Title

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Journal

Annals of Oncology, 26(7)

ISSN

0923-7534

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Publication Date

2015-07-01

DOI

10.1093/annonc/mdv206

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Annals of Oncology 26: 1446–1451, 2015
doi:10.1093/annonc/mdv206
Published online 28 April 2015

Association of proinflammatory cytokines and chemotherapy-associated cognitive impairment in breast cancer patients: a multi-centered, prospective, cohort study†

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Received 13 November 2014; revised 17 April 2015; accepted 20 April 2015

Background: Existing evidence suggests that proinflammatory cytokines play an intermediary role in postchemotherapy cognitive impairment. This is one of the largest multicentered, cohort studies conducted in Singapore to evaluate the prevalence and proinflammatory biomarkers associated with cognitive impairment in breast cancer patients.

Patients and methods: Chemotherapy-receiving breast cancer patients (stages I–III) were recruited. Proinflammatory plasma cytokines concentrations [interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, granulocyte–macrophage colony-stimulating factor, interferon-γ and tumor necrosis factor-α] were evaluated at 3 time points (before chemotherapy, 6 and 12 weeks after chemotherapy initiation). The FACT-Cog (version 3) was utilized to evaluate patients' self-perceived cognitive disturbances and a computerized neuropsychological assessment (Headminder™) was administered to evaluate

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†This study was presented as an oral presentation at the 2014 International Cognition and Cancer Task Force (ICCTF) conference in Seattle, USA; and as an oral presentation (with Merit Award) at the 2013 American Society of Clinical Oncology (ASCO) meeting in Chicago, USA.

patients' memory, attention, response speed and processing speed. Changes of cognition throughout chemotherapy treatment were compared against the baseline. Linear mixed-effects models were applied to test the relationships of clinical variables and cytokine concentrations on self-perceived cognitive disturbances and each objective cognitive domain.

Results: Ninety-nine patients were included (age 50.5 ± 8.4 years; 81.8% Chinese; mean duration of education = 10.8 ± 3.3 years). Higher plasma IL-1β was associated with poorer response speed performance (estimate: −0.78; 95% confidence interval (CI) −1.34 to −0.03; $P = 0.023$), and a higher concentration of IL-4 was associated with better response speed performance ($P = 0.022$). Higher concentrations of IL-1β and IL-6 were associated with more severe self-perceived cognitive disturbances ($P = 0.018$ and 0.001, respectively). Patients with higher concentrations of IL-4 also reported less severe cognitive disturbances ($P = 0.022$).

Conclusions: While elevated concentrations of IL-6 and IL-1β were observed in patients with poorer response speed performance and perceived cognitive disturbances, IL-4 may be protective against chemotherapy-associated cognitive impairment. This study is important because cytokines would potentially be mechanistic mediators of chemotherapy-associated cognitive changes.

Key words: breast cancer, cognitive disturbance, chemobrain, cytokines, cognitive impairment, FACT-Cog

introduction

Commonly known in the literature as 'chemobrain', chemotherapy-associated cognitive impairment is prevalent among early-stage breast cancer survivors. Although chemotherapeutic agents are unlikely to cross the blood–brain barrier (BBB) due to their molecular size, it has been alleged that the occurrence of neurotoxicity is linked to the proinflammatory cytokine pathways. In the brain, cytokines can cause local inflammation through oxidative and nitrosative processes, especially in the hippocampus and the regions of the brain where cytokine receptors are abundant. These reactions would consequently lead to the clinical symptoms of cognitive impairment, including lapses in memory, attention, processing speed and response speed.

Experimental studies have further demonstrated that proinflammatory cytokines may be mediators of chemotherapy-associated cognitive changes, and the fluctuations of circulating cytokines have been suggested to mediate 'sickness behavior' in patients with severe infections or cancer. Proinflammatory markers have also been purported to give rise to a cluster of other cancer-related or treatment-related symptoms, including pain and fatigue. Our research group has recently conducted a review to evaluate the associations between proinflammatory biomarkers and cognition in cancer patients who were treated with chemotherapy [1]; currently, evidence suggests that interleukin (IL)-1β, IL-6, IL-8 and TNF-α contribute to chemotherapy-associated cognitive impairment. Numerous studies have also evaluated the association between IL-2, IL-4, IL-10, granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ and cognition, with majority of results being inconclusive. Furthermore, the role of cytokines in postchemotherapy-cognitive impairment is still controversial because several studies have reported conflicting results with regard to the strength and direction of the association between changes in cytokine concentrations and cognition [2–4]. To gain a better understanding of the role that cytokines play in chemotherapy-associated cognitive changes, we designed a robust study to evaluate the effect of chemotherapy-induced inflammatory response on breast cancer patients' cognitive function, as reflected by the changes in plasma cytokine concentrations.

patients and methods

study design and settings

This was a multicenter prospective cohort study conducted at the two largest ambulatory cancer institutions in Singapore, where ∼70% of the cancer patients in this country are treated. Singapore is a multiracial country with a majority population of Chinese (74.2%), Malay (13.2%) and Indian (9.2%), with English and Chinese being most commonly spoken. The study was approved by the Institutional Review Board and written informed consent was obtained from all participants.

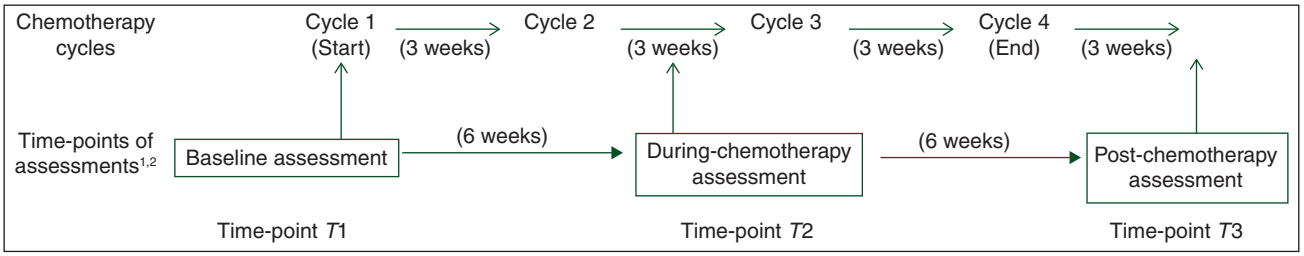
patients

Eligible patients: (i) were newly diagnosed with early-stage breast cancer by a medical oncologist (within 12 weeks of diagnosis), (ii) had no prior exposure to chemotherapy and radiation treatments, (iii) were scheduled to begin treatment on a standard adjuvant chemotherapy [anthracycline-based (a single-day chemotherapy regimen comprising 60 mg/m^2 doxorubicin and 600 mg/m^2 cyclophosphamide) or taxane-based (75 mg/m^2 docetaxel and 600 mg/m^2 cyclophosphamide)], (iv) were ambulatory and had good performance status (Eastern Cooperative Oncology Group score of 0 or 1), (v) were capable of giving informed consent and (vi) could speak either English or Chinese. Patients were excluded from the study if breast cancer was a secondary malignancy, or if they exhibited evidence of brain metastasis, psychosis or any underlying neuropsychiatric illness that might impair their cognitive abilities.

study procedure

Data collection was carried out at three time points at intervals of ∼6 weeks (Figure 1). For all patients, the first time point (*T1*) was at baseline before the initiation of chemotherapy. The second time point (*T2*) was timed at ∼6 weeks after *T1* and also coincided with the first day of the third cycle of chemotherapy. The third time point (*T3*) was ∼12 weeks after *T1* when the standard chemotherapy had been completed. Overall, the approximate duration between each time point of assessment was 6 weeks.

At *T1*, baseline demographic data were collected through existing electronic databases and through patient interviews. At each time point for data collection, the patients completed both objective and subjective self-reported neuropsychological assessments. All data collection tools [Headminder™, The Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog) (version 3), Beck Anxiety Inventory (BAI) and Brief Fatigue Inventory (BFI)] were available in English or Chinese and were administered by



[1]The first time-point (*T1*) was conducted at baseline, within 12 weeks of diagnosis, before the patients received chemotherapy. The second time-point (*T2*) occurred approximately 6 weeks after *T1* which also coincided with Cycle 3 of chemotherapy. The third time-point (*T3*) was conducted approximately 6 weeks after *T1* upon the completion of standard chemotherapy.
[2]At each time-point, patients completed a computerised neuropsychological battery (Headminder™), the Functional Assessment of Cancer Therapy-Cognitive Function Brief Fatigue Inventory and Beck Anxiety Inventory. A 10 ml tube of blood was drawn at each time-point.

Figure 1. Study procedure.

trained bilingual interviewers. Details of the assessment tools are included in supplementary Material S1, available at *Annals of Oncology* online.

cytokine analysis

At each time point, a 10-ml sample of blood was drawn from the patients before the administration of chemotherapy, placed in ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 1140 *g* (2500 rpm) for 30 min within 30–40 min of collection. Plasma was divided into aliquots and stored aseptically at −80°C until analysis. A panel of nine plasma cytokines—TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF and IFN-γ—was quantified for each 50 µl of plasma sample using a highly sensitive multiplex immunoassay (Luminex®) in triplicate. Details of the immunoassay are included in supplementary Material S2, available at *Annals of Oncology* online.

statistical analysis

All statistical analyses were carried out using the SPSS version 20. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. A simple Friedman's test was used to compare the changes in cytokine concentrations within patients across the three time points. For the evaluation of cognitive changes within individual breast cancer patients, a reliable change index (RCI) was computed for Headminder™ scores based on the repeated normative mean and standard error of the difference to adjust for the practice effect calculated from a reference group of 28 breast cancer patients who did not receive chemotherapy (supplementary Table S1, available at *Annals of Oncology* online). The RCI compares the changes in individual test scores with changes in a reference group to determine whether the changes are greater than those that would be expected from measurement error alone [5], is calculated by subtracting the score for an earlier assessment (e.g. *T1*) and the practice effect from a later assessment (e.g. *T2*), and then divide the result by the standard error of the difference in the reference population. RCI was calculated for the changes of test scores from *T1* to *T2*, *T2* to *T3* and overall *T1* to *T3*.

The degrees of change in the individual cognitive domains were defined as follows: (i) an RCI of greater than −1.5 as 'no change'; (ii) an RCI ranging from −1.5 to −2.5 as 'mild impairment' and (iii) an RCI of less than −2.5 as 'severe impairment'. This method of evaluating cognitive changes on objective neuropsychological tests has been widely adopted by numerous studies and is recommended by international guidelines [6–8].

For the evaluation of perceived cognitive changes within individual breast cancer patients, a drop of 10.6 points in the total FACT-Cog score was considered to be perceived cognitive impairment. This definition was adopted based on our predetermined 'minimal clinically important difference' (MCID) of FACT-Cog in our cancer patients [9].

A linear mixed-model analysis was used to examine the relationship between each proinflammatory cytokines with the objective and self-perceived cognitive impairment over the 12-week study period (from *T1* to *T3*). The analyses were conducted for the dependent variables in the Headminder™ scores for the four cognitive domains: processing speed, response speed, memory and attention. For self-perceived cognitive changes, the dependent variables referred to the total FACT-Cog score. Other than the cytokines of interest, documented variables that might affect cognitive function were also included a priori into the mixed model as fixed effects; these variables were age, years of education, baseline body mass index, fatigue and anxiety [7, 10–13] and the intercept varied as a random effect by each subject. Model selection for the linear mixed-effects models was conducted using the Akaike information criterion. Visual graphical inspection (skewness, kurtosis, histograms and normal Q-Q plots) was conducted to ensure that the dependent variables resemble a normal distribution and can be fitted into the linear mixed model.

results

patients' characteristics

Ninety-nine breast cancer patients were included in this analysis (supplementary Table S2, available at *Annals of Oncology* online). The mean age of the patients was 50.5 ± 8.4 years. The majority were Chinese (81.8%), postmenopausal (49.5%), early-stage breast cancer patients who received anthracycline-based chemotherapy (70.7%). Before recruitment, majority (90.9%) of the patients had received surgery (lumpectomy or mastectomy) on the affected breast. Surgery occurred 36 (±12) days before recruitment.

Plasma concentrations of cytokines across all three time points are presented in supplementary Table S3, available at *Annals of Oncology* online. No statistically significant differences among the cytokine concentrations across the three time points were observed, with the exception of IL-6. Overall, an increasing trend in the concentrations of IL-6 was observed from *T1* to *T3* ($P < 0.0001$). Notably, a substantial proportion of patients had IL-2 (53.5%–57.6%), IL-10 (54.5%–63.6%), GM-CSF (37.4%–45.5%) and IFN-γ (72.7%–82.8%) concentrations that were below the detection limit throughout *T1* to *T3*, and large inter- and intrapatient variations were found for GM-CSF (range: 0.00–439.34 pg/ml) and IL-2 (range: 0.00–43.45 pg/ml) concentrations. As the concentrations of IL-2, IL-10 and IFN-γ were mostly below detection limit, the CV of these measurements were considerably higher (over 40%).

prevalence of objective and self-perceived cognitive impairment

Overall, with reference to the baseline at *T1*, a higher proportion of patients experienced impairments in memory (13.2%) and

Table 1. Prevalence of objective and subjective cognitive impairment ($N = 99$)

Cognitive domains	Proportion of patients with impairment, *N* (%)		
	From *T1* to *T2*	From *T2* to *T3*	Overall From *T1* to *T3*
Processing speed			
Mild impairment[a]	1 (1.0)	0	2 (2.2)
Severe impairment[b]	2 (2.0)	1 (1.0)	0
Total[c]	3 (3.0)	1 (1.0)	2 (2.2)
Response speed			
Mild impairment[a]	4 (4.0)	8 (8.1)	2 (2.1)
Severe impairment[b]	0	1 (1.0)	2 (2.1)
Total[c]	4 (4.0)	9 (9.1)	4 (4.2)
Memory			
Mild impairment[a]	12 (12.1)	5 (5.1)	8 (8.1)
Severe impairment[b]	1 (1.0)	4 (4.0)	5 (5.1)
Total[c]	13 (13.1)	9 (9.1)	13 (13.2)
Attention			
Mild impairment[a]	5 (5.1)	4 (4.0)	5 (5.2)
Severe impairment[b]	2 (2.0)	0	2 (2.1)
Total[c]	7 (7.1)	4 (4.0)	7 (7.3)
FACT-Cog total score impairment[d]	18 (18.2)	24 (24.2)	29 (29.3)

[a]Defined as a reliable change index of −1.5 to −2.5.
[b]Defined as a reliable change index of lower than −2.5.
[c]Total impairment refers to the number (proportion) of patients with both mild and severe impairment in that particular cognitive domain.
[d]Subjective cognitive impairment is defined as a decrease in the Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog) score of 10.6 points or more, based on the minimal clinically important difference in FACT-Cog established in a previous study [9].

attention (7.3%) after the completion of chemotherapy. Fewer patients seemed to have suffered from processing speed (2.2%) and response speed (4.2%) impairments (Table 1). Based on the MCID of FACT-Cog, a total of 29 patients (29.3%) were classified as experiencing clinically significant perceived cognitive impairment. The cross-sectional raw scores of the tests from *T1* to *T3* are presented in supplementary Table S4, available at *Annals of Oncology* online.

biological determinants of objective cognitive function

Every unit increase in plasma IL-1β was associated with a 0.78 decrease in the response speed performance [95% confidence interval (CI) −1.34 to −0.03; $P = 0.023$], and a higher concentration of IL-4 was associated with better response speed performance ($P = 0.022$). No biological predictor for changes in processing speed, memory and attention was identified (Table 2).

Overall, age and years of education were associated with objective impairment in the domains of processing speed, response speed and memory. Patients who were older or less highly educated experienced more severe impairment in cognitive function.

biological determinants of self-perceived cognitive function

Higher concentrations of IL-1β and IL-6 were associated with more severe self-perceived cognitive disturbances ($P = 0.018$ and 0.001, respectively). Every unit increase in IL-4 concentration was associated with an estimated 0.95 increase of the FACT-Cog total score, indicating that patients with higher concentrations of IL-4 reported less severe cognitive disturbances ($P = 0.022$) (Table 2).

Both anxiety and fatigue were also strong determinants of self-perceived cognitive impairment; patients with higher levels

Table 2. Estimates and standard errors for determinants of objective and subjective cognitive impairment ($N = 99$)

Parameters	Objective measures								Subjective measure	
	Processing speed		Response speed		Memory		Attention		FACT-Cog	
	Est (SE)	*p*-value	Est (SE)	*p*-value	Est (SE)	*p*-value	Est (SE)	*p*-value	Est (SE)	*p*-value
Intercept	104.61 (7.73)	<0.0001	79.76 (12.5)	<0.0001	85.15 (14.6)	<0.0001	117.53 (12.0)	<0.0001	120.19 (11.7)	<0.0001
Clinical determinants										
Age	−0.096 (0.10)	0.349	−0.514 (0.17)	0.002	0.120 (0.19)	0.535	−0.393 (0.16)	0.014	0.273 (0.15)	0.079
Years of education	1.199 (0.26)	<0.0001	0.629 (0.42)	0.140	1.199 (0.49)	0.017	1.152 (0.40)	0.005	0.390 (0.39)	0.323
Body mass index	−0.375 (0.18)	0.045	−0.307 (0.30)	0.309	−0.106 (0.35)	0.762	−0.554 (0.29)	0.056	0.035 (0.30)	0.901
Psychosocial determinants										
Anxiety	−0.014 (0.07)	0.844	0.068 (0.12)	0.564	0.029 (0.16)	0.856	−0.148 (0.11)	0.897	−0.744 (0.14)	<0.0001
Fatigue	0.334 (0.25)	0.190	−0.124 (0.42)	0.765	−0.396 (0.56)	0.477	0.394 (0.40)	0.326	−1.568 (0.50)	0.002
Biological determinants										
IL-1β	−0.218 (0.22)	0.320	−0.778 (0.34)	0.023	−0.206 (0.48)	0.665	−0.240 (0.33)	0.471	−0.915 (0.38)	0.018
IL-4	0.234 (0.20)	0.249	0.760 (0.33)	0.022	0.040 (0.44)	0.933	0.105 (0.30)	0.729	0.949 (0.41)	0.022
IL-6	0.002 (0.01)	0.799	0.013 (0.01)	0.173	0.006 (0.01)	0.671	−0.006 (0.01)	0.579	−0.440 (0.01)	0.001
IL-8	0.004 (0.01)	0.566	0.006 (0.01)	0.581	−0.003 (0.02)	0.877	−0.020 (0.01)	0.078	0.0120 (0.014)	0.387
TNF-α	−0.015 (0.09)	0.870	0.103 (0.16)	0.528	0.039 (0.21)	0.856	0.090 (0.15)	0.556	−0.226 (0.19)	0.240

Est, estimate; SE, standard error; FACT-Cog, Functional Assessment of Cancer Therapy-Cognitive Function; IL, interleukin; TNF, tumor necrosis factor.

of anxiety and fatigue reported more cognitive disturbances ($P < 0.0001$ and 0.002, respectively).

discussion

This was one of the largest studies to evaluate the role of proinflammatory cytokines in chemotherapy-associated cognitive impairment. An increased concentration of IL-1β was associated with poorer response speed performance, and it has been widely reported in the literature that elevated IL-1β concentrations have been found in patients who manifest neurodegenerative diseases, such as Alzheimer's disease and dementia [14, 15]. Increased serum IL-1β concentrations have also been proposed as a stage marker of ongoing brain neurodegeneration in the continuum between normal aging and mild cognitive impairment [14]. In animals that were administered IL-1β endogenously, interference in cognitive processes and associated mood changes were found to be due to the hippocampal production of IL-1β [16]. IL-6 was also associated with self-perceived cognitive disturbances. These findings were similar to the results of another study; significant increases in the concentrations of IL-6 and IL-8 were observed in patients who received anthracycline-based chemotherapy [4]. These patients experienced more severe self-reported symptoms which included heavy-headedness, difficulty in thinking and problems with concentration. In this study, an elevated concentration of IL-6 was also associated with greater perceived cognitive disturbances, which measure the patients' perceived lapses in memory, concentration and mental acuity.

Interestingly, our results suggested that an elevated concentration of IL-4 was associated with improved response speed performance and self-reported cognitive function. IL-4 plays a critical role in the higher functions, such as executive functions and learning, of the brain. The neuroprotective role of IL-4 in cognitive impairment has been supported by animal studies [17]. While increases in the concentrations of cytokines such as IL-1β and IL-6 were observed in aging animals, studies have shown that this is accompanied by a decrease in the hippocampal production of IL-4 [18]. However, data from human studies are inconsistent, with two studies unable to depict any relationships between IL-4 and FACT-Cog scores [19, 20]. Furthermore, these studies were limited by the small size of the cohort of cancer patients who received different chemotherapeutic regimens. The potential neuroprotective role of IL-4 should be explored in future studies.

The exact mechanisms behind chemotherapy-induced cytokines and cognitive function are lacking in the literature. Researchers have proposed that cytokines can penetrate the BBB readily by active transport through the circumventricular regions in the brain. In the brain, cytokines may bind to the endothelial receptors in the brain vasculature to stimulate the release of other inflammatory mediators, such as cell adhesion molecules, chemokines, nitric oxide and prostaglandins, which impede the integrity of the BBB and cause structural damage to the brain [21, 22]. One study observed lower left hippocampal volume in breast cancer patients; the structural changes were also associated with higher levels of TNF-α and poorer verbal memory [3]. To examine the direct effect of cytokines on the brain, one can assess the concentrations of cytokines in the cerebrospinal fluid which has direct contact with the brain, however, this approach may not be feasible in breast cancer survivors.

A key limitation of this study was the lack of concurrent cancer controls not receiving chemotherapy; thus, a correlation between cytokine dysregulation and cognitive impairment, and the progression of cancer was not possible. Nevertheless, the finding of an association among exposure to chemotherapy, plasma concentrations of IL-1β, IL-4 and IL-6 and cognitive performance in longitudinal outcomes provided support for the continued examination of postchemotherapy inflammation as an influential factor in this phenomenon. It would also be logical to assume that the progression of cancer was less likely to cause a significant fluctuation in cytokine concentrations among these patients who were diagnosed with early-stage breast cancer. For future studies, it might be more appropriate to include both nonchemotherapy-receiving breast cancer controls and healthy controls as references in order to serve as comparison [7, 8]. By including both types of controls, the effects of the cancer itself, aging and the chemotherapy treatment on cognitive changes can be taken into account. Other factors that could potentially affect cognition were not evaluated in detail due to the constraints of resources and time within a clinical setting. Depression, physical activity, the type of surgical procedures and concurrent medications might contribute to changes in patients' cytokine levels.

These findings were only suggestive of a potential association between cytokines and cognitive function, and the identified associations did not equate with causation. In addition to statistical methods, the results must be interpreted based on information in the current literature. As discussed in the previous section, similar studies have identified the role of IL-1β, IL-4 and IL-8 in neuropsychiatric conditions and chemotherapy-associated cognitive changes [2–4, 23–25]. Our findings have paralleled a proposed mechanism behind the oxidative stress induced by cytotoxic chemotherapeutic drugs on brain cells that leads to the clinical presentation of cognitive impairment in animal models. Results from this exploratory study provide directions for future research, such as examining whether the cognitive symptoms observed during chemotherapy treatment are mediated by inflammatory responses.

In conclusion, elevated plasma concentrations of IL-1β might be associated with poorer respond speed performance during objective neuropsychological assessments. Elevated concentrations of IL-6 and IL-1β were also observed in patients with perceived cognitive disturbances. Our results have also suggested that elevated plasma concentrations of IL-4 are protective against cognitive impairment. These results are important because they suggested that cytokines would potentially be mechanistic mediators of chemotherapy-associated cognitive changes. With this knowledge, future studies can be focused on establishing the potential relationship between cytokine dysregulation and other behavioral outcomes, such as fatigue, depression and anxiety.

acknowledgements

The authors acknowledge the contributions of all of the study participants. We also thank our research assistants (Yuan Chuan Kee and Yanxiang Gan) for their assistance in data

collection, and field application specialists of Bio-Rad Laboratories (Singapore) Pte Ltd (Dr Kenny Lim, Dr Sean Tan, Dr Fu Ling Soo and Charmaine Ng) for their support.

funding

This study was financed by research grants awarded by the National University of Singapore (R-148-000-166-112), the National Cancer Centre Singapore (NRFCB12131) and the National Medical Research Council Singapore (NMRC/CIRG/1386/2014).

disclosure

The authors have declared no conflicts of interest.

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