to HL-group ($p \leqslant 0.01$).
[b] Significantly higher compared to both NHL and HL group ($p \leqslant 0.01$).

Table 5
Cytokines and CF, total and pr. diagnosis (Hamre H: Serum cytokines and chronic fatigue).

	Total (N = 232)		NHL (N = 42)		HL (N = 73)		ALL (N = 117)	
	Non-CF	CF	Non-CF	CF	Non-CF	CF	Non-CF	CF
IL-1ra	98(31–918)	99(41–428)	92(52–918)	114(77–428)	95(43–418)	94(48–210)	105(31–582)	105(41–184)
IL-6	7(2–341)	7(2–293)	6(2–109)	8(5–293)	7(3–341)	7(2–13)	8(3–186)	9(3–29)
IL-7	8(0–64)	9(0–23)	7(4–14)	9(5–18)	10(1–33)	10(3–23)	5(0–64)	7(0–18)
IL-8/CXCL8	12(1–28)	14(3–36)	12(2–23)	15(7–36)	15(6–28)	15(3–25)	9(1–27)	12(3–23)
IL-9	27(1–1631)	28(3–1289)	24(1–99)	39[b](12–1289)	25(1–91)	20(3–213)	30(7–1631)	26(8–131)
IL-10	10(0–360)	10(0–64)	9(2–28)	14(3–28)	9(0–31)	7(1–23)	10(0–360)	110–64
IL-12	18(1–420)	21(0–73)	19(2–39)	26(9–73)	18(2–50)	20(2–59)	18(1–420)	20(0–65)
FGF	18(0–75)	23(0–94)	19(1–49)	31[b](0–95)	21(0–52)	20(0–37)	11(0–75)	25(0–44)
Eotaxin/CCL11	116(0–2764)	126(0–3337)	125(0–378)	192[a](7–3337)	100(0–2764)	87(0–208)	119(9–421)	127(38–341)
IP-10/CXCL10	1094(273–6656)	1160(334–1.1 × 10^4)	1147(336–4091)	1269(515–2287)	965(273–3518)	665(336–11112)	1150(498–6656)	1165(512–4416)
MCP-1/CCL2	32(13–123)	37(11–101)	30(13–87)	37(17–92)	30(13–123)	33(11–83)	37(15–109)	43(15–100)
MIP-1 β/CCL4	82(23–254)	84(28–291)	95(29–182)	74(54–222)	78(32–154)	75(28–291)	82(23–254)	94(44–199)
RANTES/CCL5	22211(1950–7.0 × 10^5)	26370(2327–7.0 × 10^5)	17253(1950–2.5 × 10^5)	17487(2326–6.9 × 10^5)	19798(2212–7.0x10^5)	18905(3292–4.0 x10^5)	26815(4120–1.1x10^5)	29628(6596–8.5x10^4)
PDGF	11598(95–2.6 × 10^4)	12305(63–3.2 × 10^4)	10150(95–2.0 × 10^4)	13094[a](8704–2.2 × 10^4)	10753(502–2.6 × 10^4)	10754(63–3.2 × 10^4)	12911(5330–2.3 × 10^4)	12315(3444–2.5 × 10^4)
TNF-α	43(0–363)	44(0–83)	35(0–253)	46(12–83)	44(4–141)	43(0–66)	47(1–363)	49(5–79)
VEGF	67(0–350)	63(0–316)	65(0–177)	64(26–170)	55(6–222)	47(0–315)	80(5–350)	72(0–298)
IFN-γ	112(25–3390)	103(28–281)	82(26–319)	100(52–160)	91(31–218)	82(29–281)	134(44–1390)	135(28–219)

Significant associations given in bold letters, all values given as pg/mL, median (range).
[a] $p < 0.05$.
[b] $p < 0.01$.

Table 6
Univariate and multivariate analysis of cytokine level's association to CF. Odds ratio (OR) reported with 95% confidence interval (CI). N = 232, whereof N_{CF} = 66, $N_{non\text{-}CF}$ = 166. Levels of cytokines applied as pg/mL:100. (Hamre H: Serum cytokines and chronic fatigue).

	Univariate		Multivariate	
	OR	p-value	OR[a]	p-value
IL-1ra	0.842 (0.6–1.3)	0.4	0.9 (0.6–1.3)	0.5
IL-6	1.09 (0.5–2.6)	0.8	1.0 (0.5–2.4)	0.9
IL-7	2.4 (0.04–160)	0.7	2.1 (0.02–224)	0.7
IL-8/CXCL8	51.8 (0.46–5809)	0.1	32.2 (0.2–5346)	0.2
IL-9	1.03 (0.9–1.2)	0.7	1.0 (0.8–1.2)	0.9
IL-10	0.6 (0.1–3.0)	0.6	0.5 (0.06–3.3)	0.4
IL-12	0.8 (0.3–2.1)	0.7	0.7 (0.2–2.0)	0.5
FGF	5.1 (0.8–33.2)	0.1	5.2 (0.6–43.6)	0.1
Eotaxin/CCL11	1.04 (0.9–1.1)	0.4	1.0 (0.9–1.1)	0.5
IP-10/CXCL10	1.01 (1.0–1.0)	0.2	1.0 (0.9–1.1)	0.3
MCP-1/CCL2	14 (0.4–5.7)	0.6	1.7 (0.3–8.5)	0.5
MIP-1 β/CCL4	2.18 (1.0–4.7)	0.04	1.8 (0.8–4.1)	0.2
RANTES/CCL5	1.0 (1.0–1.0)	0.3	1. 0 (1.0–1.0)	0.3
PDGF	1.0 (0.9–1.0	0.2	1.0 (1.0–1.0)	0.3
TNF-α	0.64 (0.3–1.6)	0.4	0.6 (0.2–1.7)	0.3
VEGF	0.9 (0.5–1.4)	0.6	0.8 (0.5–1.3)	0.4
IFN-γ	0.7 (0.5–1.1)	0.2	0.7 (0.4–1.3)	0.3

[a] Adjusted for diagnosis, age, gender, BMI, reduced heart function.

We did not state a formal hypothesis upfront but aimed at observing some findings which would indicate or even confirm former results. Even though we were not able to demonstrate major associations between cytokine levels and CF among these childhood lymphoma and ALL survivors, a possible association between cytokines and CF among long-term cancer survivors cannot be ruled out based upon these findings. Firstly, cytokines are pleiotrophic of origin, and their biological activities are known to be context specific. Small aberrances in single cytokine levels may imply clinically relevant homeostatic changes that may not be traceable with rough statistical methods as applied in our study (Saligan and Kim, 2012). Next, cytokines act at the cellular levels and lack of differences in systemic levels does not rule out differences in the cellular microenvironment. In addition, CF is defined as a subjective experience, and the symptom is best regarded as multifactorial (Stone and Minton, 2008b). Thus, potentially diverse mechanisms may have influenced with mechanisms of inflammatory origin among our unselected population of survivors. Finally, the long observation time imply that CF may have been caused by other factors than in a cohort studied much closer to active cancer disease and anti-cancer treatment.

In order to standardize the definition of cancer-related fatigue, diagnostic criteria have been proposed (Cella et al., 1998). However, Donovan et al. demonstrated in a recent review that there was a lack of consistency in how the criteria for cancer-related fatigue have been applied (Donovan et al., 2012), and a broader revision of the criteria was asked for. The case definition of CFS includes the presence of chronic fatigue but also requires additional symptoms (musculoskeletal pain and influenza-like manifestations) that are not observed in chronic fatigued cancer survivors (Bennett et al., 2007). The exclusion criteria for CFS have moved towards a more exhaustive recommendation (Fukuda et al., 1994; Reeves et al., 2003). The latest recommendations allow patients with minor medical conditions to be included, while patients with major, permanent medical and psychiatric conditions are excluded. As compared to sufferers of CFS, the additional symptoms were not specifically assessed in the present cohort, but our exclusion of pregnant survivors and those with a second cancer is judged to be in line with present recommendations for CFS.

4.1. Limitations

The inter-group variation between the three diagnostic groups of immunologic activity was not foreseen and may have weakened the power of the univariate analysis of CF and cytokine levels. However, the separate analyses revealed some interesting findings in the NHL group which would have been overlooked otherwise.

In order to deepen the understanding of the unexpected variation of cytokine activity across the diagnostic groups, results from a control group consisting of healthy individuals without a history of cancer would have been warranted. Furthermore, even if the pre-analytic conditions were standardized across the sample, we cannot rule out pre-analytic circumstances that may have influenced upon the fast decomposition of cytokines. The lack of resolution of the assay with respect to low levels of 10 of 27 cytokines may have interfered with a possibility of performing relevant analyses of these cytokines. However, the majority of the values of IL-2, IL-15 and IL-17 were above lower detection limit, but displayed very low values, and, at least in our opinion, should be regarded as unreliable. Moreover, even if these data were included in the analyses, we found no association between the cytokines and CF in any of the patient subgroups or in the patient group as a whole (data not shown).

The lack of detailed information of the use of NSAIDSs should be regarded as a limitation of the study.

Some potential factors that may have impacted on the cytokine levels, such as alcohol intake and physical activity, were not considered.

5. Conclusions

Except for a possible association between high IL-9 levels and CF in NHL survivors, we could not find any association between a wide range of cytokine and related mediators and CF in a large population of adult survivors after childhood ALL and lymphoma. Further investigation of this frequent and distressing late effect after cancer is necessary in order to identify explicative mechanisms.

6. Conflict of interest statement

All authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2013.01.006.

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