The effect of Qigong in natural killer cells in colorectal cancer patients undergoing chemotherapy - A protocol Study

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Abstract
Colorectal cancer (CRC) is the 3rd most common form of cancer in the world. Several studies have demonstrated the highly relation between the cancer and the modulation of the immune system recognized the existence of a highly complex relationship between cancer and the immune system - immunoediting theory - this theory tell us that the immunogenic phenotype of the tumour can be frequently shaped by immunological factor. Cells like NK-cells are associated with immune-surveillance and their low levels in the human body are now related with a significant increase in predisposition to develop cancer. Some physiologic models propose that Qìgōng have biomechanical effects such as in the autonomic and somatic nervous system, helping in the reduction of physical and emotional tension and improving the immune system work. Therefore there is a growing need to explore the effect of Qìgōng exercises in the quality of life in CRC patients such as its influences in emotional balance and in the reduce of side effects of the chemotherapy.

Key words: Traditional Chinese Medicine, Qìgōng, colo-rectal cancer, Immunology, NK cells, Quality of Life.

Abbreviations
ACTH Adrenocorticotropic hormone
CAM Complementary and Alternative Medicine
CBT-I Cognitive behavioural therapy for insomnia
CRC Colorectal cancer
CSFs Colony Stimulating Factors
EORTC QLQ 30 European Organization for Research and Treatment of Cancer
Quality of Life Questionnaire
FCy Fragment Crystallizable
GM-CSF Granulocyte-macrophage colony-stimulating factor
HADS Hospital Anxiety and Depression
Scales
IFN-γ Interferons gamma
ILT2 Ig-like transcript 2
IgG Immunoglobulina G
KIRs Killer-cell immunoglobulin-like receptors
MQ Medical Qìgōng
NK Natural Killer
PBMC Peripheral blood mononuclear cells
PNI Psychoneuroimmunology
QOL Quality of life
RANTES Regulated on Activation, Normal T cell expressed and secreted
TCM Traditional Chinese Medicine
TNFα Tumor-Necrosis-Factor alfa
TSH Thyroid-stimulating hormone
WHO World Health Organization

Introduction
According to the World Health Organization (WHO), the CRC is the most common cancer in the European Union [1] affecting more people above 50 years old in both sexes but specially in men [2] often shows a mucinous histology and mismatch repair gene changes. Effective screening permits discovery of colorectal cancer at an early highly treatable stage and allows for detection and removal of premalignant colorectal adenomas. Screening methods that focus on cancer detection use fecal assays for the presence of blood or altered DNA, those for detection of adenomas (and early carcinoma) lesions [3]–[5] it is the second most common cancer in women and the third most common cancer in men in the world.
Experimental Pathology and Health Sciences

Cancer in Traditional Chinese Medicine

Traditional Chinese medicine can be a complementary and supportive care of cancer patients. This theory proposes a synergy between specific therapeutic intervention area (acupuncture, Chinese Herbs, diet, Tuī Ná and Qìgōng based on a diagnostic [19]. Combining TCM with western practise of oncology (surgery, radiotherapy chemotherapy and pharmaceuticals) seems to have potential advantages through the synergy of biomodulation. Biomodulation means modification of tumour response and reduction of adverse effects such as modulation of immunity, prevention of cancer progression and symptom control to increase survival and to improve the quality of life [17]. The body-mind information system is regulated by the sympathetic and parasympathetic autonomic nervous system. This corresponds to the traditional Chinese concept of a Yīn and Yáng (balance which represents the relation between the structure and the function (body substance/functional system) of the Horreal/Aulici (Zàng/Fǔ) connected by a complex and dynamical of channels where interactive information flow. When the person is healthy the communication between the systems flows freely producing a physiological interaction.

So, malignant tumour is associated with stagnation of Qì and Xuè. Qì in a Western view is an abnormality of signal transduction, cell contact and electrophysiology of cancer cells. The microcirculation within a tumour is very sluggish in some areas of the nodules and this stagnation leads poor oxygenation in the tumour [20], [21]. In TCM cancer is associated with disturbance in information flow manifested by an excessive effect of constructive Qì (Yīn-somatic structure) imbalance with the defensive Qì (Yáng immunologic functions). The constructive Qì is comparable with the capillary flow. It is a part of Xuè and this is controlled by the Hepatic orb. Defensive Qì is a part of extermal Qì controlled by the pulmonary orb. This function is to dispel accumulations of Constructive Qì in other words not permit new neoplastic cells formation and stimulate the immune system to destroy the neoplastic cells. It describes an imbalance between the pulmonary and hepatic orb or on an emotional level this is an imbalance between sadness (maeror) and anger (ira) [21]. There are scientific evidences that repressed anger with supressed immune system can increase the risk of cancer development because the liver in under-function and it affects the movement of Qì. The stress can influence both function as structure of the nervous system influencing immunity a resistance to cancer [20]. It is call Chinese psychosomatic theory on the origin of the cancer as a disturbed symbiosis and partnership. According with this daily life events and emotional disturbances such as partnership love, family love, supressed autonomy, supressed “ira” and “anger” for a prolonged period of time can induce the disease [21].

in men. Currently, treatment options for this debilitating disease are limited and surgical resection is the only curative treatment available. Despite rapid advances in surgery, as well as in adjuvant therapies such as radiotherapy and chemotherapy, there has been only a relatively modest improvement in mortality. The majority of colorectal cancers are epithelial-derived adenocarcinomas and arise from benign adenomas through the gain of mutations in key genes. Gastrin, an important polypeptide hormone, responsible for gastric acid secretion has been found to be involved in tumourigenesis in the gastrointestinal tract. When aberrantly expressed, the gastrin and gastrin/ CCK-2 receptor genes can mediate powerful down stream events; the gastrin gene can impart anti-apoptotic properties while the gastrin/ CCK-2 receptor can activate the transcription of a number of factors including ligands of the epidermal growth factor (EGF. Tumour growth is a complex process that depends on several interactions specially on the immune system function and the capacity to infiltrating lymphocytes in the tumour location [7]. Studies show that NK cells can control both local tumour growth and metastasis by exerting direct cellular cytotoxicity and secreting immune stimulatory cytokines [8]. NK cells are a cytotoxic innate immune lymphocyte cell type and they are about 15% of peripheral blood mononuclear cells (PBMC) [9], [10]. There are several subtypes of NK cells in humans but the most important and best studied NK subtypes present in human PBMC are CD56dim CD16+ and CD56bright CD16+. CD56dim CD16+ NK subtype cells comprise the majority of PBMC being up them 90% and it is considered the most cytotoxic cells. CD16 is the FCy receptor III, and it can connect with the FC portion of IgG antibodies mediating an antibody cytototoxicity response. This subset also exhibits lower expression of KIRs and ILT2 but higher expression of NKG2A/CD94 compared with CD56bright NK cells. The expression of granzyme B and perforin is high in this subset. Recently was demonstrated that this subtype of cells working in the producing of cytokines including IFNγ after activation. CD56brightCD16+/− NK subtype cells only comprise up to 10% of NK cells in PBMC, but is the major NK subtype in tissues and secondary lymphoid organs. This subset is conventionally known as the cytokine producing subset of NK cells such as cytokines and chemokines including IFNγ, TNFα, GM-CSF, and RANTES after activation [11], [12]. The current treatment of CRC are supported by surgery, chemotherapy, radiotherapy and target therapy [13]–[16]. The carcinogenesis perspective in Traditional Chinese Medicine (TCM) is different from the west. TCM recognize that the human functions are such as body-mind network that are regulated by the sympathetic and parasympathetic autonomic nervous system [17]. The Qìgōng is a tradicional form of neurovegetative biofeedback that acts in physiological relaxation response according to the psychoneuroimmunology theory. It contributes for the reduction of physical and emotional tension and improves the immune system.
Psychoneuroimmunology and cancer

Psychoneuroimmunology (PNI) studies the dynamic interaction between the mind, nervous system, endocrine system, and immunity. The interaction of emotions and immunocytes is made through neurotransmitters, neuropeptides, and cytokines through electrophysiological changes in the nervous system. Interaction with the brain stem, hypothalamus, limbic system, and autonomic nervous system occurs through either stimulating or suppressing the activity of afferent peripheral nerves [20], [22] mainly via the pineal gland and brain opioid system, which may stimulate and inhibit antitumor immunity respectively. Cancer-related immunosuppression does not depend only on functional damage of immune cells, but also on alterations of systems responsible for the neuroimmunomodulation, the most frequent of which is a decline in blood levels of the pineal hormone melatonin (MLT). The application of the Psychomotor-somatic-relaxation response according to the new functional map probably reduces the effects of the autonomic nervous system. It also regulates the autonomic nervous system of the body, allowing it to adapt to physical and emotional stress. Therefore, the combination of physical postural exercises, breathing movements, and meditation with vegetative stabilizing properties can be used as a form of Qìgōng. In a biological context, Qìgōng causes a physiological relaxation response according to the new knowledge related to psychoneuroimmunology theory. This model reports that the effectiveness of Qìgōng seems to be associated with hypothalamic-pituitary-adrenal axis. There is evidence to suggest relations between mood disorders and function of the immune system. Indeed, the experience of pain and suffering is intimately connected to immunity. A mood disorder such as helplessness and hopelessness may lead to a depressed immune system, too. Some investigators found the relationship between the immune system and the endocrine system through the existence of NK cell receptors for specific neurotransmitters or hormonal mediators such as catecholamine, cortisol, prolactin, ACTH, TSH, growth hormone or endogenous opiates. Another connecting factor between these two systems is the existence of sympathetic and parasympathetic nerves of lymphoid organs [22], [23] mainly via the pineal gland and brain opioid system, which may stimulate and inhibit antitumor immunity respectively. Cancer-related immuno-suppression does not depend only on functional damage of immune cells, but also on alterations of systems responsible for the neuroimmunomodulation, the most frequent of which is a decline in blood levels of the pineal hormone melatonin (MLT). The application of the Psychoneuroimmunology to medical oncology does not relate only to cancer therapy but also to the physiopathological interpretation of the neoplastic disease and the mechanism of its progression. This theory says that the immunosuppressive status depends not only on the primary damage to immune cells but also on the alterations of the psychoneuroendocrine regulation and on anticancer immune response [22] mainly via the pineal gland and brain opioid system, which may stimulate and inhibit antitumor immunity respectively. Cancer-related immuno-suppression does not depend only on functional damage of immune cells, but also on alterations of systems responsible for the neuroimmunomodulation, the most frequent of which is a decline in blood levels of the pineal hormone melatonin (MLT).

Qìgōng Definition

Greten, J, defines Qìgōng as the combination of two ideas: Qì is a vegetative capacity to function of a tissue or an organ which may cause the sensation of pressure tearing or flow, and Gōng is the skill of working with the Qì. Medical Qìgōng of health and healing consist in meditation, physical movements and breathing exercises [24]. Qìgōng is a traditional form of nervous system biofeedback. The patient can voluntarily control and have conscious about his body processes by interacting with the natural energies and integrating postural exercises, breathing movements and meditation with vegetative stabilizing properties to self-regulation the biological body systems. It is a form to stabilize body mind and spirit [18]. In a biomedicar perspective, the Qìgōng causes a physiological relaxation response according to the new knowledge related to psychoneuroimmunology theory. This model reports that the effectiveness of Qìgōng seems to be associated with hypothalamic-pituitary-adrenal axis.
and Drugs. Taken together these studies suggest that practicing Qìgōng exercises favourably affect many functions of the body, permit reduction of the dosage of the drugs for healthy maintenance. For hypertensive patients, combining drugs therapy and Qìgōng resulted in reduced incidence of stroke mortality and reduced dosage of the drugs required for blood pressure. For asthma patients, the combination therapy reduces the side effects of cancer therapy. Qìgōng also helps to rehabilitate drug addicts [33].

**Objectives**

1) Evaluate the effect of Qìgōng (“Happy Qìgōng”) on the immune system, specifically in NK cells in colorectal cancer (CRC); 2) Correlate the effect of Qìgōng on the immune system with patient’s psychoemotional status as well as quality of life; 3) Identify the Qìgōng exercises how it influences in the decreases the side effects of chemotherapy and the indices of stress and anxiety.

**Methods**

Patients will be recruited in the Oncology Center of Centro Hospitalar do Porto – Hospital de S. António. According to the following inclusion criteria: patients with CRC after have done surgery and now undergoing chemotherapy, age ≥18 years old, ability to give informed consent; no use of Colony Stimulating Factors (CSFs) or Hematopoietics. Patients will be randomized in three groups: true Qìgōng group (n=10); the placebo Qìgōng group (n=10) and the control group (n=10). The true Qìgōng group will receive 6 sessions of Qìgōng therapy, twice a week between the chemotherapy protocol. Blood samples will be collect at baseline (T0) on the day prior to do Qìgōng and the following samples will be collect in the second week after starting the Qìgōng protocol (T1) and in the end of the Qìgōng protocol (T2). The placebo Qìgōng group will do the same protocol (T2).
protocol of the verum Qìgōng group but not doing the real form of Qìgōng. The control group only will collect the blood sampling for the study (T0) without receiving any Qìgōng treatment.

**Measurements**

The following cellular components are: leukocyte, neutrophil, total lymphocytes, T and B lymphocytes and NK cells and they will be quantified in all patients of the study.

The analyses necessary to the study will be held at Cytometry Laboratory in the Immunology Unit of Centro Hospitalar do Porto.

Socio-demographic and clinical questionnaire - The sample will be characterized on the basis of socio-demographic and clinical questionnaire including sociodemographic variables (gender, age, occupation, educational level, marital status, area of residence, current employment status, religion and household) and clinical variables (clinical diagnosis, time since diagnosis and medical treatment).

• Hospital Anxiety and Depression Scales" (HADS)

In order to evaluate the levels of anxiety and depression as well as quality of life of patients instruments are used: “Hospital Anxiety and Depression Scales” (HADS), Portuguese version validated by Pais Ribeiro et al (J. Pais Ribeiro et al, 2007). Scale developed by Zigmond e Snaith (1994), in order to assess levels of anxiety and depression in patients with physical disorders and outpatient treatment. This scale is constituted by a questionnaire that takes little time to respond. It consists of 14 items which are divided into two subscales: anxiety (7 items) and depression (7 items). The items reflect a generalized anxiety state of anxiety effects of the disease and the items of depression are associated with an anhedonia state. The response options vary between zero and three, on a scale of Likert constituted by four points. The values that are between 0 and 7 indicate absence of depression or anxiety; between 8 and 10 suggest the presence of one of its psychological states; values between 11 and 14 mean anxiety or mild depression and values between 15 and 21 are indicative of a high gravity.

Table 1: Experiment flow-chart

![Experiment flow-chart](image)
we reproduce oblique exploratory factor analysis and use confirmatory factor analysis. We explore the sensibility of the questionnaire. The validation process of the Portuguese HADS version shows metric properties similar to those in international studies, suggesting that it measures the same constructs, in the same way, as the original HADS form."

“European Organization for Research and Treatment of Cancer Quality of Life Questionnaire” (EORTC QLQ 30). - This is an instrument created by European Organization for Research and Treatment of Cancer Quality of Life. QLQ-C30. It aims to assess relevant to any cancer patient regardless of neoplasia to assess not responding to the specific situation in each type of cancer. It consists of five functional scales (physical, performance, cognitive, emotional and social); three symptom scales (fatigue, pain, nausea and vomiting); global health status six simple items to assess symptoms or additional problems (dyspnea, loss of appetite, insomnia, financial difficulties, constipation and diarrhea). Items translate into worse QLQ.

Experimental protocol
All patients will participate into a pre-intervention phase. In this phase the patients will be informed about the study design and the risks of the use of needles (hematoma, infection) in order to obtain the written consent.

In T0 (first day) all of them will answer to the questionnaires and will collect a sample of blood (for evaluate leukocyte, neutrophil, total lymphocytes, T and B lymphocytes and NK cells total, NK CD 56 bright, NK CD 56 Dim by a qualified professional on Centro Hospitalar do Porto.

After, the patients will be randomized into three groups: the true or verum Qìgōng group; the placebo Qìgōng group and the control group. The control group will only participate in T0 procedures.

The verum Qìgōng group will receive the sessions of “Happy Qìgōng” therapy, twice a week with the guide of the study staff; Placebo group (placebo Qìgōng) will do a “standing” position exercise while watching a television program of their choice.

In T1 at 15th day of the study the 2nd sample blood will be collected.

In T2 at 25th day of the study the 3rd sample blood will be collected and the patients will answer again to the same questionnaires.

Expected Results
Based on previous study and theoretical considerations, this study looks for:

- Confirm an improvement in immune status and more specifically in NK cells as a result of a reduction of the autonomous nerves system dysbalance.
- Clinical value of Qìgōng in treatment colorectal cancer patients.
- Improvement of quality of life in the patients with cancer.
- Evaluation cost-effectiveness from health-care systems.

Conclusion
Taken under consideration of all these investigation this study wants to confirm that, the effects of Qìgōng can be measure objectively by a variation in the immune system, specifically in NK cells.

Also the use of the psychological questionnaires will permit to evaluate the value of Qìgōng in improving the quality of life for patients.

If the results prove the hypotheses it would be advisable to integrate Qìgōng in health system, due to the benefits of low cost without risks and allowing to reduce the side effects of cancer therapies including chemotherapy.

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References


