Mental fatigue after allogeneic hematopoietic stem cell transplantation is associated with cognitive dysfunction, but not central nervous system inflammation

For long-term survivors of allogeneic hematopoietic stem cell transplantation (aHSCT), persisting fatigue remains a significant problem 5 years after treatment.[1] Fatigue is a subjective feeling of exhaustion with physical, mental and emotional manifestations, not relieved by rest.[2] In addition to fatigue, a large proportion of cancer patients suffer from cognitive dysfunction (CD), affecting processing speed, memory and executive function which limit work capacity and everyday life.[3]

Elevated peripheral inflammatory cytokine levels correlate with fatigue after solid tumor treatment.[4] Likewise, increased interleukin (IL)-6 and soluble tumor necrosis factor α have been reported in the peripheral blood of aHSCT survivors with fatigue.[5] However, mechanistic studies as to the contribution of central nervous system (CNS) inflammation in the pathophysiology of fatigue remain largely absent.[4]

Current treatment for persistent fatigue and CD after cancer therapy is cognitive behavioral therapy, physical activity and education programs, aiming to reduce symptom burden.[2] In order to detect patients that would benefit from such interventions, systematic measurement of fatigue and CD during follow-up after cancer therapy and aHSCT is necessary.

The aim of this study was to correlate quantitatively assessed mental fatigue and CD to CNS inflammation. The impact of the symptoms was evaluated through measurement of quality of life and degree of employment. Importantly, we applied instruments that can be used without previous neuropsychological training.

The study was conducted at Karolinska University Hospital (KUS), Huddinge, Sweden, with patients classified as 1) self-reported symptoms of mental fatigue and a men-

Table 1. Clinical characteristics of the study population.

	Fatigue (n=14)	No Fatigue (n=13)	*P*
Age: mean (range)	51.7 (22-69)	55.5 (23-73)	0.24[#]
Sex (male): n (%)	5 (36%)	7 (54%)	0.45[†]
BMI at time of transplant: mean (range)	26.17 (19.05-28-41)	23.41 (21.24-32.54)	0.019*[#]
Highest education level: n (%)			
Elementary school	0 (0%)	1 (8%)	0.20[†]
High school	6 (43%)	2 (15%)	
College/University	7 (50%)	9 (69%)	
Premorbid IQ[c]: mean (range)	111.1 (106.6-122.2)	115.2 (96.2-122.2)	0.45[#]
Time from transplant to inclusion (months): mean (range)	30.6 (14-62)	30.7 (12-61)	0.98[#]
Donor type: n			
MUD	9 (64%)	8 (62%)	1[†]
SIB	4 (29%)	4 (31%)	
Haploidentical	1 (7%)	1 (8%)	
Underlying disease: n			
AML	5 (36%)	11 (85%)	0.079[†]
CML	1 (7%)	0 (0%)	
MDS	2 (14%)	2 (15%)	
PMF	3 (21%)	0 (0%)	
Myeloma	1 (7%)	0 (0%)	
CLL	1 (7%)	0 (0%)	
Sickle cell anemia	1 (7%)	0 (0%)	
Conditioning regimen			
Busulfan: n (%)	9 (64%)	10 (76%)	0.68[†]
Cyclophosphamide: n (%)	3 (21%)	2 (15%)	1[†]
Fludarabine: n (%)	11 (79%)	11 (85%)	1[†]
Treosulfan: n (%)	5 (36%)	3 (23%)	0.68[†]
Thiotepa: n (%)	2 (14%)	0 (0%)	0.48[†]
Immune reconstitution			
Days from transplant to neutrophils > 0.5 x 10^9/L: Days (range)	17.36 (12-28)	16.23 (11-19)	0.83[#]
CMV and EBV			
CMV mismatch: n (%)	5 (36%)	3 (23%)	0.68[†]
CMV reactivationd: n (%)	7 (50%)	4 (31%)	0.44[†]
EBV mismatch: n (%)	0 (0%)	1 (8%)	0.48[†]
EBV reactivatione: n (%)	2 (14%)	1 (8%)	1[†]

GvHD prophylaxis: n (%)			
ATG	10 (71%)	8 (62%)	0.69†
Ciclosporin	9 (64%)	10 (76%)	0.47†
Tacrolimus	4 (29%)	1 (8%)	
Tacrolimus + sirolimus	1 (7%)	2 (15%)	
GvHD: n (%)			
Acute[a]	9 (64%)	7 (54%)	0.70†
Chronic[b]	5 (36%)	4 (31%)	1†
Systemic immunosuppressive treatment at time of sampling: n (%)			
Corticosteroids	5 (36%)	2 (15%)	0.38†
Calcineurin inhibitors	3 (21%)	1 (8%)	0.59†
Other	1 (7%)	0 (0%)	1†
Any	5 (36%)	2 (15%)	0.38†
Regular opioid, antidepressive or anxiolytic medication: n (%)			
SSRI	3 (21%)	0 (0%)	0.22†
Opioids	2 (14%)	0 (0%)	0.48†

[a]Previous acute GvHD requiring steroid treatment. [b]Previous/current chronic GvHD. [c]Swedish National adult reading test (SWE-NART) score. [d]Defined as CMV DNA >1,000 copies/mL. [e]Defined as EBV DNA >1,000 copies/mL. [a]Wilcoxon Rank Sum Test (continuous data with non-parametric distribution according to the Shapiro-Wilk test), †Fisher's exact test (categorical data). *P<0.05. BMI: body mass index; IQ: intelligence quotient; MUD: matched unrelated donor; SIB: matched sibling donor; Haplo: haploidentical donor; AML: acute myeloid leukemia; CML: chronic myeliod leukemia; MDS: myelodysplastic syndrome; PMF: primary myelofibrosis; CLL: chronic lymphocytic leukemia; CMV: cytomegalovirus; EBV: Epstein Barr virus; GvHD: graft-*versus*-host disease; ATG: anti-thymocyte globulin; SSRI: selective serotonin reuptake inhibitor.

tal fatigue scale [MFS] ≥14 points or 2) absence of self-reported symptoms of mental fatigue and a MFS ≤10 points. Patients were in complete hematological remission, fluent in Swedish, aged >18 years, and underwent aHSCT 1-5 years ago (*Online Supplemental Table S1*). The study was approved by the regional ethics committee, Stockholm and all patients provided written consent. Brain magnetic resonance imaging (MRI) excluded other causes of mental fatigue, or CD, and signs of increased intracranial pressure, prior to lumbar puncture (LP) and peripheral blood sampling.

The patient medical history was obtained from hospital medical records, and occupational status and education from patient self-reports. Extent of mental fatigue and classification of participants were assessed using the MFS6. The fatigue severity scale (FSS) assessed impact of fatigue on functioning.[7] Quality of life was measured using the functional assessment of cancer therapy – bone marrow transplant (FACT-BMT) scale.[8] Patients with significant depression were excluded.

To analyze cognitive function we selected five tests, assessing executive function, visual memory and attention/processing speed, (*Online Supplementary Table S3*) from the computer-based Cambridge Neuropsychological Test Automated Battery (CANTAB).[9] This allowed a clinically convenient test duration (30-40 minutes), with no previous neuropsychological training of the administrator. Premorbid IQ-levels were estimated using the Swedish national adult reading test (SWE-NART). Z-scores were derived from normative data, and matched for age and SWE-NART score, before conversion to a deficit score (DS; 0-5). DS were averaged to derive a global deficit score (GDS), reflecting overall performance.[10] CD was defined as GDS>0.5.

Clinical CSF analyses were performed by the Department of Clinical Chemistry, KUS. Analyses of viral DNA was performed by PCR (Department of Clinical Microbiology, KUS). Protein microarray analysis was conducted by Sciomics GmbH (Heidelberg, Germany; see the *Online Supplementary Materials and Methods*). Flow cytometry of CSF was performed by the Department of Pathology, KUS (see the *Online Supplementary Table S4*). Peripheral blood mononuclear cells were isolated and immunophenotyping of T ($CD3^+CD56^-$), B ($CD19^+$), natural killer (NK) ($CD3^-CD56^+$) and NKT cell ($CD3^+CD56^+$) was performed (see the *Online Supplementary Materials* and *Methods and Online Supplementary Table S4*). Naïve and memory T-cell subsets were separated by CD45RO expression (CSF) and CD45RA and CD27 expression (blood). T and NK cells were classified as activated based on CD25 expression.

Of 31 study patients, four pateints were omitted early after inclusion (one with CSF and MRI findings indicating demyelinating disease, one with remnants of intracerebral hemorrhage detected on MRI, one that experienced a hematological relapse after inclusion and one that chose to withdraw from the study), with the remaining 27 including 14 with mental fatigue ("fatigued") and 13 without mental fatigue ("non-fatigued"). One fatigued patient declined MRI and LP. The only difference in baseline characteristics was a higher body mass index at time of transplant in fatigued patients (P=0.019; Table 1). Seven fatigued patients were classified as having CD (Figure 1A). No patient in the non-fatigued group had CD. The greatest difference was in mean simple reaction time, where significantly more fatigued patients showed impairment (P=0.006; Figure 1B) compared to the non-fatigued. Since only fatigued patients had CD, they were subdivided into fatigued patients +/- CD (n=7/group).

The impact of fatigue on functioning was more evident in the fatigued group (P=0.0001; Figure 1C), with a significantly worse quality of life (P=0.0003; Figure 1D). Following exclusion of patients aged ≥65 years (retirement age), fatigued patients had a lower rate of employment (55% *vs.* 93% of full-time, P=0.01; Figure 1E[i]) compared to non-fatigued patients. Interestingly, only fatigued patients with CD had a lower rate of employment compared to non-fatigued controls (Figure 1E[ii]).

MRI revealed unspecific white matter lesions in several fatigued and non-fatigued patients. A few patients in both groups exhibited vascular degeneration and/or subclinical ischemic lesions. CSF analyses were comparable between fatigued and non-fatigued patients, however, significantly

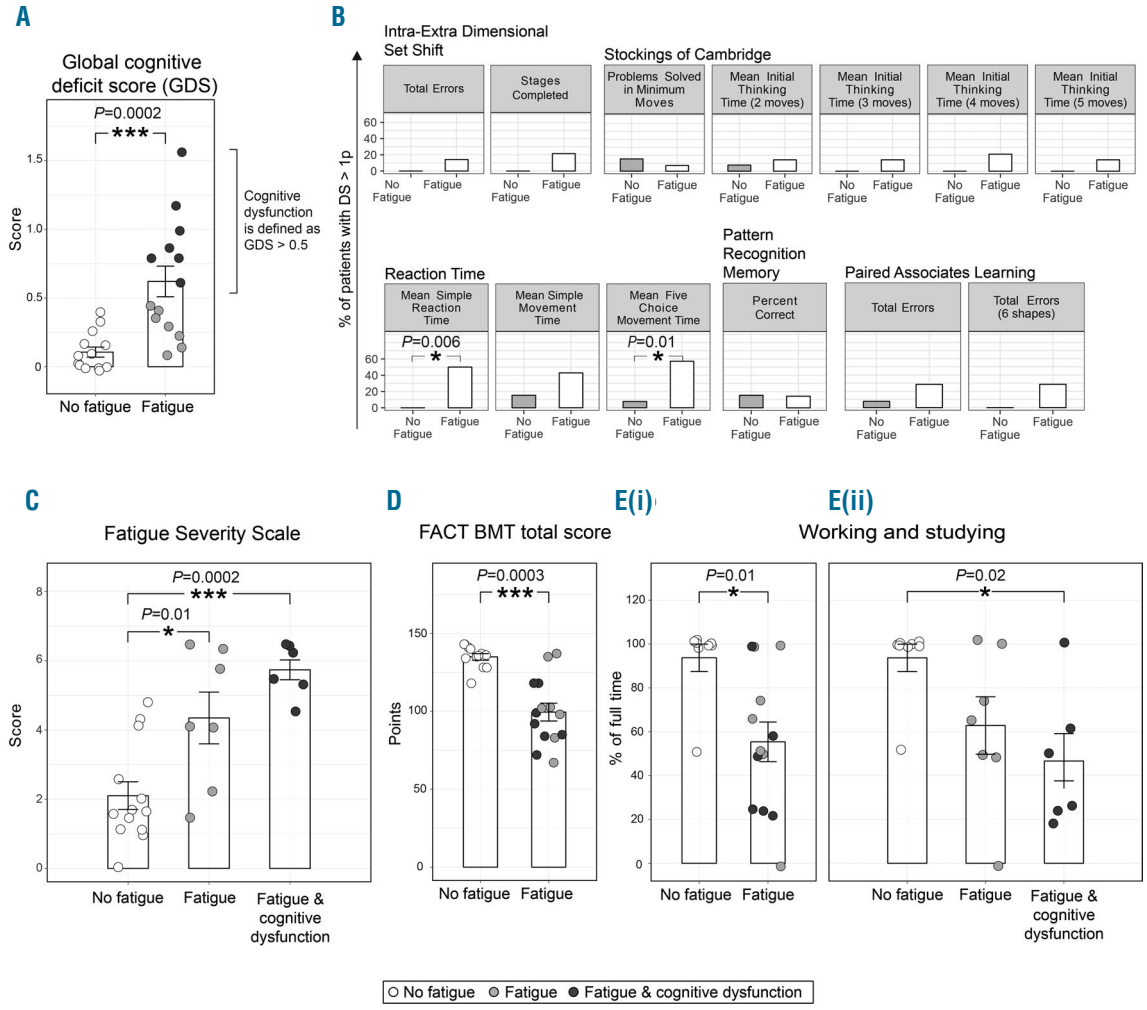

Figure 1. Patients with persistent fatigue have a decreased quality of life, cognitive function and degree of employment compared to patients without fatigue. (A) Seven patients in the fatigue group and no patients in the control group had a GDS≥0.5 hence classified as having cognitive dysfunction. (B) Cognitive impairment on individual tests (DS≥1 or Z-score≤-1) were mostly distributed across domains tested, but a larger number of patients in the fatigue group had impairment in "Mean simple reaction time" and "Mean five choice movement time". (C) Patients with fatigue had higher fatigue severity scale (FSS) scores, compared to the control patients, indicating a higher degree of impact on functioning from fatigue. (D) Functional assessment of cancer therapy – bone marrow transplant (FACT-BMT) scores were lower in the fatigue group, suggesting lower quality of life compared to the control group. (E[i]) The rate of employment among patients under the retirement age in Sweden (65 years) was lower in the fatigue group compared to the control group. (E[ii]) Further, when dividing the fatigue group based on cognitive function, only the patients with cognitive dysfunction had a lower rate of employment compared to the control group. *P*-values using Wilcoxon Rank Sum test or Kruskal Wallis test. **P*<0.05, ***P*<0.005, ****P*<0.0005. GDS: global deficit score; DS: deficit score.

more non-fatigued patients had S100B levels outside of the normal range (8 *vs.* 3; *P*=0.047; *Online Supplementary Tables S6-7*). A few patients within both groups had slightly abnormal levels of IgG index, albumin, CSF/P-albumin ratio, chemokine (C-X-C motif) ligand (CXCL)13, IL-8, tau/phospho-tau or neurofilament light chain (*Online Supplementary Table S8*), but underlying neurological disease was ruled out with clinical and MRI examinations. No significant differences between fatigued and non-fatigued patients were evident with microarray analysis of CSF (Figure 2A-B and *Online Supplementary Table S11*), or flow cytometry assessment of immune cell subsets and activation markers in CSF and blood (Figure 2C-F and *Online Supplementary Table S8-9*). *Post hoc* power calculations indicated that a larger sample size is required to detect the small differences in immune cell levels measured (*Online Supplementary Table S5*). In addition, a hypothesis-driven analysis of the data was performed, where the flow cytometry results were adjusted for age, sex and time since transplant using linear regression models, and where the correction for multiple comparisons was removed from the cytokine analysis. This analysis most notably revealed significantly higher levels of double negative T cells ($CD4^-CD8^-$) in the blood and CSF (*Online Supplementary Table S10*), as well as increased levels of IL-37 in CSF (*Online Supplementary Table S11*), of fatigued patients with cognitive dysfunction. For a detailed discussion, refer to the *Online Supplementary Materials and Methods.*

Fatigue and CD are long-term consequences of cancer therapy and aHSCT, yet often overlooked during clinical follow-up.[11] Structured measurements of these disabilities are important for appropriate and timely targeted rehabilitation and workplace adjustments. Furthermore, it increases the understanding and acceptance of these symptoms, both among care-workers and affected patients. Where cognitive problems prevent affected individuals from working, it may also facilitate communication with the proper authorities, and qualification for health insurance pay-outs. In this study, fatigue questionnaires and CANTAB testing identified patients with mental fatigue and CD, where MRI

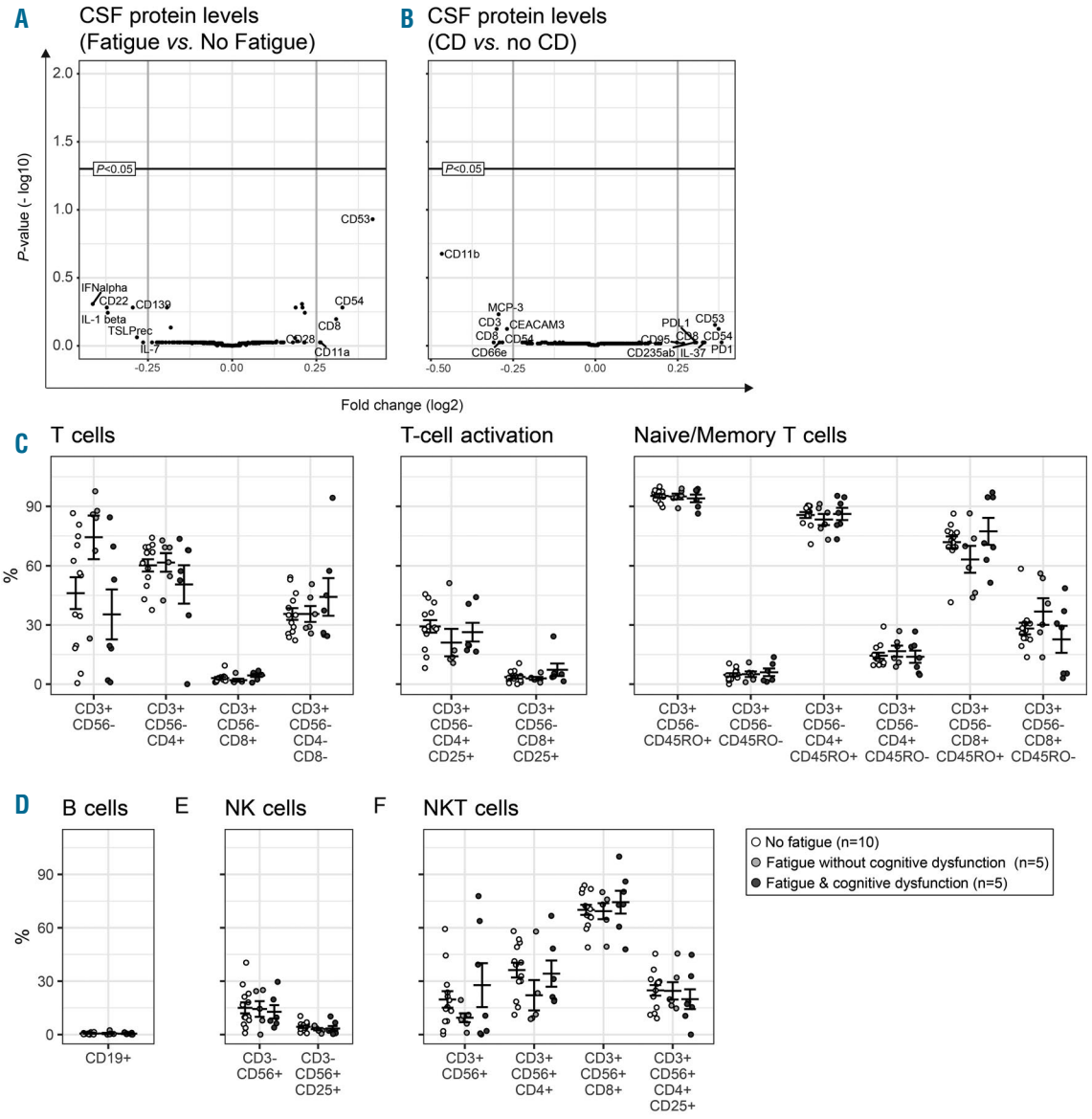

Figure 2. No difference in CSF protein or immune cell subsets in patients with and without fatigue or cognitive dysfunction. (A) and (B) The levels of 25 cytokines/chemokines and 93 cell surface markers were evaluated in a multiplex microarray analysis on cerebrospinal fluid (CSF) from 20 study participants. Data are presented as volcano-plots of all proteins evaluated, where a lower *P*-value is represented higher up on the y-axis and a greater distance from 0 on the x-axis represents a greater magnitude of difference between the groups. Any protein located above the indicated lines would be statistically significant different between the groups. No significant differences were found between patients with or without fatigue or cognitive dysfunction (CD) for any protein in the array. (C-F) CSF flow cytometry analysis revealed no differences in the levels of T (C), B (D), NK (E) and NKT cells (F). The analysis included naïve/memory T cells, as well as activation of T, NK and NKT cells assessed by CD25 expression. *P*-values with Wilcoxon rank-sum test for non-parametric data (according to Shapiro-Wilk test) and Student's t-test for parametric data. SEM: standard error of the mean; IL: interleukin; MCP: monocyte chemoattractant protein; PD: programmed cell death; PDL: programmed death ligand; GM-CSF: granulocyte macrophage colony stimulating factor; IFN: interferon; TNF: tumor necrosis factor; LIF: leukemia inhibitory factor; TSLP: thymic stromal lymphopoietin; CCL: CC chemokine ligand; NK cell: natural killer cell; NKT cell: natural killer T cell.

and CSF analyses have excluded other symptom-associated conditions. To make evaluation of cognitive function a part of follow-up, easily accessible and ready-to-use assessment tools are required. The use of screening tools has been questioned by expert groups such as the national comprehensive cancer network based on concerns that they may not be sensitive enough to address subtle cognitive decline.[12] CANTAB was able to classify a subgroup of aHSCT survivors as having mild cognitive impairment in this study. These individuals had the lowest degree of employment, emphasizing that these tests selected those with relevant disability. Cognitive impairments varied between individuals, a result that highlights the importance of using GDS. Using a neuropsychologist to interpret the results of the cognitive tests may provide deeper insights in future studies. CD has not consistently been shown to correlate with reduced ability to return to work after cancer therapy.[13] This may be explained by the use of self-reported assessments. Performance-based measures are considered the "gold standard" for measuring cognitive function, however, their results often do not strongly correlate with self-perceived cognitive function.[13] In contrast to previous studies on solid tumor patients, our study found a striking correlation between fatigue and CD.[14-16] This is likely due to our use of the MFS, which specifically measures mental fatigue, and is known to correlate to objectively measured

information processing speed.[17] Alternatively, the correlation may be due to a different pathophysiological mechanism causing fatigue and CD in aHSCT recipients compared to solid tumor patients. The fact that the fatigued patients had a variety of underlying hematological disorders, points to aHSCT as the common denominator in causing mental fatigue.

Previous studies of long-term fatigue and CD after hematological malignancies and aHSCT treatment have evaluated peripheral inflammation.[5,18] This is the first time that such an analysis of the CSF immune compartment has been conducted with respect to fatigue post-aHSCT. While the limited study size and cross-sectional design prevents us from drawing firm conclusions, and studying cause and effect, some speculation can be made as to why we did not detect ongoing inflammation. Patient samples were taken on average 30 months post-HSCT, evaluating long-term chronic CNS inflammation. The absence of chronic CNS inflammation seen in our study, could suggest an acute inflammatory response, after aHSCT, that resolves over time, yet causes irreversible CNS damage that results in persistent mental fatigue. aHSCT conditioning, as well as, post-transplant infections and graft-*versus*-host disease result in acute inflammation with increased levels of peripheral blood cytokines.[19] Alternatively, as seen in previous studies, the mental fatigue may be caused by persisting peripheral cytokines that indirectly affect the CNS through vagus nerve activation.[2]

In summary, we report, for the first time, a combinatory evaluation of neuropsychological testing with biological analyses to assess the role of CNS inflammation in mental fatigue after aHSCT. The absence of a correlation between chronic CNS inflammation and fatigue suggests that, if inflammation is involved, acute inflammatory insults, rather than persisting activity should be evaluated. Notably, the hypothesis-driven analyses suggest a difference in biology between fatigued patients with and without CD, a finding warranting further investigation. Larger prospective studies should be initiated early on after transplant in order to study immune activity changes in parallel with cognitive symptoms and fatigue development. The current study shows that fatigue questionnaires and cognitive screening batteries are adequate to identify persistent fatigue and CD in long-term survivors after aHSCT. We therefore emphasize their implication in clinical practice to allow early detection and interventions to improve the reduced quality of life associated with these symptoms.

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