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Distinct cytokine profiles across trajectories of self-perceived cognitive impairment among early-stage breast cancer survivors

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ABSTRACT

The aim of the current study is to identify distinct cytokine profiles in relation to self-perceived cognitive trajectories. In our study cohort (n = 128), early-stage breast cancer patients were categorized into no impairment reported, acute, delayed, persistent and intermittent cognitive decline respectively. Pro-inflammatory cytokines were elevated compared to baseline; with TNF-α implicated in the acute cognitive trajectory while IL-6 and IL-8 were involved in the persistent cognitive trajectory. Our findings help to further our understanding of cytokine profiles implicated in cancer-related cognitive impairment (CRCI) and support the use of cytokine levels as biomarkers of cognitive decline over time.

1. Introduction

Cancer-related cognitive impairment (CRCI) is a problem widely reported by cancer patients prior, during and after treatment, with a detrimental impact on their quality of life (Janelsins et al., 2014; Wefel et al., 2015). While the underlying etiology of CRCI is multifactorial, immune function has often been proposed as a key contributing factor (Olson and Marks, 2019). The upregulation of intrathecal cytokine production and immune cell recruitment in combination with a disruption of the blood brain barrier (BBB) occurs during neuroinflammation (Lepennetier et al., 2019). Cytokines which play an important role in cell signaling to regulate immune response is of research interest (Ren et al., 2017). They may elicit local inflammation through oxidative and nitrosative processes or induce a state of chronic inflammation which may affect neuronal and glial cell functioning (Lange et al., 2019; Olson and Marks, 2019). Given that most of the current chemotherapeutic drugs are not known to penetrate the blood brain barrier (BBB) due to their large molecular sizes, it has been postulated that tumor biology and/or cancer treatment itself trigger downstream pro-inflammatory pathways that lead to neurological changes via cytokines (Cheung et al., 2013). Alternatively, chemotherapy-induced oxidative stress may have altered the structure and integrity of the BBB, leading to an amplified release of cytokines (Gaman et al., 2016). Such a candidate mechanism for change has also been supported by animal studies, in which neurobiological changes that underlie cognitive changes are found to be a result of increased peripheral inflammation and disrupted BBB in rodent tumor models (Santos and Pyter, 2018). In the context of inflammation, peripheral cytokine dysregulation has been shown to induce cognitive changes (Craig J Wilson et al., 2002).

To date, several clinical studies conducted in breast cancer patient cohorts (Cohen et al., 2019; Lyon et al., 2016; Patel et al., 2015; Williams et al., 2018) and imaging studies (Kesler et al., 2013; Pomykala et al., 2013) had reported elevated levels of pro-inflammatory cytokines to be positively associated with cognitive deficits. Cytokines appeared to have a role in cell-signaling in neural communication relating to cognitive processes in other neurological disease states such as Alzheimer's disease (Taipa et al., 2019). Distinct cytokine profiles had been reported for patients who showed resilience to Alzheimer's disease, in which there had been an upregulation of cytokines in the affected brain regions, suggesting an inflammation resolving microenvironment (Barroeta-Espar et al., 2019). In particular, our research group had previously reported elevated concentrations of IL-6 and IL-1β to be observed in breast cancer patients with poorer response speed performance and perceived cognitive disturbances in a cohort study (Cheung et al., 2014a). While many studies had shown how cytokine levels correlate with cognition, there had been wide inter-individual variability and the implicated cytokines identified tend to differ between studies. To bridge the gap in our understanding, there presents a need to evaluate the cytokines beyond their inflammatory nature.

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Given that the degree of CRCI had been found to differ over time and during patients' treatment trajectories, our research group had previously characterized self-perceived cognitive trajectories into five distinctive types (Ng et al., 2018). There were patients who did not report any self-perceived cognitive impairment during the study period compared to patients who experienced cognitive impairment during varying phases of treatment ranging from the early phase, after the end of treatment to during survivorship respectively. Cognitive decline of some sub-group of patients also did not follow any patterns of treatment trajectory. The existence of heterogenous cognitive trajectories seemed to suggest that the biomechanisms underlying different CRCI trajectories may differ. In order to evaluate the role for cytokines as peripheral inflammatory mediators that may directly or indirectly influence cognition, this study aims to identify distinct cytokine profiles across the varying self-perceived cognitive trajectories. Identifying the cytokines that correlate with CRCI over time may serve as potential biomarkers and may help us understand how cytokines may influence neuroinflammation and contribute to CRCI. These findings will have clinical application in allowing us to devise targeted management strategies to mitigate CRCI, considering that a large proportion of cancer patients are long-term survivors.

2. Material and methods

2.1. Study design

This was a multicentre prospective cohort study conducted between 2014 and 2017. The study was approved by the Singhealth Institutional Review Board (CIRB 2014/754/B) and written informed consent was obtained from all participants.

Patients were eligible if they satisfied the following inclusion criteria: (i) at least 21 years of age, (ii) diagnosed with early-stage breast cancer, (iii) scheduled to receive chemotherapy with curative intent, (iv) had no prior history of chemotherapy and/or radiation therapy and (v) able to understand either English or Chinese. Patients who had incomplete blood samples, did not complete questionnaires at all the assessed time points, or clinically diagnosed with neurocognitive disorders such as Alzheimer's disease, dementia, depression and/or anxiety disorder, were excluded from data analysis.

2.2. Study procedure

Patients were tasked to complete the study questionnaire at the following time points: baseline, prior to start of chemotherapy (T1), approximately 6 weeks after chemotherapy initiation (T2), approximately 12 weeks after chemotherapy (T3) and a post 1-year follow-up after end of treatment (T4). Relevant demographic and clinical data were collected through patient interviews and electronic health records. A 10-mL blood sample was drawn from each patient at T1, T2 and T3.

2.3. Cognitive impairment

For the evaluation of cognitive function, Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog) (version 3) was used. It is a validated questionnaire used to assess the impact on patient's quality of life within the past 7 days (Vardy et al., 2006). The minimal clinical important difference (MCID) approach was used to categorize patients into status of cognitive impairment, based on a difference of 10.6 points reduction in the global FACT-Cog score between the assessed time points (Cheung et al., 2014b). Each of the patient was categorized into five distinct types of cognitive trajectories: no clinically significant cognitive impairment reported during the study period, acute (clinically significant cognitive decline reported at either T2 or T3, but not at T4), delayed (clinically significant cognitive decline reported at only T4), persistent (clinically significant cognitive decline reported at both T3 and T4) and intermittent decline (clinically significant cognitive decline reported at T2 and T4, but not at T3) (Ng et al., 2018).

2.4. Quantification of cytokines

At each assessed time point, a 10-mL blood sample was collected in ethylenediaminetetraacetic acid (EDTA) tube in the morning. The timing of blood collection had been standardized in order to minimize diurnal variation from affecting the fluctuation of measured cytokine levels (Haack et al., 2004). The blood sample was centrifuged at 2500 rpm for 10 min within 30 min of collection and stored at −80 °C until further analysis. A multiplex panel of cytokine consisting granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10 was quantified using 50 μL of each sample via the Luminex immunoassay, in duplicate readings. The readings with coefficient of variation above 15% were not included, while samples that were below the lower limit of quantification were replaced with zeros (Cheung et al., 2014a). Cytokines were classified based on their immunological function: Pro-inflammatory cytokines would include IFN-γ, TNF-α, IL-1β, IL-6 and IL-8 while those belonging to the anti-inflammatory category include GM-CSF, IL-2, IL-4 and IL-10 (Cohen et al., 2019; Henneghan et al., 2018).

2.5. Statistical analysis

Descriptive statistics was used to summarize the demographics, clinical characteristics and prevalence of cognitive impairment of the study cohort. The Kolmogorov-Smirnov test was conducted to test for the normality of the distribution of the dependent variables. The cytokine levels across time points were subsequently trended by their immunological function and trajectories, in medians (interquartile range, presented as first quartile - third quartile). Friedman test was used to compare the change in levels of the respective cytokine levels across the time points, followed by post-hoc Wilcoxon sign rank tests between the time points, with Bonferroni correction. The non-parametric Mann-Whitney *U* test was used to compare difference in cytokine levels between cognitively impaired and non-cognitively impaired group, where appropriate. All statistical analyses were carried out using SPSS version 23.0, with two-sided *p*-values less than 0.05 considered as statistically significant.

3. Results

3.1. Clinical and demographic characteristics

Out of the total of 217 participants recruited, 128 patients with complete blood samples and questionnaires were analyzed (Fig. 1). The age and years of education, expressed in mean ± standard deviation (SD), were 51.8 ± 8.9 and 10.8 ± 3.4 years respectively. Majority of the patients were Chinese (82.8%), had stage II breast cancer (68.7%) and received anthracycline-based chemotherapy (68.0%) (Table 1). With reference to baseline, there was a greater proportion of patients with self-perceived cognitive impairment with time: 15.6% at T2, 18.8% at T3 and 32.0% at T4. The proportion of patients with self-perceived impairment by the varying trajectories were as follows: 59.4% reported no cognitive decline, 15.6% reported delayed impairment, 12.5% reported persistent impairment, 8.6% reported acute impairment while 3.9% reported intermittent impairment (Table 2). There were no major differences in baseline demographic between the trajectory groups (Supplementary Table 1) or treatment profiles between the cognitively impaired and non-cognitively impaired group (Supplementary Table 2).

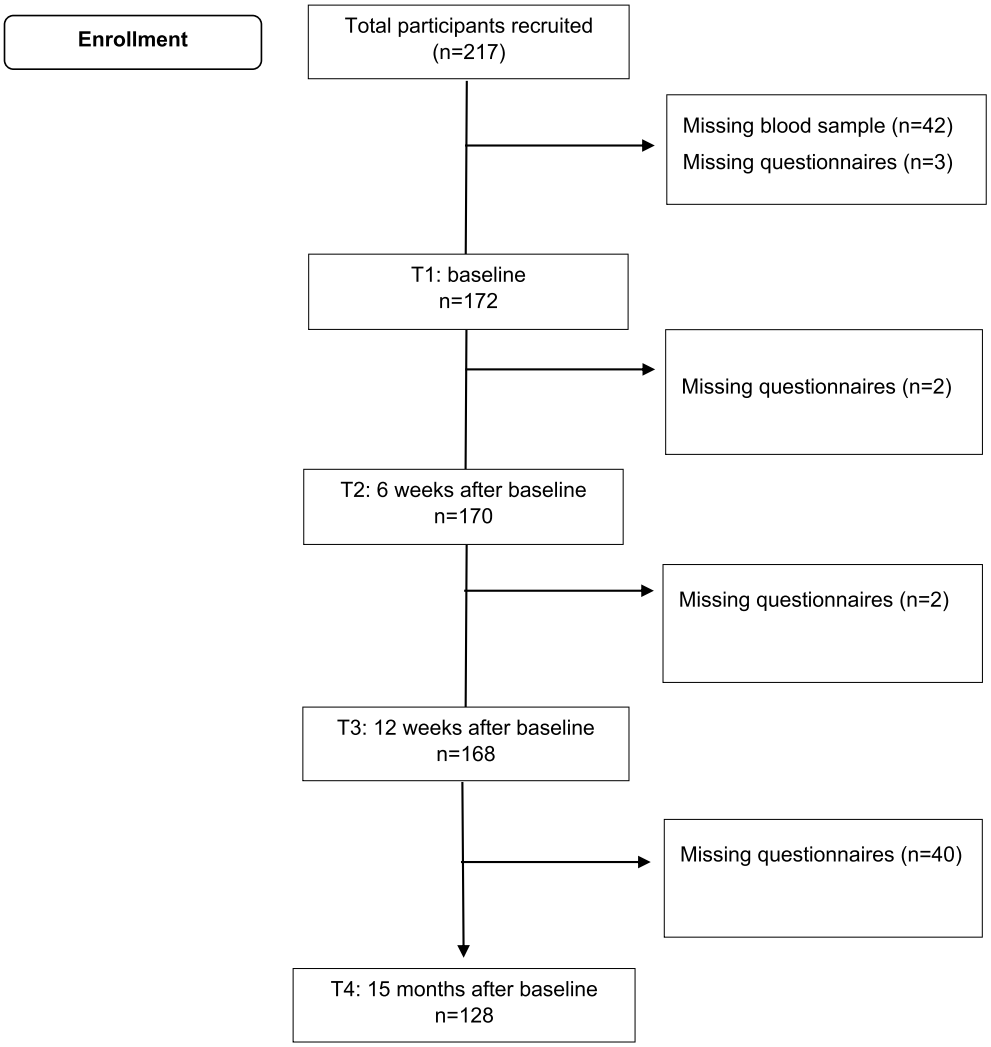

Fig. 1. Flow diagram for number of subjects analyzed (n = 128).

3.2. Respective cohort cytokine levels across time points

The plasma levels of the cohort's pro-inflammatory cytokines were plotted across timepoints (Supplementary Fig. 1). In terms of pro-inflammatory cytokines, significant changes over time were identified in median levels (interquartile range) of IFN-γ (T1: 2.29 (0.18–13.15) pg/mL, T2: 1.60 (0.00–13.30) pg/mL and T3: 2.20 (0.09–17.32) pg/mL, p = .039), TNF-α (T1: 6.07 (1.94–14.65) pg/mL, T2: 5.50 (1.30–17.78) pg/mL and T3: 6.80 (1.77–19.40) pg/mL, p < .001), IL-6 (T1: 0.43 (0.00–1.13) pg/mL, T2: 0.57 (0.00–1.76) pg/mL and T3: 0.64 (0.00–1.87) pg/mL, p = .003) and IL-8 respectively (T1: 3.59 (2.16–4.91) pg/mL, T2: 3.38 (2.14–5.20) pg/mL and T3: 3.67 (2.40–5.33) pg/mL, p = .002) (Table 3). For post-hoc p-values, changes in cytokine levels were significant at T2 vs T1 for IL-6 (p = .001); at T3 vs T2 for TNF-α (p < .001) and IL-8 (p = .002); at T3 vs T1 for TNF-α (p = .007) and IL-6 (p = .001).

Conversely, for anti-inflammatory cytokines, significant changes in median levels of GM-CSF were detected over time (T1: 0.00 (0.00–0.09) pg/mL, T2: 0.00 (0.00–0.79) pg/mL and T3: 0.00 (0.00–4.58) pg/mL, p < .001); IL-4 (T1: 0.08 (0.00–0.31) pg/mL, T2: 0.10 (0.00–0.39) pg/mL and T3: 0.24 (0.00–0.54) pg/mL, p < .001) and IL-10 (T1: 0.00 (0.00–0.00) pg/mL, T2: 0.00 (0.00–0.00) pg/mL and T3: 0.00 (0.00–0.00) pg/mL, p = .011). For post-hoc p-values, changes in cytokine levels were significant at T3 vs T2 for IL-4 (p = .008) and IL-10 (p = .015); at T3 vs T1 for GM-CSF (p = .001) and IL-4 (p < .001).

3.3. Cytokine levels by self-perceived cognitive trajectories

The median cytokine levels for pro-inflammatory TNF-α, IL-6 and IL-8 were plotted for the self-perceived cognitive trajectories that had been implicated in the impaired group (Fig. 2).

In terms of cognitive trajectories, for patients who did not report any impairment during the study period, there were statistically significant changes over time identified in median levels (interquartile range) of the following cytokines: GM-CSF (T1: 0.00 (0.00–0.34) pg/mL, T2: 0.00 (0.00–1.54) pg/mL and T3: 0.03 (0.00–7.18) pg/mL, p = .005); TNF-α (T1: 6.97 (1.94–16.69) pg/mL, T2: 7.13 (1.05–19.05) pg/mL and T3: 8.53 (1.92–22.80) pg/mL, p = .015); IL-4 (T1: 0.12 (0.00–0.29) pg/mL, T2: 0.17 (0.00–0.44) pg/mL and T3: 0.27 (0.00–0.56) pg/mL, p = .011); IL-6 (T1: 0.49 (0.00–1.20) pg/mL, T2: 0.57 (0.00–1.67) pg/mL and T3: 0.61 (0.04–2.32) pg/mL, p = .032); IL-8 (T1: 3.89 (2.12–5.18) pg/mL, T2: 3.65 (2.15–5.28) pg/mL and T3: 4.09 (2.44–5.55) pg/mL, p = .045) and IL-10 (T1: 0.00 (0.00–0.00) pg/mL, T2: 0.00 (0.00–0.00) pg/mL and T3: 0.00 (0.00–0.00) pg/mL, p = .025) (Table 4). For post-hoc p-values, changes in cytokine levels were significant at T3 vs T2 for TNF-α (p = .011), IL-8 (p = .012), at T3 vs T1 for IL-4 (p < .001).

For patients in the acute impairment trajectory, there were statistically significant changes identified in median levels of pro-inflammatory TNF-α (p = .017). In comparison to the patient group who did not report cognitive impairment, the absolute levels for those in

Table 1
Demographics and clinical information of patients (n = 128).

Characteristic		Data (mean ± SD or n (Frequency in %))
Age (years)		51.8 ± 8.9
Body mass index (kg/m^2)		24.4 ± 4.3
Education (years)		10.8 ± 3.4
Education (levels)	None	3 (2.3)
	Primary	15 (11.7)
	Secondary	62 (48.4)
	Pre-University	24 (18.8)
	Graduate and above	24 (18.8)
Ethnicity	Chinese	106 (82.8)
	Malay	12 (9.4)
	Indian	5 (3.9)
	Others	5 (3.9)
Marital status	Single	26 (20.3)
	Married	90 (70.3)
	Divorced	10 (7.8)
	Widowed	2 (1.6)
Breast cancer stage	I	14 (10.9)
	II	88 (68.7)
	III	26 (20.3)
ECOG performance status	0	124 (96.9)
	1	4 (3.1)
Chemotherapy regimen	Anthracycline based	87 (68.0)
	Taxane based	41 (32.0)
Radiation exposure	Exposure to radiotherapy	87 (68.0)
	No exposure to radiotherapy	41 (32.0)
Hormonal therapy	On Hormonal Therapy	106 (82.8)
	Not on hormonal therapy	22 (17.2)
Menopausal status	Pre-menopausal	66 (51.6)
	Post-menopausal	62 (48.4)

Table 2
Prevalence of self-perceived cognitive impairment by time points relative to baseline.

	Proportion of patients reporting self-perceived impairment based on FACT-Cog Total score, N (%)		
Timepoint	At T2	At T3	At T4
Number of patients	20 (15.6)	24 (18.8)	41 (32.0)
Trajectory		**From T1-T4**	
None		76 (59.4)	
Acute		11 (8.6)	
Delayed		20 (15.6)	
Persistent		16 (12.5)	
Intermittent		5 (3.9)	

Each of the patient was categorized into five distinct types of cognitive trajectories: none (no clinically significant cognitive impairment reported during the study period), acute (clinically significant cognitive decline reported at either T2 or T3, but not at T4), delayed (clinically significant cognitive decline reported at only T4), persistent (clinically significant cognitive decline reported at both T3 and T4) and intermittent decline (clinically significant cognitive decline reported at T2 and T4, but not at T3).

acute impairment trajectory (referred earlier) were lower across all three time points (T1: 3.75 vs 6.97 pg/mL, p = .47; T2: 3.30 vs 7.13 pg/mL, p = .61; T3: 5.11 vs 8.53 pg/mL, p = .66). For post-hoc p-values, change in cytokine levels between timepoints was not found to be significant.

For patients in the persistent impairment trajectory, there were changes over time identified for the following cytokines: GM-CSF (p = .013), IL-1β (p = .017), IL-4 (p < .001), IL-6 (p = .002) and IL-8 (p = .028). The changes in pro-inflammatory IL-1β levels (T1: 0.14 (0.00–0.40) pg/mL, T2: 0.13 (0.02–0.26) pg/mL and T3: 0.29 (0.00–6.01) pg/mL); IL-6 (T1: 0.35 (0.00–1.29) pg/mL, T2: 0.77 (0.13–1.96) pg/mL and T3: 1.21 (0.50–2.82) pg/mL) and IL-8 (T1: 3.14 (2.72–4.31) pg/mL, T2: 3.12 (2.18–4.52) pg/mL and T3: 3.28 (2.69–5.35) pg/mL) were reported to be elevated. For post-hoc p-values, changes in cytokine levels were significant at T2 vs T1 for IL-6 (p = .013); T3 vs T2 for IL-4 (p = .010); T3 vs T1 for IL-4 (p = .002) and IL-6 (p = .002).

In comparison to the non-cognitively impaired patient group, the differences between cytokine levels for persistent impairment trajectory (referred earlier) were not statistically significant: anti-inflammatory GM-CSF (T1: 0.00 vs 0.00 pg/mL, p = .56, T2: 0.00 vs 0.00 pg/mL, p = .66 and T3: 0.00 vs 0.03 pg/mL, p = .76); anti-inflammatory IL-4 (T1: 0.01 vs 0.12 pg/mL, p = .53, T2: 0.00 vs 0.17 pg/mL, p = .17 and T3: 0.29 vs 0.27 pg/mL, p = .84); IL-6 (T1: 0.35 vs 0.49 pg/mL, p = .68, T2: 0.77 vs 0.57 pg/mL, p = .44 and T3: 1.21 vs 0.61 pg/mL, p = .14) and IL-8 (T1: 3.14 vs 3.89 pg/mL, p = .50 T2: 3.12 vs 3.65 pg/mL, p = .38 and T3: 3.28 vs 4.09 pg/mL, p = .67).

For cognitive impairment that were of delayed onset and intermittent trajectories, no statistically significant changes were observed with cytokine levels over time.

For visual comparison, the cytokine levels were plotted on the logarithmic scale across the five self-perceived cognitive trajectory (Supplementary Fig. 2) and for the individual cytokines (Supplementary Fig. 3).

4. Discussion

In our cohort of early-stage breast cancer patients, 40.6% of patients (n = 52) had reported some type of self-perceived cognitive impairment during the study period. We had observed significant changes in cytokine levels across the different phases of the patient's treatment. Among the participants who had reported cognitive impairment, median cytokine levels were elevated at T3 compared to baseline for: pro-inflammatory TNF-α (T1: 5.12 pg/mL, T2: 5.53 pg/mL and T3: 5.31 pg/mL), IL-6 (T1: 0.53 pg/mL, T2: 0.87 pg/mL and T3: 0.93 pg/mL) and anti-inflammatory IL-4 (T1: 0.31 pg/mL, T2: 0.41 pg/mL and T3: 0.45 pg/mL). Levels of pro-inflammatory IL-8 was elevated for persistent cognitive impairment group (T1: 3.14 pg/mL, T2: 3.12 pg/mL and T3: 3.28 pg/mL).

TNF-α is known to trigger signaling in astrocytes and hippocampal synaptic alteration (Habbas et al., 2015) and higher levels of soluble TNF-α receptor I and II had been found to be associated with poorer performance in visual memory (Williams et al., 2018). Likewise, IL-6 triggers inflammation, has been implicated in systemic inflammation in various diseases and aging (Cohen et al., 2019) and its circulating levels are suggested to be indicator for cognitive health in healthy adults (Bradburn et al., 2017). In literature, elevated plasma levels of IL-6 had been linked to worsening cognitive function, in the domain of executive function (Meyers et al., 2005). In particular, elevated plasma IL-6 levels were found to cause hippocampal inflammation that lead to memory decline experienced by breast cancer patients undergoing radiotherapy (Shibayama et al., 2019). This finding is also consistent with a previous study conducted by our research group which showed elevated IL-6 concentration being associated with greater extent of self-perceived cognitive disturbance and may be attributed as physiological inflammatory response to chemotherapy (Cheung et al., 2014a). Our findings of cytokine elevations in participants who had reported self-perceived cognitive impairment corroborated with how CRCI is known to be associated with elevated levels of pro-inflammatory cytokines (Olson and Marks, 2019).

In our cohort, levels of anti-inflammatory IL-4 were also elevated compared to baseline. Although IL-4 had been found to be associated with both immediate and delayed recall in domain of verbal memory (Henneghan et al., 2018), our previous findings suggested higher concentration of IL-4 to be associated with better response speed performance over 12 weeks, post-chemotherapy (Cheung et al., 2014a). From this, we gained an understanding that it will be imperative for us to consider the balance and/or ratio of the quantitative cytokine levels rather than evaluating solely based on the nature of the cytokines. In

Table 3
Plasma cytokine levels with significant change over time points (n = 128).

	Concentration (pg/mL) Median (interquartile range)			Friedman Test	Post-hoc Wilcoxon Sign Rank Test		
	T1	T2	T3	p-value*	T2 versus T1, p-value	T3 versus T2, p-value	T3 versus T1, p-value
Pro-inflammatory							
IFN-γ	2.29 (0.18–13.15)	1.60 (0.00–13.30)	2.20 (0.09–17.32)	**0.039**	0.918	0.029	0.102
TNFα	6.07 (1.94–14.65)	5.50 (1.30–17.78)	6.80 (1.77–19.40)	**0.001**	0.754	< **0.001**	**0.007**
IL-6	0.43 (0.00–1.13)	0.57 (0.00–1.76)	0.64 (0.00–1.87)	**0.003**	**0.001**	0.139	**0.001**
IL-8	3.59 (2.16–4.91)	3.38 (2.14–5.20)	3.67(2.40–5.33)	**0.002**	0.803	**0.002**	0.027
Anti-inflammatory							
GM-CSF	0.00 (0.00–0.09)	0.00 (0.00–0.79)	0.00 (0.00–4.58)	< **0.001**	0.033	0.101	**0.001**
IL-4	0.08 (0.00–0.31)	0.10 (0.00–0.39)	0.24 (0.00–0.54)	< **0.001**	0.029	**0.008**	< **0.001**
IL-10	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	**0.011**	0.112	**0.015**	0.512

* Bolded are *p*-values that showed statistical significance. For post-hoc test, corrected *p*-values are set as < 0.016.

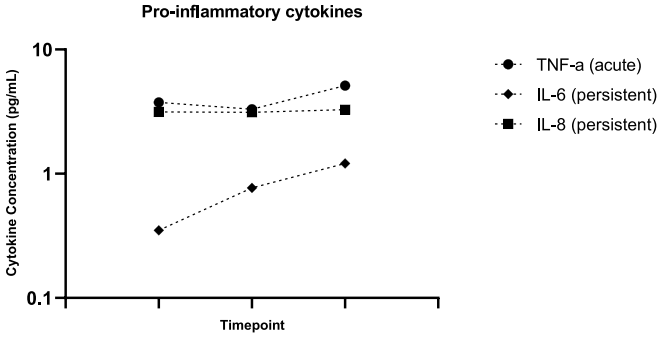

Fig. 2. Pro-inflammatory cytokine levels for affected self-perceived cognitive trajectories.
The median cytokine levels are plotted along a logarithmic axis. Timepoint 1, 2, 3 and 4 refers to baseline assessment, approximately 6 weeks after chemotherapy initiation, approximately 12 weeks after chemotherapy and post-year follow-up after end of treatment respectively. Dashed lines indicate *p*-values for change of cytokine level across time points showed statistical significance ($p < .05$).

the literature, the relationship between cytokine specificity with neural networks underlying cognitive function has been proposed to be non-linear (Henneghan et al., 2018). These other parameters are especially relevant given how cytokines may interact with other neurodegenerative biomarkers to influence cognitive functioning (Henneghan et al., 2020) and work in a network of physiological pathways to elicit downstream response. In order to fully appreciate the neurobiological mechanisms underpinning CRCI, differences in changes in cytokine levels will need to be further delineated.

When stratified by self-perceived cognitive trajectories, TNF-α was found to differ between acute impaired and non-impaired patient group, with a higher percentage of elevation in cytokine levels for the former group. For those who reported acute impairment, the elevation in cytokine levels between T3 vs T1 was 36.2% compared to 22.3% in non-impaired group. Between non-impaired and persistent patient group, anti-inflammatory GM-CSF and IL-4 as well as pro-inflammatory IL-6 and IL-8 were recurring cytokines affected in both groups. Although these differences in cytokine levels were not found to be statistically significant, there remains value in observing the temporal trends in cytokine levels. The time points at which significant changes in cytokine levels occur (upon post-hoc corrected tests) were not found to follow a consistent trend. However, elevations in cytokine levels for those belonging to persistent cognitive impaired group were highlighted: at T2 vs T1, increase in IL-6 was significant ($p = .013$); at T3 vs T2, increase in IL-4 levels was found to be significant ($p = .010$); at T3 vs T1, increase in both IL-4 ($p = .002$) and IL-6 ($p = .002$) were significant. Upon further investigation, the cytokines implicated in the different cognitive trajectory profiles appear to share immune cell profiling as there are similarities in the immunomodulatory pathways in which they are involved in.

Table 4
Plasma cytokine levels presented by self-perceived cognitive trajectories.

Trajectory	Concentration (pg/mL) Median (interquartile range)			Friedman Test	Post-hoc Wilcoxon Sign Rank Test		
	T1	T2	T3	p-value*	T2 versus T1, p-value	T3 versus T2, p-value	T3 versus T1, p-value
Non-impaired (*n* = 76)							
GM-CSF	0.00 (0.00–0.34)	0.00 (0.00–1.54)	0.03 (0.00–7.18)	**0.005**	0.076	0.225	0.018
TNF-α	6.97 (1.94–16.69)	7.13 (1.05–19.05)	8.53 (1.92–22.80)	**0.015**	0.510	**0.011**	0.018
IL-4	0.12 (0.00–0.29)	0.17 (0.00–0.44)	0.27 (0.00–0.56)	**0.011**	0.046	0.103	< **0.001**
IL-6	0.49 (0.00–1.20)	0.57 (0.00–1.67)	0.61 (0.04–2.32)	**0.032**	0.066	0.090	0.021
IL-8	3.89 (2.12–5.18)	3.65 (2.14–5.28)	4.09 (2.44–5.55)	**0.045**	0.706	**0.012**	0.040
IL-10	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	**0.025**	0.339	0.023	0.140
Acute (*n* = 11)							
TNF-α	3.75 (0.62–11.65)	3.30 (2.00–13.46)	5.11 (0.91–17.68)	**0.017**	0.284	0.037	0.075
Persistent (*n* = 16)							
GM-CSF	0.00 (0.00–0.00)	0.00 (0.00–1.66)	0.00 (0.00–6.01)	**0.013**	0.893	0.128	0.018
IL-1β	0.14 (0.00–0.40)	0.13 (0.02–0.26)	0.29 (0.05–0.48)	**0.017**	0.451	0.036	0.060
IL-4	0.01 (0.00–0.22)	0.00 (0.00–0.24)	0.29 (0.02–0.49)	< **0.001**	0.155	**0.010**	**0.002**
IL-6	0.35 (0.00–1.29)	0.77 (0.13–1.96)	1.21 (0.50–2.82)	**0.002**	**0.013**	0.088	**0.002**
IL-8	3.14 (2.72–4.31)	3.12 (2.18–4.52)	3.28 (2.69–5.35)	**0.028**	0.469	0.039	0.134

* Bolded are p-values that showed statistical significance. For post-hoc test, corrected p-values are set as < 0.016.

We had observed that pro-inflammatory cytokines implicated in acute cognitive trajectory (TNF-α) belong to T helper (Th)1 group while those implicated in the persistent (IL-6, IL-8 and anti-inflammatory GM-CSF) seemed to belong to Th17 (Kothur et al., 2016). Cytokines belonging to Th1 group are known to help with cell-mediated immunity and act on intra-cellular pathogens which may be processes that take precedence in the acute phases of immune response. Conversely, cytokines within the Th17 category are typically involved with neutrophilic inflammation in which they recruit other inflammatory cells such as monocytes by GM-CSF secretion and macrophages by IL-1β signaling. Th17 cells are also important for clearance of extracellular pathogens, regulation of autoimmunity and are often implicated in chronic inflammatory disease (Kothur et al., 2016). However, Th17 cells appear to be more stable under acute inflammation whereas under chronic inflammation, the cells would be vulnerable to display plasticity and a mixed phenotype (Maddur et al., 2012). It is proposed that cytokines of Th1 origin are more directly involved in the acute phase of CRCI while those that are implicated in the persistent impairment trajectory may be influenced by inflammatory environment and other factors that may cause a switch to alternative inflammatory cytokines in Th17 cells. Thus, understanding Th-mediated immunity and the effector cells related to the cytokines secretion may provide us with additional insights into the immunopathology of these disorders, and how immune alterations may contribute to cognitive deficits (Kothur et al., 2016). In fact, data from one study showed that distinct Th1-and Th17-related cytokines were able to differentiate the different phenotypes of multiple sclerosis, a chronic inflammatory disease of the central nervous system (Arellano et al., 2017). This may imply the value of distinguishing cytokine profiles and their relation to immune cell subtypes during neuroinflammation, in the context of CRCI.

In other studies looking at breast, colon and hematological malignancy population, some studies had reported no significant associations between pro-inflammatory cytokines such as IL-6 with cognitive function (Williams et al., 2018). Such discrepancy observed could be due to various factors. Firstly, given that biomarker levels exhibit high inter-individual variability, we may have to consider cytokine levels in relation to patient related factors like type of cancer and chemotherapy regimen received as well as biological factors such as levels of available receptors and physiological fluctuations (Olson and Marks, 2019). The type of chemotherapy regimen and exposure to certain drugs had been cited as a contributing factor, in which IL-6 levels were increased in patients undergoing doxorubicin-based chemotherapy group but were decreased in the patients receiving cyclophosphamide, methotrexate and fluorouracil chemotherapy (Janelsins et al., 2012). Secondly, the cognitive domains assessed may also be a factor. For instance, although IL-8 was classified as pro-inflammatory and expected to be associated with cognitive decline, its levels had been reported to be positively correlated with memory (Olson and Marks, 2019) and optimal levels of executive function in other studies (Henneghan et al., 2018). Apart from identifying the cytokine implicated in CRCI, it may be clinically meaningful for future studies to investigate the extent of difference or threshold cytokine levels that needed to be exceeded for certain signs and symptoms of CRCI to manifest. A strength of our study is that we have considered different trajectories and accounted for baseline measurements of cytokine levels. The temporal relationship between cytokines and CRCI, supported by how different cytokines had been implicated in acute and persistent impairment, further substantiated our use of cognitive trajectories as a way of classification. A major limitation of our study is the lack of patients' blood samples at T4 that corresponded with their cognitive outcome, which made the correlation of the changes observed in cytokine levels for both delayed and intermittent cognitive trajectories less straightforward. However, there remain potential clinical applications in which baseline cytokine levels may be predictive of cognitive status that take place after a time delay.The use of objective measures could also have been employed for a more holistic assessment of cognitive performance (Wefel et al., 2011). Our sample size has been further reduced in number when we further classified patients into the numerous cognitive trajectories. As such, our findings may be interpreted as being exploratory in nature and further studies will be needed to validate our findings in independent patient cohorts. Future work or more sophisticated statistical analysis may be needed to further entangle the differences in changes in cytokine levels and consider how cytokines act in complex networks and/or pathway.

5. Conclusion

This is one of the first study that evaluated changes in cytokines levels in relation to the self-perceived cognitive trajectories. Pro-inflammatory cytokines implicated in the acute (TNF-α) and persistent cognitive trajectories (IL-6 and IL-8) seemed to share a common trait in the immune effector cells they are involved in, belonging to Th1 and Th17 functional groups respectively. Our findings help to further current understanding of cytokine profiles implicated in CRCI and support the use of cytokine levels as predictors of cognitive decline over time.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2020.577196.

References

Arellano, G., Acuna, E., Reyes, L.I., Ottum, P.A., De Sarno, P., Villarroel, L., et al., 2017. Th1 and Th17 cells and associated cytokines discriminate among clinically isolated syndrome and multiple sclerosis phenotypes. Front. Immunol. 8, 753.

Barroeta-Espar, I., Weinstock, L.D., Perez-Nievas, B.G., Meltzer, A.C., Siao Tick Chong, M., Amaral, A.C., et al., 2019. Distinct cytokine profiles in human brains resilient to Alzheimer's pathology. Neurobiol. Dis. 121, 327–337.

Bradburn, S., Sarginson, J., Murgatroyd, C.A., 2017. Association of peripheral Interleukin-6 with global cognitive decline in non-demented adults: a meta-analysis of prospective studies. Front. Aging Neurosci. 9, 438.

Cheung, Y.T., Lim, S.R., Ho, H.K., Chan, A., 2013. Cytokines as mediators of chemotherapy-associated cognitive changes: current evidence, limitations and directions for future research. PLoS One 8, e81234.

Cheung, Y., Ng, T., Shwe, M., Ho, H.K., Foo, K.M., Cham, M.T., et al., 2014a. Association of proinflammatory cytokines and chemotherapy-associated cognitive impairment in breast cancer patients: a multi-centered, prospective, cohort study. Ann. Oncol. 1446–1451.

Cheung, Y.T., Foo, Y.L., Shwe, M., Tan, Y.P., Fan, G., Yong, W.S., et al., 2014b. Minimal clinically important difference (MCID) for the functional assessment of cancer therapy: cognitive function (FACT-Cog) in breast cancer patients. J. Clin. Epidemiol. 67, 811–820.

Cohen, R.A., Gullett, J.M., Woods, A.J., Porges, E.C., Starkweather, A., Jackson-Cook, C.K., et al., 2019. Cytokine-associated fatigue prior to, during, and post-chemotherapy for breast cancer. J. Neuroimmunol. 334, 577001.

Gaman, A.M., Uzoni, A., Popa-Wagner, A., Andrei, A., Petcu, E.B., 2016. The role of oxidative stress in etiopathogenesis of chemotherapy induced cognitive impairment (CICI)-"Chemobrain". Aging Dis. 7, 307–317.

Haack, M., Pollmacher, T., Mullington, J.M., 2004. Diurnal and sleep-wake dependent variations of soluble TNF- and IL-2 receptors in healthy volunteers. Brain Behav. Immun. 18, 361–367.

Habbas, S., Santello, M., Becker, D., Stubbe, H., Zappia, G., Liaudet, N., et al., 2015. Neuroinflammatory TNFalpha impairs memory via astrocyte signaling. Cell. 163, 1730–1741.

Henneghan, A., Palesh, O., Harrison, M., Kesler, S.R., 2018. Identifying cytokine predictors of cognitive functioning in breast cancer survivors up to 10years post chemotherapy using machine learning. J. Neuroimmunol. 320, 38–47.

Henneghan, A., Haley, A.P., Kesler, S., 2020. Exploring relationships among peripheral amyloid Beta, tau, cytokines, cognitive function, and psychosomatic symptoms in breast cancer survivors. Biol Res Nurs. 22, 126–138 1099800419887230.

Janelsins, M.C., Mustian, K.M., Palesh, O.G., Mohile, S.G., Peppone, L.J., Sprod, L.K., et al., 2012. Differential expression of cytokines in breast cancer patients receiving

different chemotherapies: implications for cognitive impairment research. Support Care Cancer 20, 831–839.
Janelsins, M.C., Kesler, S.R., Ahles, T.A., Morrow, G.R., 2014. Prevalence, mechanisms, and management of cancer-related cognitive impairment. Int Rev Psychiatry. 26, 102–113.
Kesler, S., Janelsins, M., Koovakkattu, D., Palesh, O., Mustian, K., Morrow, G., et al., 2013. Reduced hippocampal volume and verbal memory performance associated with interleukin-6 and tumor necrosis factor-alpha levels in chemotherapy-treated breast cancer survivors. Brain Behav. Immun. 30 (Suppl), S109–S116.
Kothur, K., Wienholt, L., Brilot, F., Dale, R.C., 2016. CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: a systematic review. Cytokine 77, 227–237.
Lange, M., Joly, F., Vardy, J., Ahles, T., Dubois, M., Tron, L., et al., 2019. Cancer-related cognitive impairment: An update on state of the art, detection, and management strategies in cancer survivors. Ann. Oncol. 30, 1925–1940.
Lepennetier, G., Hracsko, Z., Unger, M., Van Griensven, M., Grummel, V., Krumbholz, M., et al., 2019. Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. J. Neuroinflammation 16, 219.
Lyon, D.E., Cohen, R., Chen, H., Kelly, D.L., McCain, N.L., Starkweather, A., et al., 2016. Relationship of systemic cytokine concentrations to cognitive function over two years in women with early stage breast cancer. J. Neuroimmunol. 301, 74–82.
Maddur, M.S., Miossec, P., Kaveri, S.V., Bayry, J., 2012. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am. J. Pathol. 181, 8–18.
Meyers, C.A., Albitar, M., Estey, E., 2005. Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer 104, 788–793.
Ng, T., Dorajoo, S., Cheung, Y., Lam, Y.C., Yeo, H.L., Shwe, M., et al., 2018. Distinct and heterogeneous trajectories of self-perceived cognitive impairment among Asian breast cancer survivors. Psychooncology 27, 1185–1192.
Olson, B., Marks, D.L., 2019. Pretreatment cancer-related cognitive impairment-mechanisms and outlook. Cancers (Basel) 11.
Patel, S.K., Wong, A.L., Wong, F.L., Breen, E.C., Hurria, A., Smith, M., et al., 2015. Inflammatory biomarkers, comorbidity, and Neurocognition in women with newly diagnosed breast cancer. J. Natl. Cancer Inst. 107.
Pomykala, K.L., Ganz, P.A., Bower, J.E., Kwan, L., Castellon, S.A., Mallam, S., et al., 2013. The association between pro-inflammatory cytokines, regional cerebral metabolism, and cognitive complaints following adjuvant chemotherapy for breast cancer. Brain Imaging Behav. 7, 511–523.
Ren, X., St Clair, D.K., Butterfield, D.A., 2017. Dysregulation of cytokine mediated chemotherapy induced cognitive impairment. Pharmacol. Res. 117, 267–273.
Santos, J.C., Pyter, L.M., 2018. Neuroimmunology of behavioral comorbidities associated with cancer and cancer treatments. Front. Immunol. 9, 1195.
Shibayama, O., Yoshiuchi, K., Inagaki, M., Matsuoka, Y., Yoshikawa, E., Sugawara, Y., et al., 2019. Long-term influence of adjuvant breast radiotherapy on cognitive function in breast cancer patients treated with conservation therapy. Int. J. Clin. Oncol. 24, 68–77.
Taipa, R., das Neves, S.P., Sousa, A.L., Fernandes, J., Pinto, C., Correia, A.P., et al., 2019. Proinflammatory and anti-inflammatory cytokines in the CSF of patients with Alzheimer's disease and their correlation with cognitive decline. Neurobiol. Aging 76, 125–132.
Vardy, J., Wong, K., Yi, Q.L., Park, A., Maruff, P., Wagner, L., et al., 2006. Assessing cognitive function in cancer patients. Support Care Cancer 14, 1111–1118.
Wefel, J.S., Vardy, J., Ahles, T., Schagen, S.B., 2011. International cognition and cancer task force recommendations to harmonise studies of cognitive function in patients with cancer. Lancet Oncol. 12, 703–708.
Wefel, J.S., Kesler, S.R., Noll, K.R., Schagen, S.B., 2015. Clinical characteristics, pathophysiology, and management of noncentral nervous system cancer-related cognitive impairment in adults. CA Cancer J. Clin. 65, 123–138.
Williams, A., Shah, R., Shayne, M., Huston, A.J., Krebs, M., Murray, N., et al., 2018. Associations between inflammatory markers and cognitive function in breast cancer patients receiving chemotherapy. J. Neuroimmunol. 314, 17–23.
Wilson, C.J., Finch, C.E., Cohen, H.J., 2002. Cytokines and cognition–the case for a head-to-toe inflammatory paradigm. J. Am. Geriatr. Soc. 50, 2041–2056.