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2015

**EFFECT OF *QIGONG* IN NATURAL KILLER CELLS  
ON COLO-RECTAL CANCER PATIENTS UNDERGOING  
FOR CHEMOTHERAPY:  
A PROSPECTIVE, RANDOMIZED AND CONTROLLED  
STUDY**

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TESE DE MESTRADO

Apresentada ao Instituto de Ciências Biomédicas

Abel Salazar - Universidade do Porto

MESTRADO EM MEDICINA TRADICIONAL CHINESA

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**EFFECT OF *QIGONG* IN NATURAL KILLER CELLS ON COLO-RECTAL  
CANCER PATIENTS UNDERGOING FOR CHEMOTHERAPY:  
A PROSPECTIVE, RANDOMIZED AND CONTROLLED STUDY**

Dissertação de Candidatura ao grau de Mestre em  
Medicina Tradicional Chinesa submetida ao Instituto de  
Ciências Biomédicas de Abel Salazar da Universidade do  
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2015



*"I know that my work is a drop in the ocean, but without it, the ocean would be smaller"*

Madre Teresa de Calcutá

## RESUMO

Os doentes com cancro apresentam um enfraquecimento do sistema imunológico, onde a imunidade inata, responsável pela deteção precoce e eliminação das células malignas se encontra deficiente. Estudos recentes sobre as células NK, que parecem constituir a primeira linha de defesa contra as células tumorais, apontam para uma correlação entre a diminuição da sua atividade e a presença de um pior prognóstico. De acordo com Chen (2002), existe uma correlação positiva entre a prática de *Qigong* e o melhoramento do sistema imunitário, traduzindo-se numa melhoria da deficiência imunológica apresentada pela maioria dos doentes com cancro.

*Qigong*, um dos métodos terapêuticos da Medicina Tradicional Chinesa, apresenta-se como uma terapia de *biofeedback vegetativo*. É uma prática corpo-mente desenvolvida há mais de 5000 anos, que surge através da combinação de exercícios físicos e meditação, no sentido de harmonizar o corpo, mente e espírito. Tem efeitos ao nível emocional e psíquico assim como ao nível físico e imunológico.

**Objetivos:** : 1) Avaliar o efeito do *Qigong* no sistema imunitário, especificamente nas células NK e na qualidade de vida dos doentes com CCR. 2) Fundamentar a aplicação de técnicas de *Qigong* bem como a eficácia de um exercício específico de *Qigong* ("Happy *Qigong*") na estimulação do sistema imunitário; 3) Demonstrar a viabilidade desta terapia em ambiente hospitalar e em doentes com CCR.

**Métodos:** Estudo prospetivo, randomizado e controlado.

Foram recrutados doentes com CCR através da Clínica de Patologia Digestiva integrada no Instituto Português de Oncologia do Porto (IPO). Os doentes foram randomizados em dois grupos; intervenção de (n = 10) e grupo control (n = 10). Os doentes do grupo experimental receberam 6 sessões de terapia de *Qigong*, duas vezes por semana durante os tratamentos de quimioterapia. A terapia de *Qigong* aplicada é baseada no Modelo de Heidelberg de MTC. As amostras de sangue foram colhidas no início do estudo (T0) que corresponde ao sétimo dia após a sessão de quimioterapia e as seguintes amostras são colhidas uma vez por semana durante um período de 3 semanas. O grupo de control teve apenas de recolher as amostras de sangue para o estudo sem receber qualquer tratamento de *Qigong*.

**Parâmetros primários:** Hemograma completo; linfócitos T e B e células NK quantificados por citometria de fluxo.

**Parâmetros secundários:** Avaliação dos níveis de ansiedade e depressão bem como a qualidade de vida (QOL).

**Análise estatística:** Os resultados serão analisados dentro de cada grupo e entre os grupos (antes e depois da intervenção).

**Discussão:** Serão comparados dados acerca da quantificação das células NK nos dois grupos. Os dados sugerem que o *Qigong* pode ter um papel mieloprotetor, reduzindo as taxas de leucopenia, neutropenia e do estado psicoemocional nos pacientes submetidos a quimioterapia com CCR.

**Conclusão:** O tratamento de *Qigong* baseado no Modelo de Heidelberg fornece indícios que pode promover o aumento das células NK, o qual possivelmente se poderá traduzir na melhoria do sistema imunológico, bem como, numa melhoria da qualidade de vida nos doentes com CCR.

**Palavras-chave:** Cancro colo-retal, Quimioterapia, Medicina Chinesa, *Qigong*, Sistema imunitário.

## ABSTRACT

Cancer patients suffer from a weakened immune system where innate immunity, responsible for the early detection and elimination of malignant cells, is inefficient. The development of NK cells, which appears as the first line of defense against tumor cells, points to a correlation between the reduction in their activity and the presence of a worse prognosis. Accordingly to Chen (2002) and Cai et al( 2001) *Qigong* produce an improvement of the immune function, which may enhance the immune deficiency experienced by most of cancer patients and the improve of the microcirculatory functions.

*Qigong*, one of the therapeutic methods of Traditional Chinese Medicine, presents itself as a vegetative biofeedback therapy. It is a mind-body practice developed more than 5000 years, which are the combination of physical exercises and meditation in order to harmonize body, mind and spirit. Has specific emotional control, fatigue, nausea, stimulation of the immune system, among other exercises.

**Objective:** 1) To evaluate the effect of *Qigong* on the immune system, specifically in NK cells and the quality of life of patients with Colo- rectal cancer (CRC). 2) Justify the application of techniques of *Qigong* and the effectiveness of specific exercise *Qigong* ('*Qigong* Happy ") in the stimulation of the immune system; 3) Demonstrate the feasibility of this therapy in the hospital in patients with CRC.

**Methods:** A prospective, randomized and controlled study. CRC patients recruited from Clinic of Digestive Pathology integrated in the Portuguese Oncology Institute of Porto( IPO). They were randomized in two group intervention (n=10) and control group (n=10). Patients in the experimental group received 6 sessions of *Qigong* therapy, twice a week between the chemotherapy protocol. Treatment protocol based on the Heidelberg Model of TCM. Blood samples will be collected at baseline (T0) on the day prior to chemotherapy and the following samples are collected every once a week until next chemotherapy (CT) regimen. The control group just been collecting the blood sampling for the study without receiving any *Qigong* treatment.

**Primary Outcome:** Complete Blood counts, T and B lymphocytes, NK cells by flow cytometry.

**Secondary Outcomes:** Assessment of anxiety and depression levels as well as quality of life.

**Statistical analyses:** Results will be analyzed within each group (before and after intervention) and co- relational comparison between groups.

**Discussion:** Two studies have addressed on NK cells subset activity in cancer patients. Based on our results, they suggest that *Qigong* may have a myeloprotective role

by reducing the rates of leukopenia and neutropenia in CRC patients undergoing chemotherapy.

**Conclusion:** The *Qigong* treatment based on Heidelberg Model provides evidence that can promote the increase of NK cells, which possibly can be translated in improving the immune system, as well as an improvement of quality of life in patients with CRC.

**Keywords:** Colorectal Cancer, chemotherapy, Chinese medicine, *Qigong*, immune system.

## **ACKNOWLEDGEMENTS**

There are things in life that without the support of some special people, would never be possible to be performed. This is one them.

To my family, especially my boyfriend, who always believes in me and gives the support I need to continue this journey.

To Portuguese Oncology Institute of Porto (IPO), for making this research possible. For me, this place is more than a hospital, is where I learn how to become a better nurse and a better person every day of my life.

To all workers of Clinic of Digestive Pathology, Day Hospital Unit and Central Blood sampling of IPO Porto, I would like to express my gratitude for their help, friendship and cooperation during my research. I would like to specially thank to Dr<sup>a</sup>. Maria Fragoso, who believes in me and in this work since the beginning.

To my supervisors of master's degree, Irene Pais, for the precious help during all process of this study. Really an amazing support.

To Prof. Greten, for all the knowledge transmitted and for teaching me to see the world through the eyes of TCM.

To Prof. Maria João and Prof. Jorge Machado for their constant enthusiastic support.

And the last but not the least, to all my patients who participated in this program. They teach me more than I teach them.

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## ACRONYMS

ICBAS - Instituto de Ciências Biomédicas  
Abel Salazar

CCR - Cancro Colo Retal

CRC - Colo- Retal Cancer

NK - Natural Killer

CT - Chemotherapy

WHO - World Health Organization

IPO - Institute Portuguese of Oncology

PMN - polymorphonucleocytes

TNF- Tumor necrosis factor

IL - Interleucine

DC - Denditric cells

QLQ - Quality of Life Questionnaire

QOL- Quality of life

TCM - Traditional Chinese Medicine

DCBE - Double-contrast barium enema

CT- Virtual Colonoscopy

CEA - Carcinoembryonic antigen

HADS - *Hospital Anxiety and Depression Scales*

MC - Microcirculation

ANC - Absolute Neutrophil Counts

APC - Adenomatous polyposis

DNA - Desoxirribonucleic Acid

WBC - White Blood Cells

IARC - International Agency for  
Research on Cancer

FAP- familial adenomatous  
polyposis

HADS - Hospital Anxiety and  
Depression Scales

ALT - Algor Laedens Theory

## **I – THEORETICAL BACKGROUND**

## INTRODUCTION

According to an estimate of the World Health Organization (WHO), cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012, whereas colorectal cancer occupies the 3rd position in the ranking, with the highest rate of deaths in Portugal [1]. In Portugal, the incidence of CRC is almost similar in both genders, although Colon cancer is slightly more common in women and Rectal cancer in men [2]. According to data retrieved from the Northern Regional Cancer Registry, in 2006, from the 5171 diagnosed malignant tumors, 311 (6%) were colon and 387 (7.5%) rectal diagnosis [3].

The choice of this study's subject results from the fact that cancer is one of the most common chronic diseases and is currently considered one of the main health problems of the twenty-first century and it will be the next global concern regarding the health of the population. Furthermore, cancer will be in the top of the scientific research and technology advances. The current available practices for CRC treatment include: surgery, chemotherapy, radiotherapy as well as new antiangiogenic drugs, which are not always 100% effective.

It is common sense that chemotherapy (CT) induces immunosuppression, local or systemic, and that may adversely affect the formation, recruitment and action of effector cells of the immune system and thus precipitate a therapeutic failure.

Some studies have confirmed the effectiveness of a multidisciplinary treatment of oncological diseases; it seems that combining treatments is more successful than the proposed one-dimensional treatment. It is important for patients to have at their disposal multidisciplinary team acting in different ways and perspectives, so they can have an integrated view of the patient as a whole. For instance, Graner (2010) considered that, complementary and alternative therapies aim to reduce symptoms and promote quality of life in different stages of treatment concomitantly to clinical usual. [4]

In this context, arises the importance of *Qigong* in cancer treatment because many studies suggest that *Qigong* therapy and *Qigong* practice may help cancer patients improve their immune functions as well as improving the physical and psychological well-being. In fact, health promotion and disease prevention have been the basis for life conditions improvement.

The main purpose of this study is to evaluate the effects of *Qigong* in the immune system, particularly NK cell and quality of life of patients with CRC.

This work is divided in two parts. The first part contextualizes the problem, which presents the theoretical background since its concept; the second part presents the methodological framework and research results.



## 1. CANCER - IMMUNOLOGICAL ASPECTS

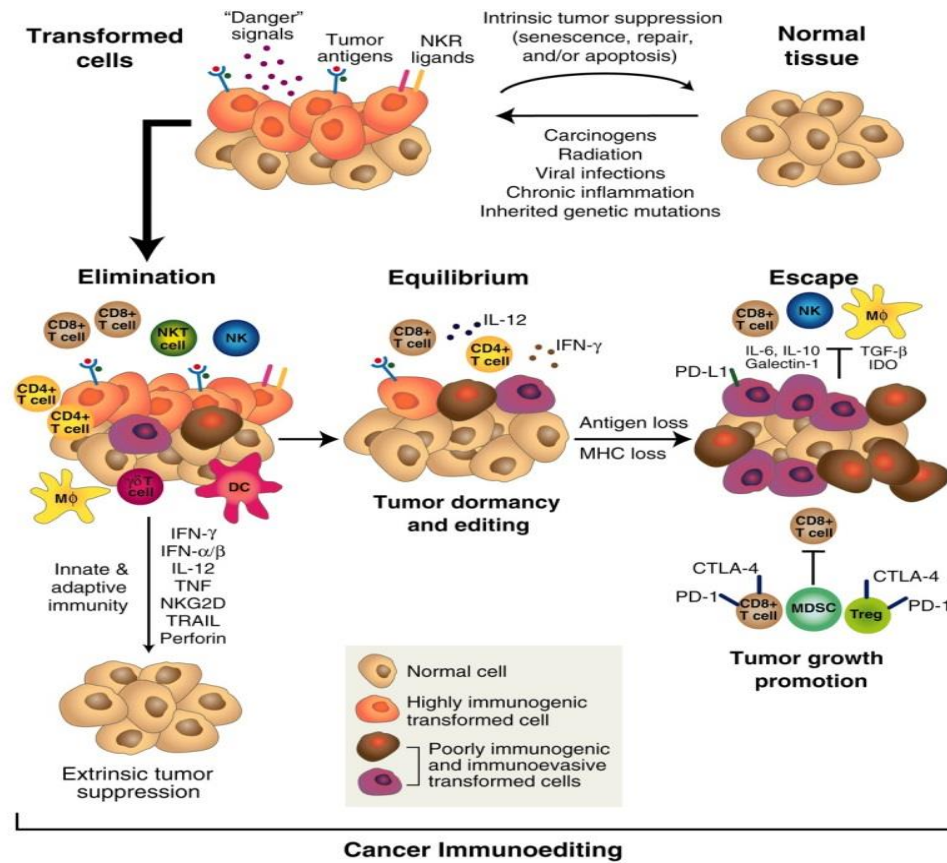
The last 20 years have seen a reemergence of interest for the immunological aspects of cancer and how the immune system behaves.

The concept that the Immune System protects the host against cancer was first proposed by Erlich in 1909 and modified in the 1950s by Burnet and Thomas.

Cancer is widely considered to be a cell-autonomous genetic disease that results from alterations in oncogenes, tumor-suppressor and genome-stability genes. However, the tumor-cell microenvironment and immunity also have a major role in cancer process. Four classes of cells have been established to have key roles in the immune response against tumors, and consequently the Immune system is totally involved in this action. These cells are: Natural Killer (NK) cells, that provide innate immune response; CD8+ T lymphocytes, that represent the adaptive immune response and NK cells.

To win the fight against cancer, it is necessary not only to develop strategies to kill all cancer (stem) cells efficiently, by using the correct combination and schedule of chemotherapeutic agents, but also to attempt to stimulate an immune response so that the immune system can keep residual tumor cells in check.

It is supported by a strong experimental data derived holds that the immune system not only protects the host against development of primary nonviral cancers but also scupts tumor immunogenicity [5]. Cancer immunoediting is a tumor suppressor mechanism that engages only after cellular transformation has occurred and intrinsic tumor suppressor mechanisms have failed. In its most complex form, cancer immunoediting consists of three phases: a) elimination phase, in which cancer cells are recognized by the immune system and may be eradicated; b) equilibrium phase appears if elimination is unsuccessful, and immune system and cancer cells achieve an equilibrium in which cancer is contained but not eliminated and c) the escape phase appears when the tumor evades the effector mechanisms of the immune system and proliferates without control [6].



**Figure 1: Cancer immunoediting theory.** Diverse immune cells involved in the different phases. Data from Robert Strausberg et al. (2011) [7]

The immune system's capacity to destroy and shape cancer is a process that makes cancer under control for long periods of time by a process called *equilibrium*. This is a component of cancer immunoediting where cells have a highly immunogenic and this process occurs between elimination and escape phases. Whereas elimination requires elements from both the innate and the adaptive immune response attenuated immunogenicity [7].

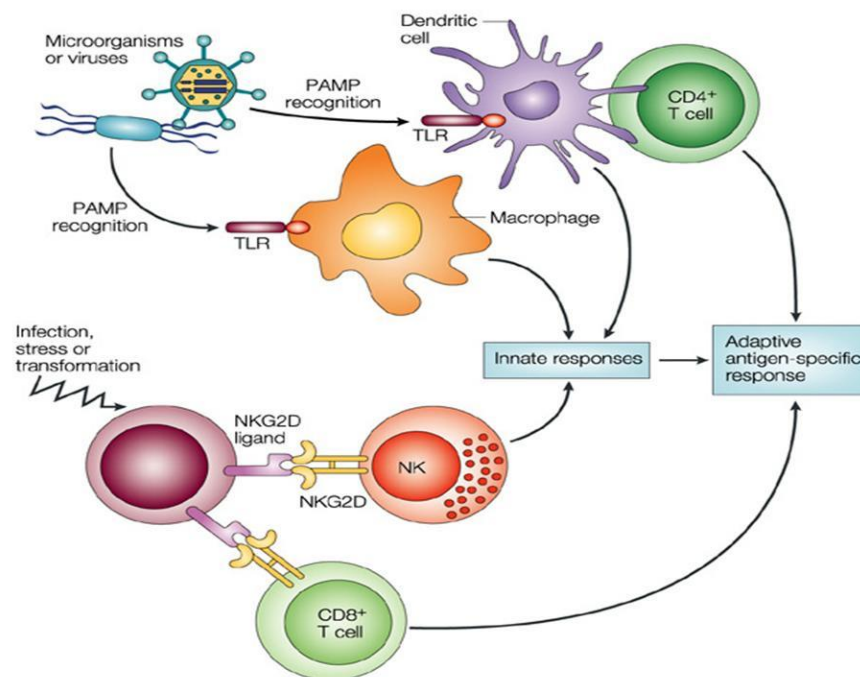
### 1.1 - NK CELLS AND THE IMMUNE SYSTEM

The immune system is classically divided into innate and adaptive immunity. The innate system is composed mainly of NK cells, polymorphonucleocytes (PMN) and macrophages, and is most directly involved in tumour immunology. These cells also

participate in the adaptive response and form an important and vital bridge between the two arms of the immune system.

Natural killer (NK) cells “sit” between innate and adaptive immunity and represent a subgroup of white blood cells and were originally defined as effector lymphocytes of innate immunity with constitutive cytolytic functions. NK cells are constantly patrolling the body. As effectors members of the innate immunity, they can directly kill target cells without prior activation, priming or assistance by cytokines. NK cells have been recognized as major producers of cytokines in many physiological and pathological conditions, such as IFN $\gamma$ , tumour necrosis factor (TNF  $\alpha$ ) and interleucine (IL)-10.

The production of IFN $\gamma$  is related with the modulation of T cell responses possibly by a direct interaction between T cells and NK cells migrating from inflamed peripheral tissues to secondary lymphoid compartments. NK cells modulate emerging B and T cells and are also regulatory cells engaged in reciprocal interactions with Dendritic cells (DC), macrophages, T cells and endothelial cells. In consequence, they achieve their full effector potential, highlighting the intimate regulatory interactions between NK cells and other components of immune response.



**Figure 2: The biological functions of NK cells**

Data from Robert D. Schreiber (2001) [8].

In resume, they are an effective mechanism for controlling potential infections with a sophisticated biological function and preventing cancer progression.

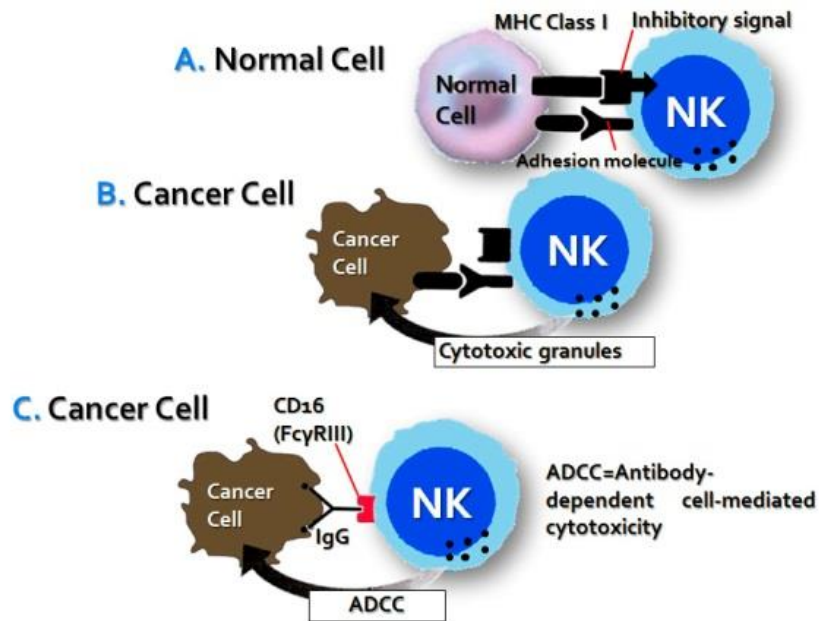
## 1.2 - NK CELLS IN CANCER IMMUNITY

In 1909, a scientist by the name of Paul Ehrlich proposed that the incidence of cancer would be much greater were it not for the vigilance of our immune defense system in identifying and eliminating tumor cells. The immune system has a major role in the prevention of tumors. Initially, the immune system can protect the host from virus-induced tumors by eliminating or suppressing viral infections. Consequently, the timely elimination of pathogens and prompt resolution of inflammation can prevent the establishment of an inflammatory environment conducive to tumorigenesis, so the immune system can specifically identify and eliminate tumor cells on the basis of their expression of tumor-specific antigens or molecules induced by cellular stress.

Several studies verified that the lack of NK cells or molecules associated with NK cells recognition or effector function in mice was associated with an increase of tumor incidence [9].

NK cells are considered to represent a first line of defense against the metastatic spread of tumor cells. This idea is supported by the report of an association between the decreased activity or low numbers of circulating NK cells with progression of cancers; and a correlation between an absolute decrease in the activity of the NK cells and an absolute decrease in the lytic potential of these cells [9]. Consequently, NK cells are the first cells identified for their ability to kill tumor cells without deliberate immunization or activation [10].

Some activating NK cell receptors and costimulatory molecules have been identified that permit these cells to recognize tumors and virus-infected cells and destroy them. Two major mechanisms are used to induce target cells apoptosis: granule exocytosis and death receptor stimulation. Granule exocytosis involves the release of perforin and granzymes while the death receptor pathway is largely mediated by apoptosis inducing members such as TNF (*alfa*) and LT [11]. When NK cells detect an infected or tumor cell, they secrete granules that contain perforin, creating a pore in the target cell; granzymes then pass through these pores, degrading cellular proteins, causing cells to undergo apoptosis.



**Fig.3: Schematic presentation of cytotoxicity of NK role in cancer cells**

Data from: Medical Research Council [70].

These cells are modulated by an inhibitory receptors that sense the levels of major histocompatibility complex (MHC) class I on prospective target cells to prevent unwanted destruction of healthy tissues. Their cytotoxic ability can be enhanced by cytokines, such as interleukin (IL) -2, IL-12, IL-15 and interferon alpha/beta (IFN-alpha/beta) [10].

In conclusion, data review show that the loss of immune competence, more precisely the lower levels and/ or activity of NK cells, is considered an important cancer risk factor and allows to conclude that NK cells participates in immunosurveillance against certain types of tumors [10].

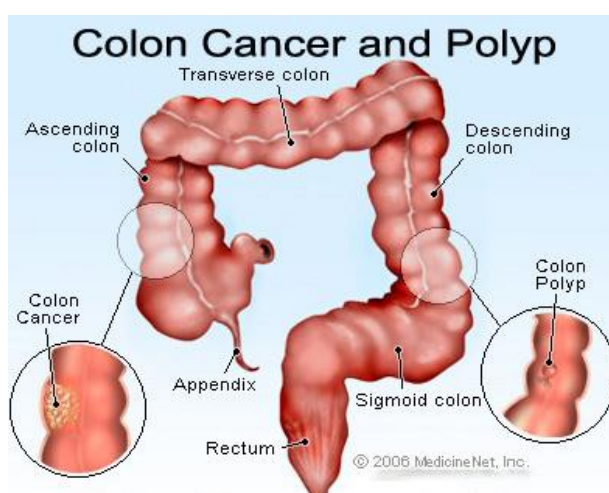
### 1.3 - IMMUNE PARAMETERS CORRELATED WITH THE EFFICACY OF CHEMOTHERAPY

Conventional anticancer chemotherapy has been historically thought to act through direct killing of tumor cells. Accumulating evidence indicates that the innate and adaptive immune systems make a crucial influence to the antitumor effects of conventional CT cancer treatment. This concept stems from the fact that cytotoxic drugs interfere with DNA synthesis and replication. Several direct effects of cytotoxic drugs have been described for macrophages, DC and NK cells. Earlier studies on NK cells function in cancer patients undergoing cytotoxic CT have shown variable effects, especially in correlation with the clinical outcome [12]. However, this fact indicates that the antitumor activities of CT also

rely on several off-target effects, especially directed to the host immune system, that cooperate for successful tumor eradication [13]. Beyond cancer-cell-intrinsic factors that determine the cytotoxic or cytostatic response, as well as the potential immunogenicity of tumor cells, the functional state of the host immune system has a major prognostic and predictive impact on the future of cancer patients treated with conventional or targeted chemotherapies [14]. Chemotherapeutic agents stimulate both the innate and adaptive arms of the immune system. The outcomes of CT can promote specific adjustments on dying tumor cells, which render them visible to the immune system. It is becoming evident that CT agents can deeply have an impact on both tumor and host immune system. Some cytotoxic drugs have been shown to induce an immunogenic type of cell death in tumor cells, resulting in the emission of specific signals that trigger phagocytosis of cell debris and promote the maturation of dendritic cells, ultimately resulting in the induction of potent antitumor responses. [13]

## 2. COLO RECTAL CANCER OVERVIEW

Colorectal cancer is one of the most common malignancies in developed countries. In the vast majority of cases, it is sporadic, but can also be hereditary, as familial adenomatous polyposis and colorectal cancer hereditary non polyposis. Colon cancer mostly arises from adenomas, recognized as colonic polyps, but may occasionally arise from the sessile serrated adenoma. Adenomatous polyposis coli (APC) gene mutation is the key molecular step in adenoma formation.



**Fig 4- Colon rectal cancer overview**

Data from Medical Research Council [70].

Those with a personal history of adenomas or CRC are at increased risk. Individuals with a family history of CRC or adenomas, various genetic polyposis and nonpolyposis syndromes and inflammatory bowel disease are also at higher risk of developing CRC. Progression from adenomas to colon cancer is a multistep process. In fact, when the process of the normal replacement of lining cells goes awry, some mistakes in mucosal cell division frequently occur that escape from our editing systems. When this occurs, these cells begin to divide independently of the normal checks and balances that control growth. Over 95% of colon and rectal cancers are adenocarcinomas and most of the patients have no identifiable genetic risk factors [15]. These are cancers that start in gland cells, like the cells that line the inside of the colon and rectum. There are some other, more rare, types of tumors of the colon and rectum that we discuss below.

## 2.1 - EPIDEMIOLOGY, ETHIOLOGY AND PATHOPHYSIOLOGY

Colorectal cancer is the 3rd most common form of cancer worldwide, with approximately one million new cases diagnosed each year. Data from GLOBOCAN, 2008 report by the International Agency for Research on Cancer (IARC) shows that colorectal cancer is the third most common form of cancer in men (663,000 cases, 10% of the total) and the second most common in women (570,000 cases 9.4% of total) worldwide [16].

According to the WHO, the CRC is the most common cancer in the European Union. About 4% of European men and 3% of European women develop CCR up to 75 years [1].

The CRC it is a global health problem with an annual incidence of approximately 1000000 cases and more than 500,000 cases per year of mortality [18]. In Portugal, the incidence of CRC is almost similar in both sexes, although the colon cancer is slightly more common in women and cancer of the rectum in men [17]. According to the Northern Regional Cancer Registry (RORENO) in 2006 of malignant tumors diagnosed 5171, 311 (6%) were colon and 387 (7.5%) of the rectum [19]. In concern to mortality, the country with the highest rate of mortality is Hungary and Greece to the lowest for both sexes. Portugal is in 7th position for males and sixth for females [20].

In Portugal CRC mortality has increased significantly in recent decades, with an average annual rate of 4%. In 1999 the CRC was responsible for 2828 deaths and considered the leading cause of cancer death in that year [21]. According to statistics, in 2005 the mortality rate for CCR was 14.6%, corresponding to 3319 deaths per year and more than nine deaths per day. CCR is currently the leading cause of death from malignancy in Portugal, only that the Western world [22].

In fact, in most countries there has been a decrease in the rate with the exception of Korea, Portugal, Slovenia, Poland, Mexico, Greece, Chile and Estonia [23].

The progress of CRC has been associated with several important risk factors, which can be grouped in two main groups: the "environmental" factor group and the genetics factor group [24]. The findings from epidemiologic studies that physical inactivity, excess of body weight and a central accumulation of adiposity have an influence on CRC risk, as well as the ethnic and racial differences in the CRC and studies on migrants suggest that an environmental factor plays an important role in a etiology of this disease.

In fact, diet is considered an important factor of increased risk of the development of CRC, playing a significant role in determining the incidence of CRC in the general population. Compelling evidence suggests a strong dose-related association between red meat and fat intake and the development of CRC; and higher cholesterol values correlate significantly with the later tumor development. On the other hand, diets rich in vegetables

and high fiber grains as well as the consumption of fish and skinless chicken demonstrate a protective effect in the pathophysiology of CRC.

As a genetic factor group, patients who have familial adenomatous polyposis (FAP), who have medical history of adenomatous colonic polyps, inevitably develop colon cancer if colectomy is not performed [25]. The FAP syndrome accounts for approximately 1% of all CRC cases and patients will most likely present with adenocarcinoma before the age of 40. This syndrome is inherited as a classic Mendelian single autosomal dominant gene. Patients who have FAP carry this germline mutation in one allele in all somatic cells, including colonocytes. Therefore, prophylactic colectomy is recommended at an early age for FAP patients. Gardner's syndrome is a rare phenotypic variant of FAP, both caused by mutation in the APC gene. In addition to colon polyposis, Gardner patients acquire extra-colonic tumours including osteomas, thyroid cancer, epidermoid cysts, fibromas, sebaceous cysts and desmoids tumours. The disease is not curable and life expectancy with the condition is 35-45 years [26]. Patients with inflammatory bowel disease with long evolution have also more prone to develop CRC and have the indication for periodic surveillance [27].

## **2.2- CLINICAL PRESENTATION – SIGNS AND SYMPTOMS**

Patients with CRC may present in three ways: patients with suspicious symptoms and/or signs; asymptomatic individuals discovered by routine screening; and emergency admission with intestinal obstruction, peritonitis, or rarely, an acute gastrointestinal bleed.

Colon cancer also tends not to produce signs until advanced [28]. Some clinical signs are anemia from gastrointestinal bleeding that produce pallor, iron deficiency anemia, spooned nails, glossitis manifested by lingual erythema and papillae loss. Hypoalbuminemia may manifest clinically as peripheral edema, ascites, or anasarca. Rectal cancer may be palpable by digital rectal examination. Although colon cancer previously was believed to frequently cause fecal occult blood as detected by stool guaiac tests.

Symptoms are common and prominent late in colon cancer when the prognosis is poor but are less common and less obvious early in the disease. Common symptoms are listed in Table 1. Less common symptoms include nausea and vomiting, malaise, anorexia, and abdominal distention [29]. Although colon cancer can present with diarrhea or constipation, a recent change in bowel habits more likely is from colon cancer than chronically abnormal bowel habits.

<b>Symptoms associated with colon cancer</b>	
<u>Symptoms</u>	<u>Frequency</u>
Abdominal Pain	44%
Change in bowel habit	43%
Hematochezia or melena	40%
Weakness or malaise	20%
Involuntary weight loss	16%

**Table 1. Symptoms associated with CRC and respectively frequency**

Extrated from Campell MS.(1992) [28].

Symptoms depend on the cancer location, size, and presence of metastases. A change in bowel habits is generally an indicator of left-sided than right-sided CRCs because fecal contents are liquid in the proximal colon and the lumen caliber is larger, and they are therefore less likely to be associated with obstructive symptoms; Hematochezia is more often caused by rectosigmoid than right-sided colon cancer; Iron deficiency anemia is more common with right-sided CRCs. Ascending colon tumors have a fourfold higher mean daily blood loss (approximately 9 ml/day) than tumors at other colonic sites [28-30].

Abdominal pain can occur with tumors arising at all sites; it can be caused by a partial obstruction, peritoneal dissemination, or intestinal perforation leading to generalized peritonitis. Advanced cancer, particularly when metastatic, can involuntary weight loss, anorexia, muscle weakness, and a feeling of poor health.

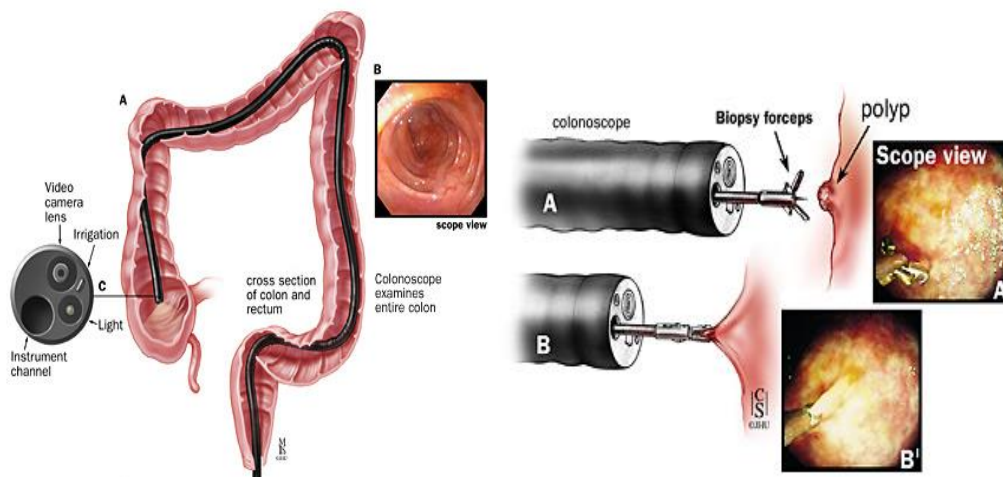
### **2.3 - SCREENING AND PREVENTION**

The CRC may be suspected from one or more of the symptoms and signs described above or may be asymptomatic and discovered by routine screening of average and high-risk subjects. Screening for the prevention and early detection of CRC is crucial to improve

one's chances against colon cancer. Screening has led to a decline in the number of deaths from colon cancer over the last 20 years. Early detection is essential to ensure survival if cancer is found.

**FOBT** is a non-invasive mean of diagnosing and relatively simple used in clinical practice. The main drawbacks are the low sensitivity of the method, that allows the diagnosis in an advanced stage, since bleeding tends to occur lately in the progression of the disease and does not allow to distinguish the origin of the hematic loss. Since the bleeding may be intermittent, FOBT may be negative in 50% of patients with CRC, this creates a limitation in this technique. However, a patient with positive FOBT must be submitted to a study with sigmoidoscopy, barium enema and / or colonoscopy.

Once a CRC is suspected, the first line exam for the study of the CRC is the colonoscopy. A prospective study that considered 13580 colonoscopies performed by surgeons in the United States found that the rate of complications associated with the technique is low, occurring in drilling 0:02% of cases undergoing diagnostic colonoscopy, bleeding in 0:19% and perforation at 0.15% of cases with therapeutic colonoscopy [31]. This single test, which usually takes an hour or less to complete, may be both diagnostic and therapeutic. The sensitivity level of colonoscopy is such that it can detect polyps greater than or equal to one centimeter in size. More than 95% of tumors are detected during a colonoscopy. Colonoscopy is the preferred procedure for the diagnosis of symptomatic patients. At the present time, it is the best diagnostic tool for the diagnosis of colorectal neoplasia [32].



**Fig 5 . Colonoscopy – Endoscopic views and biopsy technique**

Data from Johns Hopkins Medicine [32].

**Sigmoidoscopy** is another method used in the endoscopic diagnosis of CCR, allowing visualization of the colon up to 60 cm [27]. About 70 to 85% often allows

identification of the tumor at its most usual location. However, not allowing the study of the proximal part of the large intestine.

**Barium enema** is widely available and may be used to investigate patients with symptoms suggesting of CRC. Is a radiological examination of the rectum and the entire colon and has been used for many years to diagnose polyps and colon cancers. The complication rate with the procedure is very low; the rate of perforation is one in 25,000 examinations [32]. However, the diagnostic yield of both double-contrast barium enema (DCBE) alone and the combination of DCBE plus flexible sigmoidoscopy is less than that of colonoscopy or CT colonography for the evaluation of lower tract symptoms. After detection by barium enema of a polyp or a mass, colonoscopy is recommended to establish the histology, remove the polyp, and search for synchronous lesions.

**CT colonography** (virtual colonoscopy or CT colography) provides a computer-simulated endoluminal perspective of the air-filled distended colon. CT colonography requires a mechanical bowel preparation that is similar to that needed for barium enema, since stool can simulate polyps. CT colonography should be restricted to patients who are able to pass flatus and capable of tolerating the oral preparation. For clinically obstructed patients, a gastrointestinal (GI) CT scan is a good alternative to CT colonography.

Resuming, the CT colonography provides a similarly sensitive, less invasive alternative to colonoscopy in patients presenting with symptoms suggestive of CRC. However, since colonoscopy permits removal/biopsy of the lesion and the detection of any synchronous cancers or polyps, colonoscopy seems to constitute the gold standard for investigation of symptoms suggestive of CRC.

Thus, the principal of the program of screening the CRC, have as an objective the diagnosis of the disease at early stages, taking into account the potential risk of an individual developing the disease, based on various parameters, such as age, family and personal history of CRC or prior colorectal adenoma, as well as a history of inflammatory intestinal disease [33].

The screening can be divided into two groups: in the general population (group low risk) and of individuals with increased risk of family history of CRC staff or adenomatous polyps or colorectal inflammatory intestinal disease. Various entities such as the *American Cancer Society*, the *American College of Gastroenterology* and *American Society of Colon and Rectal Surgeons* recommend, for screening individuals over 50 years with no risk factors, the annual realization of FOBT and / or flexible sigmoidoscopy every 5 years or double contrast barium enema every 5 years or colonoscopy from 10 to 10 years [34]. The rectal examination should also be done in individuals over the age of 40 years [27].

Individuals with a family history of CRC are a group of high risk. For patients with a first-degree relative who was diagnosed with the disease before age 45 or with two family

first degree affected, the risk of developing this cancer rises to 1 in 10 individuals. In these patients should be recommended screening with a low starting age 10 years earlier than that endoscopy the affected family had when he was diagnosed [35].

A variety of **serum markers** have been associated with CRC, particularly carcinoembryonic antigen (CEA). Currently in Europe, the *guidelines* indicate its use only the follow-up of patients undergoing surgery as a way to detect recurrence early, and, nevertheless, a high rate of false negatives [36]. Despite being a disease with high morbidity and mortality, the process of oncogenesis is relatively long (5 to 10 years) and the presence of lesions benign precursors (normal mucosal dysplasia, adenoma, carcinoma) offer a window for therapy before the development of a malignancy, contributing to an increase in cure rates [36 e 37].

#### 2.4 - CRC STAGING

After the establishment of the diagnosis is important to determine the extent of disease either locally or at a distance [38]. Staging can be clinical or pathological. While there are several systems, Tumor-node-metastasis (TNM), defined by the *American Joint Committee on Cancer*, is the most widely used for staging of CRC [34 and 39]. The letter T represents the depth of penetration of the tumor, N defines the achievement of lymph nodes and the presence of M distant metastases[39, 40]. As is represented on the table below, the tumor is first scored with respect to the TNM variables and then assigned to stage I-IV in the CRC specifically [41- 43].

<b>TNM STAGE</b>	<b>DISEASE EXTENSION</b>
<b>T- Primary tumor</b>	
<b>Tx</b>	Primary tumour cannot be assessed
<b>T0</b>	No evidence of primary tumour
<b>Tis</b>	Carcinoma in situ: intraepithelial or invasion of lamina propria
<b>T1</b>	Tumour invades submucosa
<b>T2</b>	Tumour invades muscularis propria
<b>T3</b>	Tumour invades through the muscularis propria into pericolorectal tissues
<b>T4a)</b>	Tumor penetrates to the surface of the visceral peritoneum
<b>T4b)</b>	Tumor directly invades or is adherent to other organs or structures

**Table. 2. TNM classification System**

Data from *American Joint Committee of Cancer* (2009) [15].

<b>TNM STAGE</b>	<b>DISEASE EXTENSION</b>
<b>N-Regional Lymph Nodes</b>	
<b>Nx</b>	Regional lymph nodes cannot be assessed
<b>N0</b>	No regional lymph node metastasis
<b>N1</b>	Metastasis in 1-3 regional lymph nodes
<b>N1a)</b>	Metastasis in one regional lymph node
<b>N1b)</b>	Metastasis in 2–3 regional lymph nodes
<b>N1c)</b>	Tumor deposit(s) in the subserosa, mesentery or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis
<b>N2</b>	Metastasis in 4 or more regional lymph nodes
<b>N2a)</b>	Metastasis in 4–6 regional lymph nodes
<b>N2b)</b>	Metastasis in 7 or more regional lymph nodes

**Table 3. TNM classification System**Data from *American Joint Committee of Cancer* (2009) [15].

<b>TNM STAGE</b>	<b>DISEASE EXTENSION</b>
<b>M–Distant Metastasis</b>	
<b>M0</b>	No distant metastasis
<b>M1</b>	Distant metastasis
<b>M1a)</b>	Metastasis confined to one organ or site (for example, liver, lung, ovary, nonregional node)
<b>M1b)</b>	Metastases in more than one organ/site or the peritoneum

**Table 4. TNM classification System**

Data from *American Joint Committee of Cancer* (2009) [15].

The prognosis of patients with CRC depends on tumor invasion in the intestinal wall, the involvement of local lymph nodes and distance metastases; it is only possible to define precisely the stage of the disease in the post-operative phase, except when there is evidence of distant metastasis [41].

## 2.5- CONVENTIONAL TREATMENT APPROACH

The therapeutic approach to CRC involves, in most cases surgery, chemotherapy and radiotherapy as well as new anti-angiogenic drugs. The different therapeutic options vary according to tumor staging - size, location and metastasis, as well as the physical condition of the patient.

Cancer treatment has gone through a slow process of development. It was in the 19th and early 20th centuries that major advances were made in general surgery and cancer surgery. During the final decades of the 20th century, surgeons developed greater technical expertise in minimizing the amounts of normal tissue removed during cancer operations. This progress depends not only on understanding cancer better as a disease and on better surgical instruments, but also on combining surgery with other kinds of treatments such as chemotherapy and radiation.

### 2.5.1 - SURGERY

Surgery is the main treatment of CRC, which aims, regardless of the location of the tumor, the removal of the primary tumor adequate margins and conducting regional lymphadenectomy, and generally accepted that at least 12 nodes must be isolated for Correct staging tumor [46]. Approximately 92% of patients with colon cancer are subjected to surgery, as a first treatment option, mostly with curative intent [47]. For stage 0 (Tis N0 M0, T1 N0 M0), the treatment options include: a) local excision or simple polypectomy; b) segmentary resection for larger lesions not amenable to local excision. Treatment of stage I (T2 N0 M0) tumours consist in a wide surgical resection and anastomosis. For stage II (T3 N0 M0, T4 N0 M0) the standard treatment options include: a) wide surgical resection and anastomosis; b) after surgery, in high-risk patients adjuvant therapy could be considered. And for stage III (any T, N1 M0, any T, N2 M0) the treatment options are a) extensive surgical resection and anastomosis; b) after surgery the standard treatment is a doublet schedule with oxaliplatin and 5FU/folinic acid (LV) (FOLFOX4 or FLOX) [45]. Advances in surgical technique have increased the percentage of patients with potentially curable disease. These developments included the development of techniques such as block resection, total excision of the mesorecto for rectal carcinoma, laparoscopy and the technique of "no-touch" which aims at limiting the spread throughout the tumor vascular operative time. Laparoscopy is currently seen as a good alternative .The classical treatment, with the advantage that the need for shorter of hospital stay, less pos-operative pain and earlier recovery intestinal function. There is however, some controversy regarding possibility of intraperitoneal dissemination and hematogenous cells neoplastic with this surgical technique, with some studies pointing one increased risk of spreading when the laparoscopy is compared with the open surgery and other studies disagree with such fact [46].

Regarding colorectal cancer, the surgical treatment depends on the location of neoplasia. The proximal and middle rectal tumors are usually underwent low anterior resection with anastomosis primary, an ileostomy or colostomy may be necessary in the past temporary to facilitate healing, to divert the flow of fecal anastomosis. For tumors of the distal rectum amputation is indicated abdominoperitoneal with permanent colostomy and in cases where it is not possible to keep sphincter [39].

Advances in surgical techniques, has made it possible for patients with metastatic RCC, surgery may have a curative character. Patients with resectable liver metastases should undergo partial hepatectomy, thus achieving a survival at 5 years 25-30% [40].

### **2.5.2 - RADIOTHERAPY**

Radiotherapy is a treatment commonly used against cancer. The benefits of radiation therapy for patients with colorectal cancer include: a) killing and eliminating cancer cells and tumors; b) shrinking tumors; c) preventing cancer cells from growing and dividing. The DNA of a malignant cell is more susceptible to radiation damage than a normal cell. Radiation used before surgery to shrink a tumor can provide the best chance of successful tumor removal during surgery. Radiation administered after surgery can eliminate remaining cancer cells. Radiation therapy began with radium and with relatively low-voltage diagnostic machines. Methods and machines for delivery of radiation therapy have steadily improved. Nowadays, radiation is delivered with great precision to destroy malignant tumours and controlling damage to nearby normal tissues [15]. Radiation therapy is typically combined with chemotherapy, such as the drug, 5-FU. Adding chemotherapy to the radiation therapy improves the effectiveness of the treatment by making the tumor cells more sensitive to the radiation. This sensitivity allows the radiation to do more damage to the tumor cells. Administering the radiation and CT prior to surgery has been shown to improve the effectiveness of the surgical treatment and, also, to decrease the risk of side effects [32].

### **2.5.3 - CHEMOTHERAPY**

CT is one of the most used treatments against cancer and it's through drugs administration. Indeed, over the years, the development and use of chemotherapy have resulted in the successful treatment of many people with cancer. The ability of CT to kill cancer cells depends on its ability to halt mitosis. Usually, chemotherapy drugs work by damaging the RNA or DNA that instructs cells on how to copy themselves in division. If cells are unable to divide, they die (apoptosis). The faster that cells divide, the more likely CT is to kill the cells, causing the tumor to shrink.

CT is called a systemic treatment because it affects the entire body.

CT drugs that affect cells only when they are dividing are called cell-cycle specific. Chemotherapy drugs that affect cells at rest are called cell-cycle nonspecific. The therapeutic modalities available for the treatment of CRC include CT and can be administered in two ways: systemic chemotherapy (intravenous or oral administration with achievement in all areas of the body) and regional chemotherapy (administration artery to a particular body area). CT may also be used at different times after surgery - adjuvant chemotherapy - with a preventive aim of recurrence and tumor prior to surgery with the aim to reduce the tumor - neoadjuvant chemotherapy [39, 45- 47].

In advanced cancers, CT can also be used to help reduce tumours and relieve symptoms for cancers that have spread to other organs, such as the liver.

Agents and / or chemotherapy regimens more used in the treatment of CRC are: Xelox (capecitabine plus oxaliplatin), Folfox (5- fluorouracil (5-FU), leucovorin (LV), oxaliplatin) and Folfiri (5-FU, LV, irinotecan) [39 and 45- 47].

Type of Agent	Mechanism	Examples
<b>Alkylating</b>	Modification of nucleic acid functional groups	Cyclophosphamide, dacarbazine
<b>Antimetabolites</b>	Nucleoside analogs, alters RNA and DNA synthesis	5-FU, gemcitabine
<b>Taxanes</b>	Disruption of microtubule formation, blocks cell division	Paclitaxel, Docetaxel
<b>Anthracyclines</b>	Interfere with DNA replication process, inhibit RNA and DNA synthesis	Doxorubicin
<b>Platinum based</b>	Cross link DNA	Cisplatin, carboplatin, oxaliplatin

**Table 5. Common types of chemotherapy agents and their mechanisms.**

The main agent is 5- fluorouracil (5-FU), an inhibitor of Thymidylate synthase [38-39]. The 5-FU is commonly administered with Leucovorin (LV), which increases the affinity of agent for the therapeutic target [39-40]. There is evidence that when compared to separate administration 5-FU, the combination 5-FU and LV increases the therapeutic response. Among patients with metastatic CRC in about 20% register to a 50% reduction in tumor size, with increased survival of 6 to 12 months [39]. The 5-FU is usually administered intravenously with the infusion better tolerated than bolus with a lower incidence of side effects such as diarrhea and neutropenia [48].

In order to improve the outcome of selected patients with metastatic CRC, monoclonal antibodies against VEGF and against epidermal growth factor receptor (EGFR) should be considered in combination with chemotherapy. Bevacizumab, an anti-VEGF

antibody, increases the activity of an active cytotoxic regimen, therefore, increases the survival, progression-free survival and response rate in first-line treatments.

Nowadays, several approaches are being studied to improve the activity and to reduce the side effects of chemotherapy.

The side effects of chemotherapy depend on the type and dose of drugs given and the length of time they are taken. Common side effects drugs can include:

- Alopecia – although reversible and not endanger to patients' lives, is seen as one of the most stressful secondary effects interfering with patient's body image;
- Gastrointestinal system symptoms – anorexia, nausea, vomiting, mucositis, stomatitis, diarrhoea, taste changes, smell of food aversion;
- Neuropathies;
- Skin colour changes;
- Decreased libido;
- Fatigue, tiredness.

Data from: *National Cancer Institute*, 2011 [74].

Radiation side effects are usually mild and reversible, and include local changes in skin, fatigue, pain related to temporarily inflammation of the nerves or muscles in radiation domain [74]. Chemotherapy because induce local and systemic immunosuppression affects adversely the training, recruitment and action of effector cells of the immune system and that can cause more side effects and precipitating a treatment failure [49].

## 2.6 - PROGNOSIS

The stage of disease at diagnosis is a determinant factor in survival of these patients. As most recurrences occur in first four years post surgery, the survival rate at five years is a good indicator of healing [40]. The analysis of survival rates at 5 years of CRC shows that when the diagnosis is made early, survival is larger, decreasing while the stage progresses. For stage I, the survival exceeds 90%, falling to 70-85% in stage II to 25-80% in stage III and less than 10% in IV [40]. On average, survival rate after the detection of metastatic disease has risen from 6 to 9 months to around 24 to 30 months [40].

Although the clinical and pathological staging be fundamental, the preoperative dosing of carcinoembryonic antigen (CEA) is also very important, since it may indicate the risk of recurrence of cancer. Unlike other tumors, primary tumor size, nodal adjusted to

achievement and the degree of differentiation, not directly associated with the prognosis of the patient [40].

## 2.7- ROLE OF NK CELLS IN CRC

NK cells are believed to play an essential role in the immune surveillance against tumors and infectious diseases.

In accordance with some investigation studies that analysed the number and activity of NK cells in patients with colon cancer, lead to the suggestion that reduced NK cell activity is associated with metastatic tumour growth in patients with colon carcinoma; and, if the decreased NK cell activity precedes the development of metastasis, it could constitute a possible a marker to identify a high risk of rapid tumour progression following curative colorectal surgery [50].

According to Halama *et al* [51], the early impairment of NK cells is decisive for CRC; the same is not true for other immune cells, especially for T cells which don't have a so uniform presence during the different stages of CRC. Furthermore, they indicate a pivotal role for an escape from NK cells for CRC tumourigenesis and propose that quantification of NK cells within CRC tissue can be used as an important parameter for the detection of response therapy.

## 2.8 - FOLLOW-UP

The main objectives of the follow-up of patients with a history of CRC are the diagnosis of metachronous polyps or new primary CRC and the detection of recurrence (liver, lung) at an early stage, in which may also be candidates for a curative treatment, like what happens with liver metastases, which may undergo surgical resection [38].

Therefore, the follow-up of these patients may improve their survival rate and includes periodic medical evaluations and conduct many additional complementary exams of diagnosis [34].

Current guidelines from the *American Society of Oncology Clinical* , in relation to survival rate after curative treatment of CRC, reflect a change of attitude towards the role of TC performed routinely [15].

A colonoscopy reassessment is indicated 3 years after surgical treatment, and if negative, every 5 years [52]. Flexible sigmoidoscopy is recommended every six months for 5 years for patients with rectal cancer who haven't submitted to radiotherapy [52].

Although it continues to be one of the most common cancer in the last decades, deaths from CRC have been declining, in part very due to an effective screening and follow-up program as well as a more effective diagnosis.

## 2.9 - QUALITY OF LIFE

In recent years, an increasing awareness of interpersonal and emotional repercussions of cancer and its treatments has happened, as well as its impact on the welfare of patients. Thus, the attention has been directed also to the psychosocial needs of patients, their families and health professionals and to the impact of emotional and behavioural factors at the beginning and during the course of the disease.

The diagnosis of cancer is a major source of anxiety, depression and emotional stress which affects the quality of life of patients. Approximately 20-40% of cancer patients exhibit significant levels of depression and anxiety [77].

WHO defines QOL as an individual's perception compared to their position in life, the values and cultural context and in which the individual lives and their relationship with their objectives, expectations and concerns. This is a comprehensive concept of a complex affected by physical health, psychological state of the individual mode as well as its level of independence, and how their social environment relations, as well as by personal beliefs. The concept of quality of life and so the way to deal with cancer is also unique, personal and specific to each patient.

According Psychoneuroimmunology, psychological and emotional stress induce severe alterations in several biological responses. Activation of the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system appears to alter the cells of the immune system - reducing the number of NK cells and promote inflammation via multiple neuroendocrine and immune pathways.

According to an adaptation study of the satisfaction scale with social support to people with cancer illness diagnosis found that, the satisfaction with the social support relates to the all adaptation process to oncologic disease and results, specifically concerning to quality of life [53]. Based on the WHO definition of health as “a state of complete physical, mental and social welfare and not merely the absence of disease”, it becomes essential to see the patient as a whole, recognizing its needs in several aspects.

The ongoing research in such an important area as cancer is undoubtedly essential. More and more is known about its causes, risk factors and development. New ways to prevent, detect and treat are also being studied always focusing on improve the quality of life of patients with cancer.

## **2.10 - NOVEL APPROACH IN CANCER**

A variety of modalities can be used to improve the treatment of cancer patients. A combination of those modalities of pharmacological and non pharmacological treatment of cancer is a mainstay of supportive care to those patients as presented on the current WHO guidelines.

The study and interest in learning/understanding treatment modalities called complementary or integrative medicine has grown significantly in recent years. Highlights, the importance of therapeutic relationships between doctor and patient, focus on the whole person, including all aspects of life. The central focus is the patient's perspective, generating new strategies and multiplying treatment possibilities. According to Cassileth (2004), cancer patients usually use complementary therapies, being that about 91% of these patients used some form of complementary therapy during conventional medical treatment, in the United States [76]. Although some are expecting these therapies to have an effect on their survival time, it seems that most use these methods to improve quality of life.

One of the so called complementary therapies is the Traditional Chinese Medicine that it will discuss bellow.

### 3. TRADITIONAL CHINESE MEDICINE – OVERVIEW

Traditional Chinese Medicine (TCM) is a broad range of medicine practices sharing common concepts which have been developed in China and are based on a tradition of more 5000 years, including various forms of herbal medicine, acupuncture, massage (Tui na) exercise (*Qigong*) and dietary therapy.

The oldest findings known, where references to the most primary forms of care are shown, are the *I Ging* (The book of changes) and the *Huangdi Neijing* (Principles of Internal Medicine of the Yellow Emperor) [75]. It is thought that the book *Huangdi Neijing* was written somewhere in between the 2th century BC and the 2th century AC and establish, for the first time on the Chinese Medicine practice, some of therapeutic principles of treatment.

Many of Chinese scientific principles, since Classical China teachings and before the Yellow Emperor's *Classic of Internal Medicine*, emphasized the regulatory fluctuations through circulatory functions in a simplistic manner that is similar to a sinus wave and is part of the so-called monad (Leibniz) or Taiji sign.

Over the centuries, many compilations and reassessments of the whole field of knowledge of Chinese Medicine appeared. However, it was on the 16th and 17th centuries that the preservation and dissemination of this whole field of knowledge reached its pinnacle, with publications such as *Zhengjiu Dacheng* (Great Compendium of Acupuncture and Moxibustion). In it was the theory behind all Chinese Medicine, such as stimulation of points and channels, which have been presented so clearly that remain current until present day.

Accordingly to Hemen, it was Soulie de Morant, at France, after 30 years of study in China that contributed, in a fundamental way, to provide the theoretical basis for the diagnosis and the introduction of Chinese Medicine in the western world [54].

Western medicine, as well as any other type of corpus medicus, presents limitations both in diagnosis as in interventions. Indeed, for a long time, Western medicine has been systematically built and developed based on scientific methodology and, as a result, on the possibility of the phenomena being measured.

Chinese Medicine emerges as another medical approach, considering the human being part of a whole, being interested primarily in the expression of life, emotions and vital body functions in order to identify possible disharmony. The basic aim is not to measure or assess a specific organic change but to evaluate the condition of a patient, reporting objective and subjective their symptoms or discomforts.

Currently, there is a form of modern understanding of TCM considered crucial for their integration into the Western health system and in research. The Heidelberg model fits in this context.

### **3.1 - HEIDELBERG MODEL OF CHINESE MEDICINE**

TCM is understood as “a system of sensations and clinical signs and findings designed to define the regulatory state of the body [55].

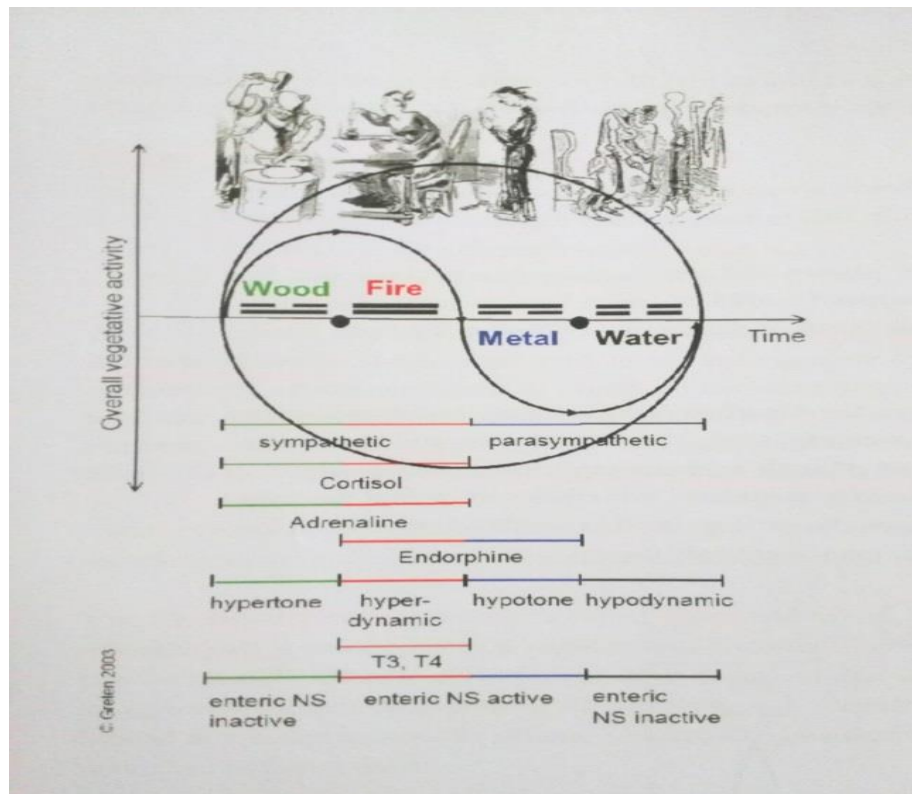
The Heidelberg Model of TCM developed by Prof. Johannes Greten was supported by the pioneering works of the medical-sinologist Prof. Manfred Porket (1974), thus adopting a Latin terminology, and also sustained by the Leibniz’ analysis of the I Ging (“The Book of Changes”).

The Heidelberg model is a scientific model that allows a rational access to Chinese medicine and it is based on symptoms that constitute the evidence of disease and pathological changes.

It is supported by a system that describes functional abnormalities through its signs and symptoms. Thus, it is understood that those signs and symptoms, as well as the feeling that the patient describes, are the result of dysfunctions in the body, particularly in the neurovegetative system.

This model of Heidelberg provides an overview of some TCM concepts by translating them as states of neurovegetative functions, being crucial to the integration of TCM in the Middle West, particularly to what regards the health system.

On the following picture it is possible to make a western physiological description of such neurovegetative functions.



**Fig 6. Western physiological description of a vegetative sinus wave**

Data from Greten 2010 [56].

The sinus wave symbolizes a sinus curve of regulation, a circular movement, as the classic circle of Yin/Yang, were are described the regulatory model of the phases (Wood, Fire, Metal and Water).

The upper part of the figure contains the Chinese way of description; the functional figures symbolize the clinical appearance of the mechanisms in terms of visible signs, the lower part contains the western description, as transmitters and neural concert of the vegetative system [56].

For instance, according to Greten (2010), stress is believed to be accompanied by a strong uprising sympathetic action, analogous to Wood [55]. At the same time, according to western medicine, vagal impulses act to counter-balance this sympathetic action and effect. In Chinese terms this is as imbalance of a vegetative functional tendency. The functional tendency, called Wood, is more or less analogous to stress and sympathetic action, adrenaline increase, muscle tension, and others. In other words, the description by groups of clinical signs in the upper part of the figure, describes the same function the western medicine describes as a transmitter and neuronal concert of vegetative system.

As a scientific medical diagnosis, besides the interpretation of symptoms, amounts to a second level of abstraction, which identifies causes and agents that can induced those changes or dysfunctions [55].

This referred state of dysfunctions can be treated in various forms. The therapy includes therefore reflex action at central and vegetative nervous system such as acupuncture and manual therapy (Tuina), as well as exercises that activate the vegetative system of biofeedback, the *Qigong*. Herbal and dietetic are also included as an effective therapy through the administration of plants, minerals and other products, but also through the food itself.

Chinese doctors look for the all active expressions of life which are seen as expression of a balance of energies that flow in a cyclic sequence along clearly defined conduits. Therefore, Chinese medicine models is the concepts of functional systems, which are establish through a systematic arrangement of its observations, representing the symptoms of disorder, different manifestations of vital forces and various phenomena of life.

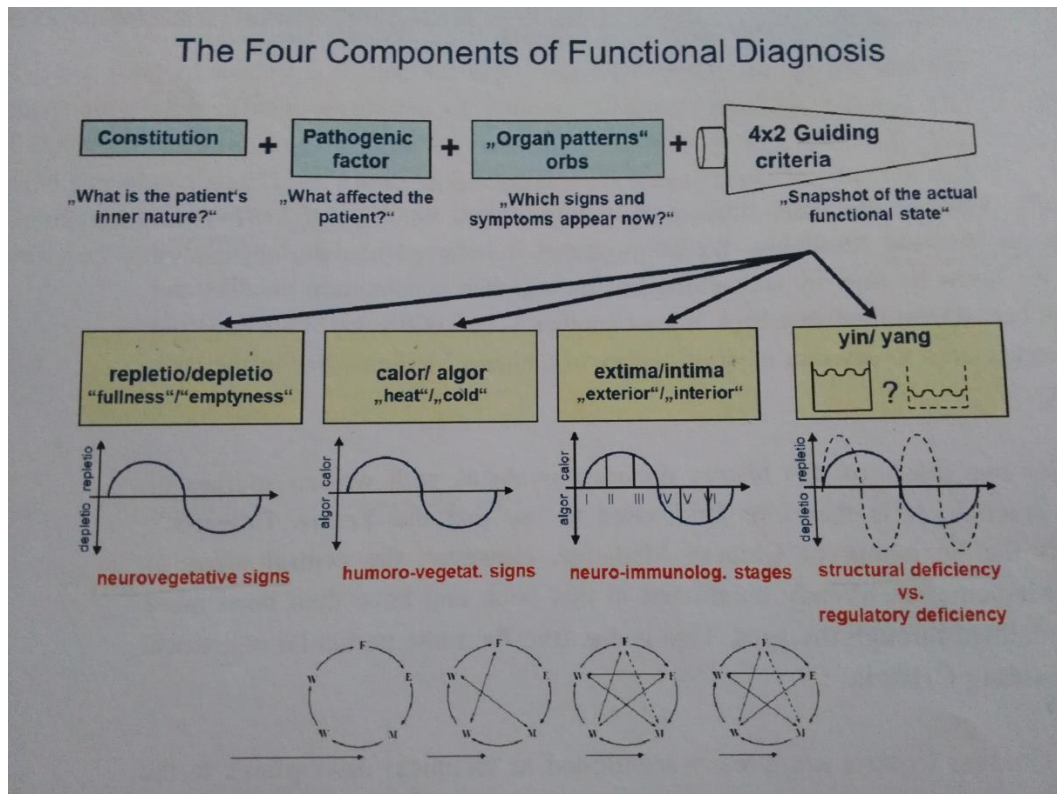
### 3.2 - DIAGNOSIS ACCORDING TO THE HEIDELBERG MODEL OF TCM

The Diagnosis is one of the most important elements in TCM. Based on Heidelberg Model functional diagnosis is composed of four main steps:

- The constitution: individual functional properties and the inner nature of the patient, while agent is regarded as a functional power that may change the individual functional properties; it produces clinical signs or induce a group of diagnostically relevant signs called orb ;
- The agent (pathogenic factor): is considered as a functional power or a “vector” that have the capacity to change the individual functional properties, producing clinical signs;
- The “orb”, are group of diagnostically relevant signs that indicate the functional state of a body island, which correlates with the functional properties of a conduit<sup>1</sup>;
- The guiding criteria, , can be understood as the teaching of body regulation. As previously described, is regarded as directional standard conventions of physiology, permit the interpretation of the actual symptoms that are a manifestation of the overall body regulation as described following.

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<sup>1</sup> Conduits refer to a connection of a group of points with effect on the clinical signs of na orb, believed to serve as a conduit for the flow of *qi* and *xue*.



**Fig 7. Four components of the functional diagnosis in TCM**

Data from Greten (2010) [55]

According to Greten, the guiding-criteria is an evaluation of clinical signs according to an underlying regulatory model of physiology and permit the interpretation of the actual symptoms that are a manifestation of the overall body regulation.

### 3.3 - THE ALGOR LAEDENS THEORY – ALT

Referring the ALT in this study becomes crucial because the topics related to cancer and chemotherapy are considered according to the Heidelberg model factors that lead to the existence of *Algor* in the body. *Algor* means the symptoms that you would experience as if you were exposed to cold. In case of CT is toxic *algor* that is a local toxic damage following extremely cold substances. That cause a process that we called ALT. The ALT is the most common pathophysiological model that describes the process of "cold-invading" diseases in TCM, which have a parallel on clinical signs induced by neuroimmunological mechanisms on a western perspective. The ALT assumes that in case of "*algor*" affecting the body system, a regional lack of microcirculation may be caused by defence reflexes to cold, by viruses (adhesion molecules, complement system, coagulation) in face of which

and a counter-reaction (“*reactive calor*”) take place, consisting on a general increase in microcirculation, inflammation, fever and sepsis.

The model course of the ALT is suggested under the basic idea that the agent invades from the *extima* to the *intima*, thereby overcoming the defence levels and revolving the flow of *Qi*<sup>2</sup> and *xue*<sup>3</sup>, and constitute a specific approach of interpretation of the guiding-criteria *extimal/intima*, as stages I to III are *extimal (yang)* and stages IV to VI are *intimal (yin)*.

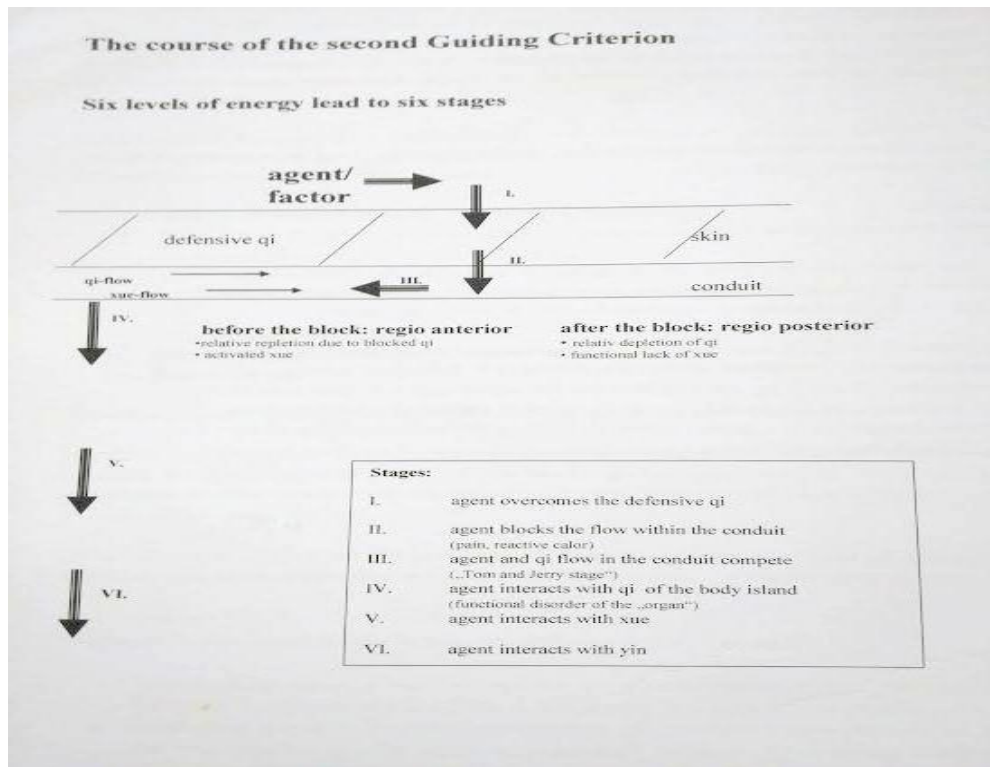
The ALT combines the language of the orbs (neurovegetatively originated signs) with the language of the system of *calor/algor*, a part of neuroimmunology, and reflects the functional activity of the defence mechanisms expressed by *calor (yang)* and *algor (yin)*.

The six stages of the ALT are syndromes composed of specific signs of two orbs, each. According to the model, the six stages form a complete circle of stages of an infectious disease. During the course of such cyclic process, an individual will overcome the infection and remain healthy thereafter. However, under certain circumstances, some signs of the six stages may remain and become chronic, such as in cases of chronic inflammation, fibromyalgia or rheumatic disorders.

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<sup>2</sup> *Qi* refers to vegetative capacity to function of a tissue or organ which may cause the sensation of pressure, tearing or flow.

<sup>3</sup> *Xue* refers to a form of functional capacity bound to body fluids with functions such as warming, moisturizing, creating qi and nutrifying a tissue.



**Fig 8. The six layers of energy.**

Data from Greten( 2010) [55].

Stage I – Agent overcomes the defensive Qi- Yang major

When the agent *algor* invades the skin, it affects the defensive *Qi*. This may induce a localized de-activation of *xue*, in western terms regional lack of microcirculation. *Yang* stands for *extima*, for activity in general and within the meaning of the phases it stands for *reactive calor* ( stage I, II, III).

Stage II – Agent blocks the flow within the conduit- Splendor Yang

Splendor Yang, the overshining yang, express that reactive calor may even be excessive and symptomatically leading. By this it is expressed that in western medicine sence the reaction to the agent *algor* or a virus produce more symptoms than the virus itself. If expelling of *algor* fails, it may proceed invading and lead to a regional block of flow of *Qi* and *xue* that causing pain.

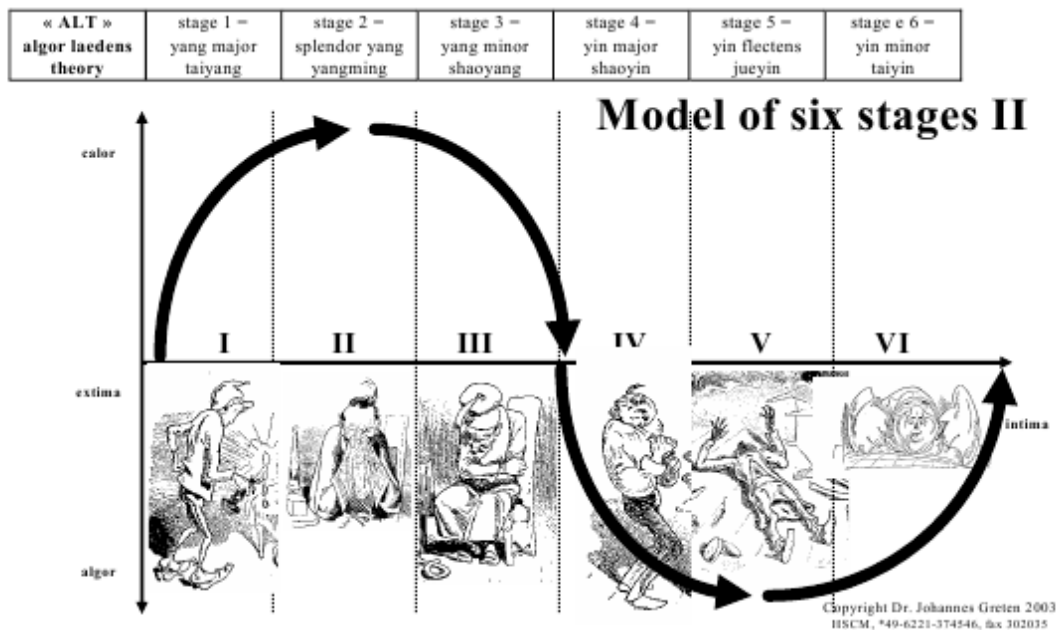
Stage III- Agent and Qi flow in the conduit compete- Yang minor

This express that the reactive calor (*yang*)is regressive so the agent is bigger and the yang is minor. *Xue* and *Qi* derive from the interior and are led through the system of conduits.If agent *algor* overcomes the *xue* flow this may lead to a reverse flow of *xue* into interior.

Stage IV- Agent interacts with Qi of the body island- Yin major

The agent *algor* invade the interior – zone of lack of microcirculation. *Algor* is in the body. The main symptoms do not come from the conduits but more frequently from the interior. The most pronounced lack of function seen on the Stage V, where the substance is used up, is called in Chinese *Jue yin* and in Latin *Yin flectens* meaning that the *yin* “goes away”, vanishes, but also bends. It represents a turning point of the disease when it becomes life-threatening, dangerous and critical.

The other two stages are, again, named in a comparative manner in relation to the acute Stage V. Thus, the Stage IV, where there is more substance blocked, more *yin* is called the bigger *yin*, *Taiyin, yin major*, and the stage where there is less substance left, the *yin* is smaller is named the smaller *yin*, *Shaoyin, Yin minor* – Stage VI. Another major aspect of these two Stages (IV and VI) is that the bigger *yin* has a downward direction, meaning that the hypofunctional state of internal *algor* is becoming more pronounced, “more *yin*” or *yin major*; in Stage VI the hypofunctional *yin* state have a upward direction comparable to the phase of water (regeneration) in the model of phases, having the capacity to regenerate to normal, is called lesser *yin* or *yin minor*



**Fig. 9. Schematic model of Algor Laedens Theory.**

Data from Greten (2010) [55].

Therefore, these six functional layers are also called the six layers of defence the body. The extimal stages of defence encompass the defensive *Qi* as first layer, followed by the *Qi* of the conduits and, finally, the xue of the conduits; and the intimal stages includes the *Qi* of the body island, followed by the xue of the body island and finally the *yin* [55].

The energy layers comprise six technically different forms of energy:

I. **Defensive Qi**, also referred as *wei Qi*, resides within the extima, outside the conduits and builds up the first barrier against external attacks.

II. **Conduit Qi** is the *Qi* within the conduits. When this second layer of defence is affected by an agent causes a block in the flow of *Qi* resulting primarily in pain and secondarily in functional disorders of the respective orb.

III. **Conduit xue**, which is driven by the conduit *Qi* and warms the conduits, “nourishes” and “moisturizes” the tissues. The warming effect on the tissue is needed e.g. to drive out the agent *algor*.

IV. **Body island Qi** is the *Qi* within the intima, a general name for the whole body’s interior, where the functions of the orbs are generated in their respective parts of the body.

V. **Body island xue**, is a substancial (yin) part of the body islands with warming, thus functionally activating and enhancing properties.

VI. **Yin**, which is the functional tissue the respective subpopulation

Therefore, it’s possible to distinguish six strategic scenarios in which the agent attempt to overcome each functional layer producing symptoms related to the invading agent (feeling of coldness) and symptoms that arise from the interaction of the agent with the layer of defence, which are specific key symptoms, thus enhancing diagnosis of the six layer model.

### 3.4 - CANCER ACCORDING TO TCM

From a Traditional Chinese Medical perspective, all cancers are considered different manifestations of constrained Liver *Qi*. Because the *Qi* commands the Blood and keeps it moving in its proper pathways, any disorder in the *Qi* will lead to a disorder in the Blood. As the circulation slows down, the Blood begins to congeal and becomes stagnant. This comprises the first stage in the potential development of a cancerous tumor [56].

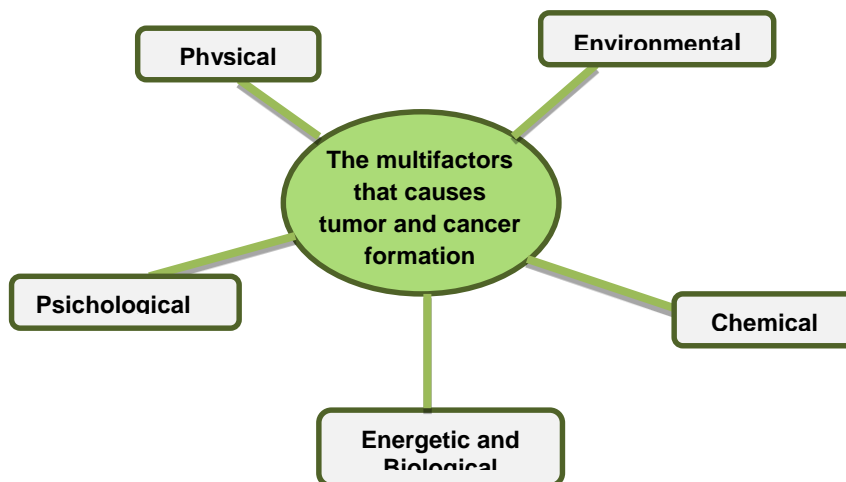
Traditional Chinese Medicine stresses that cancer formation is caused primarily from a *deficient condition* (whole body weakness), which can then develop into a localized *excess condition* (the specific cancer tissue formation). The acquired *excess condition* can also enhance the development of a *systemic deficiency* (the deterioration of the entire body), as well as destroy the entire body's immune system.

According to TCM, deficient Spleen, Lungs and Kidney *Qi* is also considered a contributing factor in the development of cancer. These specific internal organs are directly involved in stimulating and nourishing the body's True *Qi*, Righteous *Qi*, Ying *Qi*, and Wei

*Qi*. Deficient *Qi* in the Spleen, Lungs, and/or Kidneys depletes the body's natural defenses, making it easier for diseases of all types, including cancer, to take root and thrive [56].

According to Jerry Johnson (2002), the etiologies of tumors and cancers are considered multifaceted in their disease formation. They are generally viewed as being created and formed through a constant attack and weakening of the tissues, originating from imbalances in one or more of the five following factors: Environmental Factors, Chemical Factors, Physical Factors, Psychological Factors, and Energetic and Biological Factors as described the figure below [56].

Environmental Pathogenic Factors also include exposure to radiation, ultraviolet rays, industrial toxins and the six pathogenic factors (Wind, Cold, Summer Heat, Dampness, Dry-Heat, and Fire). Chemical poisoning linked to cancer formation can take place through exposure to pesticides, herbicides, growth hormones in meat and dairy products, genetically altered foods and others. A congenital tendency towards specific types of tumors and cancers (inherited weakness), involves the inability to repair weakened DNA. This leads to uncontrolled cellular replication, and hormonal imbalances. Emotional stress can originate from an inability to handle intense emotional states, causing the individual to suppress his emotions and resulting in chronic organ disharmony. Once the harmony of the body's *Qi*, Blood, Body Fluid, internal viscera, and channel circulation is disrupted, the immune system becomes overworked, which lowers the body's resistance to disease.



**Fig. 10. The causes of tumor and cancer formation according to TCM.**

Extrated from Jerry Johnson (2002) [56].

### 3.5 - CANCER ACCORDING TO THE HEIDELBERG MODEL OF TCM

Cancer, accordingly to the Heidelberg model, is understood in a comprehensive manner, as the result of a disharmony between *Qi defensivum* and *Qi constructivum*.

In conventional Western medicine, cancer is considered a process in which malignant cells outgrow environmental constraints and control mechanisms. In TCM overview, cancer results from a disturbance of smooth flow of 'Qi' (vital energy) and/or yin-yang balance and manifests as a group of syndromes in which there is a disharmony in the body–mind–environment network. In a very general way, what happens is that the referred disharmony causes, over time, a *Qi* and blood (*xue*) stagnation, which can lead to blockage states and accumulations.

According to Heidelberg model, the causal therapy for cancer is to eliminate *stasis of xue*. The cause of stasis must be diagnosed individually. Numerous disturbance and blocks may contribute to this, such as old trauma, chronic *algor* and other blocks of the conduits. Patients who don't have much *Qi* and consequently not much flow of *xue* are prone to stases.

Specifically, by local findings, this stagnation may result in three principal stages:

- Stage I – consists of local accumulation of *humour* and *pituita* (similar to edema in western medicine) that causes or makes the conduits obstruction becomes worse resulting in more *xue stasis*.
- Stage II – Named *congelatio*, situation in which an excess of a palpable substance still moves in the tissues, it can resemble a situation of existence of cysts or chronic exudates.
- Stage III – Named *concretio*, which is a local accumulation that is not mobile; These benign tumours may indicate that the tendency of block of *Qi* flow, block of *xue* flow and excessive constructive *Qi* is already present although the patient has no cancer. This is treated as benign or malignant, situations such as lipomas, fibromas or other neoplastic conditions.

The concept here involved is that the defensive *Qi* tend to disperse the accumulation of constructive *Qi* and, when this process fails, pathological situations arise [55]. So we can conclude that cancer may result from an imbalance on the Metal-Wood axis. These represents an imbalance of *Qi defensivum* and *Qi constructivum*, where *Qi defensivum* can be understood as a vegetative defence mechanism originated in the respective tissue itself and promoted by the continuous action of inspiration and expiration, as well as the vegetative rhythm induced by that; and *Qi constructivum* is derived from *xue*, which

corresponds to the western term of microcirculation (MC). This is analogous to the concept that growth factors, hormones and other factors, which will normally contribute to tissue repair, would reside in the respective tissue as a result of impaired flow of MC, possibly induced, as among other factors, by a lack of capillary outflow. From a western perspective, this is to be regarded as a vegetative theory of carcinogenesis. This implies, also, the inclusion of emotional changes and psychosocial causes of cancer by Chinese medicine.

On another point of view, according to Heidelberg model, the Chinese psychosomatic theory of the origin of cancer lead to conclude that cancer is a disease of disturbed symbiosis and partnership. On other words, is an imbalance between *maeror* and *ira* or symbiosis versus autonomy. Similarly, according to the western theory, cancer can be induced from life significant events that lead to emotional significant disturbances. This is consistent with western science which tries to catch this in the term of life-event research. In western research, the intact social and family structures prolong the life of cancer patients and positive attitudes to fighting against cancer also prolongs survival [56].

### 3.6 - CHEMOTERAPHY ACCORDING TO THE HEIDELBERG MODEL OF TCM

Chemotherapeutic agents, as previously mentionated, are considered as a form of *algor*, toxic *algor*, which leads to the occurrence of symptoms of coldness, tiredness and stiff limbs, and, therefore can be interpreted by the ALT.

Chemotherapy induces cold directly and may affect all the six levels of *Qi* in the ALT and the symptoms presented by the patient differs according to the stage of invasion.

On Stage I, the *wei Qi* is deficient, the most common *algor*-related signs are shivering and painful limbs. On Stage II, patient reveals severe and tearing pain in different regions of the body, loss of appetite, nausea and extreme sensitivity to smell. As on this stage, the *algor* affects the *Stomach orb* and patient develops a *splendour yang* syndrome with accumulation of *pituita*<sup>4</sup>, which affects the face becoming swollen. Also affects the crass intestinal, appearing gradual diarrhoea and pain in the abdomen. On Stage III, appears on the beginning of the chemotherapy, where the *xue* of the conduits is affected, and giving rise to the alternating sensations of hot/cold. In the Stage IV *toxic algor* can affect the *Qi* of *P<sup>5</sup>* and *L<sup>6</sup>* *orbs* and leads to more vulnerability to airways infections and feelings

<sup>4</sup> *Pituita*, according to Heidelberg model, refers to accumulation of humor and reactive calor during time. In western terms refers to chronic inflammation signs that may originate from pre-oedema and oedema.

<sup>5</sup> Pulmunar

<sup>6</sup> Lienal

of dullness respectively. In Stage V, the *xue* of  $H^7$  and  $PC^8$  body islands is affected, thus the muscles become inactive (weakness of arms and legs) and also may give rise to palpitations, loss of drive and weakness due to the presence of toxic *algor* in the pericardium or heart muscles. The extreme *yin* deficiency (cachexia) is due to tumour itself, but also enhanced by chemotherapy; affection of structure and function; affections of the urinary tract and affection of the brain, more precisely short-memory loss - Stage VI.

### 3.7 – QIGONG

*Qigong* is not intended to replace orthodox medicine, but rather to complement it. *Qigong* is one of therapy of TCM; the word is composed of two Chinese characters, *Qi* and *Gong*. *Qi* is often translated as energy and *Gong* as work, so to describe that it means the work on energy. It is an ancient Chinese art and science working true posture, movement, breathing and meditation.

#### 3.7.1 - OVERVIEW OF QIGONG

More than 5000 years ago, Chinese physicians came to understand that everything is composed of the same energetic substance called *Qi*. These ancient masters concluded that energetically everything is interconnected as one body, although energy may appear to take on many different forms. In modern times, the laws of physics have demonstrated that matter and energy are interchangeable, and that matter is simply another form of energy [56].

Once we understand the meaning of *Qi*, we could understand the medical capacity of *Qigong*.

As a biofeedback therapy, *Qigong* activate psychophysiological self-regulation to restore or maintain a healthy balance overall [55]. Those exercises can be done sitting, standing, moving or lying down.

According to Pedreros (1992), *Qigong* is a set of internal exercises to cultivate the *Three Treasures*, which are the hallmarks of mental and physical health of human beings. In China, health and longevity are determined by the strength, balance and cultivation of

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<sup>7</sup> Hepatic  
<sup>8</sup> Pericardiac

those three treasures called: Jing, *Qi* and Shen, representing the human being holistically [57].

According to the same author, regular practice of *Qigong* activates the internal *Qi* flow along the conduits, strengthens internal organs, maintains the vigour of body and mind, exercises the joints, muscles and tendons, prevent diseases caused by physical stress and promotes postural awareness.

There are hundreds of different forms of *Qigong* in China and, most of them are not intended to treat any specific disease but are used almost as prophylactic meditative exercises. Despite this, all share the same core principles:

- **regulation of the body** - the body is held in a stationary position or executes a series of specific movements;
- **Calm the mind** - the mind is cleared of all elements of distraction and focused on a single thought or meditative visualization of "*Qi*" to circulate through the body;
- **The "*Qi*" can be understood as life energy** and according to some authors, is considered a dynamic and ethereal with potential energy and feedback information.
- ***Qigong* is a practice of training** that leads to the development and control of "*Qi*" and its distribution across different parts of the body. It is considered a traditional therapy that integrates bio vegetative feedback postural, breathing, movement and meditation exercises with stabilizing properties vegetative and aimed at self-regulation of body biological systems (...). "

*Qigong* is, according to the model Heidelberg, a traditional therapy of vegetative biofeedback that integrates postures, breathing, movement and meditation exercises with stabilizing properties vegetative and aimed at self-regulation of the body systems [55]. This is because stimulation of autonomic reflex points, which are connected to each other through interconnection by collagen (conduits) fibers, stretch and relax is a systematic manner [56].

*Qigong* is considered a method of self practice or process by which one cultivates the *Qi* and *Yi* (consciousness or intention) with the aim of achieving an optimal state of health of body and mind [57].

For this reason, *Qigong* has the functions of activating the body's vital force (*Qi*), strengthening the blood's circulation, balancing yin and yang, stimulating the conduits and improvement psychological state [59]. In TCM the psychological capacity is a function of *Shen*. *Shen* is a functional capacity to put order into mental associativity and emotion, thus

creating “mental presence” [55]. Porkert defines *shen* as a constellating force which originates from cardiac orb [60].

In the early 1950s scientists in China began investigating the many medical benefits claimed for *Qigong*. Clinical and experimental evidence shows that *Qigong* exercise can affect various functions and organs of the body. Several studies have emerged to realize the effectiveness of *Qigong* in various medical aspects. I will discuss later the extent of research on *Qigong* and cancer.

### 3.7.2 – THE ROLE OF QIGONG IN CANCER

In the last years, in the field of modern medicine, comes an approach for treatment of cancer that considerer the condition of whole person as the environment for the cancer and to strengthen the body`s resistance to cancer. Relies on many factors such as, exercise, diet and meditative states, *Qigong* is part of this approach.

Accordingly to Sancier (1999) in a review of clinical studies on the therapeutic role of *Qigong* exercises done in conjunction with drug treatment in cancer patients, *Qigong* is able to reduce chemotherapy side effects [61].

It is known that *Qigong* can produce an increase in the absorption of oxygen through the postures, movements and breathing. A crucial explanation pointed by Dong's(1990), is that one of the important factors in cancer growth or not is the body`s content of oxygen. So if we have sufficient amount of oxygen cancer cells may die, and otherwise when the body is deficient in oxygen, cancer cells grow.

Accordingly to Chen (2002), *Qigong* maybe a beneficial option for cancer patients and a possible mechanism of interest in cancer reviews [58]. This author did a literature review of more than 50 studies of *Qigong* therapy in cancer treatment in China by dividing them into three categories: clinical studies with cancer patients, in vitro studies with cancer cells and further studies in live animals affected with cancer. Clinical studies involving observation of cancer patients who practiced *Qigong*. From the review, they found that there is a consistent trend for the groups who practiced *Qigong* in combination with conventional treatments had significant improvements and increased average life expectancy compared to those groups treated only with conventional treatments.

According to the same author, *Qigong* produce an improvement of the immune function, which may enhance the immune deficiency experienced by most of cancer patients and the improve of the microcirculatory function including changes in blood viscosity, elasticity as well as platelet functions. It is stated that through *Qigong* therapy it

can be adjust the microcirculation to a state considered optimal increasing the effectiveness of certain treatments, such as CT.

As concluded by Sun and Zhao, in a clinical study on several advanced cancers, *Qigong* is able to improve strength (82%), appetite (63%), as well as to reduce gastrointestinal symptoms (33%). Moreover, these authors found that the *Qigong* was responsible for increasing phagocytic rate of macrophages as well as the phagocytic index [62].

Another study from Shanghai Fangyi Hospital, reported changes in the immune indicators and physical health among 1,883 cancer patients after practicing *Qigong*. After practicing *Qigong* for 2 months, they reported that most patients showed remarkable improvements on white blood cells, CD20, IL-2 and NK activities. In addition, 40.8% patients reported improvement in sleep, and 36.8% reported improvement in appetite. This strongly suggests that the effect of *Qigong* therapy for cancer is not purely psychological [63].

Many studies show that the increase of NK cells and many other components of the immune system can significantly reduce the chances of infection or tumor growth [64-65].

Given the fact that most cancer therapies tend to damage or destroy patient's immune functions, which reduce the patient's overall capability of self-recovery, the indication of improvement in the immune system from *Qigong* therapy warrants further in-depth research of its therapeutic effect on cancer.

In conclusion, therapy through *Qigong* can be a valuable and indispensable in the treatment of patients with cancer, complementing conventional treatments.

## **II - CLINICAL RESEARCH PROTOCOL**

## 1. OBJECTIVES

The objectives of this study are:

### 1) Evaluate the effect of *Qigong* on NK cells.

1.1 Question 1: Will *Qigong* exercise practice a positive influence on the number of NK cells, T and B lymphocytes in patients with CRC under chemotherapy?

### 2) Evaluate the effect of *Qigong* on anxiety and depression

2.1 Question 2: Does *Qigong* improve levels of anxiety and depression in patients with CRC under chemotherapy?

### 3) Evaluate the effect of *Qigong* on quality of life of patients

3.1 Question 3: Does the practice of *Qigong* improve QOL of these patients?

4) To correlate the effect of *Qigong* on NK cells with quality of life as well as the levels of anxiety and depression in patients with CRC.

4.1 Question 4: There will be a positive correlation between the immune parameters and the QOL, levels of anxiety and depression of these patients?

## 2. METHODOLOGY

### 2.1 - SAMPLING AND RECRUITMENT PROCEDURES

#### 2.1.1 - PATIENTS SELECTION

This study was approved by the ethics commission of Portuguese Oncology Institute of Porto (IPO) on July 2014 and will be conducted at the Clinic of Digestive Pathology integrated in the IPO Porto. Written informed consent was obtained from all patients before study enrollment. Patients were eligible for inclusion as follows:

##### The inclusion criteria:

- Newly diagnosed or recurrent colorectal cancer, regardless of stage;
- Receiving myelosuppressive chemotherapy;
- No regular use of acupuncture or any TCM therapy within 120 days prior to enrollment;
- Ability to give informed consent;
- Age >18 years of age.

##### The exclusion criteria:

- Absolute neutrophil count (ANC) less than 500/ $\mu$ L;
- Platelet count less than 25,000/ $\mu$ L;
- Altered mental state;
- Clinically significant cardiac arrhythmias;
- Other unstable medical condition.

#### 2.1.2 - STUDY DESIGN

The study was an experimental, prospective, pilot clinical trial, with a randomized and controlled design.

All patients enrolled were evaluated at baseline. Patients were randomized into one of two groups: Qigong (Qi group) or non-Qigong (control group). All of the study patients, were blinded to randomization assignments. It was used the technique of simple randomization sampling: enrolled patients were assigned with consecutive numbers in order of recruitment and subsequently allocated as follows: odd numbers was allocated in the control group and even numbers in the experimental group.

All patients enrolled was evaluated at baseline. Patients was randomized into one of two groups: active *Qigong* group (*Qigroup*) or non-*Qigong* group (control group).

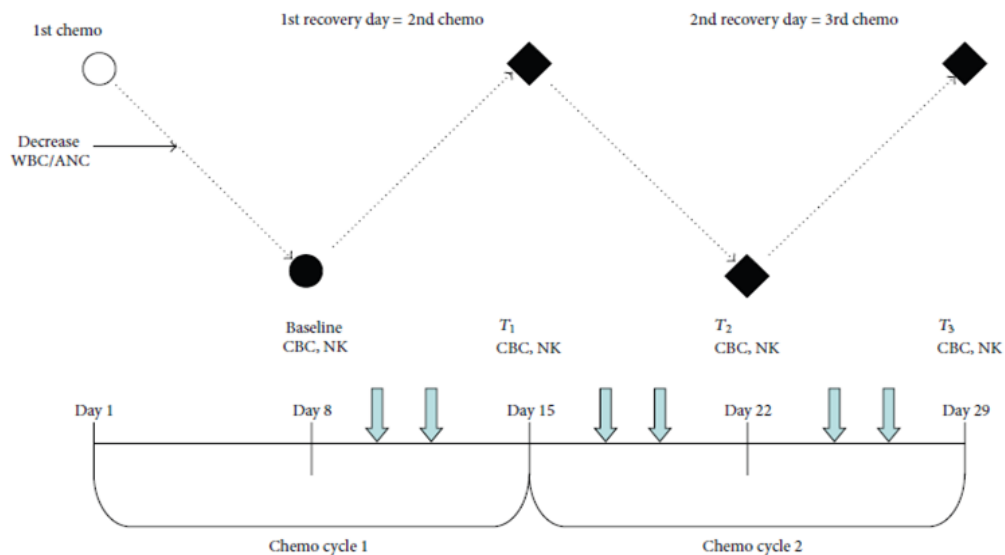
### 2.1.3 - EXPERIMENTAL GROUP

A session prior for clarification was made. Patients in the experimental group received 6 sessions of *Qigong* therapy, twice a week between the CT protocol. This treatment is started at week after the beginning of the cycle of CT and is repeated twice a week until the end of the CT regimen (Figure 1).

The method of *Qigong* therapy applied was standardized based on the model of Heidelberg Traditional Chinese Medicine advocated the teaching area in the Master of MTC ICBAS-UP.

### 2.1.4 - CONTROL GROUP

The control group consisted of patients with the same pathology and receiving the same CT regimen but they just being collecting the blood sampling for the study without receiving any *Qigong* treatment. It was offered to these patients the *Qigong* therapy protocol as a courtesy, immediately after they completed the four weeks of blood sampling.



**Figure 11. Study flow chart.** CBC, complete blood counts; NK, natural killer cells counts; WBC, white blood cells; ANC, absolut neutrophil counts. Blue Arrow, *Qigong* therapy (QG); Black circle corresponds to blood collecting samples at baseline (T0). Black diamonds (T1, T2 e T3) correspond to each subsequent 7 days blood collecting samples.

## 3. INTERVENTION

All patients enrolled were evaluate at baseline. Patients were randomize into one of two groups: active *Qigong* group (*Qigroup*) or non-*Qigong* group (control group).

### 3.1 - EXPERIMENTAL GROUP

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### 3.2 - CONTROL GROUP

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### 3.3 - QIGONG PROTOCOL INTERVENTION

The *Qigong* program runs for 3 weeks with 2 sessions per week of 30 minutes (Figure 11). The *Qigong* intervention program is a traditional *Qigong* program directed specifically to the diagnosis of cancer in specific, to strengthen the immune system; each session consisting of various exercises selected for the case.

The technique applied *Qigong*, called "Happy *Qigong*" and consists of the sequence of a set of exercises with the aim of promoting feeling relaxation, wellness and relaxation, but above all, lead to an acceleration of body energy.

The sequence of exercises begins after all participants are silent in a large room and focused on what they are asked to perform. This sequence is developed as follows:

1. Imagining one of the happiest moments in life. Feel this sense of well-being and absolute happiness. Concentration eight breaths during that time.

2. "Standing" and "white ball" - duration of 8 breaths.

- a) Standing - Feel the point Fons Scatens (R1) of the body, located in the middle of the foot.

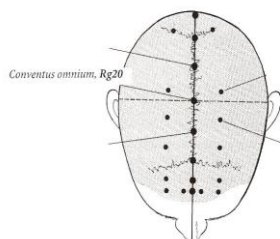


**Fig.12- Acupoint R1 ( Fons Scatens)**

Data from Porket M.,1995 [60].

Put your back into a straight position. Set the knees in order to lead to a higher concentration to the point and adjust all posture, moving it slightly forward or backward to find the balance.

After, focus attention on the point Conventus Omnium Yang (RG20), located on top of the head.



**Fig 13. Acupoint Rg 20 (Conventus Omnium Yang)**

Data from Porket M.,1995 [60].

Feel the extension of this inner shaft toward the center of the earth.

Make the connection with the two points by adjusting body posture, checking the connection RG20 and R1.

b) White ball

The completion of this exercise is done with eyes closed, putting his arms in front of her arch-shaped and set a distance of at least 20cm between both hands. Should relax , exhale deeply to improve the flow of Qi.

Turning point Medium Palmae (PC8) between one hand and the other. Remain in this position for 8 breaths .



**Fig. 14 – Acupoint PC8 ( *Medium Palmar*)**

Data from Porket M.,1995 [60]

### 3. Awaking the Qi

Raise your arms slowly in front of you with your arms straight and your palms facing down. On the head, stretch your arms, with your palms facing the sky and feel as if the body were being pulled by a rope. Feel this stretching the spine. When the arms are outstretched, lifting your heels, keeping the body weight supported as much as possible on tiptoe. Holding his breath and a quick and invigorating, so back down to keep your feet flat on the floor. Exhale and slowly down her arms. Repeating 8 times.

### 4. Shoulders roll

This exercise begins with the person standing relaxed position. *Qigong* position. Curl your shoulders slightly, palms start down the lateral aspect of both legs (felleal condui). In performs motion inspiration. When you can not extend the movement without having to bend your knees, in maximum until 90°. Back up, always with the hands to rise close to the body, this time, go up through the palms in front of the legs (stomachal conduit). This movement is executed during expiration.

When the body is practically in the standing position, slightly role your shoulders to keep your back straight and place your hands at your sides with the palms of the hands parallel to the floor. Inhale and exhale and repeat the exercise again until 8 times.

### 5. Network

Standing, legs together and knees slightly bent. Place the hip to one side, the body slightly leaning forward and to the opposite side of the hip. The elongate arms forward to the opposite side to the hip, or follow the inclination of the side of the body. With hands with palms facing up, making a motion as if pulling a rope towards the chest ( cardiac mu point). When hands lead to "rope" to the chest, repeat the exercise for the other side, not forgetting the always hip to the opposite side arm.

In the passage from one side to another exercise, the arms remain stretched forward, hands with palms facing the floor and the body rotates slowly return to starting

position, to start the movement. In this passage, when hands glide in front of him, imagine that these slip on fresh water from the sea, but never get to touch this. Imagine the feeling of freshness in the hands with this passage. Repeat the exercise until 8 times.

#### 6. Rotate the ball

Standing in *Qigong* position. Bent more the knees. Hold the *White ball*.

Turning to the body side and the right arms are attached; the right arm is above the left and underneath, keeping the arched shape of the arms as if to grab the ball. The feet on the ground to rotate the right side as well as the knees. These must be fitted, namely, the left knee fits the back of the right knee that rotated forward. The heel of the left knee raises slightly putting pressure on his right knee.

Repeat for the left side. In the passage from one side to the other feet glide on the floor and hands are always as if holding a ball. On each side we turn the arm that is on top is always the side arm that we are facing. Repeat the exercise 8 times

#### 7. Pump

Standing with one hand in a fist in front of the chest, close to the ribs. Shoulder forward. Always with the hand in the same position, dragging his hand vigorously in the body, following the intercostal line to the lumbar region. The body follows the movement and will slowly turning. In the lumbar region, always hand in a fist, down vigorously until the sacrum, always following the line of the column. Lobby movement feeling the vertebrae in its passage. When the hand reaches the sacrum, it starts movement with the opposite hand, the chest again and repeating the same cycle. Repeating 8 times to each side.

#### 8. Earthquake

Stand in *Qigong* position. This exercise is to run a vibration throughout the body and adapted and tailored to each, according to what is comfortable for each person. Allowing the whole body to perform a continuous mixing in the form of a frequency continues. Keep focused attention during the execution of the exercise at the point below the umbilicus (*dantien*) and feel as if this point were increasing slightly. After focusing the attention in the shoulder region and shoulder blades (*Crass Intestinal* passage of the conduit). Feel this region to relax. During vibration perform a technique where imagination is thought that the body is wrapped in a thick film and that this film during vibration will be broken and will dropped, with the body becoming loose.

9. Clarity of the sky

Standing with arms along the body, start the exercise by lifting them with your palms facing upwards raising the arms to the top of the head. When the arms are raised already, imagine that a very strong and bright light will be collected by us and as the arms go down, with the palms facing down, this light will envelop the whole body and brings a sense of welfare. At a later stage, explain the existence of PC8 point and repeating the same exercise focusing attention on this point, and assuming that it expands and increases with this light and provides better clarity in the entry of this same body.

10. The Horse. Standing in *Qigong* position. Palms of the hands turning to the earth in the level of the belly boton ( dantian region). Standing during 8 breathing.

11. Standing

Repetition of the second exercise.

## 4. OUTCOMES

Based on a preliminary study conducted at Hospital São João (Porto) and Hospital of Vila Nova de Gaia / Espinho, in the context of the thesis master's degree in TCM in University of Porto by Irene Pais with the Title- *Effect of Acupuncture on NK cells in patients with colorectal cancer undergoing chemotherapy*, we expect to have positive results in this study.

### 4.1 - MAIN PARAMETERS

The following cellular components were quantified in all patients, leukocyte, neutrophil, total lymphocytes, T and B lymphocytes and NK cells. Every blood sample was taken directly to the laboratory at most two hours after being harvested.

The samples of blood were collected from two different ways. When patients need to take blood samples for the hospital protocol, the investigator ask to the department of "Central de Colheitas" to take one more blood sample for the investigation study, respectively T1 and T3. In the other moments, T0 and T2, the blood was collected by the investigator (nurse in IPO) at a room on the "Clinica De Patologia Digestiva". The necessary analyses to the study was performed at the laboratory of Immunology, in Centro Hospitalar do Porto, under the supervision of Dr. Esmeralda Neves.

### 4.2 - SECONDARY PARAMETERS

It was collected data from socio-demographic and clinical questionnaires, such as diagnosis clinical time and medical treatment, as well as data from *Hospital Anxiety and Depression Scales* (HADS) – Portuguese version and from EORTC QLQ 30- and QOL-CR29. with the aim to assess the anxiety and depression levels as well as the patients' quality of life.

- *"Hospital Anxiety and Depression Scales"* (HADS) - the Portuguese version validated by Pais Ribeiro *et al.* "Hospital Anxiety and Depression Scales": Scale developed by Zigmond and Snaith (1994), with the aim of assess levels of anxiety and depression in patients with physical diseases and ambulatory treatment. It takes approximately five minutes to complete [67]. Was validated for the Portuguese context by Pais-Ribeiro *et al* (2007). It consists of 14 items which are divided into two subscales: anxiety (7 items) and depression (7 items). The items reflect a state of generalized anxiety and the depression items are associated with a state of

anhedonia. Response options vary between zero and three in a Likert scale of 4 points. [67].

- "*European Organization for Research and Treatment of Cancer Quality of Life Questionnaire*" (EORTC QLQ 30): Instrument created by the European Organization for Research and Treatment of Cancer Quality of Life [68]. The QLQ-C30 aims to assess relevant aspects for any cancer patient regardless of the neoplasia to assess, not responding to the specific situation in each type of neoplasia [71]. It consists of five functional scales (physical, performance, cognitive, emotional and social), three symptom scales (fatigue, pain, nausea and vomiting) overall health status, 6 simple items to assess additional symptoms or problems (dyspnea, loss of appetite, insomnia, financial difficulties, constipation and diarrhea) [71]. The items correspond into worse QOL [71].

#### **4.3 - STATISTICAL ANALYSIS**

This study was designed to provide preliminary data to inform the design and analysis of a subsequent large-scale, fully powered study to evaluate the effects of *Qigong* on NK cells in patients with CRC. However due to the obtained reduced sample size, it was not possible to perform statistical analyses. So it will be discussed case studies.

### **III- RESULTS**

## 5. BASELINE CHARACTERISTICS - SAMPLE CHARACTERIZATION

At baseline, all patients were characterized based on demographic and clinical characteristics (Table 6 and 7).

In the experimental group, the patients mean age is 51.7 years; 66.66% of patients are female and have colon cancer diagnosis and 33.33% are male and are diagnosed with rectal cancer. All patients are under FOLFOX CT regimen. 33.33% have a T4B cancer stage, 33.33% T3 stage with distant metastasis and T3 with local ou distant metastasis (Table 6).

The control group, the mean age is 51.7 years; 66.66% of patients are male with a diagnosis of rectal cancer and 33.33% are female and diagnosed with colon cancer. 66.66% of patients are undergoing FOLFOX CT regimen and 33.33% are under Degramon regiment. 33.33% of these patients have a T2 cancer stage and 66.66% have a T3 cancer stage with local (lymphatic nodes) metastasis. (Table 7).

	PATIENT 1	PATIENT 2	PATIENT 3
AGE	53	57	45
SEX	Female	Male	Female
RESIDENCE	Porto	Porto	Porto
DIAGNOSIS	Sigmoid colon Adenocarcinome	Retal cancer	Ascending colon adenocarcionome
TYPE OF CT	adjuvant Ct folfox	adjuvant Ct folfox	adjuvant Ct folfox
STAGE	T4B,N0,M0	T3,N2,M1	T3,N0,M0

**Table 6.** Experimental Group: Socio-demographic and clinical characteristics.

	PATIENT 1	PATIENT 2	PATIENT 3
AGE	42	51	62
SEX	Male	Male	Female
RESIDENCE	Porto	Porto	Porto
DIAGNOSIS	Retal Adenocarcinoma	Retal adenocarcinoma	Adenocarcionoma colon sigmoide
TYPE OF CT	Ct adjuvante folfox	Ct adjuvante Degramon	Ct adjuvante folfox
STAGE	T2,N0,M0	T3,N2,M0	T3,N1B,M0

**Table 7.** Control, Group: Socio-demographic and clinical characteristics.

Comparison of blood analyses at baseline showed that blood values are similar in both groups, except for B and NK cells that are in higher amounts in the control group patients (Table 8).

EXPERIMENTAL GROUP (N=3)	BASELINE BLOOD ANALYSES (MEAN)	CONTROL GROUP (N=3)
4566,6	WBC (MM3)	6266
2433,3	ANC (MM3)	2466,6
1633,3	TOTAL LYMPHOCYTE (MM3)	1666,6
219,06	B CELLS (MM3)	490
1298,3	T CELLS (MM3)	1008,6
115,54	NK CELLS (MM3)	167,15

**Table 8.** Blood analyses at baseline. Comparison between groups.

A total of 5 (83,3%) patients completed the questionnaires of QOL-CR29 and 30 and HDAS at the beginning of the study (table 9). Comparison of anxiety and depression between both groups, reveal that levels were higher in the control group, before the study. The remaining results do not allow to make a general safe comparison because of the diversity of results. However, nausea/vomit, diarrrohea and constipation do not show any symptoms in both groups.

EXPERIMENTAL GROUP (N=3) BEFORE	QOL CR 29, C30 AND HADS SCORES (MEAN)	CONTROL GROUP (N=3) BEFORE
8,33	ANXIETY	9
7	DEPRESSION	8
0	DISURIA SYMPTOMS	0
2	BLOOD IN STOOL SYMPTOMS	3,7
0	URINARY INCONTINENCE	0
1,3	ABDOMINAL PAIN	2
2	BLOATING	2
2	DRY MOUTH	2
2,3	TASTE	2
1,3	FLATULENCE	1,3
0	FAECAL INCONTINENCE	1,3
0,3	IMPOTENCE	0
0	DYSPAREUNIA	0,3
0,6	STOMA-RELATED SYMPTOMS	0
0	MALE SEXUAL FUNCTION	1,6
0	FEMALE SEXUAL FUNCTION	0,3
3	WEIGHT CONCERNS	2,6
6	BODY IMAGE	4,6
4,7	PHYSICAL FUNCTIONS	4
4,6	ROLE FUNCTION	4
7	EMOTIONAL FUNCTION	5
2	COGNITIVE FUNCTION	0,7
3,3	SOCIAL FUNCTION	4
7,3	FATIGUE	4
0	NAUSEA/VOMIT	0
3	PAIN	1,3
0,7	DYSPNOEA	0
3	INSOMNIA	1,3
0,7	LOSS OF APETITE	0
0	CONSTIPATION	0
0	DIARRHOEA	0
1,3	FINANTIAL ISSUES	2,3
9	HEALTH OWN PERCEPTION	9,7

**Table 9.** QOL CR-29, 30 and HADS scores. Comparison between groups at baseline.

## 6. RESULTS OF THE QIGONG ON THE IMMUNE SYSTEM – MAIN PARAMETERS

### Effect of Qigong on WBC

WBC were improved in two patients of the experimental group and on the control group only one patient have showed improved levels of these cells types (Figure 1 and 2). WBC improved in all patients at nadir time points T2, in comparison to nadir time point T0: patient 1, 9100cell/mm<sup>3</sup> (T2) vs 4600 cell/mm<sup>3</sup> (T0); patient 2, 4000 cell/mm<sup>3</sup> VS 3500 cell/mm<sup>3</sup> and patient 3, 6300 cell/mm<sup>3</sup> VS 5600.

In respect to recovery days (T3 vs T1), our results show that WBC were higher at T3 only in one patient (patient 2), 5600 vs 5400, suggesting that Qigong may have little effect on immunity recovery. However, when comparing WBC at the beginning and at the end of the study, only one of the patients (patient 3) in the experimental group had a slight decreased of these cells. In contrast, control group results shows that only one patient (patient 1) have had WBC higher in the end of the study than in the beginning. Moreover, one of the patients (patient 3) stopped the chemotherapy because of low values of WBC at recovery day (1800 cell/mm<sup>3</sup>), and consequently dropped out of the study.

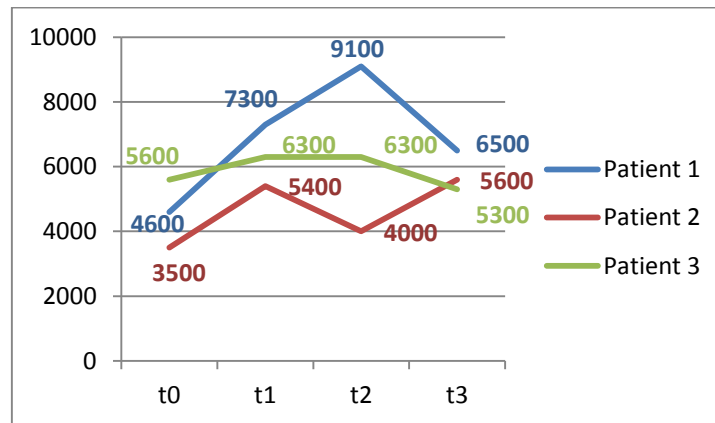


Figure 15. Experimental Group: WBC analyses

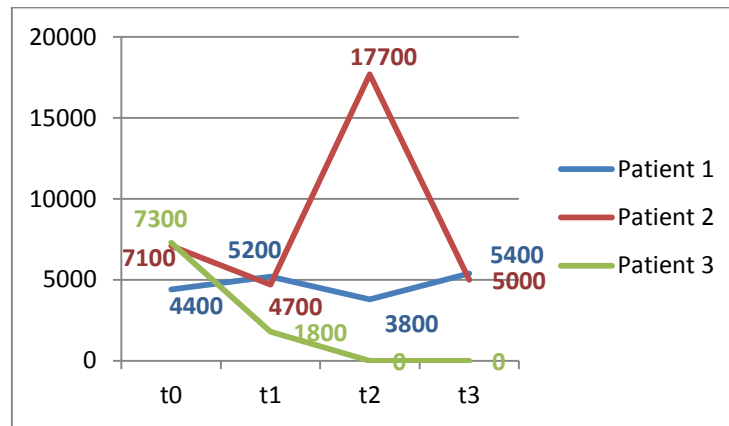


Figure 16. Control Group: WBC analyses

Moreover, when comparing experimental and control groups at baseline for WBC we found that patients on the control group have higher counts of these cells and that at the end of the study the experimental group have higher counts. Thus, it seems that Qigong have a myeloprotective effect (leukopenia).

	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	4533.3	6266
T3 (N=5)	5800	5200

Table 10. Comparison of baseline and end of study WBC counts (means-cell/mm<sup>3</sup>) between groups (experimental and control)

### Effect of Qigong on ANC

ANC were improved in two patients of the experimental group and on the control group only one patient have showed improved levels of these cells types (Figure 3 e 4). ANC at T2 were improved in all patients of experimental group and in control group only one patient showed elevated levels. At recovery day T3 no significant results on ANC were observed in the experimental group, only one patient showed higher levels of ANC comparing to T1.

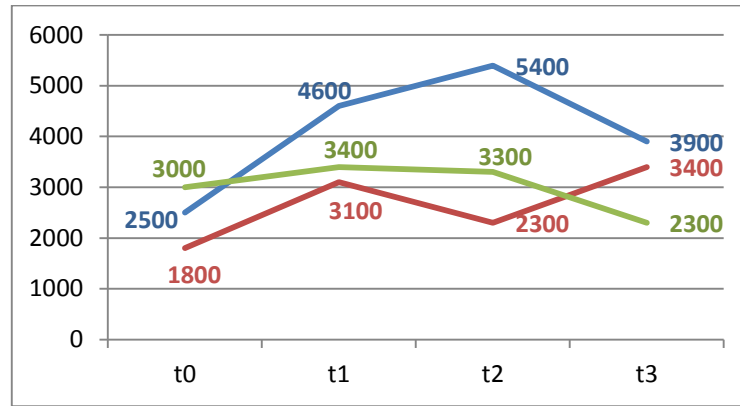


Figure 17. Experimental Group: ANC analyses.

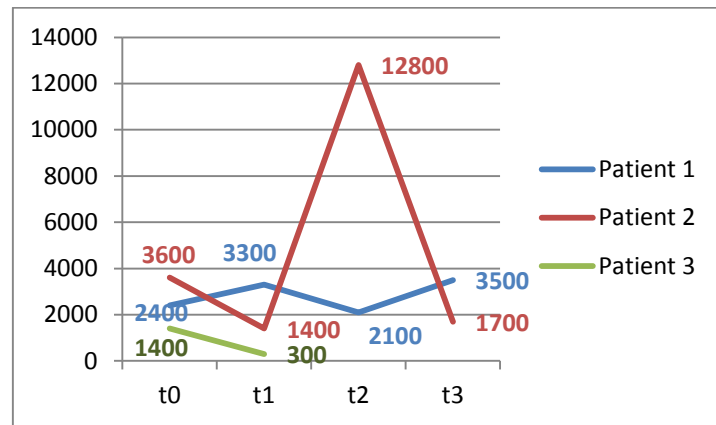


Figure 18. Control Group: ANC analyses.

Comparing the experimental and control groups at baseline for ANC we found that both groups have similar numbers of ANC. However, at T3, the experimental group has revealed a significant increase of ANC when comparing to the control group that only slightly increase of ANC.

	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	2433,3	2466,6
T3 (N=5)	3200	2600

Table 11. Comparison of baseline and end of study ANC counts (means-cell/mm<sup>3</sup>) between groups (experimental and control)

### Effect of Qigong on Total lymphocytes and subsets

Total Lymphocytes were improved in two patients (patient 1 and 2) on the experimental group comparing the results between T0 and T3. (Figure 5). The opposite happened in the control group that in two patients (patient 2 and 3) the results decrease in the same evaluation.

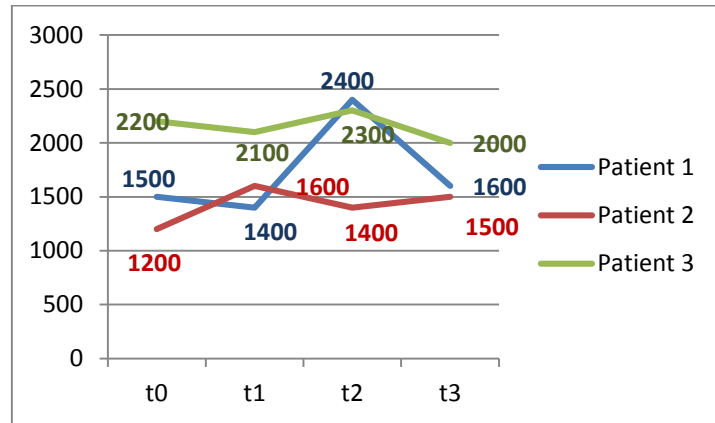


Figure 19. Experimental Group: Total lymphocytes analyses.

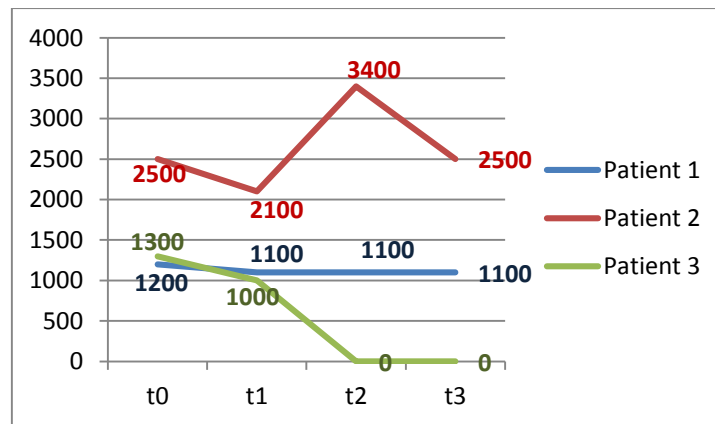


Figure 20. Control Group: Total lymphocytes analyses

Additionally, when comparing experimental and control groups at baseline (T0) and T3 for total lymphocytes, we found that both groups have had a small increase in the results. Control group have a increase of 8% and the experimental group an increase of 4%.

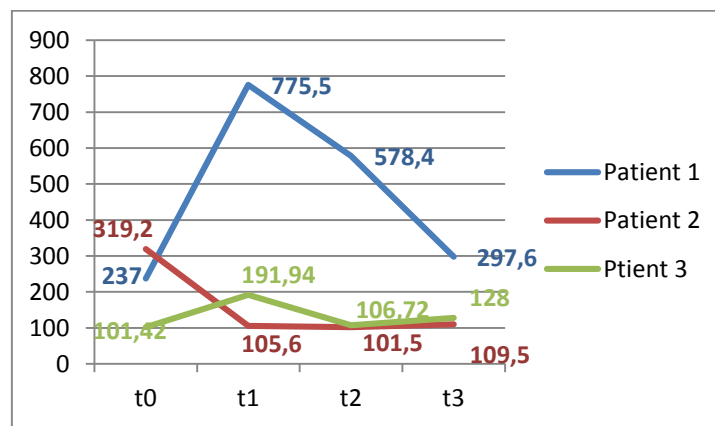
	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	1633,3	1666,6
T3 (N=5)	1700	1800

**Table 12.** Comparison of baseline and end of study total lymphocytes counts (means-cell/mm<sup>3</sup>) between groups (experimental and control)

### Effect of Qigong on B cells and subsets

In respect to B cells counts, results shows that two patients on the experimental group have higher B cells counts in the end of the study that in the beginning. Moreover, when comparing nadir time points we found that in two patients the B cells counts were higher in the second nadir (T2) relatively to the first nadir (T0). At recovery days we found that the second recovery day (T3) have lower B cells counts than the first (T1) in two patients. Only one patient (patient 2) showing higher B cells counts at T3 when compared to T1.

In the control group, patient 3 drop out of the study at T1 due to low levels of WBC. Thus only two patients were fully evaluated until the end of the study. These two patients showed decreased values of B cells at the end of the study (T3), when compared to the beginning (T0) (Figure 8).



**Figure 21.** Experimental group: B cells counts.

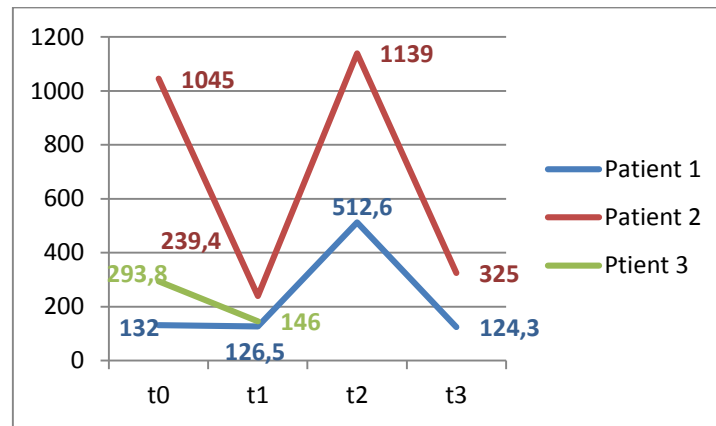


Figure 22. Control group: B cells counts.

Comparing experimental and control groups at baseline and T3 we can conclude that in both groups the B cells decrease. However, in the control group the B cells registered a decrease of 54,2%, comparing with the decrease of 18,61% obtained in the experimental group. Thus, it seems that Qigong have a protective effect on this cells.

	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	219,06	490,27
T3 (N=5)	178,3	224,65

Table 13. Comparison of baseline and end of study B cells counts (means-cell/mm<sup>3</sup>) between groups (experimental and control)

### Effect of Qigong on T cells and subsets

T cells only raised in one patient (patient 2) and kept similar in patient 1, when comparing values at the end and at the beginning of the study. At nadir time points, we found higher values in T2 relatively to T0 in all patients and at recovery days only one patient have show higher T cells counts at T3 than T1; however T cells counts on the other two patients were very similar at recovery days (Figure 9).

In the control group, only one patient showed an increased T cell counts since the beginning of the study until the end (patient 2). Patient 1 showed a slight decrease of these cells when comparing T3 to T0; however T cells counts on these patient were quit stable at nadir time points and at recovery days also (Figure 10).

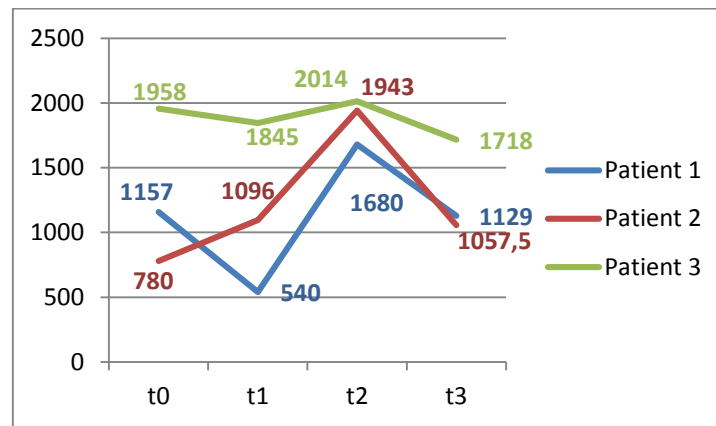


Figure 23. Experimental group: T cells counts.

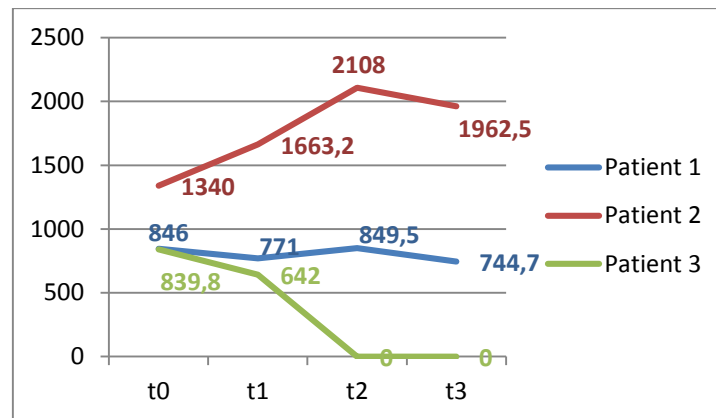


Figure 24. Control group: T cells counts.

When comparing experimental and control groups at baseline for T cells we found that patients on the control group have higher counts of these cells on T3 than in baseline. We can compare with the experimental group, that also have a increase of the results, however, in control group at the end of the study the results are slightly higher.

	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	1298,3	624,6
T3 (N=5)	1301,5	1353,6

Table 14. Comparison of baseline and end of study T cells counts (means-cell/mm<sup>3</sup>) between groups (experimental and control)

### Effect of Qigong on NK cells

Our results showed that NK cells counts were higher in the end of the study compared to the beginning. Moreover, in respect to nadir time points results shows higher counts of NK cells at T2 vs T0. At recovery days, except for patient 2, NK cells were also in higher numbers than in T1 (Figure 11).

In the control groups it was also verified an increased NK cells counts at the end of the study when compared to the beginning, in two patients (Figure 12).

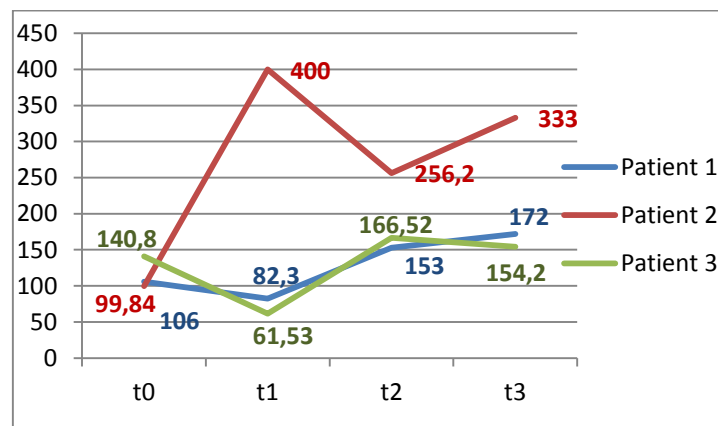


Figure 25. Experimental group: NK cells counts.

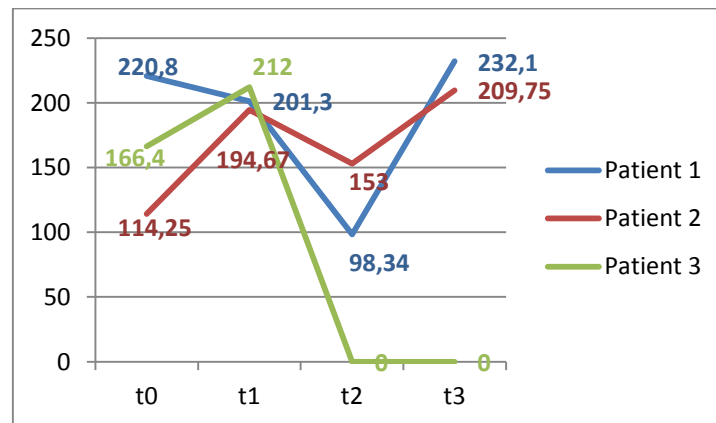


Figure 26. Control group: NK cells counts

Making the corresponding between experimental and control groups at baseline for NK cells we found that patients of the both groups have increase of these cells. However, the magnitude of the NK cells in the experimental group is much higher than in the control group, since there are a few differences on NK cells baseline counts between the

experimental group(140.8, 99.84, 106 cell/mm<sup>3</sup>) and the control group (220.8, 166.4, 114.25 cell/mm<sup>3</sup>).

	EXPERIMENTAL GROUP	CONTROL GROUP
BASELINE (N=6)	115,54	167,15
T3 (N=5)	219,73	220,92

**Table 15.** Comparison of baseline and end of study NK cells counts (means-cell/mm<sup>3</sup>) between groups.

## 7. EFFECT OF QIGONG ON PATIENTS QOL, ANXIETY AND DEPRESSION SCALES – SECONDARY PARAMETERS

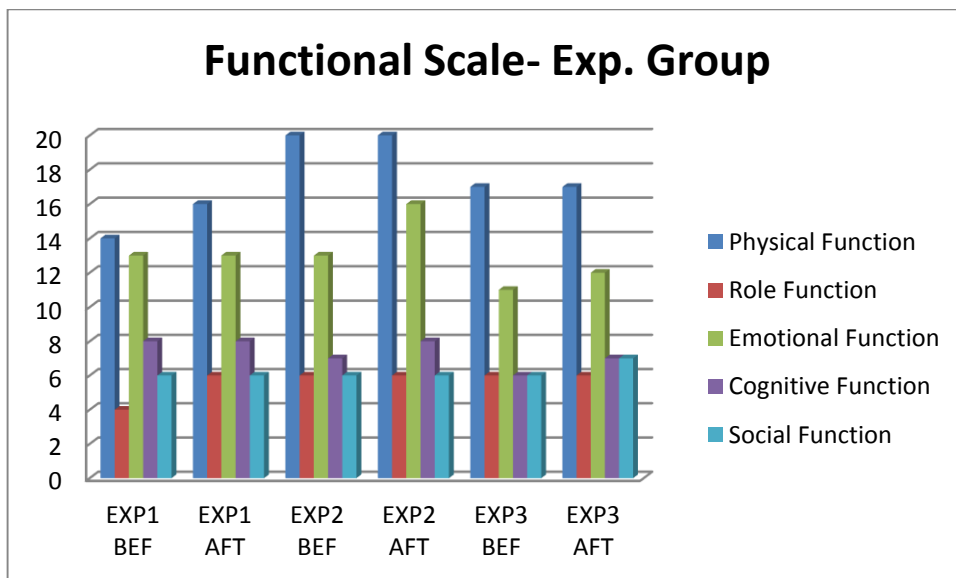
In concern of the secondary parameters of evaluation of this study, a total of 5 (83,3%) patients completed the questionnaires of QOL-CR29, QOL-CR30 and HADS at the beginning and at the end of the study.

The QLQ – CR30 is considered a questionnaire for assessing QOL in patients with cancer, consisting of 30 items, divided by a global scale, five functional scales (physical, role, emotional, cognitive and social functioning), and sub-scales of symptoms associated with cancer and treatment. It is a questionnaire considered appropriate for use with cancer patients from the moment of diagnosis to long survivorship (Table 3).

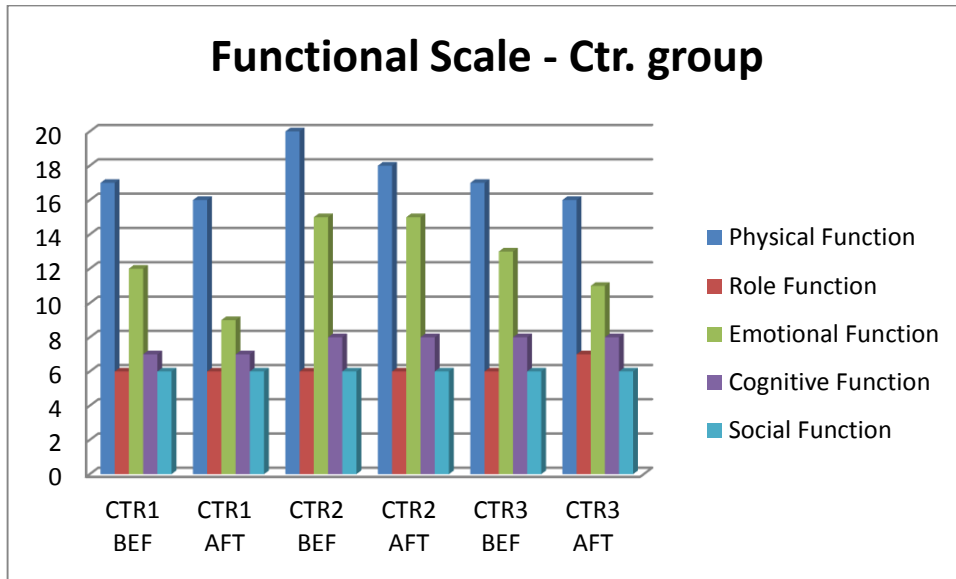
Item/subscales	Items	Total items
<b>Item/Functional subscales</b>		
Physical functioning	1, 2, 3, 4, 5	5
Occupational functioning	6, 7	2
Emotional functioning	21, 22, 23, 24	4
Cognitive functioning	20, 25	2
Social functioning	26, 27	2
<b>Items/Symptoms Subscales</b>		
Fatigue	10, 12, 18	3
Nausea and vomiting	14, 15	2
Pain	9, 19	2
Dyspnoea	8	1
Insomnia	11	1
Loss of appetite	13	1
Constipation	16	1
Diarrhoea	17	1
Financial difficulties	28	1
Overall state of health	29, 30	2

**Table 16.** Items/Subscales of QLQ-CR30 questionnaire

A. QLQ-CR30 QUESTIONNAIRE- FUNCTIONAL SCALES:



**Figure 27:** Experimental group. Functional scales assessed by QLQ-CR30 questionnaire.  
**Legend:**EXP- experimental group patient; BEF- Before; AFT- After.



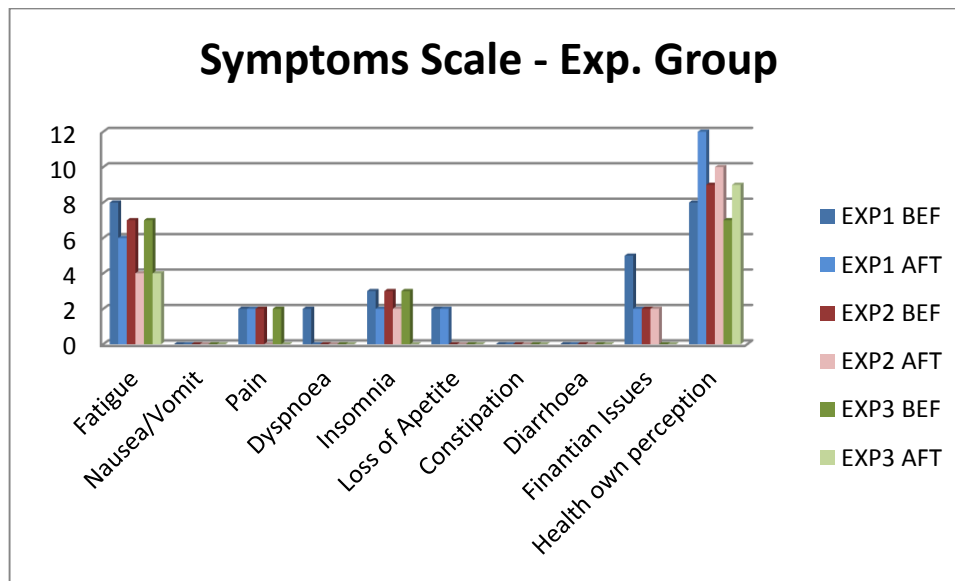
**Figure 28.** Control group. Functional scales assessed by QLQ-CR30 questionnaire.

**Legend:** CTR- Control group patient; BEF- Before; AFT- After.

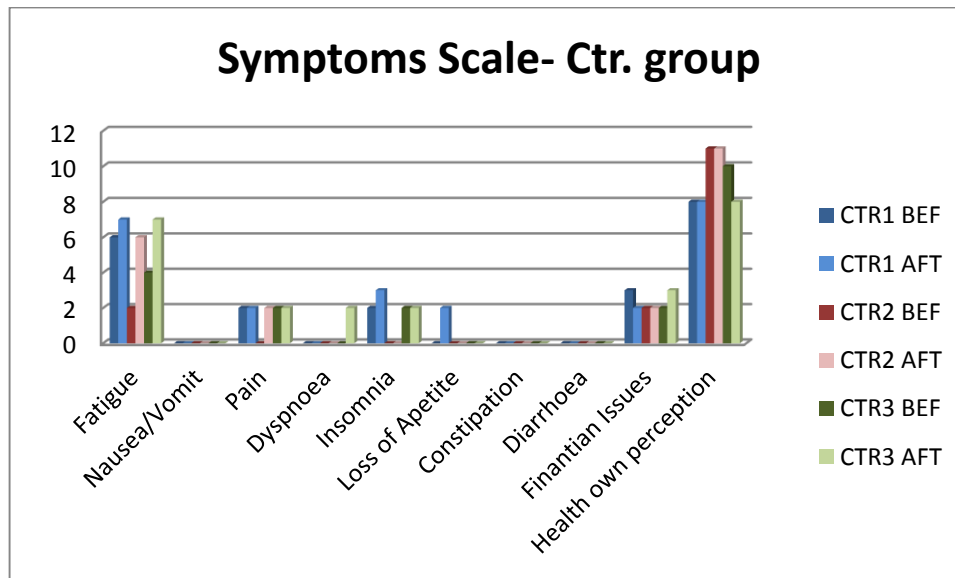
All of the functional scales in experimental group were enhanced after Qigong treatment. Emotional functions were those that presented most improvement. Social functions didn't have any change before and after the study except in patient 3 that slightly improve after the treatment. In the control group, results shows that across the time the most affected functions are in patients 1 in emotional functions that decrease and in patient 2 that physical function decrease more than the other parameters. All the other functional scales slightly decrease.

Thus, our results suggest that Qigong is able to act on cancer patients most affected functional scales (physical and emotional) as well as on improvement of cognitive and social functions.

B. QLQ-CR30 QUESTIONNAIRE- SYMPTOMS SCALES



**Figure 29.** Experimental Group , Symptoms scales assessed by QLQ-CR30 questionnaire.  
**Legend:** EXP, Experimental group patient; BEF, Before; AFT, After.



**Figure 30.** Control Group , Symptoms scales assessed by QLQ-CR30 questionnaire.  
**Legend:** CTR, Control group patient ; BEF- Before; AFT- After.

All patients of the both groups have a good health own perception experienced. Symptoms as nausea and vomit, constipation and diarrhoea don't have been manifested .

The results on the experimental group show an improvement on fatigue and all of the patients also experienced a decrease on insomnia and loss of appetite symptoms.

In contrast, symptomatic scales on the control group patients remained unchangeable for nausea/vomit, dyspnea, constipations, diarrhea. Symptoms such as fatigue are those you decrease more in the control group.

QLQ-CR29 is a specific module for colorectal cancer patients varying in disease stage and treatment modality (i.e. radiotherapy or chemoradiation, ultra-low anterior resection, minimal access surgery and new chemotherapy regimens). It is divided into two major groups of subscales (Table 4). The answer to each of these items is also given through a likert scale with four alternatives in which the extremes are “not at all” and “very much”.

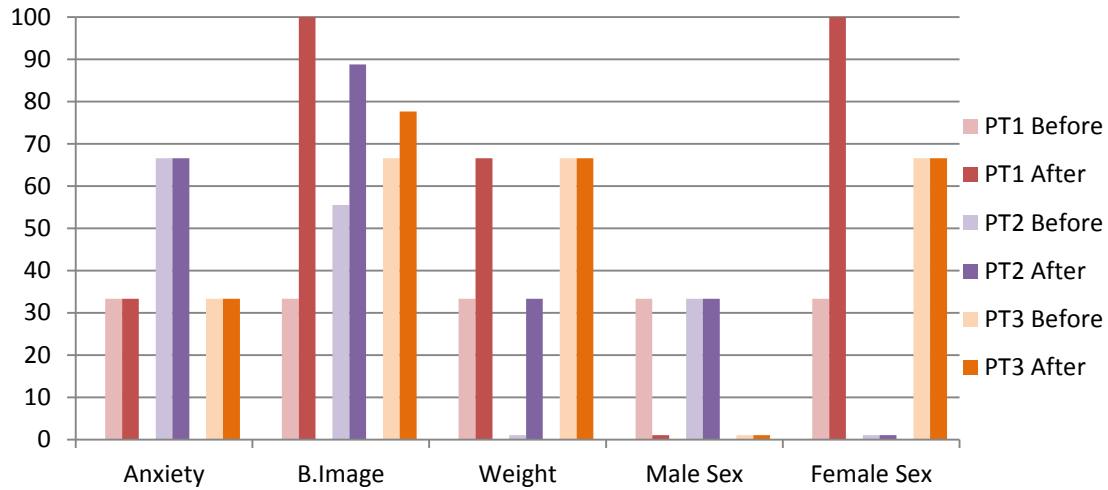
Item/ Subscales	Items	Total Items
<b>Item/ Functional Subscales</b>		
Body Image	15-17	3
Anxiety	13	1
Weight	14	1
Sexual interest (men)	26	1
Sexual interest (women)	28	1
<b>Item/ Symptom scales</b>		
Urinary frequency	1,2	2
Blood and mucus in stool	8,9	2
Stool frequency	22,23	2
Urinary incontinence	3	1
Dysuria	4	1
Abdominal pain	5	1
Buttock pain	6	1
Bloating	7	1
Dry mouth	10	1
Hair loss	11	1
Taste	12	1
Flatulence*	19	1
Faecal incontinence*	20	1
Sore skin*	21	1
Embarrassment	24	1
Stoma care problems	25	1
Impotence	27	1
Dyspareunia	29	1

**Table 17 .** Items/Subscales of QLQ-CR29 questionnaire.

### C. QLQ-CR29 QUESTIONNAIRE- FUNCTIONAL SCALES

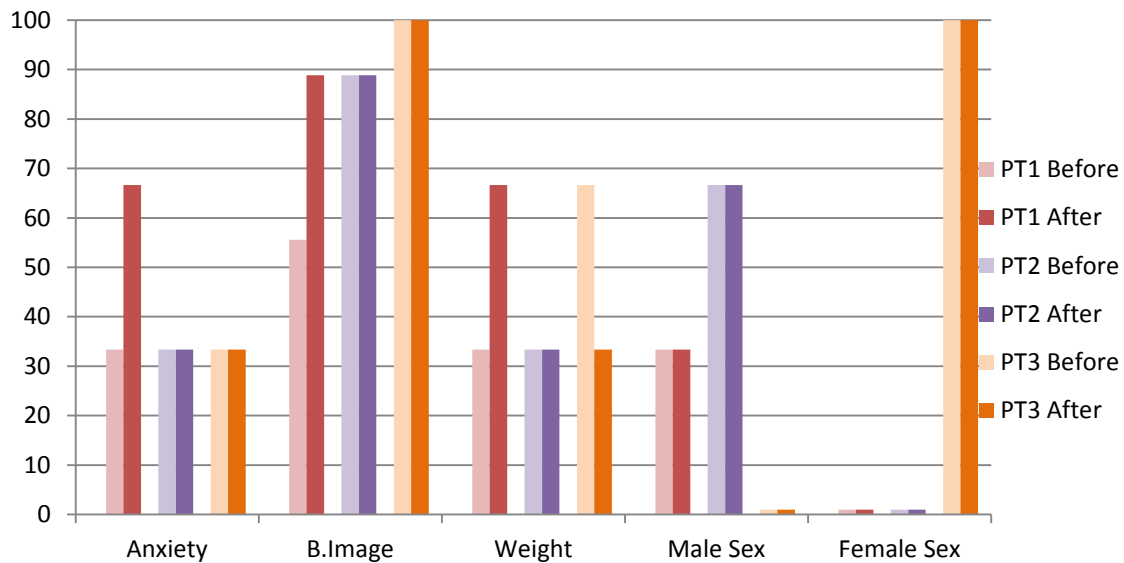
With regard to evaluation after qigong intervention, in experimental group, the score has improved in the Body image score in all of the three patients (Graphic 15). It is also at

the level of Weight functioning the score has improve in two of the tree patients. In turn, in control group (Graphic 16), the levels of anxiety are higher in one patient, comparing to the experimental group that maintained equal before and after the intervation.



**Figure 31:** Experimental group: functional scales assessed by QLQ-CR29.

**Legend:** PT, patient ; B- body



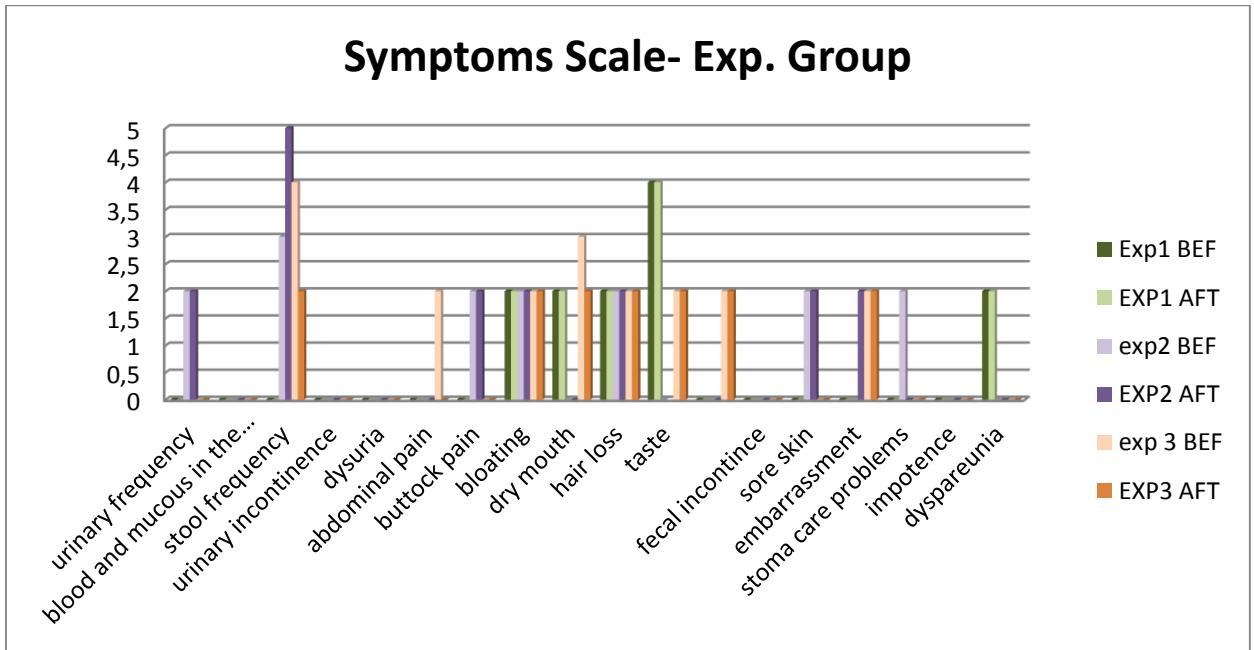
**Figure 32:** Control group: functional scales assessed by QLQ-CR29.

**Legend:** PT, patient ; B- Body

#### D .QLQ-CR29 QUESTIONNAIRE- SYMPTOMS SCALES

In concern of the evaluation of the symptoms scale of the both groups we can conclude that symptoms are not the most relevant factors that have evidence of Qigong

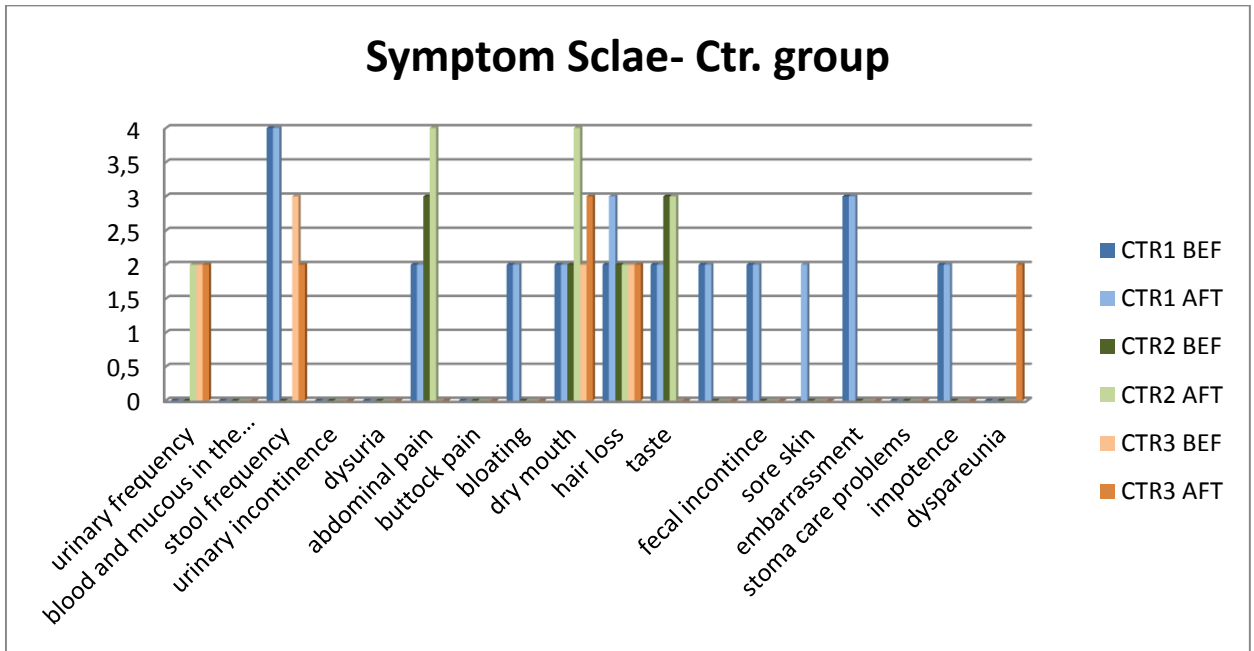
therapy. However, we could see some slightly changes between before and after the treatment in the both groups. In experimental group, the abdominal pain, stool frequency and dry moth have a significant decrease of the results (patient3). Patient 2, have a increase of the stool frequency after the study. The other symptoms maintained the same values during the study in the other patients.



**Figure 33.** Experimental group: symptomatic scales assessed by QLQ-CR29.

**Legend:** Exp, experimental group patient; AFT, after; Bef, before.

Regarding to symptoms items/subscales of the control group, it is shown that there was an aggravation of dry mouth and abdominal pain. The other symptoms maintained the al most the same values during the study in the other patients.



**Figure 34.** Control group: symptomatic scales assessed by QLQ-CR29.

**Legend:** CRT- control group patient; AFT- after; Bef - before.

#### E. EFFECTS OF QIGONG ON ANXIETY AND DEPRESSION LEVELS - HADS

The parameters of the evaluation of this scale (anxiety and depression of the patients) are significantly different from the both groups. In concern of anxiety control group show in patient 2 a increase of anxiety. The other patients maintained the same results. In experimental group the anxiety was noticeably reduced in all the patients. On the other hand, in depression scale, in control group just one patient have decrease the levels, the others increase the score of depression, comparing to the experimental group that all of the patients have decrease depression score.

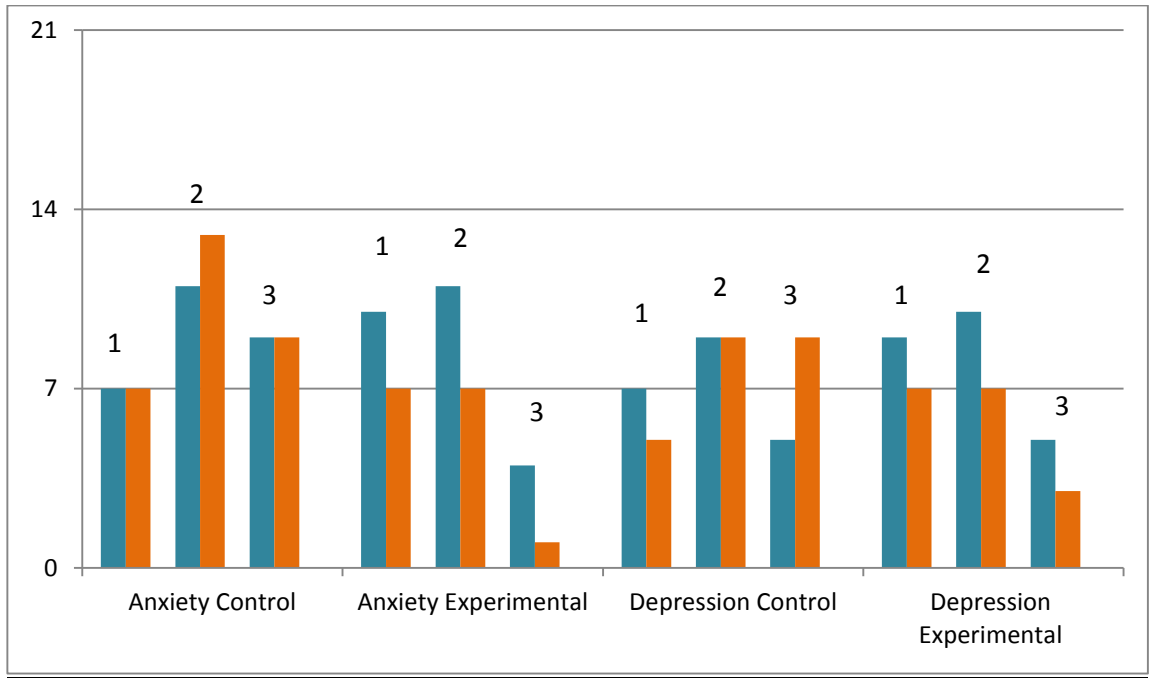


Figure 35. Anxiety and depression scale. HADS

## **IV - DISCUSSION**

## 8. DISCUSSION

This study is part of a global trend of clinical management and health research that begins to encourage the integration of complementary therapies in health care for cancer patients.

The functional state of the host immune system has a major prognostic and predictive impact on the fate of cancer patients treated with conventional or targeted chemotherapies.

Studies on CRC tumourigenesis and prognosis, showing that NK cell migration into tumour tissue is impaired in early stages of tumour development and that NK cells activity is reduced in metastatic CRC lead to conclude that NK cells have a pivot role on CRC tumourigenesis and being a first line of defense against metastasis. Moreover, Grivennikov et al revealed that the presence of NK cells in tumour immune microenvironment represents a positive prognostic marker, being the unique cells without a pro-tumourigenic role.

There is a lack of data regarding the effect of Qigong in immunity on cancer patients. In fact, only one study reported increased levels of WBC, RBC and NK cells.

To our knowledge, this is the most actual study on Qigong immunomodulation in CRC conducted in the West. Although, our results must be interpreted cautiously, due to small samples sizes, however we found trends of higher WBC and NK cells in the Qigong group relative to control group of patients with CRC undergoing chemotherapy.

Our results suggest that Qigong may have a myeloprotective role by reducing the rates of leukopenia and neutropenia in CRC patients undergoing chemotherapy. Moreover, our results suggests that Qigong is able to stimulate anticancer immunity through the augmentation of NK cells numbers on these patients.

Our results are in agreement in another one (Cai et al, 2001) that reported that qigong was able to highly improve patients immunity, specially WBC and NK cells.

Relatively to the QOL, patients show that in experimental group the anxiety and depression as well as chemotherapy effects are decrease comparing to the control group.

Further studies are needed to confirm these preliminary data as well as to compare if Qigong effects on CRC patients immunity and QOL are similar to those obtained with acupuncture and moxibustion (Pais et al 2014), namely the reduction of leukopenia and neutropenia rates in 1,5x, myeloprotective and immunoregulatory effects. And also to assess the effect of combined Qigong and acupuncture plus moxibustion on CRC patients.

We can still discuss about certain limitations that emerged along of the study and may be consider such as reduced sampling - It is necessary to carry out a study with a larger sample, to allow precise evaluation of the effect of Qigong in patients with CRC

undergoing chemotherapy, the short duration of the study did not allow more precise data on the impact of Qigong treatments in the parameters of immunity and quality of life of patients at a later stage to treatment (follow-up).

## **IV – CONCLUSION**

## 9. CONCLUSION

The present results suggest that Qigong not only improves the general condition of the patient as regards the quality of life and mental status, and is also associated with improvement of the immune system.

In this context, this clinical research protocol is unique with respect to its objectives and evaluation methods.

Our clinical research protocol is a pilot study, however revealed that *Qigong* treatment according to the Heidelberg Model of Traditional Chinese Medicine :

- may have a effect on immunity recovery;
- have a myeloprotective effect (leukopenia).
- improve the quality of life and general health of patients;
- improve the psycho-emotional state of patients, since there was a reduction in levels of anxiety and depression.

This research may have contributed to the production of new scientific knowledge in the field of complementary treatments in oncology and clarify whether a low-cost and no side effects therapy may help to improve the overall results of the multidisciplinary cancer treatment.



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