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A randomized controlled clinical study of music therapy in cancer patients with anxiety

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A randomized controlled clinical study of music therapy in cancer patients with anxiety

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Abstract

Introduction

The purpose of this study is to evaluate the effect of music therapy on the improvement of cancer-related anxiety in cancer patients.

Methods and analysis

This is a randomized, open, parallel-controlled trial with a single center. Sixty patients with malignant tumor who meet the inclusion criteria will be randomly assigned in a 1:1 ratio to either music therapy(MT) group (study group) or non-music therapy(NMT) group (control group). Personalized music therapy is used MT group. The NMT group receives routine nursing without any music intervention. State-Trait Anxiety Inventory (STAI), Distend Thermometer (DT), and Hamilton Anxiety Scale (HAMA) are the primary outcomes. Secondary outcomes include the QLQ-C30 questionnaire and blood index (serum TNF-β, INF- γ , IL-2R, IL-4, IL-6, IL-8 and IL-10).

Ethics and dissemination

On August 5, 2020, the Research Ethical Committee accepted the research of Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine. The findings of the experiment will be published in peer-reviewed publications and presented at appropriate conferences.

Trial registration number CTR2000035244

STRENGTHS AND LIMITATIONS OF THIS STUDY

1.All interventions will be instructor-led, the music therapist will play a personalized music clip based on the patient's type of music preference.

2.One limitation is that the trial implemented in only one hospital, which may limit its generalisability.

3.The study protocol duration of 12 weeks may be seen as limitation of the study design.

Introduction

Cancer endangers human health. Clinical treatment methods such as surgical resection,

chemotherapy, targeted therapy and immunotherapy are often used at the moment. However, as a result of disease and adverse effect of anti-tumor treatments patients often have psychological stress reactions and negative emotions such as anxiety and depression, which affect the quality of their lives[1, 2]. A cross-sectional, prospective study suggested the prevalence of moderate to severe depressive in patients with advanced solid tumors was 29.2%[3]. The National Comprehensive Cancer Network (NCCN) classifies oncology patients' variable psychological problems as "psychosocial distress", which includes anxiety, indicating that psychological problems can interfere with the ability to cope with disease and and have a negative impact on physical symptoms[4]. Although the somatic effects of cancer and treatment have been reported in literatures, interventions for psychological problems remain on the periphery of oncology treatment[5], and they are frequently not treated effectively[6]. Since positive coping has a significant protective effect on both physical and mental health, it is important to investigate an effective anxiety intervention.

Currently, the treatment of cancer anxiety is frequently focused on the primary tumor. Despite this, few studies have focused on treating anxiety disorders caused by it, and even fewer have focused on non-pharmacological treatments. Music therapy is an emerging, marginal interdisciplinary discipline that integrates music, medicine, and psychology and extends the role of music beyond the traditional fields of art appreciation and aesthetics. In the case of cancer, music therapy can help patients improve their positive attitude toward the disease by regulating their emotions and managing their symptoms[7, 8]. Music therapy has also been shown in cancer patients to reduce anxiety associated with the primary disease or treatment-related anxiety, thereby improving the efficacy of subsequent treatment[9, 10].

We hypothesized that music therapy significantly improves anxiety performance in cancer patients who are also anxious. As a result, the purpose of this study is to assess the effect of music therapy on the reduction of cancer-related anxiety in cancer patients and to provide a theoretical foundation for the clinical application of music therapy in the comprehensive treatment of tumors.

Materials and Methods

Study design

This is an RCT (Randomized Controlled Trial, RCT). This study will enroll 60 cancer patients who are experiencing cancer-related anxiety. For 12 weeks, the patients will be randomly divided into two groups and treated with music therapy or routine nursing without music treatment. The research will be carried out at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, between 10 August 2022 and 31 December 2022. Figure 1 depicts the trial design flowchart based on the Standard Protocol Items.

Inclusion criteria

Subjects who meet the following inclusion criteria are included in this study: (1) malignant tumor confirmed by histopathology or cytology (2) cancer-related anxiety treatment or cancer itself >= 2 months and <= 1 year; (2) Meet the standard of anxiety in HAMA >= 7 and <= 56; (3) Aged 18 to 74 years old, no gender restrictions; (4) No history of other mental disorders; Do not smoke or drink; (5) Conscious, behaving normally, hearing normally, no professional music background; Heart, liver, kidneys, blood tests, and other vital signs are all normal; (6) No anti-anxiety medications within four weeks prior to study entry; (7) Expected survival time of more than 6 months; (8) Agreeing to take part in the study and signing the informed consent form.

Exclusion criteria

Those who meet any of the following exclusion criteria are barred from participating in the study: (1) Participating in other clinical studies or clinical trials; (2) Having other serious diseases, such as infection, liver and kidney failure, that the project leader or researchers consider unable to tolerate the treatment regimen of this study; (3) Primary or metastatic brain tumors confirmed clinically or radiologically; (4) Pregnant or lactating women; (5) Having received music therapy within 3 months prior to the study; (6) Taking other anxiety medications or medications that affect anxiety.

Rejection, suspension and shedding criteria

(1) Wrong inclusion or exclusion; (2) Patients with poor compliance, and fail to follow up as required; (3) Incomplete medical records; (4) Patients withdraw from the study by themselves; (5) Patients who are not suitable for the continued study due to serious deterioration of disease, severe complications or special physiological changes; (6) Patients who are not considered suitable to continue the study by investigator.

Participant recruitment

This study aims to include 60 cancer patients who are experiencing cancer-related anxiety. Patients were recruited at the Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine Recruitment advertisements are posted on the hospital's website and on posters, with a brief description of the study's aims, requirements, and ways to experiment in this study. All participants will provide informed consent. The recruitment process began in August 2022 and will last until December 2022.

Informed consent

All study processes were explained to participants prior to the start of the study. Participants were also informed that their participation in the trial was entirely voluntary and that they could opt out at any time. Each participant is informed and signs a written informed consent form before receiving any intervention.

Randomization and allocation concealment

Patients were randomly assigned to the treatment and control groups in a 1:1 ratio at

the time of enrollment. SPSS 21.0 software was used in practice to generate a set of randomized numbers, which were then randomized into opaque envelopes. The order in which the patients entered the study determined the grouping in the corresponding envelopes for allocation.

Intervention

Patients in NMT group received only a nursing routine and were not allowed to receive any treatment for anxiety state intervention. They were observed for one week and scored on the STAI, DT, and QLQ-C30 scales.

Patients in MT group were given music therapy under the supervision of a therapist after enrollment, three times a week for one week, and detailed records were kept. The music therapist played a personalized music clip created on-site for 20 minutes based on the patient's type of music preference. Simultaneously, another music therapist used a psycho-educational approach in conjunction with verbal instruction. Music therapy requires the following conditions: (1) no bright light interference; (2) the patient is in a resting or inactive state; (3) music is played through speakers, and the volume is controlled between 45-65%.

Data collection

The reviewers of the Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine evaluated and collected data from inpatients at screening and baseline, interventional and follow-up periods.

Enrolment and baseline

Patients were screened at admission using inclusion-exclusion criteria, and those who passed the screening provided information on important demographic characteristics (e.g., age, sex, education, marital status, type of disease, etc.). They were also evaluated using the State-Trait Anxiety Inventory, which consists of 40 items and employs a 4-point scale to calculate cumulative scores for the S-AI and T-AI scales, with a minimum score of 20 and a maximum score of 80. The total S-AI score reflects the severity of the subject's current anxiety symptoms, whereas the total T-AI score reflects the subject's consistent or usual anxiety. The DT uses distress scores to determine the patient's level of psychological distress. The HAMA consists of 14 items. Symptoms ranging from mild to severe were represented on a 5-point scale from 0 to 4.0 indicates no symptoms; 1 indicates mild symptoms; 2 indicates moderate symptoms; 3 indicates severe symptoms; and 4 indicates extremely severe symptoms. The cut-off value for HAMA was a total score of 14. A total score $\geqslant$29 may indicate severe anxiety; a total score $\geqslant$ 21indicates significant pressure; a total score $\geqslant$14 indicates anxiety; a total score $\geqslant$7 indicates probable anxiety; and a total score $<$ 7 indicates no anxiety symptoms.

Adherence and follow up

During the treatment period, patients were hospitalized at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University

of Traditional Chinese Medicine, to facilitate follow-up and telephone follow-up by a research assistant if inpatient follow-up was not performed. The schedules of participants are shown in table 1.

Table 1 Clinical trial procedure

	Tial period			
	Allocation	**Interventio n start**	**Intervention finish**	**Follow-up**
Day	D0	D1	D 7	D14
Enrolment				
Informed consent	√			
Inclusion/exclusion criteria	√			
Exclusion criteria	√			
Basic information	√			
Previous History	√			
Validity observation				
STAI	√	√	√	
DT	√	√	√	
HAMA	√	√	√	
QLQ-C30	√	√	√	
IL -1β、IL-2、IL-4、IL-6、IL-10、INF-γ、TNF-β	√	√	√	
Safety observation				
Vital signs	√			
Blood routine, liver and kidney function	√			
Adverse event		√	√	√
Other Tasks				
Adherence evaluation		√	√	
Analysis of shedding causes		√	√	
Efficacy evaluation			√	

Outcome measures

The primary and secondary outcomes are shown in table 2.

Table 2 Primary and secondary outcomes

Outcome measure
Primary outcome

To Assess the effect of music therapy on subjective symptoms of anxiety in cancer patients with anxiety	1. STAT will be used to detect the usual anxiety and the severity of anxiety symptoms in cancer patients. 2. DT will be used to determine the level of psychological distress in cancer patients 3. HAMA will be used to evaluate the severity of anxiety symptoms in cancer patients
Secondary outcomes	
To Assess the effect of music therapy on quality of life and immune-related blood index in in cancer patients with anxiety	1. ORTC QLQ-C30 will be used to assess the quality of life and overall health status of cancer patients with anxiety 2. Blood index will be used to observe changes in cytokine levels (serum TNF-β, INF-γ, IL-2R, IL-4, IL-6, IL-8 and IL-10) in cancer patients with anxiety before and after treatment.

Quality control

To ensure the accuracy of the experiments, research assistants check study documents, informed consent forms, case report forms (CRFs), and data records on a regular basis.

Data management and statistical methods

The subject leader and research assistant will review the CRF and scales before handing them over to data management staff for data entry and administration. The original CRF and all scales (including consent forms) will be kept at the Department of Oncology , Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine.

Patients and public involvement

Patients and the general public will not be involved in the study's design or conduct, or in the outcome measures, and no attempt will be made to assess the burden of the intervention on the patients themselves.

Sample size estimate

Combined with previous studies, the level of STAI improvement was used as the main efficacy index, and the difference value of 5.43 was taken as the primary outcome, =0.05 (unilateral) and 1-=0.9. The PASS 15.0 software was used to calculate the sample size, and the total sample size was 48 cases, including 24 cases in the treatment group and 24 cases in the control group, and the rate of missed visits in each group was estimated to be 20%, so the sample size for each group was at least 30

and 30 people, for a total of 60 people The formula used for calculating the sample size is,

$$n1=n2=\frac{[u_{\alpha/2}\sqrt{2\bar{p}(1-\bar{p})}+u_{\beta}\sqrt{p_1(1-p_1)+p_2(1-p_2)}]}{(p_1-p_2)^2}.$$

Statistical analysis

The t-test was used to examine data with a normal distribution and chi-squared. The Mann-Whitney rank-sum test was used to analyze data with non-normal distribution and chi-squared and was considered statistically significant at $P<0.05$. Individual data points were superimposed on a box-line plot to calculate all data. The SPSS 20.0 (IBM North America, New York, NY, USA) two-way analysis of variance (ANOVA) method was used to analyze ELISA, PCR, and behavioral data. At $P<0.05$, all were considered statistically significant.

Ethical issues

The study will adhere to the Helsinki Declaration and the Ethical Guidelines for Clinical Research. The study protocol of Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, has been approved by the Research Ethical Committee, (approval Number: 2019−092).

Discussion

Studies in recent years have shown that anxiety and depression are associated with cancer mortality and survival rate[11]. With a focus on life quality of oncology patients, their emotional symptoms have received attention. Over the last few decades, music therapy has evolved from a specialized field of work to a method of treating a wide range of conditions[12], including perioperative oncology anxiety[13], anxiety in breast cancer patients [14], anxiety in lung cancer patients[15], etc.

Currently, music therapy played an important role in clinical practice as an adjunct[16], which could reduce anxiety and improve the quality of life of cancer patients[17, 18]. The overactive hypothalamic-pituitary-adrenal axis (HPA axis), the action of inflammatory mediators, and immune factors were discovered to be the primary mechanisms of concomitant anxiety and depression in tumor patients[19, 20]. Cancer patients were more likely to develop depressive symptoms and had a worse prognosis than healthy people. Development and treatment of cancer caused pro-inflammatory cytokine (IL-6, IL-10, TNF-α)-mediated inflammation, which dysregulated HPA axis activity and results in depression-like behaviors. Depression, on the other hand, activates the HPA axis, resulting in the release of endogenous glucocorticoids, which can cause depressive symptoms in cancer patients[21]. Correspondingly music could modulate salivary stress markers, physiological markers of HPA axis[22] and reduce depressive symptoms[23]. Meanwhile, the results of an experiment showed that exposure to music in mice affects the expression of neurotrophic factor (BDNF) in the

hypothalamus[24]. The study finds that BDNF is associated with tumor development[25, 26], And high BDNF expression indicates poor cancer prognosis[27, 28]. Therefore, there is significant value in researching the use of music therapy for oncology anxiety. The current study is a randomized controlled trial of music therapy for cancer anxiety patients in order to further establish the "precision" of music therapy and to provide a clearer direction for clinical music therapy research.

Since current studies lack precise indicators of music therapy, such as frequency, duration, and type of music, as well as a clear assessment of efficacy, we conducted a randomized controlled study to determine the effectiveness of music therapy in reducing cancer-related anxiety in patients by analyzing anxiety-related scales and indicators in cancer patients after music therapy and providing a foundation for the clinical application of music therapy in cancer anxiety.

Trial status

The first participant will be enrolled in August 2022 and the study is expected to end in July 2023.

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Competing interests

None declared.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Not applicable.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplemental material

Not applicable

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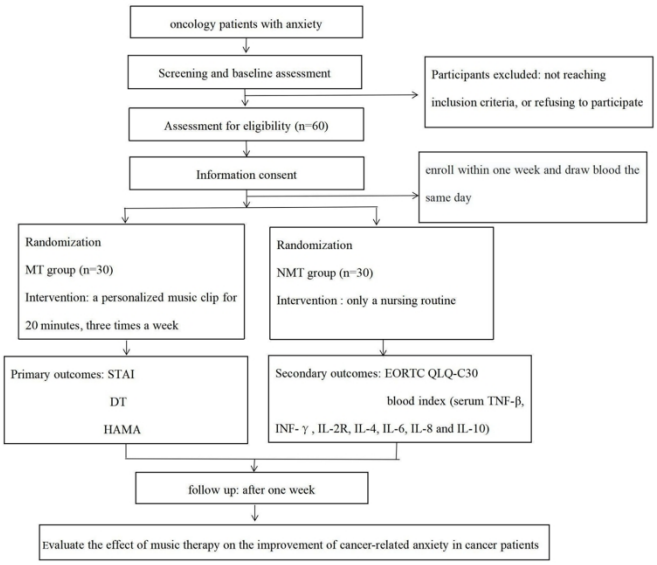
Fig. 1 Flowchart of the trial design, based on the Standard Protocol Items



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Effects of music therapy on anxiety in cancer patients: study protocol of a randomised controlled trial

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Abstract

Background

Although music therapy has been found to reduce anxiety in cancer patients and delay tumor progression to some extent, its mechanism of action has not been determined. Music therapy may reduce anxiety by reducing the concentrations of pro-inflammatory cytokines. The present study was designed to evaluate the effects of music therapy on anxiety and cytokine levels in cancer patients

Methods and analysis

This randomized, open, single-center parallel-controlled trial will randomize 60 patients with malignant tumors who meet the inclusion criteria in a 1:1 ratio to either a music therapy (MT) group or a non-music therapy (NMT) group. Patients in the MT group will receive individualized receptive music therapy for 1 week, whereas patients in the NMT group will receive emotional nursing care alone. Primary outcomes will include scores on the State-Trait Anxiety Inventory (STAI), Distress Thermometer (DT), and Hamilton Anxiety Scale (HAMA). Secondary outcomes will include scores on the Quality of Life Questionnaire C30 (QLQ-C30), serum concentrations of the cytokines IL-1β， TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10,serum concentrations of the neurotransmitters 5-HT, DA, NE, ACTH and GABA, and determination of gut microbiota populations.

Ethics and dissemination

On August 5, 2020, the study protocol was approved by the Research Ethics Committee of the Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine of the Shanghai University of Traditional Chinese Medicine. The findings of this study will be published in peer-reviewed publications and presented at appropriate conferences.

Trial registration number CTR2000035244

Protocol version Version identifier: 2.0 (November 20, 2022)

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. All interventions will be instructor-directed, with music therapists playing personalized music clips based on each patient's type of preferred music.
2. The study will evaluate cytokine levels, gut microbiota and neurotransmitters to

explore the clinical evaluation of the efficacy of music therapy.

3. One limitation of this study will be its inclusion of patients from a single hospital, which may limit the generalizability of the study results.

4. A second limitation may be that the 1-week duration of music therapy may be regarded as too short.

Introduction

Current cancer treatments include surgical resection, chemotherapy, targeted therapy and immunotherapy. These treatments, as well as the disease itself, may have adverse psychological effects on patients, including psychological stress reactions and negative emotions such as anxiety and depression, negatively affecting patient quality of life[1-2]. A cross-sectional, prospective study suggested that the prevalence of moderate to severe depression in patients with advanced solid tumors was 29.2%[3]. The National Comprehensive Cancer Network (NCCN) has classified psychological problems in cancer patients, such as anxiety and depression, as "psychosocial distress"[4]. These psychological problems can interfere with patients' ability to cope with disease and have a negative impact on physical symptoms[4]. Reducing anxiety in cancer patients may therefore have positive effects on their physical and mental health.

Current treatment of cancer-associated anxiety is frequently focused on the primary tumor. Few studies to date have focused on treating anxiety disorders caused by cancer, with even fewer focusing on non-pharmacological treatments. Music therapy has been defined by the American Music Therapy Association (AMTA) as the "clinical and evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program"[5]. Music therapy includes active methods, such as singing or playing instruments, and receptive methods, which involves the playing of prerecorded music under the guidance of a certified music therapist [6]. Over the last few decades, music therapy has evolved from a specialized field to a method of treating a wide range of conditions[7], including perioperative cancer-associated anxiety[8] and anxiety in patients with breast [9] and lung [10] cancer. Evidence has shown that music therapy could help patients improve their positive attitude toward the disease by regulating their emotions and managing their symptoms[11-12]. Notably, the Clinical Practice Guidelines on the Evidence-Based Use of Integrative Therapies During and After Breast Cancer Treatment have recommended the use of music therapy to improve the quality of life and physical functioning of breast cancer patients [13].

The pathophysiological basis of anxiety disorders has not yet been determined, although structural brain abnormalities, neurobiochemical abnormalities, and genetic factors are thought to be involved[14]. The primary mechanisms of concomitant anxiety and depression in tumor patients are thought to involve an overactive hypothalamic-pituitary-adrenal (HPA) axis, inflammatory mediators, and immune factors [15-16]. Cancer patients were found more likely to develop depressive symptoms and had a poorer prognosis than healthy individuals. The development and treatment of cancers have been found to increase inflammation medicated by pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. This in turn, resulted in dysregulation of the HPA axis and leading to depression-like behaviors. Conversely, depression, was shown to

activate the HPA axis, resulting in the release of endogenous glucocorticoids, which can cause depressive symptoms in cancer patients[17]. The Microbiome-Gut-Brain Axis (MGBA) theory has suggested alternative pathways for the pathogenesis of tumor anxiety, especially anxiety associated with intestinal cancers. The intestinal flora were shown to regulate brain function via neural pathways involving the enteric and vagus nerves; endocrine pathways involving intestinal hormones; and immune pathways involving immune cells and cytokines [18], thereby affecting mood, behavior, and neuroinflammation [19]. Many gut microbiota, including Candida, Streptococcus, Enterococcus and Bacillus species and Escherichia coli, have been shown to produce neurotransmitters, such as 5-hydroxytryptamine (5-HT)[20-21].

In addition to directly influencing tumor development by regulating angiogenesis and the tumor growth microenvironment, chronic stress can indirectly affect tumor development by altering human hormone levels[22]. Music therapy may affect tumor anxiety by altering neuroendocrine factors and factors associated with the cellular immune system and the gut-brain axis. For example, music was shown to modulate salivary stress markers and physiological markers of the HPA axis[23] and to reduce depressive symptoms[24]. Exposure of mice to music was found to alter the expression of brain-derived neurotrophic factor (BDNF) in the hypothalamus[25]. BDNF has been associated with tumor development[26-27], with high levels of BDNF expression in cancer indicating poor prognosis[28-29]. In depressed mice, music therapy increased serum 5-HT levels, decreased monoamine oxidase levels in hippocampal tissue and malondialdehyde levels in liver tissue, and improved depression[30]. Improved mood has been shown to reduce anxiety and depression by influencing metabolism and ultimately inhibiting tumor development[31]. However, despite evidence showing that music therapy can reduce anxiety in oncology patients and delay tumor progression to some extent, its exact mechanism of action is still unknown.

The present study will evaluate the effects on cancer patients of receptive and individualized music therapy under the guidance of a music therapist. This randomized controlled study will assess the ability of individualized music therapy to reduce cancer-related anxiety in patients by analyzing anxiety-related scales after music therapy. In addition, this study will evaluate cytokine levels, gut microbiota and neurotransmitters to explore the clinical evaluation of the efficacy of music therapy.

Materials and Methods

Study design

This randomized controlled trial (RCT) will enroll 60 cancer patients who are experiencing cancer-related anxiety. Patients will be randomly divided 1:1 into two groups, with patients in the music therapy group receiving individualized receptive music therapy (MT group) and patients in the non-music therapy group receiving emotional nursing care alone (NMT group) for 1 week. The study will be performed at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, between 10 December 2022 and 31 December 2023. Figure 1 shows the design of the trial based on standard protocol

items. The SPIRIT checklist is shown in supplemental appendix 1.

Inclusion criteria

Subjects will be included if they (1) have a malignant tumor, as confirmed by histopathology or cytology; (2) have been treated for cancer-related anxiety or cancer itself for ≥ 2 months and ≤ 1 year; (2) meet the standard criteria for anxiety, including HAMA scores ≥7 and ≤28 and STAI sores ≥ 20 and ≤ 80; (3) are aged 18 to 74 years, with no gender restrictions; (4) have no history of other mental disorders and do not smoke or drink; (5) are conscious, behave and hear normally, and do not have a professional music background; (6) have normal heart, liver, kidney, and blood test results, with all other vital signs being normal; (7) have not taken anti-anxiety medications within four weeks prior to study entry; (8) have an expected survival time >6 months; and (8) provide written informed consent to study participation.

Exclusion criteria

Subjects will be excluded if they (1) are currently participating in other clinical studies or clinical trials; (2) have other serious diseases, such as infection, liver or kidney failure, making them unable, in the opinion of the project leader or researchers, to tolerate the treatment regimen of this study; (3) have primary or metastatic brain tumors, as confirmed clinically or radiologically; (4) are pregnant or lactating women; (5) have received music therapy within 3 months prior to the study; or (6) are taking anxiety medications or medications that can affect anxiety.

Rejection, suspension and shedding criteria

Patients will be discontinued from the study if they (1) are found not to meet the above inclusion and exclusion criteria; (2) show poor compliance or fail to follow up as required; (3) have incomplete medical records; (4) withdraw voluntarily from the study; (5) are regarded as unsuitable to continue the study due to serious deterioration of disease, severe complications or special physiological changes; or (6) are regarded by the investigator as unsuitable to continue the study. In addition, patients in the control group will be discontinued if they listen to music during the study period.

Participant recruitment

This study aims to include 60 cancer patients who are experiencing cancer-related anxiety. Patients at the Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, will be recruited by posting advertisements on the hospital's website and on posters. These advertisements will include brief descriptions of the study aims, requirements, and methods. All participants will provide written informed consent. Patients will be recruited from December 2022 to December 2023.

Informed consent

All study processes will be explained to participants prior to the start of the study. Participants will also be informed that their participation in this trial is entirely voluntary and that they can opt out at any time. Each participant will be required to sign a written informed consent form before receiving any intervention. The informed consent is shown in supplemental appendix 1.

Randomization and concealment of allocation

Patients will be randomly assigned to the treatment and control groups in a 1:1 ratio at

the time of enrollment. A set of randomized numbers will be generated by SPSS 22.0 software, with each number randomized into opaque envelopes. The order in which patients enter the study will be determined by the grouping of the corresponding envelopes which allocated by the researcher.

Intervention

Patients in the NMT group will receive emotional nursing care but not be allowed to receive any treatment for anxiety or music listening. Emotional nursing care includes symptoms inquiry and timely communication when emotional distress occurs. Inquiry and communication will be carried out while the MT group performs the treatment. If treatment is required due to worsening anxiety, it will be recorded and excluded. Patients who requested to participate in the music therapy sessions randomly assigned to the NMT group will receive the same music therapy as the MT group after completing the prescribed follow-up time (D14 and D28).

Patients in the MT group will receive individualized receptive music therapy supervised by a music therapist, along with required anti-cancer medications. Prior to starting music therapy, patients in the MT group will be introduced to the music therapy process for 5-10 minutes by the music therapist. The treatment room will be soundproofed, with patients in a resting or sitting position with eyes closed and relaxed. Prior to music therapy, patients in the MT group will be exposed to various types of music to determine their preferences. The repertoire for music therapy which is nature-based sound, lasting 10-15 minutes, will be composed by the Department of Sound Engineering of Shanghai Conservatory of Music. After recording each patient's music preferences during playback, no more than three music clips from each genre will be chosen for individualized music clips of total length about 20 minutes, increasing individual patient comfort and orientation and reducing anxiety. Subsequently, the music therapist will play the personalized music clip created for 20 minutes, at 15:00-16:00, once a day for one week. Simultaneously, another music therapist will use a psycho-educational approach in conjunction with verbal instructions. Conditions for music therapy will include: (1) no bright light interference; (2) the patient in a resting or inactive state; and (3) music played through speakers at a volume controlled between 45-65%.

All participants will be required to complete State-Trait Anxiety Inventory (STAI), Distress Thermometer (DT), Hamilton Anxiety Scale (HAMA) and Quality of Life Questionnaire C30 (QLQ-C30) instruments on the day before and the day after treatment, as well as 14 and 28 days after treatment. The total scores of these items will be calculated and their differences in the MT and NMT groups will be compared. Blood samples will be collected for enzyme-linked immuno sorbent assay(Elisa) analysis of cytokine levels (serum IL-1β， TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10), neurotransmitter levels (5-HT、DA、NE、ACTH and GABA) . Gut microbiota will be collected on the day after treatment, then be analysed by 16sRNA. Changes in primary disease condition will also be recorded. Music Intervention flow is shown in Table 1.

Table 1 Music Intervention

Groups	Intervention	Time point	Scales	Indicators
NMT Groups	Emotional nursing care: Symptoms inquiry, timely communication when emotional distress occurs	Inquiry and communication:20minutes, at 15:00-16:00, once a day for one week.	STAI DT HAMA QLQ-C30	Blood test: cytokines and neurotransmitters; Gut microbiota
MT Groups	Individualized receptive music therapy: according to patient's music preferences	Music therapy: 20 minutes, at 15:00-16:00, once a day for one week.	STAI DT HAMA QLQ-C30	Blood test: cytokines and neurotransmitters; Gut microbiota

Data collection

Members of the Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine will collect and evaluate data from inpatients at screening and baseline, as well as during and after intervention and at follow-up periods.

Enrollment and baseline

Patients will be screened at admission using inclusion and exclusion criteria. Information collected from patients who qualified will include important demographic characteristics, such as age, sex, education, and marital status, and general clinical characteristics, including type of disease, disease stage, surgical history, current treatment, and previous treatment. All included patients will complete the STAI, DT, HAMA, and QLQ-C30. The STAI consists of 40 items, each of which is graded on a 4-point scale with S-AI levels of 1 for not at all, 2 for somewhat, 3 for moderately, and 4 for very significantly; and T-AI levels of 1 for almost never, 2 for occasionally, 3 for frequently, and 4 for almost always; with 10 reverse scores. Reverse scoring will be given in order 4, 3, 2, and 1 to calculate cumulative scores for the S-AI and T-AI scales, with a minimum score of 20 and a maximum score of 80. The total S-AI score will reflect the severity of each subject's current anxiety symptoms, whereas the total T-AI score will reflect each subject's consistent or usual anxiety, with higher scores indicating more severe anxiety[32-33], and no specifific cut-offs exist[34]. Referring to previous studies, we defined "high anxiety" as a score of STAI ≥40[35]. The DT uses distress scores to determine each patient's level of psychological distress which includes a VAS score and a problem list[36-38]. The HAMA consists of 14 items, with symptoms graded on a 5-point scale from 0 to 4, with 0 indicating no symptoms; 1 indicating mild symptoms; 2 indicating moderate symptoms; 3 indicating severe symptoms; and 4 indicating extremely severe symptoms. The cut-off value for HAMA was a total score of 14. Total scores ≥29, ≥21, ≥14, ≥7, and <7 were indicative of severe

anxiety, significant pressure, anxiety, probable anxiety, and no anxiety, respectively[39]. The QLQ-C30, which will be utilized to assess quality of life, has shown high reliability and validity in cancer patients. The QLQ-C30 questionnaire was given as a self-assessment, with a total of 30 entries, including 5 functional subscales: somatic functioning (PF), role functioning (RF), cognitive functioning (CF), emotional functioning (EF), and social functioning (SF), 3 symptom subscales: fatigue (FA), pain (PA), and nausea and vomiting (NV), and 6 single symptom measures dyspnea (DY), insomnia (SL), loss of appetite (AL), constipation (CO), diarrhea (DI), economic hardship (FD), and an overall quality of life scale (CQL). A higher functional scale score indicates a better quality of life, whereas a higher symptom scale score indicates a worse quality of life[40].

Adherence and follow up

During the treatment period, patients will be hospitalized at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, to facilitate follow-up; patients unable to be followed-up as in patient will undergo telephone follow-up by a research assistant. Patient schedules are shown in Table 2.

Table 2 Clinical trial procedure

	Trial period				
	Allocation	**Interventio n start**	**Intervention finish**	**Follow-up**	
Day	D0	D1	D7	D14	D28
Enrollment					
Informed consent	√				
Inclusion	√				
Exclusion criteria	√				
Basic information	√				
Previous history	√				
Efficacy indicators					
STAI		√	√	√	√
DT		√	√	√	√
HAMA		√	√	√	√
QLQ-C30		√	√	√	√
IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10		√	√		
5-HT、DA、NE、ACTH and GABA		√	√		
Gut microbiota		√	√		
Safety indicators					
Vital signs	√		√		
Blood, liver and	√		√		

kidney functions			
Adverse events	√	√	√
Other parameters			
Adherence evaluation	√		
Analysis of shedding causes	√		
Efficacy evaluation	√		

Outcome measures

The primary and secondary outcomes are shown in Table 3.

Table 3 Primary and secondary outcomes

Outcome measures	
Primary outcomes	
Assessment of the effects of music therapy on subjective symptoms of anxiety in cancer patients with anxiety	1. STAI will be used to detect usual anxiety and the severity of anxiety symptoms in cancer patients 2. HAMA will be used to evaluate the severity of anxiety symptoms in cancer patients 3. DT will be used to determine the level of psychological distress in cancer patients
Secondary outcomes	
Assessment of the effects of music therapy on quality of life and immune-related blood indices in cancer patients with anxiety	1. QLQ-C30 will be used to assess the quality of life and overall health status of cancer patients with anxiety 2. Blood index and physiological indicators will be used to assess changes in serum concentrations of cytokines (IL-1β, TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10) and neurotransmitters (5-HT, DA, NE, ACTH and GABA) in cancer patients with anxiety from before to after treatment 3. Gut microbiota will be collected to compare the differences in diversity, enterobacteriaceae, and short-chain fatty acids.

Quality control

To ensure the accuracy of the experiments, research assistants will review study

documents, informed consent forms, case report forms (CRFs), and data records on a regular basis.

Data management

The team leader and research assistant will review the CRFs and scales before handing them over to data management staff for data entry and administration. The original CRFs and all scales (including consent forms) will be kept at the Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine.

The safety of this study will be monitored by the Data and Safety Monitoring Board (DSMB) of the Clinical Assessment Center at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine will monitor the safety of the study every month, which is made up of clinical experts and statisticians.

Patients and public involvement

Patients and the general public will not be involved in the design of the study or in the determination of outcome measures. No attempt will be made to assess the burden of the intervention on the patients themselves.

Sample size

Based on previous studies, the level of STAI improvement will be regarded as the main indicator of efficacy, with differences of 5.43 regarded as the primary outcome, with a unilateral P=0.05. Sample size calculation with PASS 15.0 software using the formula:

$$n1=n2=\frac{\left|u_{a/2}\sqrt{2\bar{p}(1-\bar{p})}+u\beta\sqrt{p_1(1-p_1)+p_2(1-p_2)}\right|}{(p_1-p_2)2}$$

The minimum total sample size was calcu ated to be 48 patients. Including a 20% withdrawal rate, the minimum total sample size was 60 patients, or 30 in each group based on 1:1 randomization.

Statistical analysis

Normally distributed continuous variables will be compared by t-tests and non-normally distributed continuous variables by Mann-Whitney rank-sum tests. Categorical variables will be compared by chi-squared tests. spss 22.0 will be used to generate a normal probability graph and perform a hypothesis test to check whether the observed values obeyed a normal distribution. Individual data points will be superimposed on a box-line plot for calculations. The results of anxiety correlation scales and Elisa will be compared by t-tests or Mann-Whitney rank-sum tests according to whether the normal distribution is met or not. The results of 16sRNA will be classified by the RDP reference database (http://www.mothur.org/wiki/RDP_reference_files) to calculate the relative abundance of microbial communities at different levels. Then, the differences between samples (groups) will be calculated by Principal Component Analysis (PCA), Principal Co-ordinates Analysis (PCoA), Non-Metric Multi-Dimensional Scaling (NMDS),

Unweighted Pair-group Method with Arithmetic Means (UPGMA), and Beta Diversity Index Inter-roup Difference Analysis. All statistical analyses will be performed by SPSS 22.0 software, with $P<0.05$ considered statistically significant. Patients who used other drugs or therapies on cancer, will be stratified in statistical analysis.

Ethical issues

The study will adhere to the Helsinki Declaration and the Ethical Guidelines for Clinical Research. The study protocol has been approved by the Research Ethical Committee of Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine (approval Number: 2019-092).

Discussion

Anxiety and depression are associated with cancer mortality and survival rates[41]. The increased focus on quality of life of cancer patients has increased interest in their emotional symptoms. Music therapy may reduce anxiety and improve the quality of life of cancer patients[42-43]. Music therapy is a risk-free, long-term, and cost-effective intervention that may improve anxiety in cancer patients. The randomized control trial described in this study will test whether this non-pharmacological intervention can reduce anxiety in cancer patients. Findings of this study may serve as a reference for trials determining whether other non-pharmacological methods could improve anxiety symptoms in oncology patients.

A major strength of the present trial is its method of intervention, in which individual patients are exposed to a personalized music clip based on each patient's preference. Outcomes will be measured using the STAI, DT, HAMA and QLQ-C30 instruments. HAMA scores are measured by physicians, making them more objective. STAI can respond to both short-term and long-term emotional traits. The STAI and HAMA scales will therefore be combined to reduce bias and increase the reliability of the results. Moreover, this study will measure the levels of neurotransmitters, gut microbiota and cytokines in tumor patients to explore the mechanisms by which music therapy improves anxiety in tumor patients. A meta-analysis reported that high levels of IL-8 and IL-6 were significantly associated with the prognosis of cancer patients treated with immune checkpoint inhibitors[44]. Moreover, serum IL-6 levels can be used as a biomarker to predict the outcome of treatment with antidepressants [45]. A case-control study found that fecal microbiota signatures differed in gastrointestinal cancer patients with and without anxiety and depression[46]. The gut-brain-microbiota-axis could modulate depression and anxiety induced by chronic stress through ileal immune regulation[47]. In addition, music therapy could increase salivary immunoglobulin A levels and reduce cortisol levels of cancer patients[48]. Music therapy can significantly increase NK cell count and activity[49]. Therefore, we will assess the effects of music therapy on the efficacy of immunotherapy in cancer patients in the future studies.

This study, however, is still subject to some limitations. The major limitation is the single-center nature of this trial, which may limit the generalizability of study results. In addition, the intervention period will be only 1 week, which may be too short to determine the effects of music therapy on immune function and gut microbiota. Future studies should be designed to test whether longer periods of music therapy are of greater

benefit in cancer patients.

Trial status

The first participant will be enrolled in December 2022 and the study is expected to end in December 2023.

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Contributors

CS and SS design the study. YZ, XN and HY wrote the manuscript. GY and XL recruited the patients and refined the protocol.All authors approved the final version.

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Competing interests

None declared.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplemental material

This content has been supplied by the author(s).

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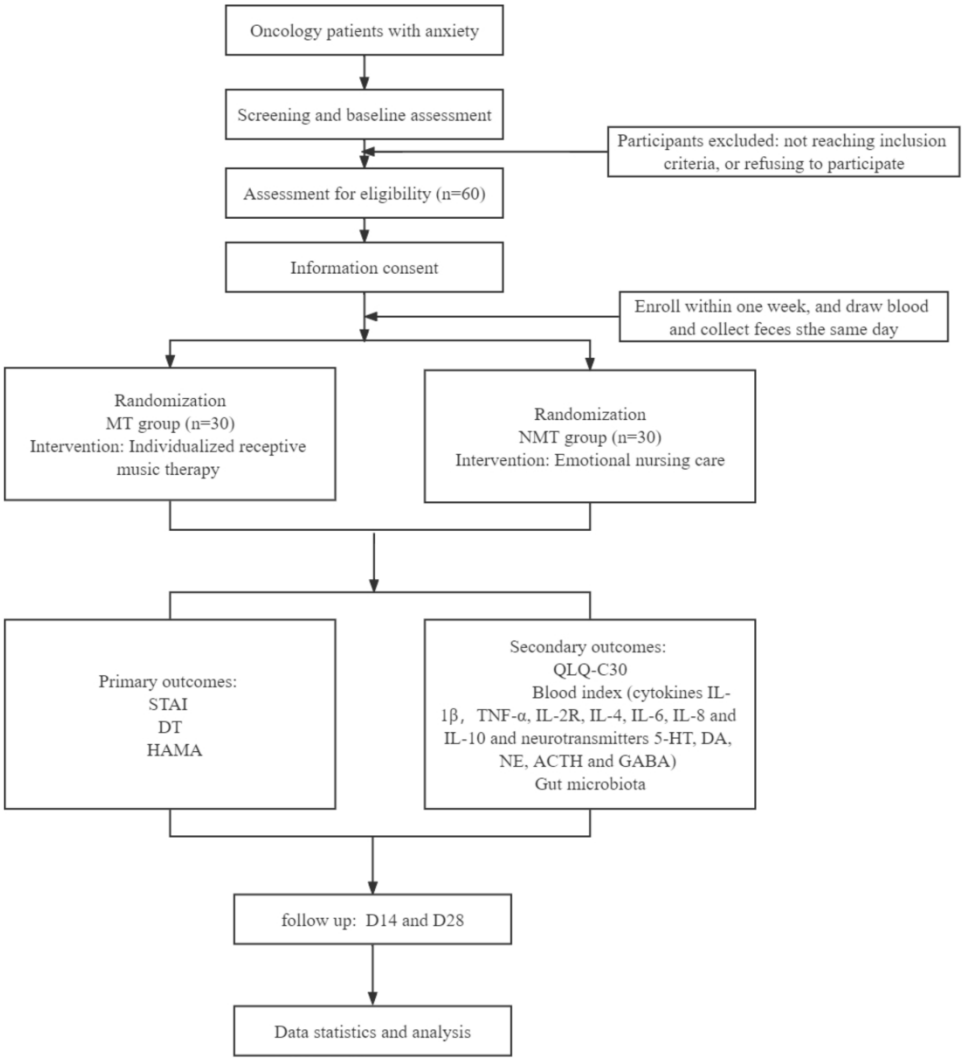
Fig. 1 Flowchart of the trial design, based on standard protocol items.



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Informed Consent • Informed Notice page

Project Name: Effects of music therapy on anxiety in cancer patients：A randomized controlled trial

Research Unit: Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine of the Shanghai University of Traditional Chinese Medicine

Principal: Ling Xu Yabin Gong Chenbing Sun

Address: 110 Ganhe Road, Shanghai, China

Dear patient,

Your doctor has diagnosed you with a malignant neoplastic disease.

We will invite you to participate in a randomized controlled trial of the effects of music therapy on anxiety in cancer patients.

Before you decide whether or not to participate in the study, please read the following as carefully as possible to help you understand the study and why it is being conducted, the procedure and duration of the study, and the benefits, risks, and discomfort that may result from participating in the study. If you wish, you can also discuss it with your relatives and friends, or ask your doctor for an explanation to help you make a decision.

1. Research Background:

Current cancer treatments include surgical resection, chemotherapy, targeted therapy and immunotherapy. These treatments, as well as the disease itself, may have adverse psychological effects on patients, including psychological stress reactions and negative emotions such as anxiety and depression, negatively affecting patient quality of life.

Current treatment of cancer-associated anxiety is frequently focused on the primary tumor. Few studies to date have focused on treating anxiety disorders caused by cancer, with even fewer focusing on non-pharmacological treatments. Music therapy has been defined by the American Music Therapy Association (AMTA) as the "clinical and evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program". Over the last few decades, music therapy has evolved from a specialized field to a method of treating a wide range of conditions, including perioperative cancer-associated anxiety and anxiety in patients with breast and lung cancer. The pathophysiological basis of anxiety disorders has not yet been determined, although structural brain abnormalities, neurobiochemical abnormalities, and genetic factors are thought to be involved. The primary mechanisms of concomitant anxiety and depression in tumor patients are thought to involve an overactive hypothalamic-pituitary-adrenal (HPA) axis, inflammatory mediators, and immune factors.

2. purpose of research:

This randomized controlled study will assess the ability of individualized music therapy to reduce cancer-related anxiety in patients by analyzing anxiety-related scales after music therapy. In addition, this study will evaluate cytokine

concentrations, gut microbiota and neurotransmitter-related indicators to explore the link their associations in cancer patients. The study will be conducted at Yueyang Hospital of Integrated Traditional Chinese and Western Medicine affiliated to Shanghai University of Traditional Chinese Medicine, and 60 participants are expected to participate voluntarily. The Ethics Committee of Yueyang Hospital has considered that the study is in line with the principles of the "Measures for Ethical Review of Biomedical Research Involving Humans" and the "Declaration of Helsinki", and in line with medical ethics.

3. Inclusion criteria

Subjects will be included if they (1) have a malignant tumor, as confirmed by histopathology or cytology; (2) have been treated for cancer-related anxiety or cancer itself for ≥ 2 months and ≤ 1 year; (2) meet the standard criteria for anxiety, including HAMA scores ≥7 and ≤28 and STAI sores ≥ 20 and ≤ 80; (3) are aged 18 to 74 years, with no gender restrictions; (4) have no history of other mental disorders and do not smoke or drink; (5) are conscious, behave and hear normally, and do not have a professional music background; (6) have normal heart, liver, kidney, and blood test results, with all other vital signs being normal; (7) have not taken anti-anxiety medications within four weeks prior to study entry; (8) have an expected survival time >6 months; and (8) provide written informed consent to study participation.

4. Exclusion criteria

Subjects will be excluded if they (1) are currently participating in other clinical studies or clinical trials; (2) have other serious diseases, such as infection, liver or kidney failure, making them unable, in the opinion of the project leader or researchers, to tolerate the treatment regimen of this study; (3) have primary or metastatic brain tumors, as confirmed clinically or radiologically; (4) are pregnant or lactating women; (5) have received music therapy within 3 months prior to the study; or (6) are taking anxiety medications or medications that can affect anxiety.

5. Rejection, suspension and shedding criteria

Patients will be discontinued from the study if they (1) are found not to meet the above inclusion and exclusion criteria; (2) show poor compliance or fail to follow up as required; (3) have incomplete medical records; (4) withdraw voluntarily from the study; (5) are regarded as unsuitable to continue the study due to serious deterioration of disease, severe complications or special physiological changes; or (6) are regarded by the investigator as unsuitable to continue the study. In addition, patients in the control group will be discontinued if they listen to music during the study period.

6. If you participate in the study, you will need to do the following work:

(1) Before you are enrolled in the study, you will undergo the following tests to determine whether you can participate in the study. Your doctor will ask you, take your medical history, and perform a physical examination. You need to do the cytokines IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10, serum concentrations of the neurotransmitters 5-HT, DA, NE, ACTH and GABA, and determination of gut microbiota populations.

(2) If you meet the eligibility criteria, you will be studied according to the following steps:

At the beginning of the study, a random number will determine whether you will be placed in the control or treatment group. Patients in the study each had a 50% chance of being assigned to one of the two different treatment groups. Neither you nor your doctor can know and choose any treatment in advance. The treatment will last for 1 weeks.

7. Intervention

Patients in the NMT group will receive emotional nursing care but not be allowed to receive any treatment for anxiety. If treatment is required due to worsening anxiety, it will be recorded and excluded. Patients who requested to participate in the music therapy sessions randomly assigned to the NMT group will receive the same music therapy as the MT group after completing the prescribed follow-up time.

Patients in the MT group will receive individualized receptive music therapy supervised by a music therapist, along with routine nursing care as well as required medications. Prior to starting music therapy, patients in the MT group will be introduced to the music therapy process for 5-10 minutes by the music therapist. The treatment room will be soundproofed, with patients in a resting or sitting position with eyes closed and relaxed.

Prior to music therapy, patients in the MT group will be exposed to various types of music to determine their preferences. The repertoire for music therapy which is nature-based sound, lasting 10-15 minutes, will be composed by the Department of Sound Engineering of Shanghai Conservatory of Music. After recording each patient's music preferences during playback, no more than three music clips from each genre will be chosen for individualized music clips of total length about 20 minutes, increasing individual patient comfort and orientation and reducing anxiety. Subsequently, the music therapist will play the personalized music clip created for 20 minutes, once a day for one week. Simultaneously, another music therapist will use a psycho-educational approach in conjunction with verbal instructions. Conditions for music therapy will include: (1) no bright light interference; (2) the patient in a resting or inactive state; and (3) music played through speakers at a volume controlled between 45-65%.

All participants will be required to complete State-Trait Anxiety Inventory (STAI), Distend Thermometer (DT), Hamilton Anxiety Scale (HAMA) and Quality of Life Questionnaire C30 (QLQ-C30) instruments on the day before and the day after treatment, as well as 14 and 28 days after treatment. The total scores of these items will be calculated and their differences in the MT and NMT groups will be compared. Blood samples will be collected for analysis of cytokine levels (serum IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10), neurotransmitter levels (5-HT、DA、NE、ACTH and GABA) and gut microbiota on the day after treatment. Changes in primary disease condition will also be recorded.

8. Other matters requiring your cooperation

You must follow your doctor's instructions for treatment. If you need any other treatment, please contact your doctor in advance.

9. Possible benefits of participating in a study

You and society will probably benefit from this research. Such benefits include the

possibility that your condition may or may not improve. In addition, this study may help evaluate the effectiveness of music therapy in relieving cancer patients with anxiety, and form a comprehensive music therapy program for tumor patients with anxiety symptoms, which can be used for other patients with similar conditions.

10. Possible adverse reactions, risks, discomfort and inconvenience of participating in the study

The treatment process may have emotional abnormalities and other adverse reactions. We will strictly regulate the treatment process and operation of music therapy to avoid the above things.

If you experience any discomfort during the study, or if you experience any new changes in your condition, or if anything unexpected, whether or not it is related to your treatment, you should inform your doctor promptly and he/she will make a judgment and take medical treatment.

The researchers will do their best to prevent and treat the adverse reactions that may result from this study. If an adverse event occurs during a clinical trial, a committee of medical experts will determine whether it is related to the treatment. As determined by the expert committee, the adverse event is related to the treatment, and the research group will provide the cost of treatment and corresponding financial compensation for the trial-related damage.

During the study period, you need to go to the hospital for follow-up visit on time and do some physical and chemical examinations such as interleukin energy, which may cause you trouble or inconvenience.

11. Qualified tuition and related expenses

The costs of the studies related to this study that you undergo during your study are at your own expense, but these tests themselves are also required for the assessment of your primary disease. Tumor related immune indicators (cytokines and neurotransmitters) and gut microbiota were detected once before and after treatment.

12. Is personal information confidential?

Your medical records (research record / CRF, physical and chemical examination report, etc.) will be completely stored in the hospital, and the doctor will record the results of the laboratory examination in your outpatient medical record. The investigator, sponsor representative, and ethics committee will be granted access to your medical records. Any public report on the results of this study will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical data to the extent permitted by law.

Your biological specimen will be kept in the hospital laboratory as required. In addition to this study, it may be used again in other studies in the future. You may now refuse to use your biological specimens for research other than this study.

13. How to get more information?

You may ask any questions about this study at any time. Your doctor will give you his/her phone number so he/she can answer your questions.

Your doctor will inform you if there is any important new information during the study that may affect your willingness to continue participating in the study.

14. You can voluntarily opt in and out of the study

Participation in the study is entirely up to you. You may decline to participate in the study or withdraw from the study at any time during the study without affecting your relationship with your physician or any loss of medical or other benefits.

Your physician or investigator may, in your best interest, discontinue you from the study at any time.

If you do not participate in the study or drop out of the study, there are many alternative treatments, such as anti-anxiety medications. You do not have to choose to participate in this study in order to treat your disease.

15. What should we do now?

It is up to you to decide whether to participate in this study. You can discuss it with your family or friends before making a decision.

Before you make the decision to participate in a study, please ask your doctor as many questions as possible until you fully understand the study. Thank you for reading the above. If you decide to participate in this study, please let your doctor know and he/she will arrange everything for you.

16. The experimental protocol is approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine. Subjects can directly consult or complain to the Ethics Committee if there is any question or situation during the experiment.

Please keep this information.

Informed Consent · Signature page

Clinical Research Project Name: Effects of music therapy on anxiety in cancer patients：A randomized controlled trial

Sponsor: Ling Xu Yabin Gong Sunchenbing

Trial registration number: CTR2000035244

Declaration of consent

I have read the above introduction about this study and fully understand the full contents of the informed consent form, and I have had the opportunity to discuss and ask questions about this study with my doctor. All my questions were answered satisfactorily.

I am aware of the possible risks and benefits of participating in this study. I understand that participation in the study is voluntary, I confirm that I have had sufficient time to consider it, and I understand that:

I can always ask the doctor for more information.

I can withdraw from the study at any time without discrimination or retaliation, and my medical treatment and rights will not be affected.

I also know that if I drop out of the study, especially due to side effects, I need to tell my doctor about any changes in my condition and complete the appropriate physical and physical examination, which will be very beneficial to me and the whole study.

If I need to take any other medication due to a change in my condition, I will either consult my doctor beforehand or tell my doctor afterwards.

I allow the Ethics Committee or the sponsor's representative to access my research data.

I consent to or refuse to use my medical records and biological specimens for research other than this study.

I will be provided with a signed and dated copy of the informed consent. In the end, I decided to agree to participate in the study and try to follow the doctor's advice.

Date of subject's signature (handwritten)

Subject's contact Number

Subject's legal guardian's signature (if necessary), (handwritten) **Date**

Guardian's contact Number

Doctor's statement

I confirm that I have explained the details of the trial, including its powers and possible benefits and risks, and have given the patient a signed copy of the informed consent.

The investigator (informing subject) **signed** (handwritten) **Date**

Researcher contact Number

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	None
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	11
	5b	Name and contact information for the trial sponsor	None
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	11
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	None

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-3
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	3
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	4-5
		Methods: Assignment of interventions (for controlled trials)	
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	None

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	None
		Methods: Data collection, management, and analysis	
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6-7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
		Methods: Monitoring	
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	9

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	4
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	4
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	None
		Ethics and dissemination	
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	10
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	None
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	None
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	5
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	None
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	5
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	None

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Effects of music therapy on anxiety in cancer patients: study protocol of a randomised controlled trial

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Effects of music therapy on anxiety in cancer patients: study protocol of a randomised controlled trial

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Abstract

Background

Although music therapy has been found to reduce anxiety in cancer patients and delay tumor progression to some extent, its mechanism of action has not been determined. Music therapy may reduce anxiety by reducing the concentrations of pro-inflammatory cytokines. The present study was designed to evaluate the effects of music therapy on anxiety and cytokine levels in cancer patients.

Methods and analysis

This randomized, open, single-center parallel-controlled trial will randomize 60 patients with malignant tumors who meet the inclusion criteria in a 1:1 ratio to either a music therapy (MT) group or a non-music therapy (NMT) group. Patients in the MT group will receive emotional nursing care and individualized receptive music therapy for 1 week, whereas patients in the NMT group will receive emotional nursing care alone. Primary outcomes will include scores on the State-Trait Anxiety Inventory (STAI), Distress Thermometer (DT), and Hamilton Anxiety Scale (HAMA). Secondary outcomes will include scores on the Quality of Life Questionnaire C30 (QLQ-C30), serum concentrations of the cytokines IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10,serum concentrations of the neurotransmitters 5-HT, DA, NE, ACTH and GABA, and determination of gut microbiota populations.

Ethics and dissemination

On August 5, 2020, the study protocol was approved by the Research Ethics Committee of the Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine of the Shanghai University of Traditional Chinese Medicine. The findings of this study will be published in peer-reviewed publications and presented at appropriate conferences.

Trial registration number CTR2000035244

Protocol version Version identifier: 2.0 (November 20, 2022)

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. All interventions will be instructor-directed, with music therapists playing personalized music clips based on each patient's type of preferred music.
2. The study will evaluate cytokine levels, gut microbiota and neurotransmitters to

explore the clinical evaluation of the efficacy of music therapy.

3. One limitation of this study will be its inclusion of patients from a single hospital, which may limit the generalizability of the study results.

4. A second limitation may be that the 1-week duration of music therapy may be regarded as too short.

Introduction

Current cancer treatments include surgical resection, chemotherapy, targeted therapy and immunotherapy. These treatments, as well as the disease itself, may have adverse psychological effects on patients, including psychological stress reactions and negative emotions such as anxiety and depression, negatively affecting patient quality of life[1-2]. A cross-sectional, prospective study suggested that the prevalence of moderate to severe depression in patients with advanced solid tumors was 29.2%[3]. The National Comprehensive Cancer Network (NCCN) has classified psychological problems in cancer patients, such as anxiety and depression, as "psychosocial distress"[4]. These psychological problems can interfere with patients' ability to cope with disease and have a negative impact on physical symptoms[4]. Reducing anxiety in cancer patients may therefore have positive effects on their physical and mental health.

Current treatment of cancer-associated anxiety is frequently focused on the primary tumor. Few studies to date have focused on treating anxiety disorders caused by cancer, with even fewer focusing on non-pharmacological treatments. Music therapy has been defined by the American Music Therapy Association (AMTA) as the "clinical and evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program"[5]. Music therapy includes active methods, such as singing or playing instruments, and receptive methods, which involves the playing of prerecorded music under the guidance of a certified music therapist [6]. Over the last few decades, music therapy has evolved from a specialized field to a method of treating a wide range of conditions[7], including perioperative cancer-associated anxiety[8] and anxiety in patients with breast [9] and lung [10] cancer. Evidence has shown that music therapy could help patients improve their positive attitude toward the disease by regulating their emotions and managing their symptoms[11-12]. Notably, the Clinical Practice Guidelines on the Evidence-Based Use of Integrative Therapies During and After Breast Cancer Treatment have recommended the use of music therapy to improve the quality of life and physical functioning of breast cancer patients [13].

The pathophysiological basis of anxiety disorders has not yet been determined, although structural brain abnormalities, neurobiochemical abnormalities, and genetic factors are thought to be involved[14]. The primary mechanisms of concomitant anxiety and depression in tumor patients are thought to involve an overactive hypothalamic-pituitary-adrenal (HPA) axis, inflammatory mediators, and immune factors [15-16]. Cancer patients were found more likely to develop depressive symptoms and had a poorer prognosis than healthy individuals. The development and treatment of cancers have been found to increase inflammation medicated by pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. This in turn, resulted in dysregulation of the HPA axis and leading to depression-like behaviors. Conversely, depression, was shown to

activate the HPA axis, resulting in the release of endogenous glucocorticoids, which can cause depressive symptoms in cancer patients[17]. The Microbiome-Gut-Brain Axis (MGBA) theory has suggested alternative pathways for the pathogenesis of tumor anxiety, especially anxiety associated with intestinal cancers. The intestinal flora were shown to regulate brain function via neural pathways involving the enteric and vagus nerves; endocrine pathways involving intestinal hormones; and immune pathways involving immune cells and cytokines [18], thereby affecting mood, behavior, and neuroinflammation [19]. Many gut microbiota, including Candida, Streptococcus, Enterococcus and Bacillus species and Escherichia coli, have been shown to produce neurotransmitters, such as 5-hydroxytryptamine (5-HT)[20-21].

In addition to directly influencing tumor development by regulating angiogenesis and the tumor growth microenvironment, chronic stress can indirectly affect tumor development by altering human hormone levels[22]. Music therapy may affect tumor anxiety by altering neuroendocrine factors and factors associated with the cellular immune system and the gut-brain axis. For example, music was shown to modulate salivary stress markers and physiological markers of the HPA axis[23] and to reduce depressive symptoms[24]. Exposure of mice to music was found to alter the expression of brain-derived neurotrophic factor (BDNF) in the hypothalamus[25]. BDNF has been associated with tumor development[26-27], with high levels of BDNF expression in cancer indicating poor prognosis[28-29]. In depressed mice, music therapy increased serum 5-HT levels, decreased monoamine oxidase levels in hippocampal tissue and malondialdehyde levels in liver tissue, and improved depression[30]. Improved mood has been shown to reduce anxiety and depression by influencing metabolism and ultimately inhibiting tumor development[31]. However, despite evidence showing that music therapy can reduce anxiety in oncology patients and delay tumor progression to some extent, its exact mechanism of action is still unknown.

The present study will evaluate the effects on cancer patients of receptive and individualized music therapy under the guidance of a music therapist. This randomized controlled study will assess the ability of individualized music therapy to reduce cancer-related anxiety in patients by analyzing anxiety-related scales after music therapy. In addition, this study will evaluate cytokine levels, gut microbiota and neurotransmitters to explore the clinical evaluation of the efficacy of music therapy.

Materials and Methods

Study design

This randomized controlled trial (RCT) will enroll 60 cancer patients who are experiencing cancer-related anxiety. Patients will be randomly divided 1:1 into two groups, with patients in the music therapy group receiving emotional nursing care and individualized receptive music therapy (MT group) and patients in the non-music therapy group receiving emotional nursing care alone (NMT group) for 1 week. The study will be performed at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, between 10 December 2022 and 31 December 2023. Figure 1 shows the design of the trial based on standard protocol items. The SPIRIT checklist is shown in supplemental

appendix 1.

Inclusion criteria

Subjects will be included if they (1) have a malignant tumor, as confirmed by histopathology or cytology; (2) have been treated for cancer-related anxiety or cancer itself for ≥ 2 months and ≤ 1 year; (2) meet the standard criteria for anxiety, including HAMA scores ≥7 and ≤28 and STAI sores ≥ 20 and ≤ 80; (3) are aged 18 to 74 years, with no gender restrictions; (4) do not smoke or drink; (5) without psychiatric symptoms; (6) hear normally, and do not have a professional music background; (7) have normal heart, liver, kidney, and blood test results, with all other vital signs being normal; (8) have not taken anti-anxiety medications within four weeks prior to study entry; (9) have an expected survival time >6 months; and (10) provide written informed consent to study participation.

Exclusion criteria

Subjects will be excluded if they (1) are currently participating in other clinical studies or clinical trials; (2) have other serious diseases, such as infection, liver or kidney failure, making them unable, in the opinion of the project leader or researchers, to tolerate the treatment regimen of this study; (3) have primary or metastatic brain tumors, as confirmed clinically or radiologically; (4) are pregnant or lactating women; (5) have received music therapy within 3 months prior to the study; ~~or~~ (6) are taking anxiety medications or medications that can affect anxiety.

Rejection, suspension and shedding criteria

Patients will be discontinued from the study if they (1) are found not to meet the above inclusion and exclusion criteria; (2) show poor compliance or fail to follow up as required; (3) have incomplete medical records; (4) withdraw voluntarily from the study; (5) are regarded as unsuitable to continue the study due to serious deterioration of disease, severe complications or special physiological changes; or (6) are regarded by the investigator as unsuitable to continue the study. In addition, patients in the control group will be discontinued if they listen to music during the study period.

Participant recruitment

This study aims to include 60 cancer patients who are experiencing cancer-related anxiety. Patients at the Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, will be recruited by posting advertisements on the hospital's website and on posters. These advertisements will include brief descriptions of the study aims, requirements, and methods. All participants will provide written informed consent. Patients will be recruited from December 2022 to December 2023.

Informed consent

All study processes will be explained to participants prior to the start of the study. Participants will also be informed that their participation in this trial is entirely voluntary and that they can opt out at any time. Each participant will be required to sign a written informed consent form before receiving any intervention. The informed consent is shown in supplemental appendix 1.

Randomization and concealment of allocation

Patients will be randomly assigned to the treatment and control groups in a 1:1 ratio at

the time of enrollment. A set of randomized numbers will be generated by SPSS 22.0 software, with each number randomized into opaque envelopes. The order in which patients enter the study will be determined by the grouping of the corresponding envelopes which allocated by the researcher.

Intervention

Patients in the NMT group will receive emotional nursing care but not be allowed to receive any treatment for anxiety or music listening. Emotional nursing care includes symptoms inquiry and timely communication when emotional distress occurs. Inquiry and communication will be carried out while the MT group performs the treatment. If treatment is required due to worsening anxiety, it will be recorded and excluded. Patients who requested to participate in the music therapy sessions randomly assigned to the NMT group will receive the same music therapy as the MT group after completing the prescribed follow-up time (D14 and D28).

Patients in the MT group will receive individualized receptive music therapy supervised by a music therapist, along with required anti-cancer medications. Prior to starting music therapy, patients in the MT group will be introduced to the music therapy process for 5-10 minutes by the music therapist. The treatment room will be soundproofed, with patients in a resting or sitting position with eyes closed and relaxed. Prior to music therapy, patients in the MT group will be exposed to various types of music to determine their preferences. The repertoire for music therapy which is nature-based sound, lasting 10-15 minutes, will be composed by the Department of Sound Engineering of Shanghai Conservatory of Music. After recording each patient's music preferences during playback, no more than three music clips from each genre will be chosen for individualized music clips of total length about 20 minutes, increasing individual patient comfort and orientation and reducing anxiety. Subsequently, the music therapist will play the personalized music clip created for 20 minutes, at 15:00-16:00, once a day for one week. Studies have shown that 1-3 day intervention with music therapy 30-60 minutes per day is effective for depression and anxiety of various cancer patients[32-34]. Simultaneously, another music therapist will use a psycho-educational approach in conjunction with verbal instructions. The psycho-educational approach in conjunction with verbal instruction include: 1) informing the patient of the purpose and duration of the treatment; 2) adjusting the appropriate volume; 3) instructing the patient how to adjust breathing and relax methodically from head to feet to the rhythm of the music. Conditions for music therapy will include: (1) no bright light interference; (2) the patient in a resting or inactive state; ~~and~~ (3) music played through speakers at a volume controlled between 45-65%.

All participants will be required to complete State-Trait Anxiety Inventory (STAI), Distress Thermometer (DT), Hamilton Anxiety Scale (HAMA) and Quality of Life Questionnaire C30 (QLQ-C30) instruments on the day before and the day after treatment, as well as 14 and 28 days after treatment. The total scores of these items will be calculated and their differences in the MT and NMT groups will be compared. Blood samples will be collected for enzyme-linked immuno sorbent assay(Elisa) analysis of cytokine levels (serum IL-1β， TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10), neurotransmitter levels (5-HT、DA、NE、ACTH and GABA) . Gut microbiota will

be collected on the day after treatment, then be analysed by 16sRNA. Changes in primary disease condition will also be recorded. Music Intervention flow is shown in Table 1.

Table 1 Music Intervention

Groups	Intervention	Time point	Scales	Indicators
NMT Groups	Emotional nursing care: Symptoms inquiry, timely communication when emotional distress occurs	Inquiry and communication: 20minutes, at 15:00-16:00, once a day for one week.	STAI DT HAMA QLQ-C30	Blood test: cytokines and neurotransmitters; Gut microbiota
MT Groups	Emotional nursing care and individualized receptive music therapy: (1) Music clips: according to patient's music preferences (2) Music therapy include the psycho-educational approach:1) informing the patient of the purpose and duration of the treatment; 2) adjusting the appropriate volume; 3) instructing the patient how to adjust breathing and relax methodically (head - face - neck - shoulders - arms, hands - chest - abdomen - low back - buttocks - legs - calves - feet) to the rhythm of the music.	Music therapy: 20 minutes, at 15:00-16:00, once a day for one week. Inquiry and communication: befor music therapy	STAI DT HAMA QLQ-C30	Blood test: cytokines and neurotransmitters; Gut microbiota

Data collection

Members of the Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine will collect and evaluate data from inpatients at screening and baseline, as well as during and after intervention and at follow-up periods.

Enrollment and baseline

Patients will be screened at admission using inclusion and exclusion criteria.

Information collected from patients who qualified will include important demographic characteristics, such as age, sex, education, and marital status, and general clinical characteristics, including type of disease, disease stage, surgical history, current treatment, and previous treatment. All included patients will complete the STAI, DT, HAMA, and QLQ-C30. The STAI consists of 40 items, each of which is graded on a 4-point scale with S-AI levels of 1 for not at all, 2 for somewhat, 3 for moderately, and 4 for very significantly; and T-AI levels of 1 for almost never, 2 for occasionally, 3 for frequently, and 4 for almost always; with 10 reverse scores. Reverse scoring will be given in order 4, 3, 2, and 1 to calculate cumulative scores for the S-AI and T-AI scales, with a minimum score of 20 and a maximum score of 80. The total S-AI score will reflect the severity of each subject's current anxiety symptoms, whereas the total T-AI score will reflect each subject's consistent or usual anxiety, with higher scores indicating more severe anxiety[35-36], and no specific cut-offs exist[6]. Referring to previous studies, we defined "high anxiety" as a score of STAI ≥40[37]. The DT uses distress scores to determine each patient's level of psychological distress which includes a VAS score and a problem list[38-40]. The HAMA consists of 14 items, with symptoms graded on a 5-point scale from 0 to 4, with 0 indicating no symptoms; 1 indicating mild symptoms; 2 indicating moderate symptoms; 3 indicating severe symptoms; and 4 indicating extremely severe symptoms. The cut-off value for HAMA was a total score of 14. Total scores ≥29, ≥21, ≥14, ≥7, and <7 were indicative of severe anxiety, significant pressure, anxiety, probable anxiety, and no anxiety, respectively[41]. The QLQ-C30, which will be utilized to assess quality of life, has shown high reliability and validity in cancer patients. The QLQ-C30 questionnaire was given as a self-assessment, with a total of 30 entries, including 5 functional subscales: somatic functioning (PF), role functioning (RF), cognitive functioning (CF), emotional functioning (EF), and social functioning (SF), 3 symptom subscales: fatigue (FA), pain (PA), and nausea and vomiting (NV), and 6 single symptom measures dyspnea (DY), insomnia (SL), loss of appetite (AL), constipation (CO), diarrhea (DI), economic hardship (FD), and an overall quality of life scale (CQL). A higher functional scale score indicates a better quality of life, whereas a higher symptom scale score indicates a worse quality of life[42].

Adherence and follow up

During the treatment period, patients will be hospitalized at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, to facilitate follow-up; patients unable to be followed-up as in patient will undergo telephone follow-up by a research assistant. Patient schedules are shown in Table 2.

Table 2 Clinical trial procedure

	Trial period				
	Allocation	**Interventio n start**	**Intervention finish**	**Follow-up**	
Day	D0	D1	D7	D14	D28

Enrollment					
Informed consent	√				
Inclusion	√				
Exclusion criteria	√				
Basic information	√				
Previous history	√				
Efficacy indicators					
STAI		√	√	√	√
DT		√	√	√	√
HAMA		√	√	√	√
QLQ-C30		√	√	√	√
IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10		√	√		
5-HT、DA、NE、ACTH and GABA		√	√		
Gut microbiota		√	√		
Safety indicators					
Vital signs	√		√		
Blood, liver and kidney functions	√		√		
Adverse events			√	√	√
Other parameters					
Adherence evaluation			√		
Analysis of shedding causes			√		
Efficacy evaluation			√		

Outcome measures

The primary and secondary outcomes are shown in Table 3.

Table 3 Primary and secondary outcomes

Outcome measures	
Primary outcomes	
Assessment of the effects of music therapy on subjective symptoms of anxiety in cancer patients with anxiety	1. STAI will be used to detect usual anxiety and the severity of anxiety symptoms in cancer patients 2. HAMA will be used to evaluate the severity of anxiety symptoms in cancer patients 3. DT will be used to determine the level of psychological distress in cancer

	patients
Secondary outcomes	
Assessment of the effects of music therapy on quality of life and immune-related blood indices in cancer patients with anxiety	1. QLQ-C30 will be used to assess the quality of life and overall health status of cancer patients with anxiety 2. Blood index and physiological indicators will be used to assess changes in serum concentrations of cytokines (IL-1β, TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10) and neurotransmitters (5-HT, DA, NE, ACTH and GABA) in cancer patients with anxiety from before to after treatment 3. Gut microbiota will be collected to compare the differences in diversity, enterobacteriaceae, and short-chain fatty acids.

Quality control

To ensure the accuracy of the experiments, research assistants will review study documents, informed consent forms, case report forms (CRFs), and data records on a regular basis.

Data management

The team leader and research assistant will review the CRFs and scales before handing them over to data management staff for data entry and administration. The original CRFs and all scales (including consent forms) will be kept at the Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine.

The safety of this study will be monitored by the Data and Safety Monitoring Board (DSMB) of the Clinical Assessment Center at Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine will monitor the safety of the study every month, which is made up of clinical experts and statisticians.

Patients and public involvement

Patients and the general public will not be involved in the design of the study or in the determination of outcome measures. No attempt will be made to assess the burden of the intervention on the patients themselves.

Sample size

Based on previous studies, the level of STAI improvement will be regarded as the main indicator of efficacy, with differences of 5.43 regarded as the primary outcome, with a unilateral P=0.05. Sample size calculation with PASS 15.0 software using the formula:

$$n1=n2=\frac{\left|u_{a/2}\sqrt{2\bar{p}(1-\bar{p})}+u\beta\sqrt{p_1(1-p_1)+p_2(1-p_2)}\right|}{(p_1-p_2)2}$$

The minimum total sample size was calculated to be 48 patients. Including a 20% withdrawal rate, the minimum total sample size was 60 patients, or 30 in each group based on 1:1 randomization.

Statistical analysis

Normally distributed continuous variables will be compared by t-tests and non-normally distributed continuous variables by Mann-Whitney rank-sum tests. Categorical variables will be compared by chi-squared tests. SPSS 22.0 will be used to generate a normal probability graph and perform a hypothesis test to check whether the observed values obeyed a normal distribution. Individual data points will be superimposed on a box-line plot for calculations. The results of anxiety correlation scales and Elisa will be compared by t-tests or Mann-Whitney rank-sum tests according to whether the normal distribution is met or not. The results of 16sRNA will be classified by the RDP reference database (http://www.mothur.org/wiki/RDP_reference_files) to calculate the relative abundance of microbial communities at different levels. Then, the differences between samples (groups) will be calculated by Principal Component Analysis (PCA), Principal Co-ordinates Analysis (PCoA), Non-Metric Multi-Dimensional Scaling (NMDS), Unweighted Pair-group Method with Arithmetic Means (UPGMA), and Beta Diversity Index Inter-roup Difference Analysis. All statistical analyses will be performed by SPSS 22.0 software, with $P<0.05$ considered statistically significant. Patients who used other drugs or therapies on cancer, will be stratified in statistical analysis.

Ethical issues

The study will adhere to the Helsinki Declaration and the Ethical Guidelines for Clinical Research. The study protocol has been approved by the Research Ethical Committee of Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine (approval number: 2019-092).

Discussion

Anxiety and depression are associated with cancer mortality and survival rates[43]. The increased focus on quality of life of cancer patients has increased interest in their emotional symptoms. Music therapy may reduce anxiety, depression and improve the quality of life of cancer patients[44-46]. Music therapy is a risk-free, flexible-operation, and cost-effective intervention that may improve anxiety in cancer patients. The randomized control trial described in this study will test whether this non-pharmacological intervention can reduce anxiety in cancer patients. Findings of this study may serve as a reference for trials determining whether other non-pharmacological methods could improve anxiety symptoms in oncology patients.

A major strength of the present trial is its method of intervention, in which individual patients are exposed to a personalized music clip based on each patient's preference. Outcomes will be measured using the STAI, DT, HAMA and QLQ-C30 instruments.

HAMA scores are measured by physicians, making them more objective. STAI can respond to both short-term and long-term emotional traits. The STAI and HAMA scales will therefore be combined to reduce bias and increase the reliability of the results. Moreover, this study will measure the levels of neurotransmitters, gut microbiota and cytokines in tumor patients to explore the mechanisms by which music therapy improves anxiety in tumor patients. A meta-analysis reported that high levels of IL-8 and IL-6 were significantly associated with the prognosis of cancer patients treated with immune checkpoint inhibitors[47]. Moreover, serum IL-6 levels can be used as a biomarker to predict the outcome of treatment with antidepressants [48]. A case-control study found that fecal microbiota signatures differed in gastrointestinal cancer patients with and without anxiety and depression[49]. The gut-brain-microbiota-axis could modulate depression and anxiety induced by chronic stress through ileal immune regulation[50]. In addition, music therapy could increase salivary immunoglobulin A levels and reduce cortisol levels of cancer patients[51]. Music therapy can significantly increase NK cell count and activity[52]. Therefore, we will assess the effects of music therapy on the efficacy of immunotherapy in cancer patients in the future studies.

This study, however, is still subject to some limitations. The major limitation is the single-center nature of this trial, which may limit the generalizability of study results. In addition, the intervention period will be only 1 week, which may be too short to determine the effects of music therapy on immune function and gut microbiota. Future studies should be designed to test whether longer periods of music therapy are of greater benefit in cancer patients.

Trial status

The first participant will be enrolled in December 2022 and the study is expected to end in December 2023.

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Contributors

CS and SS designed the study. YT, XN and HY wrote the manuscript. PZ and HL developed the details of music therapy. YG and LX recruited the patients and refined the protocol. All authors approved the final version.

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Competing interests

None declared.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplemental material

This content has been supplied by the author(s).

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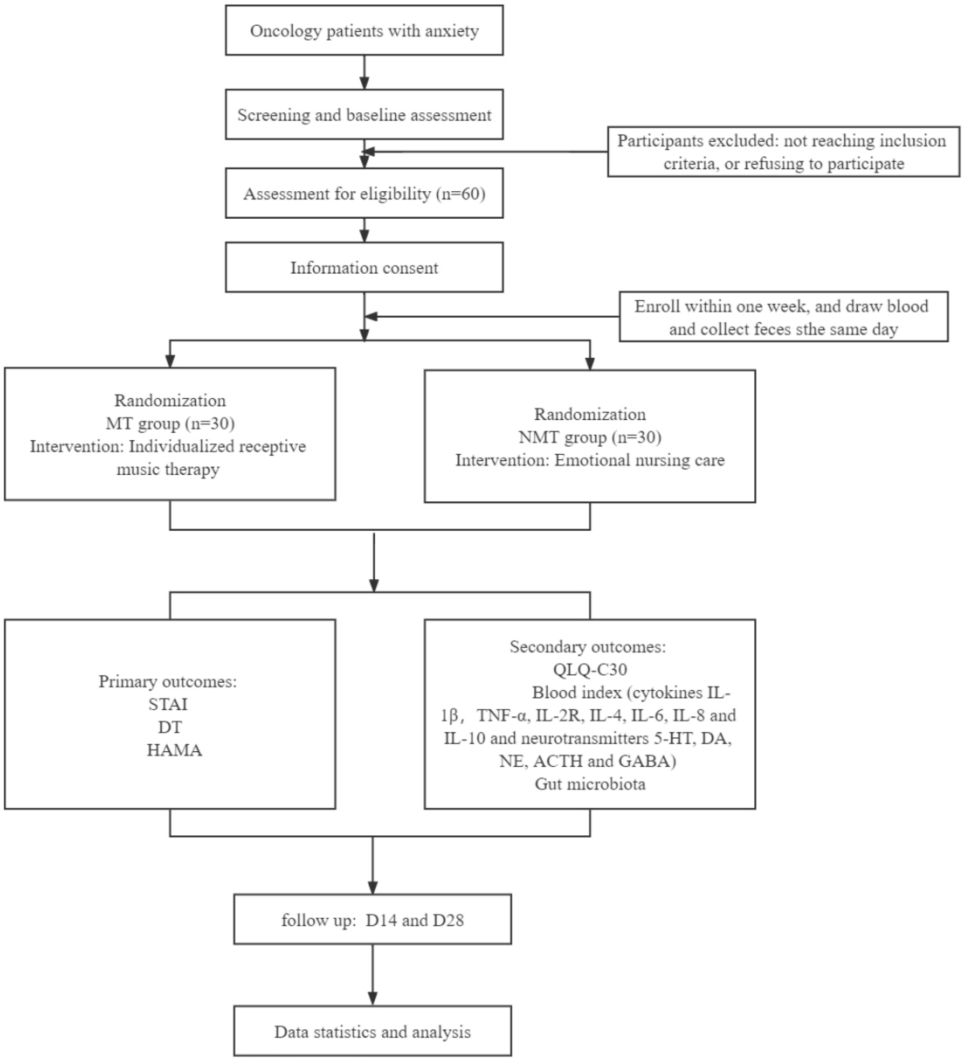
Fig. 1 Flowchart of the trial design, based on standard protocol items.



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Informed Consent • Informed Notice page

Project Name: Effects of music therapy on anxiety in cancer patients：A randomized controlled trial

Research Unit: Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine of the Shanghai University of Traditional Chinese Medicine

Principal: Ling Xu Yabin Gong Chenbing Sun

Address: 110 Ganhe Road, Shanghai, China

Dear patient,

Your doctor has diagnosed you with a malignant neoplastic disease.

We will invite you to participate in a randomized controlled trial of the effects of music therapy on anxiety in cancer patients.

Before you decide whether or not to participate in the study, please read the following as carefully as possible to help you understand the study and why it is being conducted, the procedure and duration of the study, and the benefits, risks, and discomfort that may result from participating in the study. If you wish, you can also discuss it with your relatives and friends, or ask your doctor for an explanation to help you make a decision.

1. Research Background:

Current cancer treatments include surgical resection, chemotherapy, targeted therapy and immunotherapy. These treatments, as well as the disease itself, may have adverse psychological effects on patients, including psychological stress reactions and negative emotions such as anxiety and depression, negatively affecting patient quality of life.

Current treatment of cancer-associated anxiety is frequently focused on the primary tumor. Few studies to date have focused on treating anxiety disorders caused by cancer, with even fewer focusing on non-pharmacological treatments. Music therapy has been defined by the American Music Therapy Association (AMTA) as the "clinical and evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program". Over the last few decades, music therapy has evolved from a specialized field to a method of treating a wide range of conditions, including perioperative cancer-associated anxiety and anxiety in patients with breast and lung cancer. The pathophysiological basis of anxiety disorders has not yet been determined, although structural brain abnormalities, neurobiochemical abnormalities, and genetic factors are thought to be involved. The primary mechanisms of concomitant anxiety and depression in tumor patients are thought to involve an overactive hypothalamic-pituitary-adrenal (HPA) axis, inflammatory mediators, and immune factors.

2. purpose of research:

This randomized controlled study will assess the ability of individualized music therapy to reduce cancer-related anxiety in patients by analyzing anxiety-related scales after music therapy. In addition, this study will evaluate cytokine

concentrations, gut microbiota and neurotransmitter-related indicators to explore the link their associations in cancer patients. The study will be conducted at Yueyang Hospital of Integrated Traditional Chinese and Western Medicine affiliated to Shanghai University of Traditional Chinese Medicine, and 60 participants are expected to participate voluntarily. The Ethics Committee of Yueyang Hospital has considered that the study is in line with the principles of the "Measures for Ethical Review of Biomedical Research Involving Humans" and the "Declaration of Helsinki", and in line with medical ethics.

3. Inclusion criteria

Subjects will be included if they (1) have a malignant tumor, as confirmed by histopathology or cytology; (2) have been treated for cancer-related anxiety or cancer itself for ≥ 2 months and ≤ 1 year; (2) meet the standard criteria for anxiety, including HAMA scores ≥7 and ≤28 and STAI sores ≥ 20 and ≤ 80; (3) are aged 18 to 74 years, with no gender restrictions; (4) have no history of other mental disorders and do not smoke or drink; (5) are conscious, behave and hear normally, and do not have a professional music background; (6) have normal heart, liver, kidney, and blood test results, with all other vital signs being normal; (7) have not taken anti-anxiety medications within four weeks prior to study entry; (8) have an expected survival time >6 months; and (8) provide written informed consent to study participation.

4. Exclusion criteria

Subjects will be excluded if they (1) are currently participating in other clinical studies or clinical trials; (2) have other serious diseases, such as infection, liver or kidney failure, making them unable, in the opinion of the project leader or researchers, to tolerate the treatment regimen of this study; (3) have primary or metastatic brain tumors, as confirmed clinically or radiologically; (4) are pregnant or lactating women; (5) have received music therapy within 3 months prior to the study; or (6) are taking anxiety medications or medications that can affect anxiety.

5. Rejection, suspension and shedding criteria

Patients will be discontinued from the study if they (1) are found not to meet the above inclusion and exclusion criteria; (2) show poor compliance or fail to follow up as required; (3) have incomplete medical records; (4) withdraw voluntarily from the study; (5) are regarded as unsuitable to continue the study due to serious deterioration of disease, severe complications or special physiological changes; or (6) are regarded by the investigator as unsuitable to continue the study. In addition, patients in the control group will be discontinued if they listen to music during the study period.

6. If you participate in the study, you will need to do the following work:

(1) Before you are enrolled in the study, you will undergo the following tests to determine whether you can participate in the study. Your doctor will ask you, take your medical history, and perform a physical examination. You need to do the cytokines IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10, serum concentrations of the neurotransmitters 5-HT, DA, NE, ACTH and GABA, and determination of gut microbiota populations.

(2) If you meet the eligibility criteria, you will be studied according to the following steps:

At the beginning of the study, a random number will determine whether you will be placed in the control or treatment group. Patients in the study each had a 50% chance of being assigned to one of the two different treatment groups. Neither you nor your doctor can know and choose any treatment in advance. The treatment will last for 1 weeks.

7. Intervention

Patients in the NMT group will receive emotional nursing care but not be allowed to receive any treatment for anxiety. If treatment is required due to worsening anxiety, it will be recorded and excluded. Patients who requested to participate in the music therapy sessions randomly assigned to the NMT group will receive the same music therapy as the MT group after completing the prescribed follow-up time.

Patients in the MT group will receive individualized receptive music therapy supervised by a music therapist, along with routine nursing care as well as required medications. Prior to starting music therapy, patients in the MT group will be introduced to the music therapy process for 5-10 minutes by the music therapist. The treatment room will be soundproofed, with patients in a resting or sitting position with eyes closed and relaxed.

Prior to music therapy, patients in the MT group will be exposed to various types of music to determine their preferences. The repertoire for music therapy which is nature-based sound, lasting 10-15 minutes, will be composed by the Department of Sound Engineering of Shanghai Conservatory of Music. After recording each patient's music preferences during playback, no more than three music clips from each genre will be chosen for individualized music clips of total length about 20 minutes, increasing individual patient comfort and orientation and reducing anxiety. Subsequently, the music therapist will play the personalized music clip created for 20 minutes, once a day for one week. Simultaneously, another music therapist will use a psycho-educational approach in conjunction with verbal instructions. Conditions for music therapy will include: (1) no bright light interference; (2) the patient in a resting or inactive state; and (3) music played through speakers at a volume controlled between 45-65%.

All participants will be required to complete State-Trait Anxiety Inventory (STAI), Distend Thermometer (DT), Hamilton Anxiety Scale (HAMA) and Quality of Life Questionnaire C30 (QLQ-C30) instruments on the day before and the day after treatment, as well as 14 and 28 days after treatment. The total scores of these items will be calculated and their differences in the MT and NMT groups will be compared. Blood samples will be collected for analysis of cytokine levels (serum IL-1β，TNF-α, IL-2R, IL-4, IL-6, IL-8 and IL-10), neurotransmitter levels (5-HT、DA、NE、ACTH and GABA) and gut microbiota on the day after treatment. Changes in primary disease condition will also be recorded.

8. Other matters requiring your cooperation

You must follow your doctor's instructions for treatment. If you need any other treatment, please contact your doctor in advance.

9. Possible benefits of participating in a study

You and society will probably benefit from this research. Such benefits include the

possibility that your condition may or may not improve. In addition, this study may help evaluate the effectiveness of music therapy in relieving cancer patients with anxiety, and form a comprehensive music therapy program for tumor patients with anxiety symptoms, which can be used for other patients with similar conditions.

10. Possible adverse reactions, risks, discomfort and inconvenience of participating in the study

The treatment process may have emotional abnormalities and other adverse reactions. We will strictly regulate the treatment process and operation of music therapy to avoid the above things.

If you experience any discomfort during the study, or if you experience any new changes in your condition, or if anything unexpected, whether or not it is related to your treatment, you should inform your doctor promptly and he/she will make a judgment and take medical treatment.

The researchers will do their best to prevent and treat the adverse reactions that may result from this study. If an adverse event occurs during a clinical trial, a committee of medical experts will determine whether it is related to the treatment. As determined by the expert committee, the adverse event is related to the treatment, and the research group will provide the cost of treatment and corresponding financial compensation for the trial-related damage.

During the study period, you need to go to the hospital for follow-up visit on time and do some physical and chemical examinations such as interleukin energy, which may cause you trouble or inconvenience.

11. Qualified tuition and related expenses

The costs of the studies related to this study that you undergo during your study are at your own expense, but these tests themselves are also required for the assessment of your primary disease. Tumor related immune indicators (cytokines and neurotransmitters) and gut microbiota were detected once before and after treatment.

12. Is personal information confidential?

Your medical records (research record / CRF, physical and chemical examination report, etc.) will be completely stored in the hospital, and the doctor will record the results of the laboratory examination in your outpatient medical record. The investigator, sponsor representative, and ethics committee will be granted access to your medical records. Any public report on the results of this study will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical data to the extent permitted by law.

Your biological specimen will be kept in the hospital laboratory as required. In addition to this study, it may be used again in other studies in the future. You may now refuse to use your biological specimens for research other than this study.

13. How to get more information?

You may ask any questions about this study at any time. Your doctor will give you his/her phone number so he/she can answer your questions.

Your doctor will inform you if there is any important new information during the study that may affect your willingness to continue participating in the study.

14. You can voluntarily opt in and out of the study

Participation in the study is entirely up to you. You may decline to participate in the study or withdraw from the study at any time during the study without affecting your relationship with your physician or any loss of medical or other benefits.

Your physician or investigator may, in your best interest, discontinue you from the study at any time.

If you do not participate in the study or drop out of the study, there are many alternative treatments, such as anti-anxiety medications. You do not have to choose to participate in this study in order to treat your disease.

15. What should we do now?

It is up to you to decide whether to participate in this study. You can discuss it with your family or friends before making a decision.

Before you make the decision to participate in a study, please ask your doctor as many questions as possible until you fully understand the study. Thank you for reading the above. If you decide to participate in this study, please let your doctor know and he/she will arrange everything for you.

16. The experimental protocol is approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine. Subjects can directly consult or complain to the Ethics Committee if there is any question or situation during the experiment.

Please keep this information.

Informed Consent · Signature page

Clinical Research Project Name: Effects of music therapy on anxiety in cancer patients：A randomized controlled trial

Sponsor: Ling Xu Yabin Gong Sunchenbing

Trial registration number: CTR2000035244

Declaration of consent

I have read the above introduction about this study and fully understand the full contents of the informed consent form, and I have had the opportunity to discuss and ask questions about this study with my doctor. All my questions were answered satisfactorily.

I am aware of the possible risks and benefits of participating in this study. I understand that participation in the study is voluntary, I confirm that I have had sufficient time to consider it, and I understand that:

I can always ask the doctor for more information.

I can withdraw from the study at any time without discrimination or retaliation, and my medical treatment and rights will not be affected.

I also know that if I drop out of the study, especially due to side effects, I need to tell my doctor about any changes in my condition and complete the appropriate physical and physical examination, which will be very beneficial to me and the whole study.

If I need to take any other medication due to a change in my condition, I will either consult my doctor beforehand or tell my doctor afterwards.

I allow the Ethics Committee or the sponsor's representative to access my research data.

I consent to or refuse to use my medical records and biological specimens for research other than this study.

I will be provided with a signed and dated copy of the informed consent. In the end, I decided to agree to participate in the study and try to follow the doctor's advice.

Date of subject's signature (handwritten)

Subject's contact Number

Subject's legal guardian's signature (if necessary), (handwritten) **Date**

Guardian's contact Number

Doctor's statement

I confirm that I have explained the details of the trial, including its powers and possible benefits and risks, and have given the patient a signed copy of the informed consent.

The investigator (informing subject) **signed** (handwritten) **Date**

Researcher contact Number

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	None
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	11
	5b	Name and contact information for the trial sponsor	None
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	11
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	None

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-3
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	3
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	4-5
		Methods: Assignment of interventions (for controlled trials)	
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	None

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	None
		Methods: Data collection, management, and analysis	
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6-7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
		Methods: Monitoring	
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	9

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	4
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	4
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	None
		Ethics and dissemination	
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	10
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	None
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	None
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	5
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	None
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	5
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	None

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.