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Changes in Brain Structural Networks and Cognitive Functions in Testicular Cancer Patients Receiving Cisplatin-Based Chemotherapy

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Abstract

Background: Cisplatin-based chemotherapy may have neurotoxic effects within the central nervous system. The aims of this study were 1) to longitudinally investigate the impact of cisplatin-based chemotherapy on whole-brain networks in testicular cancer patients undergoing treatment and 2) to explore whether possible changes are related to decline in cognitive functioning.

Methods: Sixty-four newly orchiectomized TC patients underwent structural magnetic resonance imaging (T1-weighted and diffusion-weighted imaging) and cognitive testing at baseline prior to further treatment and again at a six-month follow-up. At follow-up, 22 participants had received cisplatin-based chemotherapy (CT) while 42 were in active surveillance (S). Brain structural networks were constructed for each participant, and network properties were investigated using graph theory and longitudinally compared across groups. Cognitive functioning was evaluated using standardized neuropsychological tests. All statistical tests were two-sided.

Results: Compared with the S group, the CT group demonstrated altered global and local brain network properties from baseline to follow-up as evidenced by decreases in important brain network properties such as small-worldness ($P = .04$), network clustering ($P = .04$), and local efficiency ($P = .02$). In the CT group, poorer overall cognitive performance was associated with decreased small-worldness ($r = -0.46$, $P = .04$) and local efficiency ($r = -0.51$, $P = .02$), and verbal fluency was associated with decreased local efficiency ($r = -0.55$, $P = .008$).

Conclusions: Brain structural networks may be disrupted following treatment with cisplatin-based chemotherapy. Impaired brain networks may underlie poorer performance over time on both specific and nonspecific cognitive functions in patients undergoing chemotherapy. To the best of our knowledge, this is the first study to longitudinally investigate changes in structural brain networks in a cancer population, providing novel insights regarding the neurobiological mechanisms of cancer-related cognitive impairment.

Testicular cancer (TC) accounts for the majority of solid malignant tumors in young men in developed countries (1). With the introduction of cisplatin-based regimens, TC has become the

most curable solid tumor (2). Unfortunately, cisplatin-based chemotherapy has well-known side and late effects including Reynaud-like symptoms, ototoxicity, and neurotoxicity (3,4),

and furthermore, cisplatin may penetrate the brain, with adverse effects on neuronal populations (5). In our own work with TC patients, we have shown that cisplatin-based chemotherapy is associated with reduced brain gray matter density in the prefrontal cortex, as well as overall cognitive decline (6). Other studies have also reported cognitive impairment related to cisplatin-based chemotherapy (7,8). Optimal cognitive functioning, however, does not just rely on the integrity of neuronal cells, but is dependent on the successful processing and integration of information across the brain (9,10). Cortical and subcortical structures are highly interconnected by white matter (WM) pathways consisting of glial cells and myelinated axons forming a complex, yet highly efficient, structural network that has been described as a small-world network, which is characterized by high clustering in local regions while retaining relatively short path lengths across all brain regions, supporting the notion of the brain as a functionally segregated yet highly distributed and efficient system (11,12). Impairment to the structural network has been demonstrated to have detrimental effects on cognition (13,14). Furthermore, in breast cancer populations, acute and long-term impairments in WM integrity, as well as disrupted structural networks, have been demonstrated to have adverse effects on cognitive functioning (15–18). To the best of our knowledge, one cross-sectional study has previously reported changes in WM microstructure in long-term TC survivors (8). Clearly, much remains unknown about the effects of cisplatin-based chemotherapy on brain WM connectivity and networks in TC populations. The aims of the present prospective study were, thus, 1) to longitudinally investigate the impact of cisplatin-based chemotherapy on whole-brain WM networks and 2) to explore whether possible changes in brain networks are related to cognitive functioning.

To investigate these aims, we undertook diffusion tensor imaging (DTI) and applied graph theoretical analyses to investigate the organization of large-scale brain structural networks (see the Supplementary Methods, available online). The strength of a graph theoretical approach to the study of brain networks is that it allows for the quantification and assessment of the entire brain network. So far, graph theoretical approaches have been used in cross-sectional studies of survivors of breast cancer and childhood leukemia, demonstrating chemotherapy-induced alterations in both functional and structural brain networks (18–21). Possible chemotherapy-induced brain network alterations are yet to be investigated in TC populations, and there are, to the best of our knowledge, currently no longitudinal studies with cancer populations using graph analysis to investigate such brain network changes.

Methods

Participants

Recently orchiectomized TC patients were consecutively recruited from June 2012 to December 2013 at the Department of Oncology, Aarhus University Hospital (National Committee on Health Research Ethics trial registration No. 1-10-72-156-12). Applied exclusion criteria were age younger than 18 years, time since orchiectomy greater than 30 days, previous cancer and central nervous system diseases, brain metastases, known mental disorders, inability to read and understand Danish, and any contraindications for magnetic resonance imaging (MRI). Informed consent was obtained from all participants upon enrollment. The regional scientific ethical committee approved

the study, and data were handled according to The Danish Data Protection Agency guidelines.

Patients consisted of two groups: 1) a surgery-only group (S) and 2) a chemotherapy group that, in addition to surgery, received three or four cycles of chemotherapy consisting of bleomycin, etoposide, and cisplatin (CT). All patients were scheduled for two assessments: shortly after orchiectomy but prior to further treatment (baseline) and approximately six months later (follow-up), corresponding to approximately three months after completed chemotherapy for the CT group. Assessments included a structural MRI scan, blood sampling (results reported elsewhere [22]), neuropsychological testing, and a questionnaire package.

Clinical and demographic data were obtained from medical records and questionnaires.

Magnetic Resonance Imaging

Please refer to Figure 1 and the Supplementary Methods (available online) for details regarding the scan acquisition, preprocessing, and construction of brain networks.

Neuropsychological Testing

The first author and a trained research assistant undertook all neuropsychological testing. Participants were tested by the same test administrator at both time points. A neuropsychological test battery, that lasted approximately 1.5 hours and consisted of eight standardized tests, was used for the assessment of different cognitive domains: Reaction time was measured with the Lafayette reaction time panel (23); attention and working memory with the Paced Auditory Serial Addition Test and Wechsler Adult Intelligence Scale, version 4 (WAIS-IV) Digit Span (24,25); processing speed with WAIS-IV Coding and the Trail Making Test (TMT)–Part A (24,26); learning and memory with the Rey Auditory Verbal Learning Test (RAVLT) using the total learning and retention scores (27); verbal fluency with the Controlled Oral Word Association Test using the letters F, N, and S (28); and finally, executive functioning with the TMT–Part B and the Wisconsin Card Sorting Test (26,29). Premorbid intellectual functioning was estimated with the WAIS-IV Vocabulary (24). For further details regarding specific test outcomes, please refer to our previous publication (22).

Statistical Analysis

Group differences were tested with *t* tests and the Mann-Whitney test for continuous variables and chi-square tests for categorical variables. All performed statistical tests were two-sided, and a *P* value of less than .05 was considered statistically significant.

Longitudinal Brain Network Analysis

Longitudinal graph analysis was performed with the Graph Analysis Toolbox, version 1.4.1 (30), using the following procedure: First, networks were normalized by the mean network strength, and the following graph measures were quantified for the normalized networks: characteristic path length (*L*), network clustering coefficient (*C*), local and global efficiency. The small-world organization of each network was assessed by calculating a measure of its small-worldness (*SW*) defined as $SW = [C/C_{rand}]/[L/L_{rand}]$, where C_{rand} and L_{rand} are mean values

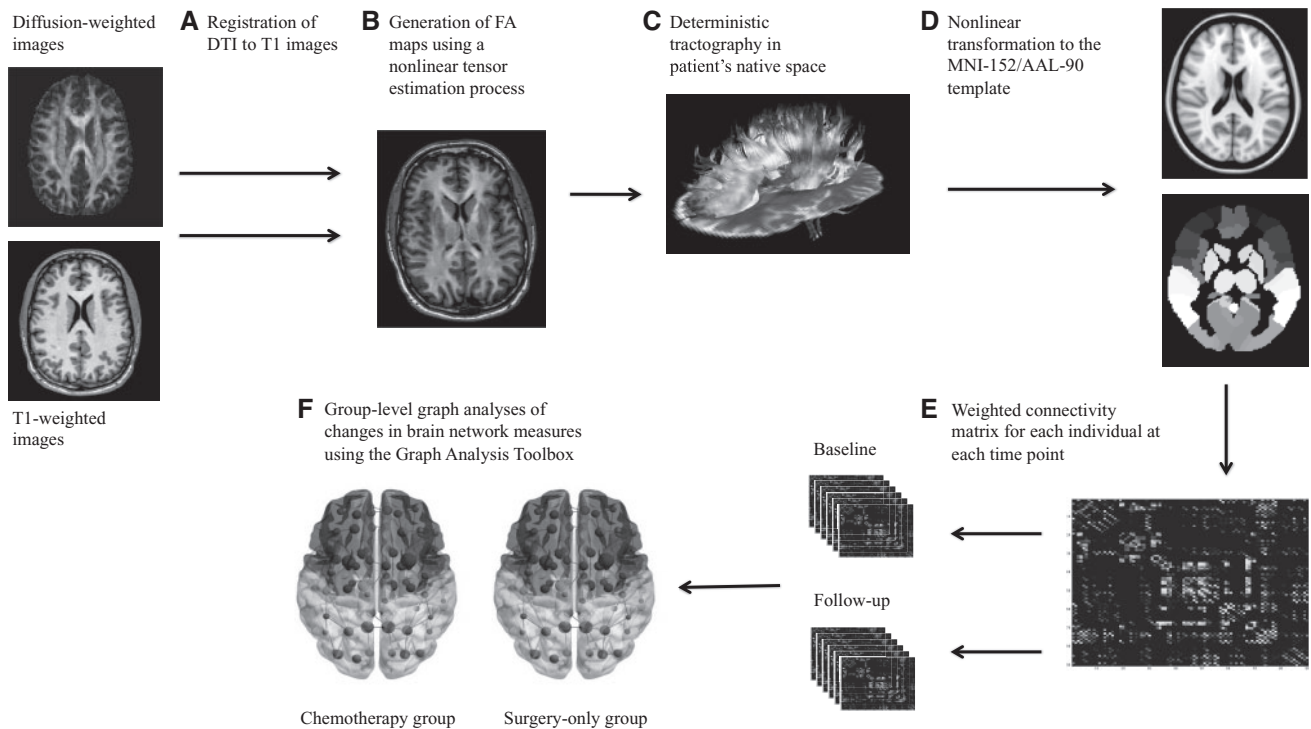


Figure 1. Brain structural network construction for longitudinal graph analysis. Postprocessing steps (A–F) to create brain structural networks for longitudinal graph analysis. **A)** Diffusion-weighted images were registered to T1-weighted images. **B)** An iterative nonlinear tensor estimation process was used to generate fractional anisotropy (FA) maps. **C)** Whole-brain tractography was performed in native space using a deterministic streamline approach. **D)** The whole-brain tractography was then parcellated using the Automated Anatomical Labeling atlas, in which the Montreal Neurological Institute brain template is divided into 90 cortical and subcortical regions of interest. **E)** For each individual, FA-weighted connectivity matrices were created for each time point. **F)** Longitudinal group-level graph analyses of changes in brain network measures were performed using the Graph Analysis Toolbox. AAL = Automated Anatomical Labeling; DTI = diffusion-weighted images; FA = fractional anisotropy; MNI = Montreal Neurological Institute.

of C and L from corresponding random networks. Networks were considered to have a small-world organization when SW was greater than 1 (31). A nonparametric permutation test with 2000 repetitions was then used to test the statistical significance of between-group differences in changes in graph metrics (slope) controlling for age, education, and intracranial volume (19). In each permutation, the calculated regional streamlines of each participant (at either time point) were randomly assigned to one of the two groups so that each randomized group had the same number of subjects as the original groups. The null distribution of between-group differences in graph metric changes (slope) across time were then calculated. The actual difference in the slope between groups was compared with the obtained permutation distribution of difference in slope between randomized groups in order to obtain a *P* value for each network metric. In order to investigate possible associations between decreased network measures and neuropsychological performance, as well as demographic variables, change scores for network measures were calculated by subtracting follow-up values from baseline values.

Neuropsychological Performance and Cognitive Decline

Changes over time in cognitive performance were investigated using a standardized regression-based (SRB) approach based on repeated neuropsychological testing of a matched healthy control group as described in the Supplementary Methods (available online). Following this approach, a global composite *z* score (GCS-*z*) was calculated capturing overall cognitive performance, while cognitive domain-level *z* scores were calculated based on

the means of domain-relevant tests as indicated above. Subsequently, participants with *z* scores of -1.64 or lower on either the GCS-*z* or on domain-level *z* scores were classified as evidencing clinically significant cognitive decline. The applied cutoff indicates scores that fall in the lowest 5% of the normal distribution (32). The GCS-*z* and cognitive domain *z* scores were also used to explore possible associations with brain network changes. For each statistically significant association, a subsequent interaction test was performed using a linear regression model with group, corresponding network measure change variable, and mean-centered group $\times$ change interaction variable entered as predictors.

Results

Participants

Of 94 eligible patients, 66 patients (70%) agreed to be enrolled with no statistically significant differences between participating and nonparticipating patients regarding histology, cancer stage, chemotherapy ordination, or occupational status (data not shown). Of the 66 enrolled participants, two patients were lost to follow-up (3.0%): One participant stopped attending his medical consultations and did not respond to our repeated notices, while another did not undergo full MRI assessment and was excluded from the present study. Mean age for the remaining sample was 36.7 years ($SD = 10.8$ years), and all participants were assessed twice with a mean test-retest interval of six months ($SD = 1.2$ years). The S group consisted of 42

participants, and the CT group consisted of 22 participants. As presented in Table 1, no statistical differences were observed between groups on any of the demographic variables except for age, where the CT group was, as expected, younger than the S group ($P = .008$). On clinical variables, the CT group had greater disease severity as indicated by cancer stage, metastatic involvement, and cancer histology (all P s $< .001$).

Brain Network Analysis

All participants demonstrated a small-world organization as indicated by SW greater than 1. For the entire group, shorter characteristic path length at baseline, which indicates more efficient network organization, was associated with higher premorbid intellectual functioning as captured by the WAIS-IV vocabulary subtest ($r = -0.26$, $P = .04$; data not shown). At baseline, groups did not differ in number of reconstructed streamlines ($P = .14$) or in any of the network measures (P s = .18–.94). Results from the longitudinal graph analysis revealed statistically significant decreases in brain network measures from baseline to follow-up in the CT group compared with the S group (Table 2). Specifically, statistically significant decreases were observed in local efficiency ($P = .02$), network clustering ($P = .04$), and small-worldness ($P = .04$).

Cognitive Decline

Frequencies of cognitive decline by group are presented in Table 3. As reported in our previous publication (6), a trend toward a greater frequency of overall cognitive decline was observed in the CT group (63.6%) compared with the S group (38.1%, $P = .07$). Furthermore, a marginally greater frequency of decline was observed in the cognitive domain of verbal learning and memory in the CT group compared with the S group (7.5% vs 28.6%, respectively, $P = .05$).

Associations Between Changes in Brain Network Measures and Cognitive Performance

Lower premorbid intellectual functioning was statistically significantly associated with decreased local and global efficiency (both r s = -0.44 , P s = .04) and decreased clustering ($r = -0.45$, $P = .03$) in the CT group, but not in the S group (all P s $> .10$), although all associations were in similar directions for this group too (data not shown). In the CT group, greater decreases in network local efficiency ($r = -0.51$, $P = .02$) and network small-worldness ($r = -0.46$, $P = .04$) were associated with poorer overall cognitive performance across time, as captured by the GCS-z. In the S group, no statistically significant associations were observed. However, formal tests of group $\times$ change interactions did not reach statistical significance levels (local efficiency: $\beta = -0.35$, $P = .07$; small-worldness: $\beta = -0.38$, $P = .05$). Furthermore, greater decrease in local efficiency was associated with poorer verbal fluency in the CT group but not in the S group ($r = -0.55$, $P = .008$). Again, a formal group $\times$ change interaction test was not statistically significant ($\beta = -0.31$, $P = .07$).

Discussion

To the best of our knowledge, this is the first study to longitudinally investigate changes in structural networks in a cancer population. Using this approach, we found that TC patients who

Table 1. Demographic and clinical characteristics of participant groups

Characteristic	Surgery only (n = 42)	Surgery+ chemotherapy (n = 22)	P*
Demographics			
Age, mean (SD), y	39.2 (10.5)	31.9 (9.4)	.008
Education, mean (SD)	14.2 (2.5)	14.1 (1.8)	.75
Premorbid cognitive functioning, mean (SD)†	10.2 (1.9)	10.1 (2.3)	.85
Ethnicity, No. (%)			–
Caucasian	42 (100.0)	22 (100.0)	
Occupational status, No. (%)			.11
Unemployed	1 (2.4)	3 (13.6)	
Occupationally engaged	41 (97.6)	19 (86.4)	
Annual income (in 100 000 DKK), mean (SD)	4.0 (1.9)	3.8 (2.2)	.68
Marital status, No. (%)			.24
Married or cohabiting	33 (78.6)	14 (63.3)	
Single	9 (21.4)	8 (36.4)	
Clinical variables			
Histology, No. (%)			<.001
Seminoma	33 (78.6)	4 (18.2)	
Nonseminoma	9 (21.4)	18 (81.8)	
Cancer stage, No. (%)			<.001
I	40 (95.2)	4 (18.2)	
II	2 (4.8)	15 (68.2)	
III	–	3 (13.6)	
Orchiectomy (unilateral), No. (%)	42 (100.0)	22 (100.0)	–
Type of anesthesia, No. (%)			–
General	42 (100.0)	22 (100.0)	
Metastatic involvement, No. (%)	0 (0.0)	22 (100.0)	<.001
Chemotherapy, No. (%)			–
BEP $\times$ 3	–	20 (90.9)	
BEP $\times$ 4	–	2 (9.1)	
Radiotherapy, No. (%)	4 (9.5)	1 (4.5)	.65

*Group differences tested with two-sided t tests or the Mann-Whitney test for continuous variables and chi-square tests for the categorical variables. BEP = bleomycin, etoposide, cisplatin; DKK = Danish Krone.

†Vocabulary subtest from Wechsler Adult Intelligence Scale, version 4.

received cisplatin-based chemotherapy evidenced changes in both global and local network measures compared with patients who did not receive such treatment. Specifically, network local efficiency, small-worldness, and clustering, all important measures of optimal network organization, were decreased at three months after completing chemotherapy. Network efficiency indicates how efficiently information may be exchanged across the entire network (33) and has been shown to be important for optimal cognitive functioning (34). Local efficiency, which is a measure of the average of local subgraphs in a network, may reveal how tolerant a network is to local failures (33). These results indicate that treatment with cisplatin-based chemotherapy may result in large-scale brain network changes. The nature of these changes may be characterized by a more random organization, which may result in reduced brain functional specificity and segregation with implications for cognitive functions.

A recent cross-sectional study also demonstrated reduced brain structural network efficiency in response to a simulated neurodegeneration in breast cancer survivors receiving

Table 2. Brain network measures from baseline to follow-up in each group

Brain network measure	Surgery only, mean (95% CI) (n = 42)		Surgery+chemotherapy, mean (95% CI) (n = 22)		P*
	Baseline (T1)	Follow-up (T2)	Baseline (T1)	Follow-up (T2)	
Reconstructed streamlines	11 767 (11 026 to 12 564)	11 649 (10 894 to 12 447)	10 852 (10 178 to 11 600)	11 253 (10 421 to 12 113)	.16
Small-worldness	1.88 (1.81 to 1.95)	1.89 (1.83 to 1.95)	1.96 (1.87 to 2.04)	1.85 (1.78 to 1.92)	.04
Characteristic path length	1.09 (1.08 to 1.10)	1.09 (1.08 to 1.10)	1.09 (1.08 to 1.10)	1.09 (1.08 to 1.11)	.68
Clustering coefficient	2.05 (1.98 to 2.11)	2.05 (1.99 to 2.11)	2.13 (2.04 to 2.22)	2.02 (1.95 to 2.08)	.04
Local efficiency	0.049 (0.047 to 0.050)	0.048 (0.047 to 0.049)	0.050 (0.048 to 0.051)	0.047 (0.046 to 0.048)	.02
Global efficiency	0.055 (0.054 to 0.056)	0.056 (0.054 to 0.056)	0.056 (0.055 to 0.057)	0.055 (0.054 to 0.056)	.18

*Group differences in changes in graph metrics were tested with two-sided nonparametric permutation tests controlling for age, education, and intracranial volume. CI = confidence interval.

Table 3. Frequency of patients with decline on cognitive domains

Cognitive domain	Surgery + chemotherapy		P*
	Surgery only No. (%)	No. (%)	
Reaction time	2 (5.1)	1 (4.5)	1.00
Processing speed	2 (4.8)	4 (18.2)	.17
Attention and working memory	5 (12.5)	3 (14.3)	1.00
Learning and memory	3 (7.5)	6 (28.6)	.05
Verbal fluency	11 (26.2)	3 (13.6)	.35
Executive functions	5 (13.2)	3 (13.6)	1.00
Overall cognitive decline	16 (38.1)	14 (63.6)	.07

*A two-sided Fisher's exact test was used to calculate the P values.

chemotherapy compared with healthy controls (18). In the survivors, lower network attack tolerance was associated with greater cognitive impairment. Another recent cross-sectional study revealed atypical structural brain networks associated with chemotherapy as evidenced by reduced small-worldness and clustering in young survivors of childhood leukemia. Furthermore, poorer network organization was found to be associated with greater cognitive impairment (21). Although the chemotherapy regimens used in the above-mentioned populations differ from those administered to TC patients, these cross-sectional studies, together with the findings reported here, indicate that changes to the brain's structural network may be an important neurobiological pathway of chemotherapy-induced cognitive impairment.

We have previously shown evidence of morphological changes in cerebral gray matter in the present sample of TC patients both related and unrelated to cisplatin-based chemotherapy (6). Chemotherapy-induced decreases in gray matter density were observed in prefrontal areas including the right paracingulate gyrus and the right frontal pole. Furthermore, these changes were associated with poorer overall cognitive performance. In the present study, we expand on these results by investigating chemotherapy-induced alterations in whole-brain WM structural networks, and, as in our previous publication, we observed a marginally higher frequency of overall cognitive decline in the CT group compared with the S group (6). In the present paper, we investigated the frequency of domain-level cognitive decline by combining test outcomes belonging to a given cognitive domain.

Cognitive impairment frequencies are often found to be higher when assessed at the test level compared with

frequencies obtained by using domain- or global-level composite scores (35). However, individual test-level outcomes tend to be more variable than domain scores, especially among those with an absence of neurological disorders (36), and may artificially inflate cognitive impairment frequencies. Applying domain- and global-level composite scores in order to assess group differences in impairment frequencies may, thus, be a more robust approach than simply using individual test-level outcomes.

Consistent with this, the previously reported decline frequencies on individual test-level outcomes of verbal learning and memory in the S group (namely RAVLT total and delayed scores) were twice the frequency of the domain-level frequency reported in the present study (7.5%) (6). For the CT group, however, this was not the case, which may demonstrate that the learning and memory decline observed in this group are more consistent across individual test outcomes. Accordingly, our results revealed a marginally higher frequency of learning and memory decline in the CT group (28.6%) compared with the S group (7.5%), which was not detected in our previous publication.

Regarding the associations between chemotherapy-induced brain network changes and cognitive performance, we observed that decreased small-worldness and local efficiency was related to poorer overall cognitive performance across time, while decreased local efficiency was also associated with poorer verbal fluency. In healthy populations, more efficiently organized networks have been related to better cognitive functioning and higher intelligence (37), supporting the notion of optimal cognitive processes being dependent on an effective network organization characterized by both high segregation and high system-level integration across brain regions (38). In accordance with this, we found that shorter characteristic path length, but not other network measures, was associated with higher levels of premorbid intellectual functioning for the entire TC group at baseline. Our results also indicated that lower premorbid intellectual functioning, a proxy for cognitive reserve, was associated with more drastic longitudinal changes in brain network organization in patients receiving chemotherapy. This lends support to the notion of cognitive reserve as a protective factor against chemotherapy-induced cognitive decline (39). Cognitive reserve refers to the brain's capacity to cope with cerebral damage, and evidence indeed suggests a relationship between brain structure and cognitive reserve proxies in both healthy and pathological aging (40). In this regard, individuals with greater cognitive reserve have a higher tolerance level for neuropathological effects before clinical manifestations may become evident.

Although the underlying molecular and cellular mechanisms of cerebral damage following cisplatin-based chemotherapy are still relatively unknown, both direct effects, through the penetration of the blood-brain barrier (5,41), and indirect neuro-inflammatory effects, through chronically activated brain microglia, have been suggested as possible pathways (42). Recent evidence has demonstrated decreases in brain WM integrity and reduced dendritic spine density and neuronal arborization, as well as cognitive dysfunction, in mice treated with cisplatin (43). Specifically, coherency of myelin basic protein fiber was suggested as the possible mechanism of the observed decrease in WM integrity. In contrast to a neuroinflammatory account of cisplatin-induced neuronal damage, the group did not observe microglial activation in cisplatin-treated mice (43). Of particular interest, the group also demonstrated that administration of the drug metformin, a common drug to treat type 2 diabetes, had protective effects against cisplatin-induced cognitive dysfunction and peripheral neuropathy although the causal mechanisms remain unknown. In another recently published study, the effects of cisplatin on neurotransmitter release were investigated at the cell level using single-cell amperometry (44). This study revealed that cisplatin, at both low and high concentrations, regulates the exocytotic ability of cells, resulting in impaired neural communication at the neurotransmitter level. Despite such novel findings, much research is still needed before the molecular and cellular mechanisms of cisplatin-induced cerebral damage are clearly understood.

In summary, we report the first longitudinal evidence of brain structural network alteration in TC patients receiving chemotherapy, providing novel insights regarding both the neurobiological change mechanisms of cancer-related cognitive impairment and further evidence of chemotherapy-induced cognitive impairment in testicular cancer patients. Our results may be used to create more awareness in health care professionals about the risk of brain and cognitive changes following cisplatin-based chemotherapy with the potential to improve patient decision-making processes, as well as potentially guiding future treatment and rehabilitation efforts in this understudied population.

Strengths of the present study include a cancer control group with similar demographic characteristics, a longitudinal design with repeated MRI assessment with the application of a longitudinal graph theoretical approach to analyze structural brain networks, and domain-level analyses of cognitive testing. Recruitment levels were satisfactory (70%), with a low dropout rate at the follow-up assessment (3%).

The main limitations of the present study are related to uncorrected multiple testing of exploratory associations between changes in network metrics and behavioral outcomes. Furthermore, unequal group sizes may have reduced our ability to detect associations with equal statistical power for both groups. However, given that most of the observed statistically significant results were found in the smaller CT group, this limitation may not necessarily apply. Finally, intrinsic clinical differences between TC groups (ie, cancer type, stage, and metastatic involvement) could potentially confound chemotherapy-specific results. Future larger longitudinal studies, preferably with long-term follow-ups, are required to replicate these results and to investigate the trajectory and potential reversibility of cisplatin-induced changes.

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Notes

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