Longitudinal Trajectory of Spontaneous Brain Activity Changes in Breast Cancer Patients Following One Circle and the Completion of Neoadjuvant Chemotherapy-a preliminary prospective study

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Research Article

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Abstract

There is growing evidence that brain activity changes in breast cancer patients after chemotherapy. However, the longitudinal changes in brain function during chemotherapy are unclear and not studied before. To assess trajectory of brain activity changes during chemotherapy, we prospectively enrolled 36 breast cancer patients and longitudinally compared amplitude of low-frequency fluctuation (ALFF) and neuropsychological tests at three time points including before neoadjuvant chemotherapy (NAC) (time point 0, TP0), before the second cycle of NAC (time point 1, TP1), and after NAC (pre-operation, time point 2, TP2). Compared with TP0, ALFF values of right orbital part of inferior frontal gyrus, left medial orbital part of the superior frontal gyrus, right insula, left medial part of superior frontal gyrus and right middle frontal gyrus decreased significantly at TP1 and TP2. Besides, the score of Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog) decreased significantly at both TP1 and TP2. The scores of Self-Rating Anxiety Scale and Perceived Cognitive Abilities have a significant decrease at TP1 and TP2, respectively. There was no statistically significant difference found between the scores on the neuropsychological tests at TP1 and TP2. And aberrant ALFF values were correlated with neuropsychological tests scores at TP0. We conclude that brain activity of breast cancer patients treated with NAC changed significantly after the first cycle of NAC and lasted until the end of chemotherapy, along with the cognitive function deterioration. Most brain regions with ALFF changes were located in the frontal lobe, which is involved in the higher-order of cognitive function.

1 Introduction

The most common cancer-related death among women is breast cancer (Ferlay et al., 2021). Patients with locally advanced breast cancer, especially those with larger tumors, are typically treated with neoadjuvant chemotherapy (NAC) (Cortazar et al., 2014). A response to NAC can result in downstaging of a tumor and therefore can improve tumor resectability, and reduce postoperative complications (Spring et al., 2017). However, one of the most common complications of chemotherapy drugs is toxicity to the central nervous system, namely, chemotherapy-related cognitive impairment (CRCI) (Jansenelsins et al., 2017). Patient care and quality of life are greatly impacted by cognitive problems, since minor changes in cognition may have a substantial impact (Fried et al., 2002). The mechanism is not entirely clear. It is crucial to elucidate the underlying neurological mechanisms of CRCI in breast cancer patients, which may enable early diagnosis and treatment.

An important method of understanding cognitive impairment caused by chemotherapy in breast cancer patients is through neuroimaging, which may clarify the underlying mechanisms. Low-frequency fluctuation amplitude (ALFF) is a measure of the intensity of spontaneous fluctuations in resting-state functional magnetic resonance imaging (rs-fMRI) signals that are based on blood oxygen levels, which can locate spontaneous neural activity and physiological states in specific brain regions (Cordes et al., 2001; Fox and Raichle, 2007). ALFF analysis for brain activity has been used to evaluate the impact of pathological conditions. One study demonstrated decreased ALFF in the left frontal lobe which was significantly associated with executive dysfunction after chemotherapy in gastric cancer patients (Kim et al., 2017). Another study in elderly breast cancer patients revealed that ALFF increased in the bilateral subcallosal gyri and right anterior cingulate gyrus after chemotherapy (Chen et al., 2019). However, none of these studies ruled out the effect of surgery. We know that surgery can affect the cognitive function of cancer patients (Admoun and Mayrovitz, 2021; Harrison et al., 2021), and it can also affect their brain activity (Feinkohl, 2022; Gu et al., 2020; Yu et al., 2020).

Chemotherapy-induced central neurotoxicity can be acute, subacute, or delayed (Taillibert et al., 2016), meaning that CRCI can occur during and just after chemotherapy, and long after chemotherapy (Jansenelsins et al., 2014; Koppelmans et al., 2012), but is most common during and just after chemotherapy (Wefel et al., 2004). According to previous longitudinal studies, approximately 75% of patients become cognitively impaired during chemotherapy, and approximately 35% experience further cognitive decline after chemotherapy (Wefel et al., 2015). Collins and colleagues
conducted neuropsychological assessments in 60 women who were evaluated at the end of each chemotherapy cycle. Each subsequent time point was marked by progressive decline in comparison with matched healthy controls (Collins et al., 2013).

However, previous studies on the mechanism of chemotherapy-induced central neurotoxicity through neuroimaging mainly focus on the late stage (Chen et al., 2019), and there is a lack of studies on acute and subacute chemotherapy-induced central neurotoxicity through neuroimaging. Numerous studies have reported changes in brain activity in breast cancer patients one month, one year or more after chemotherapy ends (Chen et al., 2019; Dumas et al., 2013). A longitudinal study showed that functional activation of the frontal gyrus decreased one month after completion of chemotherapy for breast cancer and partial returned to baseline one year later (McDonald et al., 2012). Another study found that the posterior parietal cortex was significantly less responsive to memory-encoding and executive-function tasks 10 years after chemotherapy in breast cancer patients (de Ruiter et al., 2011). The above studies all found that the brain regions with brain activity changes varies with time course in breast cancer patients receiving chemotherapy, and they were all studied after chemotherapy.

In this study, we hypothesized that spontaneous brain activity in breast cancer patients was altered during chemotherapy. We prospectively included breast cancer patients receiving NAC to observe changes in ALFF values as well as neuropsychological test scores at three time points, including before NAC, before the second cycle of NAC, and before surgery. This cohort has the advantages of being free of confounding factors such as surgery and anesthesia, and enabling early investigations of brain activity. The aim of this study was to provide a comprehensive understanding of acute and subacute chemotherapy-induced central neurotoxicity through neuroimaging.

2 Materials and methods

2.1 Participants

This prospective longitudinal study was approved by the Ethics Committee of our hospital. Between January 2021 and March 2022, 41 patients with breast cancer were recruited from our institution. Inclusion criteria for this study included: (1) Women between the ages of 30 and 70 with breast cancer, (2) NAC was planned prior to operation and (3) right-handedness. Exclusion criteria for this study included: (1) a history of brain surgery, (2) patients with organic brain abnormalities such as tumor, stroke and trauma, (3) psychiatric or neurological history (dementia, major depressive disorder, schizophrenia, etc.), (4) metal implants, (5) patients receiving hormonal therapy, and (6) contraindications to MR examinations.

This study collected neuropsychological data and brain rs-fMRI at three time points. The first assessment was performed before NAC (time point0, TP0). The second assessment was performed before the second NAC cycle (time point 1, TP1), and the last assessment was performed after the end of NAC, before surgery (time point 2, TP2). The Hospital Information System (HIS) provided information about disease stage and chemotherapy regimens.

2.2 Neuropsychological tests

We used Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog) to measure cognitive function in cancer patients (Cheung et al., 2013). Higher scores are associated with fewer cognitive complaints over the past week. Four subscales comprise the FACT-Cog: Perceived Cognitive Impairment (PCI), Impact on Quality of Life (QOL), Comments from Others (Oth), and Perceived Cognitive Abilities (PCA). Executive function and working memory are tested with Digit Span Tests (DSTs, forward and backward) (Alloway et al., 2006; Diamond, 2013; St Clair-Thompson and Allen, 2013), mental flexibility is assessed through the Verbal Fluency Test (VFT) (Diamond, 2013), and psychomotor speed and executive function are assessed by the Trail Making Test (TMT, Parts A) (Bowie and Harvey,
Self-rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS) were used to quantify anxiety and depression symptoms, respectively.

2.3 Magnetic resonance imaging

All patients were scanned using a 64-channel head-neck coil in the 3.0 Tesla Whole Body Magnetic Resonance System (MAGNETOM Prisma, Siemens, Erlangen, Germany). To reduce noise and restrict head movement, during the acquisition, participants were instructed to keep their eyes closed and focus on nothing in particular, and foam pads were used to minimize head motion. The following parameters were used for RS-fMRI acquisition, the repeat time is 2000 ms, the echo time is 30 ms, the flip angle is 70°, the field of view is 240 × 240 mm², the slices are 36 (interleaved), the matrix is 80 × 80, and the voxel size is 3 × 3 × 3 mm³. A total of 240 volumes were acquired from each participant over a period of 8 minutes and 8 seconds. We used a T1-weighted 3D-spoiled gradient recall sequence to obtain a high-resolution 3D structural image. The repetition time is 2100 ms, the echo time is 2.26 ms, the flip angle is 8°, the field of view is 256 × 256 mm², slices are 192, the matrix is 256 × 256, and the voxel size is 1 × 1 × 1 mm³. We scanned for 4 minutes and 53 seconds in total.

2.4 Neuroimaging processing

We used DPABI_V5.1 for preprocessing RS-fMRI data (Yan et al., 2016). The pre-processing steps are as follows: (1) Convert the original scanned DICOM files into neuroimaging Information Technology Program (NIfTI) format, (2) remove the first 10 time points from the functional image, (3) time difference was corrected using slice timing, (4) the head motion was then corrected using a realignment procedure, subjects with a head motion of > 2.0 mm or > 2.0° were excluded from further analysis, (5) head motion effects were regressed using the Friston 24-parameter model. As nuisance variables, white matter and cerebrospinal fluid signals were removed. Inter-subject comparisons were made based on normalized functional images from the Montreal Neurological Institute (Yan et al., 2013), (6) bandpass filters were applied (0.01–0.10 Hz) to the data, (7) to improve signal-to-noise ratio, the data were spatially smoothed using a 6-mm full-width half-maximum filter.

2.5 Statistical analysis

The numerical data was analyzed using SPSS (version 22.0). The Kolmogorov-Smirnov test was first used to determine whether the distribution was normal, and subsequent statistical analysis was conducted according to the test results. Normally distributed data were compared using Repeated measures analysis of variance (ANOVA), and nonnormal distributions were compared using Friedman’s M test. A post hoc test, corrected by the Bonferroni test, was then conducted for pairwise comparison in each analysis, revealing a significant effect.

DPABI V5.1 software offers a Statistical Analysis module that analyses ALFF maps. The distribution patterns of ALFF of each group (in comparison with 0) were first assessed with a one-sample t test. Repeated measures ANOVA was performed with DPABI software as a measure of ALFF difference among the three time points (Yan et al., 2016). We corrected all statistical analyses of image data using Gaussian random field theory (voxel P value < 0.001, cluster P value < 0.05), the clusters were then saved. Then, ALFF values were acquired by extracting signals from saved clusters. Finally, SPSS 22.0 software was used to analyze the ALFF values at different time points. Repeated measures ANOVA was used to compare the ALFF values among the three time points, and Bonferroni correction of post hoc analysis was performed at the same time.

We calculated average ALFF values in abnormal brain areas and used Spearman correlation analysis (due to non-normal distribution of neuropsychological test scores) to assess correlations between neuropsychological tests scores and ALFF values with significant differences in the post hoc test. For each pair of ALFF values and neuropsychological tests scores, we assessed the following relationships using Spearman correlation analysis: 1) ALFF values and the
neuropsychological tests scores at each time point; 2) Differences in neuropsychological tests scores with significant differences and differences in ALFF values between corresponding time points; and 3) ALFF values at TP0 and the change in neuropsychological tests scores. The significance level was \( P < 0.05 \).

3 Results

3.1 Demographic characteristics and clinical data

The study recruited 41 women with breast cancer. All of them received either 6 or 8 cycles of NAC. During the follow-up scan, five patients had head movements greater than 2 mm. Therefore, a total of 36 patients were enrolled for data analysis. The average interval between TP0 and TP1 was 28 days (range 19–46 days), the average interval between TP1 and TP2 was 118 days (range 62–171 days) and the average interval between TP0 and TP2 is 146 days (range 81–198 days). Based on the baseline clinical stage and preoperative chemotherapy regimen, Table 1 shows the characteristics of the NAC group.

3.2 Neuropsychological tests comparison

During NAC treatment, the scores of FACT-Cog, PCA, PCI, DST backward in breast cancer patients changed significantly. Compared with TP0, the score of FACT-Cog decreased significantly at both TP1 and TP2, the scores of SAS and PCA has a significant decrease at TP1 and TP2, respectively. \(( P < 0.05; \text{Table 2, Fig. 1})\). Neuropsychological tests showed no statistically significant difference between TP1 and TP2 \(( P > 0.05)\).

3.3 Group differences in ALFF

Based on the one-sample t test results, Fig. 2 shows ALFF distribution patterns for each group corrected using Gaussian random field theory. ALFF values were predominantly elevated in bilateral frontal cortex, insula, precentral gyrus, parietal cortex and postcentral gyrus (Fig. 2A – 2C).

Repeated measures ANOVA results showed that there were significant differences in ALFF values of right orbital part of inferior frontal gyrus (ORBinf.R), left medial orbital part of superior frontal gyrus (ORBsupmed.L), right insula (INS.R), left medial part of superior frontal gyrus (SFGmed.L) and right middle frontal gyrus (MFG.R) among the three time points (Fig. 2D).

3.4 Pairwise comparison of ALFF

ALFF values were obtained by extracting the signals on the above significant clusters, and then pairwise comparisons were performed in SPSS. Compared with TP0, ALFF values of the above five brain regions at TP1 and TP2, including ORBinf.R, ORBsupmed.L, INS.R, MFG.R and SFGmed.L, were significantly decreased. Compared with TP1, there was no significant change in ALFF values at TP2 (Table 3, Fig. 3). The pairwise comparisons were adjusted with Bonferroni correction.

3.4 Correlation Analysis

Our results demonstrated that ALFF values were correlated with neuropsychological tests scores at TP0, whereby the ALFF values in ORBinf.R were significantly positively correlated with the scores of DST backward \(( \rho = 0.355, P = 0.033; \text{Fig. 4A})\), ALFF values in MFG.R were significantly positively correlated with the scores of SAS \(( \rho = 0.414, P = 0.012; \text{Fig. 4B})\), and ALFF values in ORBsupmed.L were negatively correlated with the PCA scores \(( \rho = 0.419, P = 0.011; \text{Fig. 4C})\). At TP1 and TP2, there was no significant correlation between the ALFF values of the above significant brain regions and neuropsychological tests scores. Between TP1 and TP0 and between TP2 and TP0, there was no significant correlation between the difference in ALFF values in any brain region and the difference in neuropsychological tests scores.
4 Discussion

The aim of the present study was to explore trajectory of changes in brain activity and cognitive function during NAC in breast cancer patients. We observed aberrant ALFF in ORBinf.R, ORBsупmed.L, INS.R, MFG.R and SFGmed.L in breast cancer patients who was receiving NAC. And we found damage to brain activity and cognitive function mainly occurred in the early stage of chemotherapy. According to our knowledge, this is the first study of spontaneous brain activity during chemotherapy, earlier than any previous study (Chen et al., 2019; Tong et al., 2018).

We found that the neurotoxicity caused by chemotherapy was persistent, but more pronounced in the early stages of chemotherapy, which was different from previous longitudinal studies (Feng et al., 2020). Yun Feng et al. found that the functional connectivity of the brain was significantly changed one month after the end of chemotherapy, but recovered 6 months after the end of chemotherapy. We speculate that acute neurotoxicity during chemotherapy is mainly concentrated in the early stage of chemotherapy, and the late stage of chemotherapy has been tolerant to the brain oxidative stress or inflammation caused by the chemotherapy (Onzi et al., 2022), therefore, there was no further deterioration in brain function or cognitive function.

Previous studies have shown that the frontal lobe is susceptible to chemotherapy (Chen et al., 2019; Lange et al., 2019). In our study, the brain regions with significant changes during NAC, including ORBinf.R, ORBsупmed.L, MFG.R and SFGmed.L, are all components of the frontal lobe, which is consistent with previous studies. ORBinf and Orbsupmed are components of the orbitofrontal cortex. Orbital frontal cortex activity is associated with some higher-order cognitive functions, such as executive function (Nejati et al., 2018), working memory (Christophel et al., 2017; Wilson et al., 2014) and emotion regulation (Rolls et al., 2020). Our study found that the reduced ALFF values of ORBinf.R were correlated with the reduced scores of DST backward at TP0. The DST backward is related to the executive function and working memory (Peterson et al., 2016). This may indicate that the orbitofrontal cortex is the target of chemotherapy-induced neurotoxicity, and the involved orbitofrontal cortex, ORBsupmed in particular, is associated with the executive function and working memory of breast cancer patients.

We found a significant reduction of ALFF in insula during NAC. Previous studies found that the decrease in insula gray matter density and volume in breast cancer patients within 1 month after the end of chemotherapy (Chen et al., 2018; Lepage et al., 2014). Our research team has also previously found that the structure of the insula changed before the second cycle of NAC (Zhou et al., 2022). The insula is essential for neural communication between the posterior regions and prefrontal cortex (Augustine, 1996). Disruption of the insula and related regions may underlie the cognitive impairment shown in patients with chemotherapy-exposed breast cancer.

In our study, patients’ cognitive function continued to decline as the number of chemotherapy cycles increased, which is consistent with previous findings (Collins et al., 2013; Janselsins et al., 2017; van Dam et al., 1998). We found that FACT-Cog scores were significantly reduced during NAC, including PCI and PCA (represents self-reported perceived cognitive ability). It indicated that patients’ cognitive function was affected during chemotherapy. In addition, we found ALFF values in ORBsупmed.L were negatively correlated with the PCA scores at TP0. This may be due to the involvement of the orbitofrontal cortex in regulating negative emotions (Banks et al., 2007; Lebreton et al., 2009), which lead to a further decline in self-perception (Herman et al., 2018). However, we did not find a significant correlation between the difference of ALFF and the difference of PCA after chemotherapy. We speculate that this may be due to the practical effect of the neuropsychological tests, because the practical effect may be significant when patients take the same test three times in a short period of time (Bartels et al., 2010; Hausknecht et al., 2007).
In our study, anxiety was reduced at the early stages of chemotherapy, similar to the results of most previous findings (Baqutayan, 2012; Bekele et al., 2021; Schneider et al., 2016). Initially, breast cancer patients are more anxious (Voogt et al., 2005). Over time, they will come to accept the disease and adapt to its treatment. (Ng et al., 2017). Moreover, the reduction of lump size and pain relieved some patients and reduced their anxiety levels (Chintamani et al., 2011). This may be interpreted as adaptation, meaning that in the later stages of chemotherapy, patients become familiar with the treatment procedure, and therefore, the level of anxiety does not change significantly (Schneider et al., 2016). We also found that ALFF values in MFG.R were significantly positively correlated with the SAS scores at TP0. The MFG is part of the prefrontal cortex and previous studies have also shown it is associated with anxiety (Mochcovitch et al., 2014).

This study has the following limitations. First, we studied a relatively small number of breast cancer patients, which may limit our statistical ability to detect modest differences over time. Second, all patients receiving hormone therapy were excluded from this study, but the interference of estrogen changes should not be ignored. In future studies, we can expand the sample size and further perform subgroup analysis based on menstrual status. Third, practice effects can affect test results, particularly in longitudinal studies where the same tests are repeated over time on the same patients. Fourth, this study used FACT-Cog to assess cognitive function, FACT-Cog is a self-report questionnaire that is not an objective assessment of cognitive function but is more closely associated with stress, sleep, mood and fatigue. We will improve the scale design in the future. At last, our study lacked a breast cancer control group without NAC treatment and a healthy control group. Based on previous longitudinal studies, we believe that the effect of aging on these control groups is negligible during the short period of time within the NAC procedure (Chen et al., 2019). However, in future longitudinal studies, control groups should be included to explain the potential learning effects in neuropsychological tests.

5 Conclusion

In the present study, the brain activity and cognitive function of breast cancer patients changed significantly during NAC. Most brain regions with ALFF changes were located in the frontal lobe, which may be mainly involved in the higher-order of cognitive function. In addition, our preliminary findings provide evidence that the damage to brain activity and cognitive function caused by chemotherapy mainly occurred in the early stage of chemotherapy and lasted until the end of chemotherapy.

Abbreviations

ALFF Amplitude of low-frequency fluctuation
ANOVA Analysis of variance
CRCI Chemotherapy-related cognitive impairment
DST Digit Span Test
FACT-Cog Functional Assessment of Cancer Therapy-Cognitive Function
INS Insula
L Left
MFG Middle frontal gyrus
NAC Neoadjuvant chemotherapy
Declarations

Ethical Approval

This study was approved by the Ethics Committee of Chongqing University Cancer Hospital (NO. CZLS2021042-A). all participants signed informed consent before participating in the research. This work was conducted by the principles of the Declaration of Helsinki and its later amendments.

Competing interests

I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Authors’ contributions

Yixin Hu and Hong Yu contributed equally to this work, they performed the experiments and wrote the manuscript. Daihong Liu and Jiuquan Zhang contributed to study design, obtaining funding, and study supervision. Yong Lai, Jiang Liu; Yong Tan, Weiwei Lei and Jing Zhang contributed to the collection of patient samples and interpreted the results. Xiaoyu Zhou and Ying Cao contributed data and statistical analysis. All authors approved the final manuscript.

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Availability of data and materials

The data from this article cannot be shared publicly due to the privacy of the individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

References


23. Fox, M.D., Raichle, M.E. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci, 2007; 8, 700-711. DOI: 10.1038/nrn2201.


Tables
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TP0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.92 ± 9.29</td>
</tr>
<tr>
<td>Education (years)</td>
<td>9.19 ± 4.12</td>
</tr>
<tr>
<td>BMI (TP0)</td>
<td>24.52 ± 3.68</td>
</tr>
<tr>
<td>Hypertension/Nonhypertension (TP0)</td>
<td>6/30</td>
</tr>
<tr>
<td>Premenopausal/Postmenopausal (TP0)</td>
<td>16/20</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>c A/c B</td>
<td>2/4</td>
</tr>
<tr>
<td>c A/c b/c c</td>
<td>18/5/7</td>
</tr>
<tr>
<td>Subtype, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>7 (19.44%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>20 (55.56%)</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>5 (13.89%)</td>
</tr>
<tr>
<td>Basal like</td>
<td>4 (11.11%)</td>
</tr>
<tr>
<td>Chemotherapy regimen, No. (%)</td>
<td></td>
</tr>
<tr>
<td>AC-T</td>
<td>7 (19.44%)</td>
</tr>
<tr>
<td>EC-T</td>
<td>1 (2.78%)</td>
</tr>
<tr>
<td>AT</td>
<td>1 (2.78%)</td>
</tr>
<tr>
<td>TC</td>
<td>1 (2.78%)</td>
</tr>
<tr>
<td>TCbHP</td>
<td>8 (22.22%)</td>
</tr>
<tr>
<td>TCbHB</td>
<td>5 (13.89%)</td>
</tr>
<tr>
<td>TAC</td>
<td>13 (36.11%)</td>
</tr>
</tbody>
</table>

Distribution data are reported as means ± SD. The comparisons of age, education, BMI, the $P$ value was obtained using the One-way repeated measures ANOVA. Abbreviations: AC-T, doxorubicin + cyclophosphamide + docetaxel; AT, doxorubicin + docetaxel; BMI, body mass index; EC-T, epirubicin + cyclophosphamide + docetaxel; TAC, paclitaxel + doxorubicin + cyclophosphamide; TC, docetaxel + cyclophosphamide; TCbHB, docetaxel + carboplatin + trastuzumab + pyrotinib; TCbHP, docetaxel + carboplatin + trastuzumab + pertuzumab; TP, time point.
Table 2 Comparison of the neuropsychological test results

<table>
<thead>
<tr>
<th>Neuropsychological tests</th>
<th>TP0</th>
<th>TP1</th>
<th>TP2</th>
<th>P</th>
<th>TP0 vs. TP1</th>
<th>TP1 vs. TP2</th>
<th>TP0 vs. TP2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 36)</td>
<td>(n = 36)</td>
<td>(n = 36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General cognition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FACT-Cog</td>
<td>117.00 (108.08, 118.60)</td>
<td>111.15 (106.63, 116.70)</td>
<td>111.35 (105.65, 116.80)</td>
<td>0.001</td>
<td>0.004</td>
<td>1.000</td>
<td>0.004</td>
</tr>
<tr>
<td>PCI</td>
<td>63.90 (59.40, 64.80)</td>
<td>62.55 (57.83, 63.90)</td>
<td>61.20 (56.70, 64.80)</td>
<td>0.035</td>
<td>0.088</td>
<td>1.000</td>
<td>0.178</td>
</tr>
<tr>
<td>Oth</td>
<td>16.00 (16.00, 16.00)</td>
<td>16.00 (16.00, 16.00)</td>
<td>16.00 (16.00, 16.00)</td>
<td>0.827</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA</td>
<td>21.80 (18.70, 21.80)</td>
<td>19.80 (17.30, 21.80)</td>
<td>19.05 (17.30, 21.00)</td>
<td>0.001</td>
<td>0.231</td>
<td>0.231</td>
<td>0.001</td>
</tr>
<tr>
<td>QOL</td>
<td>16.00 (15.00, 16.00)</td>
<td>16.00 (15.00, 16.00)</td>
<td>16.00 (15.00, 16.00)</td>
<td>0.276</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Executive function and psychomotor speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TMT-A</td>
<td>58.50 (34.25, 90.00)</td>
<td>53.00 (30.50, 63.00)</td>
<td>48.50 (37.25, 71.25)</td>
<td>0.160</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mental flexibility</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VFT</td>
<td>37.00 (34.25, 41.75)</td>
<td>39.00 (32.25, 44.75)</td>
<td>37.50 (31.25, 41.00)</td>
<td>0.382</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DST forwards</td>
<td>7.00 (6.25, 8.00)</td>
<td>7.00 (6.00, 8.00)</td>
<td>7.00 (6.00, 8.00)</td>
<td>0.311</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DST backwards</td>
<td>5.00 (4.00, 5.00)</td>
<td>4.00 (4.00, 5.00)</td>
<td>4.00 (3.00, 5.00)</td>
<td>0.022</td>
<td>1.000</td>
<td>0.335</td>
<td>0.102</td>
</tr>
<tr>
<td>Anxiety and depression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAS</td>
<td>27.00 (26.00, 30.75)</td>
<td>26.00 (25.00, 27.00)</td>
<td>26.00 (25.00, 28.00)</td>
<td>0.016</td>
<td>0.040</td>
<td>1.000</td>
<td>0.155</td>
</tr>
<tr>
<td>SDS</td>
<td>26.00 (25.25, 28.00)</td>
<td>27.00 (25.00, 28.00)</td>
<td>26.00 (26.00, 27.00)</td>
<td>0.721</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nonnormal distribution data are reported as [M(QR)]. $P$ value was obtained using Friedman's M test. A post hoc test, corrected by the Bonferroni test, was then conducted for pairwise comparison in each analysis, revealing a significant effect. Abbreviations: DST, Digital Span Test; FACT-Cog, Functional Assessment of Cancer Therapy-Cognitive Function; Oth, Comments from Others; PCA, Perceived Cognitive Abilities; PCI, Perceived Cognitive Impairments; QOL, Impact on Quality of Life; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale; TMT-A, Trail Making Test A; TP, time point; VFT, Verbal Fluency Test.
Table 3 Repeated measures ANOVA and pairwise comparison of ALFF among the three groups

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>BA</th>
<th>t-value</th>
<th>Cluster (voxels)</th>
<th>Peak MNI coordinates</th>
<th>F value</th>
<th>P value</th>
<th>TP0 vs. TP1 (P)</th>
<th>TP1 vs. TP2 (P)</th>
<th>TP0 vs. TP2 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORBinf.R</td>
<td>47</td>
<td>12.6444</td>
<td>18</td>
<td>36 36 -18</td>
<td>24.547</td>
<td>&lt;0.001</td>
<td>**&lt;0.01</td>
<td>0.987</td>
<td>**&lt;0.01</td>
</tr>
<tr>
<td>ORBsupmed.L</td>
<td>11</td>
<td>13.4835</td>
<td>52</td>
<td>-3 51 -9</td>
<td>40.972</td>
<td>&lt;0.001</td>
<td>*&lt;0.05</td>
<td>0.292</td>
<td>****&lt;0.0001</td>
</tr>
<tr>
<td>INS.R</td>
<td>48</td>
<td>15.8532</td>
<td>25</td>
<td>45 18 -3</td>
<td>20.894</td>
<td>&lt;0.001</td>
<td>***</td>
<td>0.663</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>MFG.R</td>
<td>11</td>
<td>17.987</td>
<td>112</td>
<td>27 54 0</td>
<td>30.549</td>
<td>&lt;0.001</td>
<td>***</td>
<td>0.806</td>
<td>**&lt;0.01</td>
</tr>
<tr>
<td>SFGmed.L</td>
<td>10</td>
<td>10.7993</td>
<td>21</td>
<td>0 57 12</td>
<td>26.060</td>
<td>&lt;0.001</td>
<td>*&lt;0.05</td>
<td>0.724</td>
<td>***&lt;0.001</td>
</tr>
</tbody>
</table>

ALFF, the amplitude of low-frequency fluctuation; INS.R, right insula; MFG.R, right middle frontal gyrus; ORBinf.R, right orbital part of inferior frontal gyrus; ORBsupmed.L, left medial orbital part of Superior Frontal Gyrus; SFGmed.L, left medial part of Superior Frontal Gyrus; TP, time point. All statistical maps were corrected with Gaussian Random Field method with the significance of voxel $P < 0.001$, cluster $P < 0.05$, two-tailed. $****P < 0.0001$, $***P < 0.001$, **$P < 0.01$, *$P < 0.05$ after Bonferroni correction of post hoc analysis.

Figures
Figure 1
Comparisons of neuropsychological test scores at three time points in breast cancer patients (repeated-measures ANOVA), **\( P < 0.01 \), *\( P < 0.05 \) after Bonferroni correction of post hoc analysis. DST, Digital Span Test; FACT-Cog, Functional Assessment of Cancer Therapy-Cognitive Function; PCA, Perceived Cognitive Abilities; PCI, Perceived Cognitive Impairments; SAS, Self-Rating Anxiety Scale; TP, time point.

Figure 2
Distribution of ALFF at TP0, TP1 and TP2 (one-sample \( t \)-test) (A-C). Comparisons of ALFF among TP0, TP1 and TP2 (repeated measures ANCOVA) (D). Corrected with Gaussian random-field theory (voxel level \( P < 0.001 \), cluster level \( P < 0.05 \)). The color bar denotes the \( t \) value. R, right; L, left. ALFF, amplitude of low-frequency fluctuation; TP, time point.
Pairwise comparison of ALFF in the five brain regions. Repeated measures ANOVA was used to compare the ALFF values of the three groups, and Bonferroni correction of post hoc analysis was performed at the same time in the significant brain regions (A–E). ****\(P < 0.0001\), ***\(P < 0.001\), **\(P < 0.01\), *\(P < 0.05\) after Bonferroni correction of post hoc analysis. ALFF, the amplitude of low-frequency fluctuation; INS.R, right insula; MFG.R, right middle frontal gyrus; ORBinf.R, right orbital part of the inferior frontal gyrus; ORBsupmed.L, left medial orbital part of the superior frontal gyrus; SFGmed.L, left medial part of the superior frontal gyrus; TP, time point.
Figure 4

The correlation between ALFF values and neuropsychological tests scores. ALFF values in ORBinf.R ($\rho = 0.355$, $P = 0.033$) and MFG.R ($\rho = 0.414$, $P = 0.012$) were significantly positively correlated with the scores of DST backward and SAS, respectively (A, B). ALFF values in ORBsupmed.L were significantly negatively correlated with the PCA scores ($\rho = 0.419$, $P = 0.011$) (C). ALFF, amplitude of low-frequency fluctuation; DST, Digital Span Test; INS.R, right insula; MFG.R, right middle frontal gyrus; ORBinf.R, right orbital part of the inferior frontal gyrus; ORBsupmed.L, left medial orbital part of the superior frontal gyrus; PCA, Perceived Cognitive Abilities; SAS, Self-Rating Anxiety Scale; SFGmed.L, left medial part of the superior frontal gyrus; TP, time point.