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# Efeito da acupunctura nas células NK em doentes com cancro colorectal submetidos a quimioterapia

# - Estudo prospectivo, randomizado e controlado

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TESE DE MESTRADO APRESENTADA
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# EFEITO DA ACUPUNCTURA NAS CÉLULAS NK EM DOENTES COM CANCRO COLORECTAL SUBMETIDOS A QUIMIOTERAPIA

## - ESTUDO PROSPECTIVO, RANDOMIZADO E CONTROLADO -

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

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## DEDICATION

I dedicate this study to

my precious friends

that are

always with me.

**ACKNOWLEDGMENTS**

I would like to thank my closest family and friends for all their background support that has enabled me to develop my own education.

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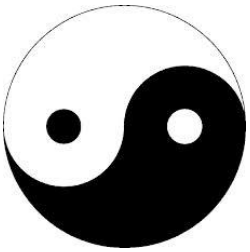

*Any medicine, to the extent that it justifies the qualification of "rational science", does not directly combat disturbances defined empirically, i.e., their symptoms, their symptomatic abnormalities. Instead, it strives to isolate and define general or basic postulates producing these disturbances"*

Prof. Dr. Manfred Porkert, 1983

*Traditional Chinese Medicine is a system of sensations and findings designed to establish a functional vegetative state.*

Prof. Dr. Henry Johannes Greten

**RESUMO**

**Introdução:** As células *Natural Killer* (NK) parecem constituir a primeira linha de defesa contra as células tumorais. A relevância das células NK é suportada pela associação entre uma diminuição da actividade, ou baixa quantidade de células NK circulantes em pacientes com progressão tumoral. No carcinoma colorectal (CRC), uma diminuição da actividade e do número de células NK parece estar associado a um pior prognóstico. De acordo com dois estudos chineses, a acupunctura e a moxabustão (AcuMoxa) poderão ter um impacto positivo nas células NK no CRC, as quais podem ser distinguidas em dois subtipos: citotóxico ($NK^{dim}$) e imunoregulador ($NK^{bright}$). Contudo, o efeito da acupunctura na sua expressão permanece por esclarecer.

**Objectivo:** Determinar os efeitos da AcuMoxa no sistema imune dos doentes com CRC, principalmente o seu efeito nas células NK e seus subtipos.

**Métodos:** Doentes com CRC serão recrutados a partir do Departamento de Oncologia do Centro Hospitalar S. João e distribuídos em dois grupos: o grupo experimental (n=9) e o grupo de controlo (lista de espera; n=9). No grupo experimental, os doentes serão submetidos a tratamento de AcuMoxa segundo o Modelo de Heidelberg de Medicina Tradicional Chinesa (8 sessões/mês). Serão efectuadas colheitas de sangue periférico em quatro tempos distintos: a primeira colheita (t=0) será efectuada na semana anterior ao tratamento de quimioterapia e as restantes três colheitas, a cada 8 dias. O tratamento de AcuMoxa consistirá dos pontos LI4, TB5, SP9, Lv3, St36, GB39, PC5 e Lu7.

**Parâmetros primários:** Hemograma completo; linfócitos T e B, células NK e subtipos $NK^{dim}$/ $NK^{bright}$ 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:** Dois estudos abordaram a modulação da actividade dos subtipos de células NK em doentes oncológicos. Baseado nestes dados sobre acupunctura e células NK, pretendemos confirmar uma melhoria do *status* imune, mais especificamente nas células NK. Adicionalmente, esperamos revelar qual o subtipo NK mais prevalente durante cada fase da QT / intervenção (T0, T1,T2 e T3) incluindo a magnitude do rácio $NK^{dim}$/ $NK^{bright}$.

**Conclusão:** O tratamento de AcuMoxa segundo o Modelo de Heidelberg poderá promover a actividade das células NK, especificamente a sua citotoxicidade, de acordo com a literatura. No futuro, este tratamento complementar poderá ter um impacto positivo no prognóstico e na QOL dos doentes com CRC.

**ABSTRACT**

**Introduction**

NK cells appear to represent a first line of defence against tumour cells. This relevance of NK cells is supported by an association between decreased activity or low numbers of circulating NK cells in patients with progression of cancers. In colorectal cancer (CRC), decreased activity and low number of NK cells appears to be associated with a worse prognosis. According to two Chinese studies, acupuncture and moxibustion may have a positive impact on NK cell, which can be distinguished in two subsets: cytotoxic ($NK^{dim}$) and immunoregulatory cells ($NK^{bright}$). However, the effect of acupuncture on its expression remains unclear.

**Objectives**: To assess the effect of acupuncture and moxibustion on the immune function of colorectal cancer patients.

**Methods**: CRC patients recruited from the Oncology department of Centro Hospitalar S. João, were randomized in two groups, intervention (n=9) and control/waiting list (n=9). In the intervention group, patients were submitted to acu-moxibustion treatment protocol based on the Heidelberg Model of TCM (8 sessions/ month). Blood samples will be collected at baseline (T0) on the week prior to chemotherapy and the following samples are collected every once a week until next chemotherapy (CT) regimen. The acupuncture/moxa treatment will consist of the points LI4, TB5, SP9, Lv3, St36, GB39, PC5 and Lu7.

**Primary Outcome:** Complete Blood counts, T and B lymphocytes, NK cells, $NK^{dim}$/ $NK^{bright}$ counts by flow cytometry.

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

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

**Discussion**: two studies have addressed modulation of NK cells subset activity in cancer patients. Based on these data on acupuncture and NK activity, we would like to confirm a improvement in immune status and more specifically in NK cells. In addition, we hope to unveil which NK subset is more prevalent during each phase of the CT / intervention (T0, T1, T2 and T3) regimen, including magnitude of $NK^{dim}$/ $^{bright}$ ratio.

**Conclusion:** Acupuncture/moxa treatment according to the Heidelberg Model may promote NK cells activity, especially cytotoxic according to literature. In the future, this complementary treatment might have a positive impact in the prognosis and QOL of CRC patients in the future.

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| | |
|---|---|
| 5-FU | 5- fluorouracil |
| ACTH | adrenocorticotropic hormone |
| ADCC | antibody-dependent cell cytotoxicity |
| ALT | algor laedens theory |
| ANC | absolute neutrophil counts |
| APC | antigen presenting cell |
| BRAF | serine/threonine-protein kinase |
| CCR7 | chemokine receptor 7 |
| CEA | carcinoembryonic antigen |
| CRC | colorectal cancer |
| CTL | cytolytic T cells |
| DC | dendritic cell |
| DCBE | double-contrast barium enema |
| DNA | desoxirribonucleic acid |
| EC | ethics committee |
| EGFR | epidermal growth factor |
| FAP | familial adenomatous polyposis |
| G-CSF | granulocyte colony-stimulating factor |
| GM-CSF | granulocyte macrophage colony-stimulating factor |
| H | Hepatic |
| HNPCC | hereditary nonpolyposis colorectal cancer |
| IBD | inflammatory bowel disease |
| IFNγ | interferon γ |
| IGF1 | insulin-like growth factor-1 |
| IGFBP-3 | insulin-like growth factor-binding protein-3 |
| IGFII | insulin-like growth factor II |
| IL | Interleukin |
| KIR | killer cell immunoglobulin-like Receptors |
| KRAS | Kirsten rat sarcoma viral oncogene homolog |
| L | Lienal |
| LAK | lymphokine-activated killer |
| LHA | lateral hypothalamic area |
| LV | leucovorin |
| MC | microcirculation |
| MHC | major histocompatibility complex |
| NCR | natural cytotoxicity receptors |
| NF-Kβ | nuclear transcription factor- Kβ |
| NK | natural killer |
| NO | nitric oxide |
| P | pulmonal |
| p53 | protein 53 |
| Pb | peripheral bllod |
| PC | pericardial |
| PDGF | platelet-derived growth factor |
| PGE2 | prostaglandin E2 |
| PMN | polymorphonucleocytes |
| PSC | primary sclerosing cholangitis |
| PSGL-1 | PEN5P-selectin glycoprotein ligand-1 |
| QOL | quality of life |
| R | renal |
| RNA | ribonucleicc acid |
| RS | respondens |
| ST | stomachal |
| TAMs | tumour-associated macrophages |
| TCM | tradicional chinese medicine |
| TGFα | tumor growth factor |
| Th | T helper |
| TK | Tricaloric |
| TME | total mesorectal excision- |
| TNF | tumour necrosis factor |
| TNM | Tumour nodules metastases |
| VEGF | vascular endothelial growth factor |
| WBC | white blood cells |
| HPA | hypothalamus-pituitary-adrenal |
| SNS | Sympathetic nervous system |

# PART ONE – THEORETICAL BACKGROUND

# CHAPTER I – IMMUNOLOGICAL ASPECTS OF CANCER

## 1. Immunoediting Theory

Cancer is widely considered to be a cell-autonomous genetic disease that results from alterations in oncogenes, tumour-suppressor genes and genome-stability genes. However, the tumour-cell microenvironment, the stroma and immunity also have a major role in cancer. Indeed, for the development of a neoplasia, cancer cells have to overcome intrinsic (cell autonomous) and extrinsic (immune mediated) barriers to oncogenesis.

Initial studies on cancer immunobiology, proposed the hypothesis of "**immunosurveillance**": the immune system is able to recognize and destroy proliferating cancer cells, which express unique antigens at surface that are by-products of the process of malignant transformation and the immune system's capacity for surveillance can be modified through both medical and lifestyle interventions. Initially, was proposed that an impaired immune system would fail to react even to a highly immunogenic tumour and this impairment was due to genetic factors, radiotherapy or immunosuppressive drugs. Nowadays, it is know that, except for hormone therapy, all conventional treatments for cancer can suppress immune response.[1]

Later studies recognized the existence of a highly complex interrelationship between cancer and the immune system and suggested the replacement of the term "immunosurveillance" by a more comprehensive term, "**immunoediting**" (figure 1). The cancer immunoediting process engenders **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 as result of constant pressure from the immune system, cancer cells can undergo genetic alterations becoming resistant to immune attack and, thus, are free to grow even in the presence of an intact immune system.[2]

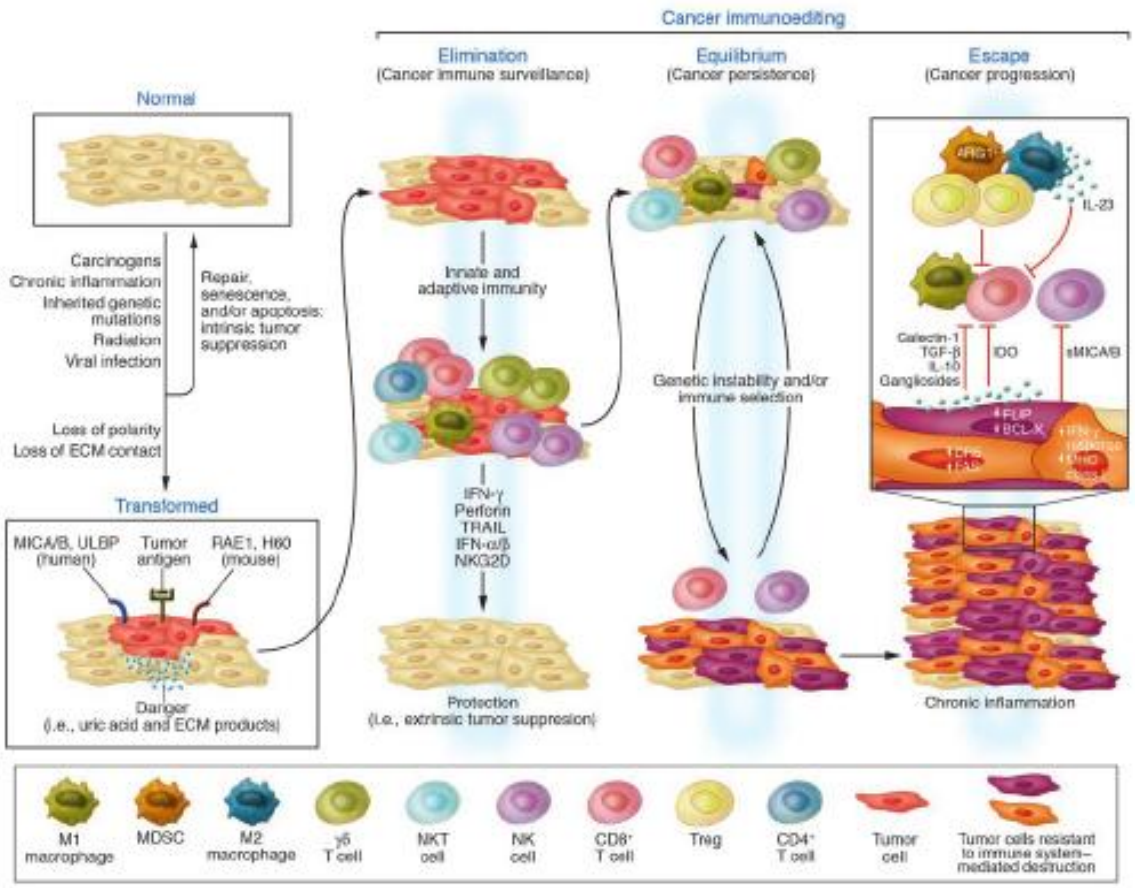



**Figure 1: Cancer immunoedting theory.** Diverse immune cells involved in the different phases. Extracted from Swann *et al*, 2007 [3]

*Elimination phase* represents the classical concept of cancer immunosurveillance. As reviewed by Dunn[4], cancer immunosurveillance has two critical effectors functions: IFNγ production and cytotoxicity. Interferon (IFN) γ facilitates development of powerful anti-tumour effector functions mediated by both adaptive and innate immunity: generation of tumour-specific $CD4^+$ Th1 T cells and cytolytic T cells (CTL) and activation of cytocidal activity in macrophages. Moreover, IFNγ has the capacity to upregulate tumour immunogenicity, explaining the effects on tumour detection and elimination in immunocompetent hosts. Perforin was identified as a critical cytolytic molecule in the primary host anti-tumour response.

The molecular definition of tumour antigens provides a firm basis for how the adaptive immune system discriminates between normal and neoplasic cells. The tumour antigens are recognized by CD8+ αβ T cells in the context of MHC class II and class I proteins, respectively.

Another important component used by both adaptive and innate cells to distinguish cancer cells from normal cells a receptor expressed on the surface of NK cells, the **NKG2D-activating receptor**. NKG2D binds to the MHC class I chain-related proteins A

an B, to the UL16 binding proteins and to the lymphocyte effector cell toxicity-activating ligand (LETAL).

According to the immunoediting hypothesis, tumour's immunogenic phenotype is continuously shaped by the immunological factors in its environment and, thus, the dynamic interaction between immunity and cancer in the equilibrium phase produces new populations of tumour cells, unstable and rapidly mutating cells. In the equilibrium phase, the tumour has three possible outcomes: eventual elimination by the immune system; permanent maintenance in the equilibrium phase by cellular and molecular controls of immunity, or escape from immune pressure and transit to the final escape phase of the immunoediting process.

In the escape phase, tumour growth proceeds unrestrained by immune pressure. To become clinically detectable in an immunocompetent host, cancer cells must circumvent both innate and adaptive immunologic defences. It has been proposed several mechanisms through which tumour escape from immune control. Tumour escape can be a) a direct consequence of alterations occurring in edited tumour targets themselves, like for instance tumour cells develop lesions in antigen processing and presentation pathways that facilitate evasion from adaptive immune recognition; b) due to the inhibition of the protective functions of the immune system; and c) due to suppression of pro-inflammatory danger signals.[4]

**Tumour microenvironment**

Robert Strausberg[2] highlights the importance of studying the genetics and phenotypes of the tumour surrounding microenvironment, as well as the cancer cells. In fact, there is compelling evidence that the innate and adaptive immune cells of the tumour microenvironment engage an extensive and dynamic crosstalk with cancer cells, through direct contact or by chemokine and cytokine production[5], leading to conclude that tumour related-inflammation and anti-tumour immunity co-exist at different points along tumour progression and that the environmental and micro environmental conditions dictate the balance between the two.

In fact, some studies[6,7,8] show that several components of the tumour microenvironment plays an important role in promoting tumour progression. Tumour stroma, containing fibroblasts, inflammatory cells and endothelial cells, plays an important role in promoting tumour progression; where myofibroblast-driven desmoplasic stromal reaction is considered to be a poor prognostic indicator in primary CRC. Several cytokines (TGF-beta, PDGF, IL4 and IGF II), that induce myofibroblastic differentiation, seem to

have pro-oncogenic functions.[6] In addition the presence of ECM molecules in tumour microenvironment means the existence of an inflammatory process, which predisposes to the acquisition of malignant characteristics for tumour cells.[7]

The microenvironment of pre-malignant and early tumour lesions is generally composed by immune cells (tumour-infiltrating lymphocytes), such as T lymphocytes, macrophages and dendritic cells (DC) – a component of an inflammatory host response. The most frequent immune cells within the tumour microenvironment are tumour-associated macrophages (TAMs) and T cells. TAMs are associated with tumour growth and may be pre-requisite for angiogenesis, invasion and metastasis, being correlated with poor prognosis. T Cells have both tumour suppressive and promoting effects. In some cancers, increased T cell numbers were correlated with better survival and, low levels of T cell seem to be associated with more susceptibility to spontaneous or chemical carcinogenesis. However, evidence found in solid tumours has showed that many of T cells subsets are involved in tumour promotion, progression and metastasis.

The *NK cells* are the unique cells without a pro-tumourigenic role.[5] In fact; recent studies revealed that the presence of NK cells in tumour immune infiltration represents a *positive prognostic marker*.

## 2. NK cells role in innate and adaptive immune system

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*. It recognizes non-self molecules according to a specific pattern. Another feature of the innate immune system is the complement, a group of inactive proteins in the blood which are activated in the presence of pathogens and non-self cells and cause cell lysis.[9]

As effectors members of the innate immunity, NK cells play a major role in anti-infection activity and tumour surveillance. NK cells can directly kill target cells to which they are capable of adhering within 1 to 4 hours without prior activation, priming or assistance by cytokines. NK cells can be triggered through various receptors depending on specific ligands presented by target cells in a given encounter.

NK cells have been recognized as major producers of cytokines in many physiological and pathological conditions, such as IFNγ, tumour necrosis factor (TNF$\alpha$)

and interleucine (IL)-10, as well as growth factors such as granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and IL3. NK cells also secrete several chemokines, which are vital for their co-localization with other hematopoietic cells such as DC in areas of inflammation. The production of IFNγ is related with the modulation of T cell responses in lymph nodes, possibly by a direct interaction between naïve T cells and NK cells migrating from inflamed peripheral tissues to secondary lymphoid compartments and by indirect effect on DC, promoting the maturation of DC by NK cell-derived IFNγ and TNFα and subsequently leading to the enhancement of antigen presentation to T cells.

In resume, NK cells modulate emerging B and T cells and are also regulatory cells engaged in reciprocal interactions with DCs, macrophages, T cells and endothelial cells. Thus NK cells are *able to limit or exacerbate immune responses* (Figure 2).[9]

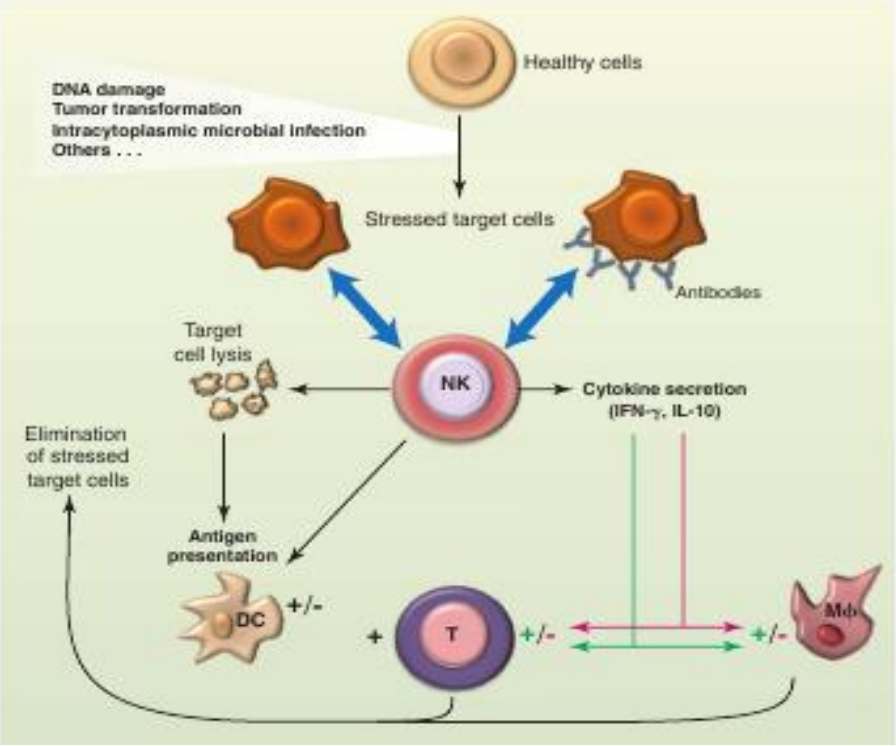



**Figure 2: The biological functions of NK cells.**
DC – dendritic cells. Mϕ - Macrophages. Extracted from Vivier *et al*, 2011[9]

In order to exert effectively their functions, cytolytic and regulatory function of the adaptive immune response, NK cells must be recruited to the site of infection or tumour and must be in closely proximity to antigen-stimulated T and/or B cells, respectively. They require triggering by several factors, such as IL15 presented by DC or macrophages, IL12 or IL18. IL 15 is an important for the maturation and survival of NK cells.

NK cells possess three main groups of receptors: the activating receptors, the inhibitory receptors and the chemotactic receptors (Figure 3). Among the activating receptors groups are included receptors that interact with cytokines (e.g. NKp46, CD16, NKG2D) and receptors that interact with cell surface molecules (e.g. FcRγ). This repertoire of receptors allows NK cells to exert their functions through various means: they can kill a variety of target cells in the absence of antibody; NK cells are able to detect antibody-coated cells through CD16 cell surface receptor and to exert antibody-dependent cell cytotoxicity (ADCC) and cytokine production. The natural cytotoxicity receptors, NKp46/NCR1; NKp44/NCR2 and NKp30/NCR3, play a crucial role in antitumoral responses.

The activating receptor NKG2D is responsible for the NK cells capacity to distinguish self molecules in conditions of cellular stress. This receptor interacts with various ligands that have low levels of expression in most tissues but are over-expressed in situations of cellular distress.

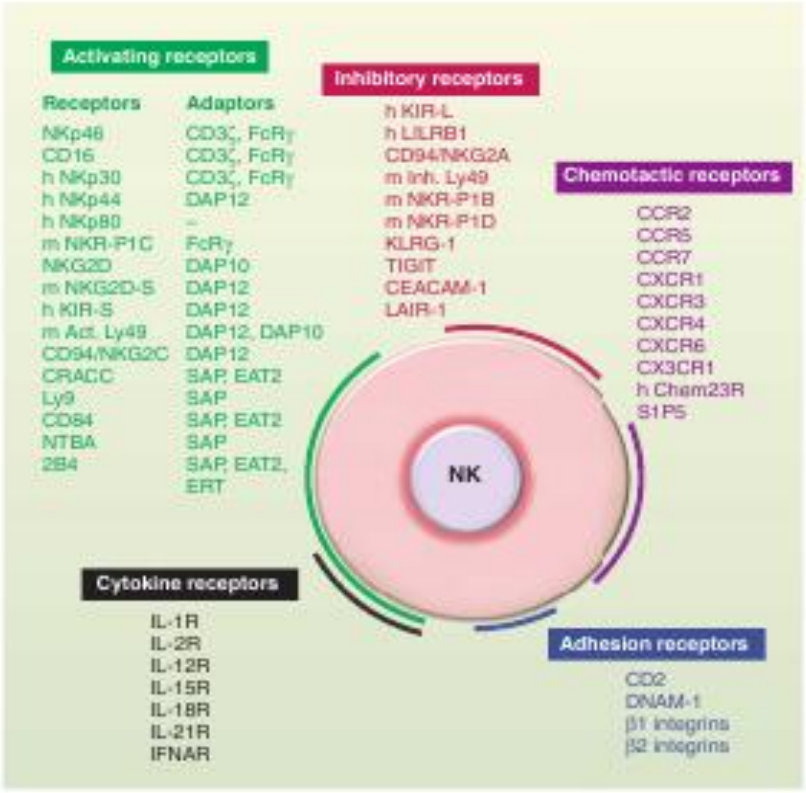



**Figure 3: NK cells receptors.**
Extracted from Vivier *et al*, 2011[65]

Another function attributed to NK cells is that they are able to detect the lack of major histocompatibility complex (MHC) class I ("missing self"), a situation that occurs during viral infections or cellular transformation (Figure 4). This is the reason why NK cells selectively kill target cells "in distress" and spare healthy cells. This is explained by the

expression on the NK cell surface of several MHC class I-specific inhibitory receptors, such as killer cell immunoglobulin-like receptors (KIRs) and CD94/NKG2D.

The array of receptors that individual NK cells express during development is largely random, therefore, some NK cells may express activating receptors for a self-ligand, but they do not express inhibitory receptors for self-MHC molecules. Thus NK cells, like T and B cells, have the potential for *auto-reactivity*. In order to avoid auto-reactivity, such NK cells acquire a state of hypo-responsiveness to self cells and also to several other stimuli, including MHC class I-deficient tumour cells or cross-linking antibodies specific activating receptors. The findings of several studies leads to the suggestion that in steady-state conditions, NK cell tuning enables NK cells with inhibitory receptors for self-MHC to rapidly eliminate MHC class I-deficient cells that arise in the environment, whereas NK cells with fewer such receptors can be mobilized by inflammatory signals that accompany pathogen infections.

The molecular mechanisms that govern responsiveness are yet not established, although it has been suggested that in the presence of high levels of activating ligands, a negative turning effect may occur (Figure 5). It has been proposed some possible mechanisms for the impaired responsiveness of NK cells: (1) the induction of an anergic state in NK cells that are not inhibited by MHC molecules, as can occur in auto-reactive T and B cells; (2) the failure of these NK cells to undergo functional maturation, which may depend on interactions between MHC and inhibitory receptors on NK cells; (3) the function of inhibitory receptors for non-MHC ligands or (4) the action of suppressor cells.

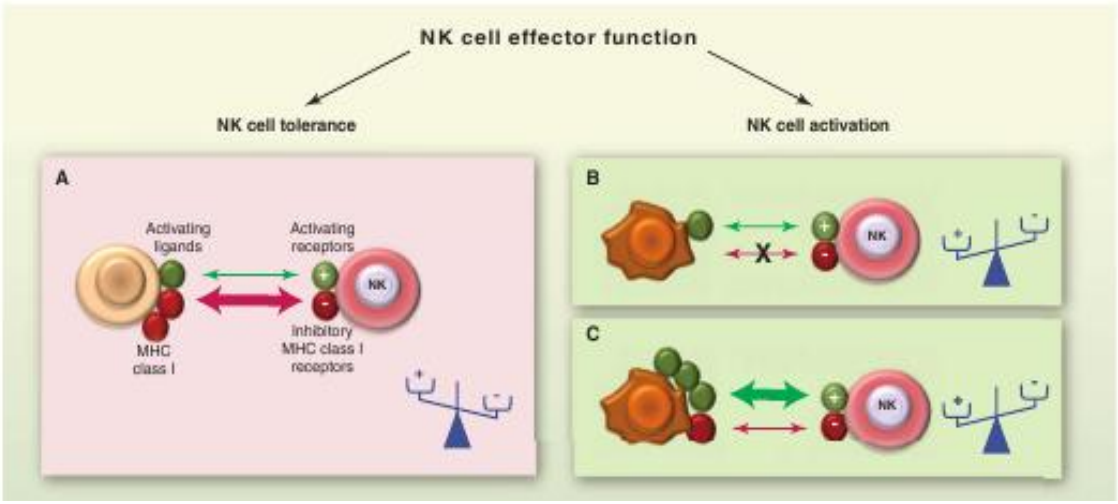



**Figure 4: The dynamic regulation of NK cell effector function.** NK cells sense the density of various cell surface molecules expressed at the surface of interacting cells. The integration of these distinct signals dictates the quality and the intensity of the NK cell response. NK cells spare healthy cells that express self-MHC class I molecules and low amounts of stress-induced self molecules (A), whereas they selectively kill target cells "in distress" that down-regulate MHC class I molecules (B) or up-regulate stress-induced self molecules (c). + activating receptors; - inhibitory receptors. Extracted from Vivier *et al*, 2011[9]

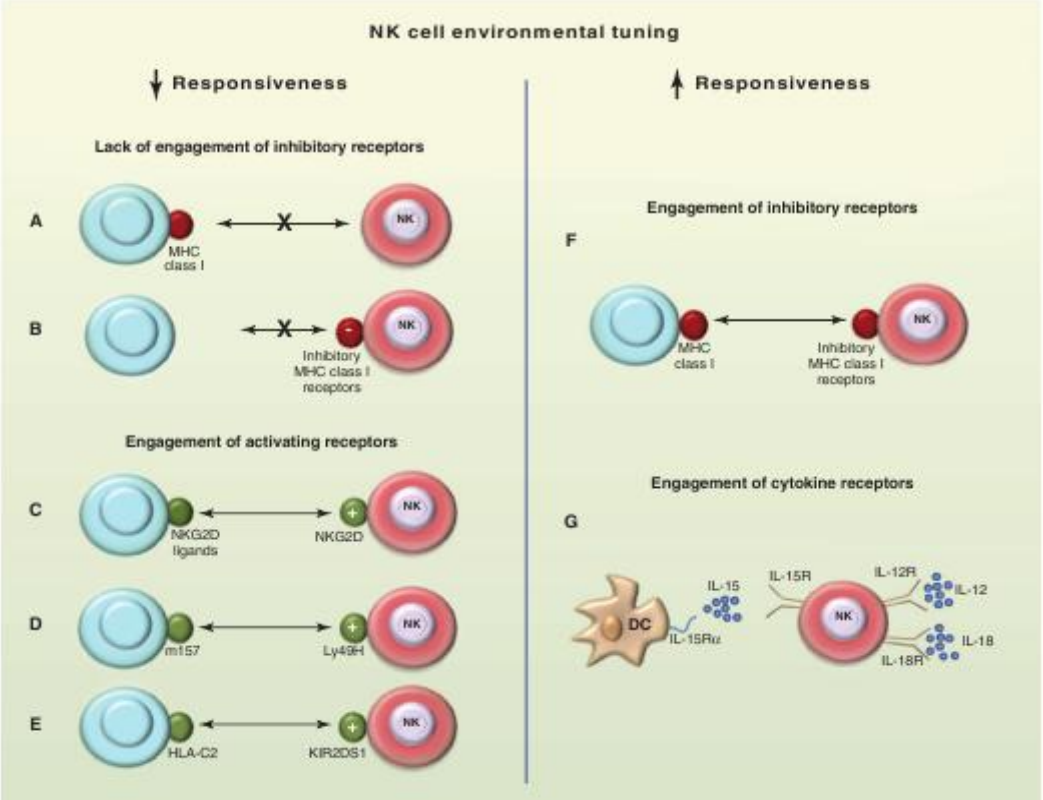



**Figure 5: NK cell tuning. Schematic experimental conditions in which NK cells show to adapt to their environment.** In the absence of detection of MHC class I (lack of MHC class I receptors) (A), or in MHC class I deficient hosts (B), NK cells are hypo-responsive at steady state. NK cells are rendered "anergic" by the chronic engagement of various activating receptors such as NKG2D (C), Ly49H in the mouse (D) or KIR2DS1 in humans (E). NK cells can be educated by MHC class I molecules via their cognate inhibitory receptors (F) and primed by cytokines (G). Extracted from Vivier *et al*, 2011[9]

## NK cells Subsets

The NK cells are lymphogranular cells having an ability to kill the intruding pathogens and are the most important cells of the system. They are found in the innate and the adaptive system as well. NK cells originate from the lymphoid progenitor and migrate to the secondary lymphoid tissues. When activated, NK cells burst forth from the tonsils, lymph nodes and spleen, and destroy infected and cancerous cells while the immune T and B cells are still mobilizing. They do not require memory from previous encounter and target virus infected cells and tumour cells and are the first line of defence.

Activity of the immune cells varies from person to person and may be genetically inherited. The ability to kill cancers cells in less than 24 hours varies from 97% to 2%.[10] NK cells have the ability to kill infected target cells and tumour cells and, also, to secrete

various effectors molecules. The physiological functions of NK cells are tightly regulated by a delicate balance between signals transmitted by activating and inhibitory receptors.

Human peripheral blood NK (pbNK) cells can be divided into two subsets based on their cell surface density of CD56 and CD16, the **CD56$^{dim}$** and **CD56$^{bright}$** subpopulations[13].

The majority of peripheral blood NK cells, about 90%, is the **CD56$^{dim}$** subset, which have low density expression of CD56 and express high levels of CD16 and has been shown to be responsible for the natural **cytotoxicity** against tumour targets.

**CD56$^{bright}$** NK cells, comprising 10% of NK cells, expresses high levels of CD56 and low levels of CD16. CD56$^{bright}$ NK cells secrete higher levels of different cytokines such as IFNγ , TNFβ, GM-CSF, IL-10 and IL-13 in response to monokine stimulation. CD56$^{bright}$ NK cells exhibit low natural cytotoxicity and ADCC, but have potent lymphokine-activated killer (LAK) activity, representing the **immunoregulatory** subset. CD56$^{bright}$ NK cells are able to regulate adaptive T and B cell responses in secondary lymphoid tissues.[11]

CD56$^{dim}$ NK cells are associated with low capacity to produce cytokines, are potent mediators of ADCC, LAK activity and natural cytotoxicity and have a more granular morphology than CD56$^{bright}$ NK cells.[12] A recent study suggest that CD56$^{dim}$ CD16$^{+}$NK cells also have their unique abilities in influencing events occurring in their microenvironment.[14]

Moreover, the two NK cell subsets can be distinguished concerning their **receptor expression profiles** (Figure 6). The CD56$^{bright}$ NK cell subset expresses high levels of CD94/NKG2A and low levels of killer Ig-like receptors (KIRs), high amounts of chemokine receptor 7 (CCR7) and L-selectin (CD62L). The expression of CCR7 and L-selectin seems to be responsible for their mobilization to secondary lymphoid tissues. The CD56$^{dim}$ NK cells subset show no expression of CCR7 and little or no L-selectin, but high levels of another adhesion molecule – PEN5P-selectin glycoprotein ligand-1(PSGL-1), suggesting that they migrate to peripheral nonlymphoid tissues. Although, after their activation at sites of inflammation in the peripheral tissues, CD56$^{dim}$ NK cells express CCR7, promoting their migration to secondary lymphoid tissues.[11,13] CD56$^{dim}$ NK cells also have high level expression of KIRs[15]

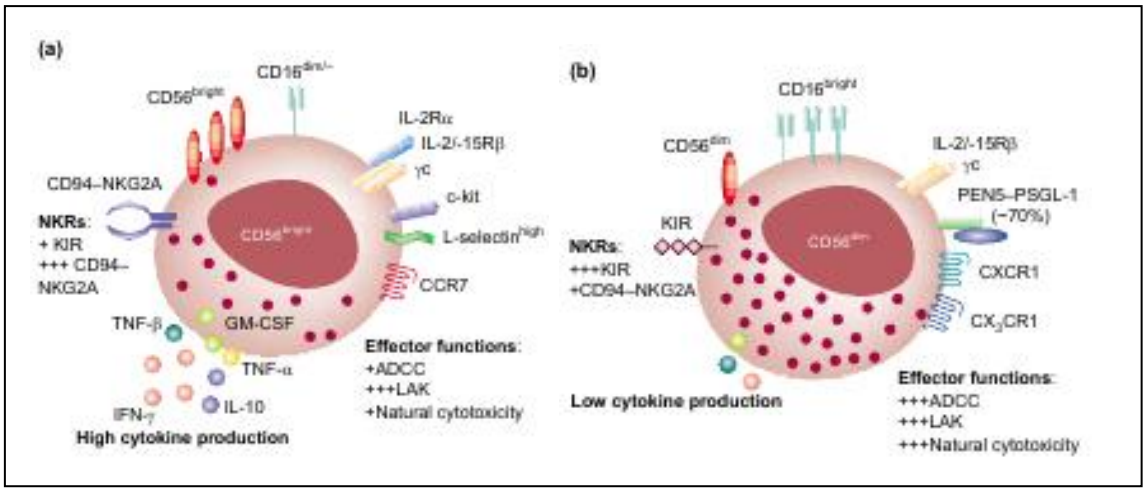



**Figure 6: NK cells subsets and surface receptors: NK$^{bright}$ (a) and NK$^{dim}$ (b).**
Extracted from Cooper 2001[13]

As previously described, they exist in both activator and inhibitory forms, and are grouped in three major superfamilies: (1) the killer cell Ig-like receptor (KIR) superfamily, (2) the C-type lectin superfamily (CD94, NKG2 receptors); and (3) natural cytotoxicity receptors (NCRs). Another class of NK' receptors have been described, the Ig-like transcripts (ILTs). NK receptors (NKRs) are crucial for distinguishing normal cells from infected and/or transformed cells by monitoring the expression levels of MHC molecules.[12]

Opposite to CD56$^{dim}$ NK cell, CD56$^{bright}$ NK cells have low or no expression of KIRs and ILT-2 (an inhibitory receptor) and high level expression of CD94-NKG2A inhibitory receptors.

Both NK cells subsets, as well as T cells, macrophages and CD8$^{+}$ T cells, largely express the activating NKG2D receptor, which has the capacity to interact with a diverse family of MHC class I-related ligands that are induced by cellular stress.[16]

KIRs control the response of NK cells by delivering inhibitory or activating signals upon recognition of MHC class I ligands on the surface of target cells. They provide an alternative means of modulating the immune response to damaged or foreign cells.[16]

The NCRs are exclusively expressed on NK cells and appear to be the main receptors involved in antitumoral responses.

Relatively to NK maturation, Yu *et al*[17] suggested that the density of CD94 surface expression on CD56$^{dim}$ NK cells identifies a functional and developmental intermediary between CD56$^{bright}$ and CD94$^{low}$CD56$^{dim}$ NK cells, and that CD56$^{bright}$ NK cells progress through a *continuum* of differentiation that ends with CD94$^{low}$CD56$^{dim}$ phenotype. [13]

### 3. NK cells in cancer immunity

Several studies verified that the lack of NK cells or molecules associated with NK cells recognition or effectors function in mice was associated with an increase of tumour incidence. In another studies the loss of immune competence, more precisely the low levels of NK cells, is considered an important cancer risk factor and allows to conclude that NK cells participates in immunosurveillence against certain types of tumours.[3]

The NK cells act in tumour immunosurveillance (Figure 7), by directly inducing tumour cells death (NK act as cytolytic effectors lymphocytes), even with the absence of surface adhesion molecules and antigenic peptides.[4,9] NK cells initially recognize certain 'stress' or 'danger' signals that are produced by tumours. NK-cell lysis of cancer cells could provide tumour antigens for DCs, which induce them to mature and present antigen to CTLs in lymph nodes. Cytokines, such as IFNγ , produced by activated NK cells, activate CTL and helper T-cell ($CD4^+$) responses. This leads to the proliferation of helper T cells and cytokine production. Activated NKT cells can also induce the antitumour activity of NK cells. Cytokines that are produced by NK cells might also regulate B-cell production of antitumour antibodies. [18]

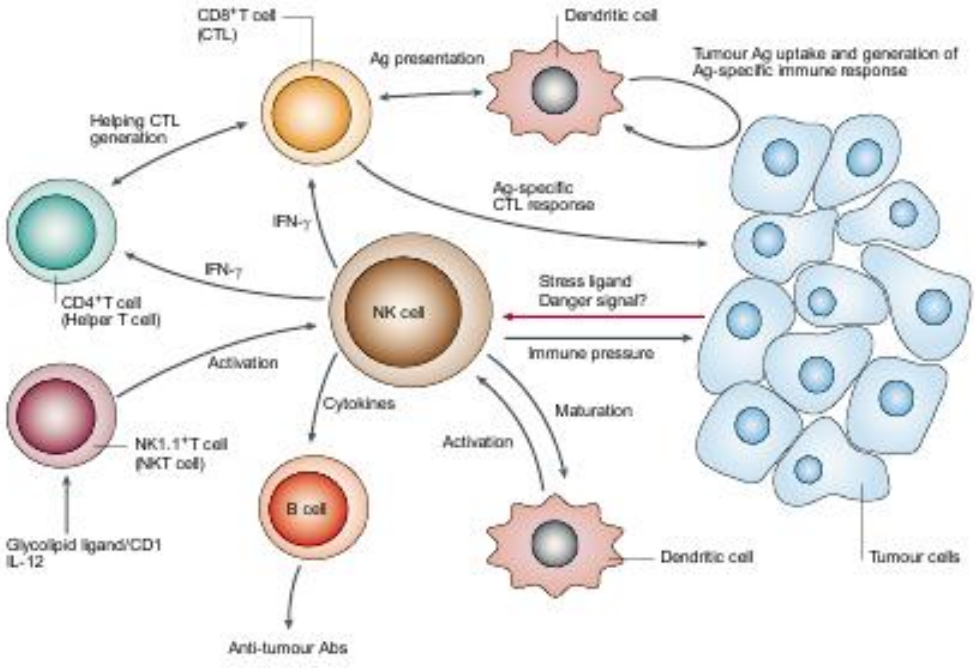



**Figure 7: Schematic presentation of NK role in immunoediting theory.**
Extracted from Smyth *et al*.[18]

According to the immunoediting theory cancer cells and immune cells reciprocally modulate each other and the two possible outcomes are: the elimination or escape of

tumour cells. NK cells are considered to represent a *first line of defence against the metastatic spread of tumour 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.[19] Recent studies of tumour-associated NK cells demonstrated a striking phenotype, supporting the notion that tumour-induced alterations of activating NK cell receptor expression may hamper immune surveillance and promote tumour progression. These tumour-induced alterations includes, in some cases the *down-regulation of activating receptors and in others, the over-expression of inhibitory receptors leading to reduced cytotoxicity*.

Studies in metastatic melanoma patients reveal decreased activity and IFN$\gamma$ production of NK cells and a redistribution of NK subsets: an increase of non-cytotoxic NK ($CD56^{bright}$) cells and a decrease of cytotoxic NK cells ($CD56^{dim}$). Additionally, they found a decreased NKG2D activating receptor and an over-expressed inhibitory NK cell receptor (CD158a), which is correlated to lower NK cell cytotoxicity.[20]

## 4. Co-stimulatory molecules that regulate NK-cell antitumour immunity and link innate and acquired immune system

NK cells and DCs have the ability to reciprocally activate one-another, through two possible distinct mechanisms: (1) cell contact involving unknown receptor-ligand pairs and (2) through soluble mediators produced by the two cells. The cytokines, TNF$\alpha$, IL-2, IL-12, IL-18 and IFNγ, have all been implicated in this process. The end result of these interactions is NK cells activated for cytotoxicity, IFNγ production, and proliferation and DC that have matured and are capable of cytokine production and T cell activation (Figure 8).[21]

NK cells can be activated by tumour-cell- or DC-surface molecules, such as CD70, CD80 or CD86. Activated NK cells proliferate and produce cytokines such as IFNγ, or kill the tumour cell or DC. IFNγ that is produced by activated NK cells can promote (a) the antitumor acquired immune response, inducing the $CD8^+$T cells to become antigen-specific CTLs, which lead to the development of immunological memory against the tumour; and (b) promote differentiation of $CD4^+$T cells towards a Th1-dominant state which promotes CTL differentiation. NK-cell activation following DC-NK-cell interaction can also result in DC maturation. These DCs can facilitate the generation of $CD8^+$T-cell

responses through their ability to internalize and present T cells with tumour-specific antigens, which were derived from NK-cell-mediated tumour-cell lysis. [18]

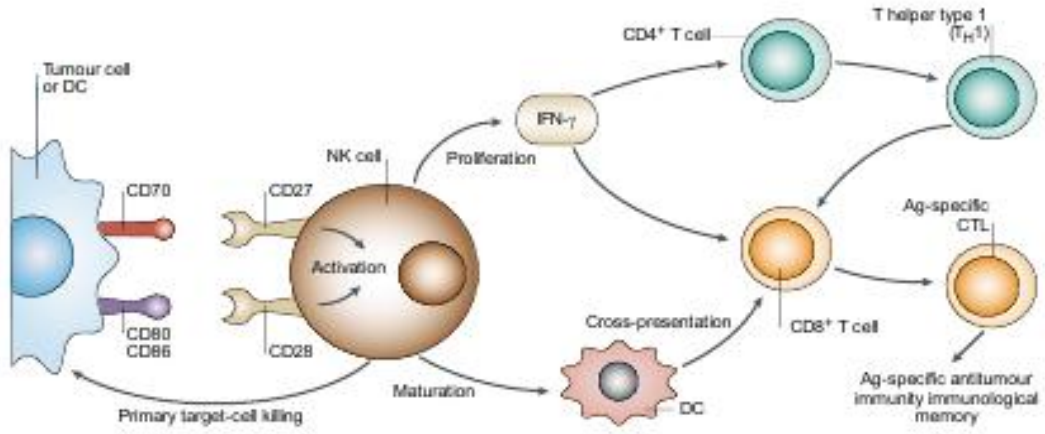



**Figure 8. Role of CD56$^{bright}$ NK cells and T cell in the interaction between adaptive and innate system.** Extracted from Smyth *et al*.[18]

Todd *et al,*[22] demonstrate that CD56$^{bright}$ NK cells and T cell are crucial cells in the interaction between adaptive and innate system. They found that endogenous T cell-derived IL-2, acting through the high affinity IL-2 receptor, co-stimulates CD56$^{bright}$ NK cells to secrete IFNγ.

## 5. Immune parameters affecting the efficacy of chemotherapy regimens

The outcome of chemotherapy can be influenced by the host immune system at multiple levels. Chemotherapy can kill cancer cells by causing them to elicit an immune response or alternatively, by increasing their susceptibility to immune attack. In addition, chemotherapy can stimulate anticancer immune effectors either in a direct fashion or by subverting immunosuppressive mechanisms. Beyond cancer-cell-intrinsic factors that determine the cytotoxic or cytostatic response, as well as the potential immunogenicity of tumour cells, 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.[23]

It was supposed that chemotherapeutic agents elicit immunogenic tumour cell-death and the release of immunogenic signals. These signals are recognized by innate immune effectors (DCs) and trigger an adaptive immune response, which includes CD8$^{+}$ T cells and IFNγ signalling, and allows the immune system to control residual tumour cells.

Assuming this supposition as real, the interruption of this pathway at any level would lead to therapeutic failure: (1) chemotherapeutic agent may be unable to trigger immunogenic cell death; (2) tumour cells may be unable to express the required set of immunogenic signals; (3) the innate immune system may be unable to recognize such immunogenic signals; and (4) local or systemic immunosuppression or tumour-dependent immunosubversion and immunoevasion can prevent the formation, recruitment or action of adaptive immune effectors.[23]

Studies performed in multiple human carcinomas suggest that DCs, M1 macrophages, cytotoxic $CD8^+$ T cells, Th1 $CD4^+$ T cells, NK cells and Th1 $CD4^+$ T cells present in the tumour bed possibly *reduce cancer growth*. And by contrast, neutrophils, M2 macrophages, myeloid-derived suppressor cells, Th2 $CD4^+$ T cells, B lymphocytes are suspected to *stimulate cancer growth*.

## 6. The immune system and the quality of life (QOL) in cancer patients

Cancer diagnosis is a significant source of anxiety, depression and emotional distress, followed by an extended period of stressful cancer treatment significantly compromise patients' QOL. About 20-40% of cancer patients exhibit significant levels of depression and anxiety, which was associated with higher frequency and severity of clinical symptoms, such as pain, fatigue, poor appetite, sleep disturbance and poor quality of life.[24]

Psychological stress may have multiple negative impacts on health outcomes, namely with a) higher incidence of infections, accelerated aging, and greater cardiovascular diseases in several populations; b) in breast cancer patients, with low physical and psychological QOL and significantly shorter disease-free interval; and with (3) a decrease of efficacy or resistance to chemotherapeutics agents, in animal models.

According to **psychoneuroimmunology**, psychological and emotional stress induces several alterations in diverse biological responses. The activation of the *hypothalamic-pituitary-adrenal axis* and the *sympathetic nervous system* may generate a change in the immune cell traffics and promotes inflammation via multiple neuroendocrine and immune pathways. Higher stress levels were associated with poorer immune responses (low NK cell activity) and the stress reduction with improvement of immune responses, in women with breast cancer. Furthermore, psychological and emotional stress has negative implications for increased symptom perception and poor quality of life,

and is significantly associated with increased neutrophil percentage but decreased lymphocyte percentage which has been associated with negative cancer outcomes.[24]

A study on early stage breast and prostate cancer found an association between the reduction of stress levels and the depressive pattern with a shift in the balance from a pro-inflammatory to an anti-inflammatory environment.[25]

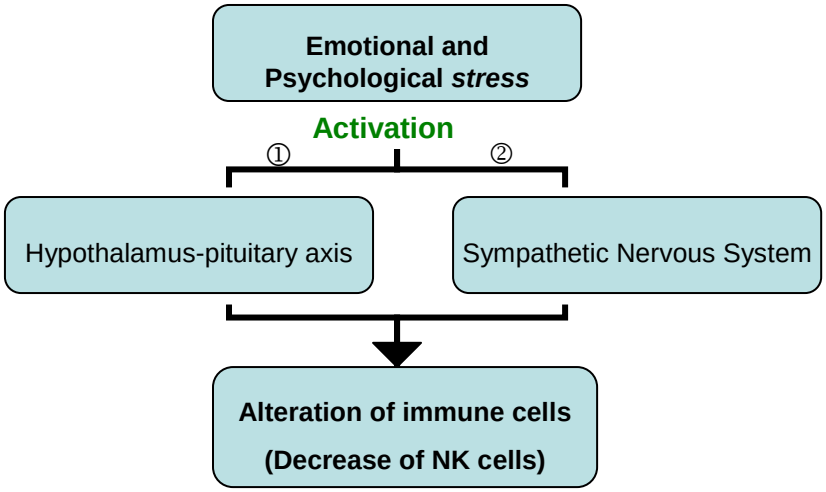
WHO defines **Quality of Life** as individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, personal beliefs and their relationship to salient features of their environment.

The effect that chemotherapy may have on QOL is extremely important. This QOL includes not only the individual's physical well-being, but their mental well-being, role functioning and levels of emotional distress as well.

The inclusion of QOL endpoints in chemotherapy trials with cancer patients started in earliest during the last decade, with the majority of studies assessing the impact of chemotherapeutic agents on symptoms such as pain and fatigue. Since then, the inclusion of QOL endpoints has become a more common outcome in chemotherapy studies, although many continue to neglect the psychological component, focusing instead on the occurrence of symptoms and their impact on physical QOL.

QOL may be considered *to be the effect of an illness and its treatment* as perceived by patients and is modified by factors such as impairments, functional stress, perceptions and social opportunities.[26]

The most widely applicable instrument to measure the QOL in cancer patients is the **EORTC QLQ-C30**.



# CHAPTER II – COLORECTAL CANCER OVERVIEW

## 1. Epidemiology and Etiopathophysiology

Colorectal cancer (CRC) is the fourth leading cause of cancer deaths worldwide in both men and women.[27] Approximately two thirds of the cases are located in the colon.

The disease is often considered as one, irrespective of location, but should probably be regarded as two distinct entities as biology, risk factors, treatment and outcome may differ.[28,29] Most CRCs (95%) are epithelial cancers (*adenocarcinoma*) that originate from glandular tissue. Other, rarer cancers in the colon and rectum are *squamous cell carcinoma and lymphoma*.

In Portugal, CRC is the second most common cancer among Portuguese men and women, with an incidence rate of 40.6 and 24.1 cases per 100,000, respectively and with a mortality rate of 19.6 and 16.4 per 100,000, respectively. [30]

In respect to CRC mortality in Europe, Portugal is the 8$^{th}$ country with highest mortality indexes, and the male rates higher than female rates; Hungary accounts with the highest mortality rates for both sexes and Greece and Cyprus accounts with the lowest rates.[31]

The development of CRC has been associated with several important risk factors, which can be aggregated in two main groups: the **"environmental" factors** group and the **genetics factors** group.[32]

The findings from epidemiologic studies that physical inactivity, excess of body weight and a central accumulation of adiposity have influence on CRC risk, as well as the ethnic and racial differences in CRC and studies on migrants suggest that an environmental factor plays an important role in aetiology of this disease. In fact, diet is considered an important factor of increased risk in 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 correlates significantly with later tumour development. On the other hand, diets rich in vegetables and high fibber grains as well as the consumption of fish and skinless chicken demonstrate a protective effect in the pathophysiology of CRC.

In fact, the modifiable lifestyle factors are thought to contribute substantially to the geographical difference in CRC incidence[33].

Mutations in the genes **Kras** and **p53** are highly relevant for CRC development and the same mutations have been linked to reduced physical activity, smoking and diets high in red meat.[34]

Regular exercise may increase the number and activity of macrophages, natural killer cells, and cytokines with the ability to kill cancer cells and thereby improve anti-tumour immune responses.[35] Furthermore, obesity may be a risk factor for CRC development independently of inactivity. Visceral adiposity is associated with low-grade chronic inflammation leading to up-regulation of the nuclear transcription factor-Kβ (NF-Kβ) with transcription of genes that promote tumourigenesis as a consequence.[36] Prostaglandin E2 (PGE2) is reduced with high levels of physical activity, possibly through insulin-like growth factor-1 (IGF-1)[37] and PGE2 affects both tumour immunity and tumourigenesis.[38] The motility of the intestine may affect CRC risk as shortened intestinal transit time reduces the exposure of the mucosa to faecal carcinogens. Exercise is thought to increase the expression of PGF2 and the vagal tone, both stimulating motility. Diets high in fiber, fresh fruits and fresh vegetables may also shorten the intestinal transit time and the formation of fiber may produce mucosa-protecting agents, thereby affecting CRC risk. However, studies regarding diet, transit time and CRC risk are contradictory and firm conclusions cannot be drawn.[39,40,41,42]

Insulin and IGF-1 are up-regulated in the absence of physical activity and in obesity.[51] Increased levels of insulin and IFG-1 are correlated with risk of CRC [52] possibly through mechanisms that activate the Wnt pathway and increase the expression of pro-angiogenic proteins such as HIF-11 and vascular endothelial growth factor (VEGF).[36] Furthermore, elevated levels of the reciprocally expressed insulin-like growth factor-binding protein-3 (IGFBP-3) have been shown to be associated with reduced risk of CRC.[53]

Increasing age[43] and a family history of CRC[44] predispose to the development of CRC, and CRC is slightly more common in men. Furthermore, **genetic conditions** such as *familial adenomatous polyposis* (FAP)[45], *Lynch syndrome* (formerly HNPCC; hereditary nonpolyposis colorectal cancer)[46] and *Gardner's syndrome* (considered a subtype of FAP)[47]are genetic risk factors. The FAP syndrome accounts for approximately 1% of all CRC cases and patients will most likely present with adenocarcinoma before the age of 40. Therefore, prophylactic colectomy is recommended at an early age for FAP patients.[48]

*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 [47]. The disease is not curable and life expectancy with the condition is 35-45 years.

*Lynch syndrome* is an autossomal dominant trait (as FAP) predisposing for CRC, the associated mutations impair genes related to DNA mismatch repair. Approximately 1 to 4% of the cases of colon cancer are related to Lynch syndrome.[46]

Patients with *inflammatory bowel disease* (IBD; ulcerative colitis and Crohn's) disease have increased risk of developing CRC and the risk is more likely a result of chronic inflammation rather than genetic predisposition.[49] The progression from adenoma to carcinoma that occurs during development of sporadic colorectal tumours appears to be a sequence of inflammation – dysplasia – carcinoma in IBD-associated CRC[49]. Primary sclerosing cholangitis (PSC) is another chronic inflammatory disease associated with CRC when concomitant with IBD.[50] Pro-inflammatory factors of the innate and adaptive immune system contributes to the development and growth of colon neoplasia.

It is too early to establish a causal relationship between *smoking* and CRC, however most reports point in the direction that smoking is a risk factor.[51,54] Furthermore, smoking may be associated with different subtypes with different mutational background than non-smokers (e.g. p53, APC and Kras).[55] Heavy alcohol intake (>45g/day) has been associated with increased risk of CRC, while lower levels were not.[56] The increased CRC risk has been suggested to be associated with lower intake of folate connected with heavy drinking.[57]

In conclusion, there is evidence that **inactivity**, **red meat**, **smoking**, **alcohol**, and **obesity** are connected to increased risk of CRC, but the quality of the observations varies and in some cases, there are contradictory reports.

### 1.1. Clinical presentation

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.

Despite the increasing usage of CRC screening allows the early diagnosis, on an asymptomatic, of CRC, the majority of CRCs is diagnosed after the occurrence of symptoms. The symptomatic presentation of CRC is normally due to growth of the tumour into the lumen or adjacent structures and usually reflects relatively advanced CRC. The symptomatic presentation can be divided in two groups: symptoms that derive from the local tumour and symptoms related to metastasis.[58]

Symptoms/signs associated with **local CRC** include hematochezia or melena, abdominal pain, otherwise unexplained iron deficiency anemia, and/or a change in bowel habits. Abdominal distention, and/or nausea and vomiting, which may be indicators of obstruction, are less common presenting symptoms. Obstructive symptoms are more common with cancers that encircle the bowel, producing the so-called "apple-core" (seen on radiologic imaging). Rectal cancer can also cause also cause tenesmus, rectal pain, and diminished caliber of stools.

These clinical manifestations differ according on tumour location: a change in bowel habits is generally 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. Cecal and ascending colon tumors have a fourfold higher mean daily blood loss (approximately 9 mL/day) than tumors at other colonic sites.

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.

The symptoms/signs of **metastatic disease** include the presence of right upper quadrant pain, abdominal distention, early satiety, supraclavicular adenopathy, or periumbilical nodules. These categories of symptoms are explained by de fact that the most common metastatic sites are regional lymph nodes, liver, lungs and peritoneum. Since the venous drainage of the intestinal tract is via the portal system, the first site of hematogenous dissemination is generally the liver, followed by the lungs, bone, and many other sites, including the brain. However, tumors arising in the distal rectum may metastasize initially to the lungs because the inferior rectal vein drains into the inferior vena cava rather than into the portal venous system.

There are also a diversity of atypical presentations of CRC, including: a) local invasion or a contained perforation causing malignant fistula formation into adjacent organs, such as bladder (resulting in pneumaturia) or small bowel; b) Fever of unknown origin, the existence of abscesses (intra abdominal, retroperitoneal, abdominal wall or intrahepatic) due to a localized perforated colon cancer; c) *Streptococcus bovis bacteremia* and *Clostridium septicum* sepsis are associated with underlying colonic malignancies in about 10% to 25% of patients. Seldom, other extra-abdominal infections caused by colonic anaerobic organisms (eg, *Bacteroides fragilis*) may be associated with CRC.

The presence of symptoms and their particular type provide some prognostic importance: symptomatic patients at time of diagnosis have a more advanced disease and a worse prognosis; however, asymptomatic patients tend to have a more favorable pathological stage and consequently better prognosis.

### 1.2 Diagnosis

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. Once a CRC is suspected, the next test can be a colonoscopy, barium enema, or CT colonography. However, examination of tissue is required to establish the diagnosis; this is usually accomplished by colonoscopy. In fact, colonoscopy is the most accurate and resourceful diagnostic test for CRC, since it can localize and biopsy lesions throughout the large bowel, detect synchronous neoplasm, and remove polyps. Synchronous CRCs, refers to the diagnosis, within six months of an initial CRC, of two or more distinct primary tumors, separated by normal bowel, and not due to direct extension or metastasis.

The vast majority of colon and rectal cancers are endoluminal masses (exophytic or polypoid) that arise from the mucosa and protrude into the lumen. Bleeding may be seen with lesions that are friable, necrotic, or ulcerated. Circumferential or near-circumferential involvement of the bowel wall correlates with the so-called "apple-core" description seen on radiologic imaging.

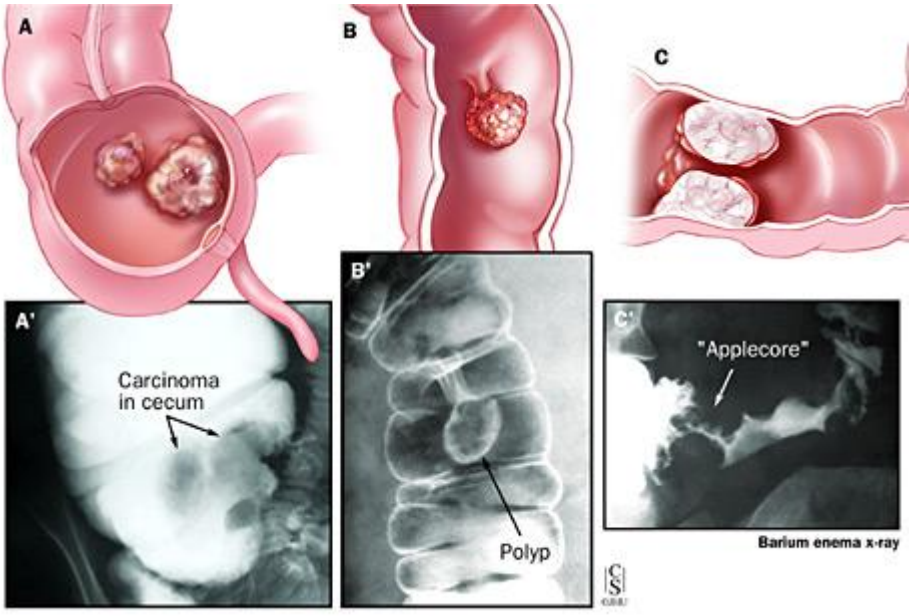



**Figure 9. Colonic features and corresponding barium enema X-rays.**
A: carcinoma in the cecum; B: pedunculated polyp; C: apple core lesion.
From: http://www.hopkinscoloncancercenter.org

A minority of neoplastic lesions in the gastrointestinal tract is nonpolypoid and relatively flat or depressed, which are more difficult to detect by colonoscopy.

If a malignant obstruction precludes a full colonoscopy preoperatively, the entire residual colon should be examined soon after resection.

**Flexible sigmoidoscopy** is generally not considered to be an adequate diagnostic study for a patient suspected of having a CRC, unless a palpable mass is felt in the rectum. In such cases, a full colonoscopy will still be needed to evaluate the remainder of the colon for synchronous polyps and cancers.

Barium enema is widely available and may be used to investigate patients with symptoms suggesting of CRC. 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.

Reasons for incompleteness include the inability of the colonoscope to reach the tumour or to visualize the mucosa proximal to the tumour for technical reasons (eg, partially or completely obstructing cancer, tortuous colon, and poor preparation) and patient intolerance of the examination. 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.

In respect to laboratory tests, although CRC is often associated with iron deficiency anemia, its absence does not reliably exclude the disease. There is no diagnostic role for other routine laboratory test, including liver function tests, which lack sensitivity for detection of liver metastases.

A variety of **serum markers** have been associated with CRC, particularly carcinoembryonic antigen (CEA). However, all these markers have a low diagnostic ability to detect primary CRC due low sensitivity for early stage disease. And on other hand, non-cancer-related causes can elevate CEA levels, including gastritis, peptic ulcer disease, diverticulitis, liver disease, chronic obstructive pulmonary disease, diabetes, and any acute or chronic inflammatory state. The American Society of Clinical Oncology (ASCO) and the European Group on Tumour Markers recommended that neither serum CEA nor CA 19.9 levels should be used as a screening or diagnostic test for colorectal cancer.

However, CEA levels do have value in the follow-up of patients with diagnosed CRC as well as on preoperative scenarios with the objective to aid in surgical treatment planning, post-treatment follow up, and in the assessment of prognosis: CEA >5 ng/mL have a worse prognosis, stage for stage, than those with lower levels; and elevated preoperative CEA levels that do not normalize following surgical resection imply the presence of persistent disease and the need for further evaluation.

Furthermore it's recommended to perform a serial assay of postoperative CEA levels for five years for patients with stage II and III disease candidate for surgery or chemotherapy (metastatic disease). A rising CEA level after surgical resection implies recurrent disease and should prompt follow-up radiologic imaging.[58]

### 1.3 CRC Staging

Two different staging systems are used to provide prognostic information and determine treatment strategy in CRC. The Duke classification system from 1932 [59] has been replaced by the more detailed TNM staging system developed and maintained by the American Joint Committee on Cancer (AJCC). Duke staging is no longer recommended for use in clinical practice and is not further discussed here. TNM staging (tumour, node and metastasis) is used on many solid cancers, adapted for CRC and is now in the 7$^{th}$ edition as of 2010. As Table I shows, the tumour is first scored with respect to the TNM variables then assigned to stage I-IV in the CRC specific (Table II). However, TNM does not adapt to recent advances in metastatic treatment. For example, the survival of a patient with resectable solitary liver metastasis is better than that of a patient with stage III disease.[60] Current observations regarding the clinical course of the patients with CRLM support emerging arguments for a new staging system in CRC. [60,61,62]

| TMN Stages | Disease extension |
|---|---|
| T – Primary Tumour | |
| Tx | primary tumour cannot be assessed |
| T0 | no evidence of primary tumour |
| Tis | carcinoma in situ: intraepithelial or invasion of lamina propria |
| T1 | tumour invades submucosa |
| T2 | tumour invades muscularis propria |
| T3 | tumour invades through muscularis propria into subserosa or into nonperitonealized pericolic or perirectal tissues |
| T4 | tumour directly invades other organs or structures and/or perforates visceral peritoneum |
| N – Regional Lymph Nodes | |
| Nx | Regional lymph nodes cannot be assessed |
| N0 | No regional lymph node metastasis |
| N1 | Metastasis in one to three regional lymph nodes |
| N2 | Metastasis in four or more regional lymph nodes |
| M – Distant Metastasis | |
| Mx | Distant metastasis cannot be assessed |
| M0 | No distant metastasis |
| M1 | Distant metastasis |

**Table 1. The 7th edition of AJCC-TNM classification system.**

| Stages | T | N | M |
|---|---|---|---|
| 0 | Tis | N0 | M0 |
| I | T1 | N0 | M0 |
| | T2 | N0 | M0 |
| II | T3 | N0 | M0 |
| | T4 | N0 | M0 |
| III | T1, T2 | N1 or N2 | M0 |
| | T3, T4 | N1 or N2 | M0 |
| IV | Any T | Any N | M1 |

**Table 2. The 7th Edition of AJCC-TNM Classification System.**

In respect to rectal cancer, the TNM staging should be used, being recommended the version 5 from 1997 over TNM version 6 (2002) and 7 (2010), due to marked interobserver variation in defining stage II and Stage II, on the version 7. And also, there is the need for further subclassification particularly of cT3 (Table 3).[63]

T1 tumours refer to cancers that are in a stalked adenoma and according to the submucosa (sm)-system in a sessile adenoma. The level of infiltration into the submucosa predicts the risk of lymph node metastases and thus the type of surgery.

| TNM | Stage | Extension to | |
|---|---|---|---|
| Tis N0 M0 | 0 | Carcinoma *in situ*: intraepithelial or invasion of lamina propria | |
| T1 N0M0 | I | Submucosa | |
| T2 N0M0 | I | *Muscularis propria* | |
| T3 N0M0 | II A | Subserosa/perirectal tissue | |
| | Substaging[a] | T3a | <1 mm |
| | | T3b | 1-5mm |
| | | T3c | 5-15mm |
| | | T3d | >15mm |
| T4 N0 M0 | II B | Perforation into visceral peritoneum (b) or invasion to other organs (a)[b] | |
| T1-2 N1 M0 | III A | 1–3 regional nodes involved | |
| T3-4 N1 M0 | III B | 1–3 regional nodes involved | |
| T1-4 N2 M0 | III C | ‡4 regional nodes involved | |
| T1-4 N1-2 M1 | IV | Distant metastases | |

**Table 3: Rectal cancer. TNM staging.**

[a] Subclassification based upon an evaluation using MRI before treatment decision is clinically valuable, and used in these recommendations. It can be used also in the histopathological classification but is not validated and not incorporated in any of the TNM versions (5–7).[b] This is the subclassification in TNM5. It has been reversed in TNM6 and 7.

## 2. Treatment management of CRC

Treatment options vary in accordance with the stage of the cancer – its size, position in the colon or rectum (Table IV), whether it has spread to other parts of the body, and the physical condition of the patient. In general, the treatment options for CRC are surgery, radiotherapy, chemotherapy and biological therapy. [64-69] Chemotherapy can be administered in different ways. **The Systemic chemotherapy** uses drugs that are injected into a vein or given by mouth. These drugs enter the bloodstream and reach all areas of the body. This treatment is useful for cancers that have metastasized. In the **Regional chemotherapy,** drugs are injected directly into an artery leading to a part of the body containing a tumour. This approach concentrates the dose of chemotherapy reaching the cancer cells. It reduces side effects by limiting the amount reaching the rest of the body.

Chemotherapy may be used at different times during the treatment of colon or rectal cancers. The chemotherapy used after surgery to remove the cancer is known as ***"adjuvant chemotherapy"***. Its goal is to prevent the cancer recurrence, by killing the

small number of cancer cells that may have been left behind at surgery or that might have escaped from the main tumour and settled in other parts of the body.

For some cancers, chemotherapy is given (sometimes with radiation) before surgery in order to reduce the cancer and make surgery easier. This is known as **"*neoadjuvant treatment*"** and is often used in treating rectal cancer.

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

Localized disease

The main treatment is **surgery.** The goal of surgery is a wide resection of the involved segment of bowel together with removal of its lymphatic drainage, and the resection should include a segment of colon of at least 5 cm on either side of the tumour, although wider margins are often included due to obligatory ligation of the arterial blood supply.

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.

The laparoscopic colectomy can be safely performed for colon cancer, particularly for left-sided cancer, with long-term oncological results similar to those of the conventional approach and with some advantages over the conventional approach, such as reduced pain, reduced length of hospitalization and reduced duration of ileus. For right-sided colonic cancers, the benefit is less obvious since anastomosis must be hand sew which requires a laparotomy.

The treatment of obstructive colorectal cancers consists in one or two stages. The two-stages procedures can include previous colostomy followed by colonic resection, or Hartmann's procedure first followed by colostomy closure and anastomosis. The one-stage procedure consists in subtotal colectomy or ileorectal anastomosis, in selected cases, segmental resection after intraoperative colonic lavage. To relieve obstruction from rectosigmoid cancer and allow subsequent one-step resection can be used an endoscopic stent.[64]

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). [64]

It have been recommended the surveillance not only of patients at high risk of recurrence as well as in early stage, which encompasses: [64]

- History and physical examination and CEA determination are advised every 3 to 6 months for 3 years and every 6 to 12 months at years 4 and 5 after surgery.
- Colonoscopy must be performed at year 1 and thereafter every 3–5 years looking for metachronous adenomas and cancers.
- CT scan of chest and abdomen every 6 to 12 months for the first 3 years can be considered in patients who are at higher risk for recurrence.
- CEUS (contrast enhancement ultrasound scan) could substitute for abdominal CT scan.
- Other laboratory and radiological examinations are of unproven benefit, being restricted to patients with suspicious symptoms.

Metastatic CRC

The majority of patients have metastatic disease that initially is not suitable for resection. Therefore it is crucial to distinguish patients in whom the metastases are suitable for resection and those with initially unresectable disease and whom the metastases can become resectable after a major response to chemotherapy. [65]

In unresectable metastatic CRC, the treatment of patients should be considered as a continuum of care in which the determination of the goal of the treatment is important: prolongation of survival, cure, improving tumour-related symptoms, stopping tumour progression and/or maintaining quality of life (QOL). In the last years the outcome of patients with metastatic CRC improved with a median survival almost reaching 24 months.[65]

The first line **palliative chemotherapy** consists of a fluoropyrimidine (intravenous 5-fluorouracil (5-FU) or oral fluoropyrimidines) in diverse combinations and schedules. The most frequently used regimens are a 48h infused regimen of 5-FU/leucovorin (LV) every 2 weeks (LV5FU2). The oral fluoropyrimidines capecitabine and uracil-ftorafur (UFT/LV) are an alternative to intravenous 5-FU/LV as monotherapy. [65]

Another chemotherapy combination can be used with higher response rates, longer progression-free survival and better survival than 5-FU/LV, the combination of 5-FU/LV/oxaliplatin (FOLFOX) or 5-FU/LV/irinotecan (FOLFORI). These two regimens, FOLFOX and FOLFORI, have similar activity, but differ on toxicity profile, where irinotecan induces more alopecia and diarrhoea and oxaliplatin induces more polyneuropathy. They

consist of a 48h administration every 2 weeks. It was showed that combination chemotherapy is not superior to sequential treatment in terms of overall survival, thus sequential therapy starting with fluoropyrimidine monotherapy is a valid option in selected and frail patients. The selection criteria of patients to enrol the monotherapy are not yet well establish, but it is estimated that about 15% of patients are treated initially with fluoropyrimidine alone.

An alternative to the combination of infused 5-FU and oxaliplatin, based on similar activity and safety, is the combination of capecitabine plus oxaliplatin (CAPOX/XELOX). [65]

The duration of chemotherapy for metastatic CRC remains controversial; however, options are a fixed treatment period, between 3-6 months, and treatment until progression or toxicity. The interruption of treatment or less intensive cytotoxic treatment should be considered in cases of cumulative toxicity, of unresectable metastases and if the disease is controlled. The maintenance treatment with fluoropyrimidine alone is advisable, prolongs the progression-free survival. In cases of tumour progression is indicated the reintroduction of combination chemotherapy.

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.

The anti-EGFR antibodies cetuximab and panitumumab are active as single agent in chemorefractory metastatic CRC. It has been shown that cetuximab improves survival of chemorefractory patients and panitumumab improves the progression-free survival. The anti-EGFR antibodies activity is confined to Kras wild-type tumours, not being its usage indicated in Kras mutant CRC. About 40% of metastatic CRCs are Kras mutant and 5% to 10% of CRC are BRAF mutant. Kras mutations and BRAF mutations are usually mutually exclusive. Thus, the anti-EGFR antibodies seem to be confined to BRAF wild-type CRC.

In patients presenting synchronously with primary colon cancer and metastases, and presenting symptoms of the primary tumour (e.g. occlusion, bleeding), it should be considered a resection of the primary tumour before starting chemotherapy. In patients with metastatic rectal cancer with symptoms of the primary tumour, it is indicated irradiation of the primary tumour, possibly combined with chemotherapy.

Resection of metastatic disease

The surgical resection is indicated for solitary or confined liver metastases, providing a 5-year survival rate ranging from 30% -35% to more than 50%. However, about 60%-70% of these patients will have a relapse following the resection of the hepatic metastases. In patients with resectable metastases, perioperative combination (3 months before and 3 months after surgery) chemotherapy with FOLFOX regimen improves the progression-free survival by 7%-8% at 3 years. In cases of lung metastases, its resection offers 25%-35% 5-year survival rates.

In patients with initially unresectable metastases, the combination chemotherapy regimens of 5-FU/LV in combination with either irinotecan (FOLFORI) or oxaliplatin (FOLFOX) seems to facilitate the resection of 7%-40%. However, about 75%-80% of these patients will relapse within 2 years of resection.

It is recommended a re-evaluation of the patient every 2-3 months if chemotherapy is continued; and during palliative chemotherapy it is recommended CEA evaluation, if initially elevated, and a CT scan of the involved regions after 2-3 months, and also the evaluation of the general conditions, the side-effects of the chemotherapy and the impact on the QOL of the patient and physical examination.[64]

Rectal cancer

From a practical point of view influencing treatment, the rectal cancers can be divided into four groups: very early (some cT1), early (cT1-2, some cT3), more advanced (cT3, some cT4) and locally advanced (cT4).[63] Therefore very early cancers normally correspond to the malignant polyps, are indicated for a local procedure, using the transanal endocopic microsurgery (TEM) technique. In the early tumours, due to the risk of recurrence or lymph node metastases is too high (>10%) it is recommended postoperative chemoradiotherapy or more safely a major surgery (total mesorectal excision-TME). When cancer diagnosis is achieved through a biopsy, a presurgical chemoradiotherapy is indicated for perform a local procedure. In more locally advanced tumours (most cT3, some cT4) is recommended radiotherapy followed by TME, reducing local recurrence rates. Even in the absence of extramural growth (cT2) in very low tumours, preoperative radiotherapy may be indicated because the distance to the mesorectal fascia is very small. In the most locally advanced, frequently non-resectable tumours (cT3, cT4) with overgrowth to organs not readily resectable (cT4a) is indicated preoperative radiochemotherapy with concomitant 5-FU, followed by radical surgery 6-8 weeks later.

The combination of radio and neoadjuvant chemotherapy has shown to be more efficient in the treatment of resectable rectal cancer, in stages II and III, resulting in lower recurrence and higher survival rates, in comparison to single treatments options (surgery or radio or chemotherapy).[66,69] The most used neoadjuvant chemoradiotherapy is the combination of capecitabine and oxaliplatin (XELOX) and radiotherapy. Capecitabine has been studied with results that suggest that may be equivalent to continuous infusion of 5-FU.[68] The efficacy of combined adjuvant therapy led to the investigation of effect of chemoradiation in the preoperative period and has been demonstrated that the combination of neoadjuvant chemoradiation provides better control of the local disease, lower toxicity and reducing the need for colostomy compared with patients at stage II or III submitted only to the adjuvant treatment.

Currently, the default schema of the treatment of Rectal Cancer in stages II and III is based on the preoperative chemoradiotherapy (XELOX) followed by surgical resection and adjuvant chemoradiotherapy (FOLFOX).[69]

### 3. Chemotherapy - common used agents and related side effects

The history of chemotherapy dates from the 20$^{th}$ century, being used to treat cancer only in the beginning of 1930. Paul Ehrlich introduced the term “chemotherapy” to describe the chemical treatment of disease. Chemotherapy has a great curative potential in some types of advanced cancer, such as acute lymphoblastic and acute myelogenous leukemia, Hodgkin’s and non-Hodgkin’s lymphoma, germ cell cancer, lung cancer, ovarian cancer and choriocarcinoma, among others. However, the curative potential is not always observed, nevertheless, it promotes significant improvement in progression-free and overall survival.[77]

The most common types of chemotherapy agents are alkylating agents, antimetabolites, taxanes, anthracyclines and platinum based (Table V).

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

**Table 4 Common types of chemotherapy agents and their mechanisms.**

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 hair loss, mouth sores, loss of appetite, nausea and vomiting, and low blood counts. As chemotherapy affect the blood forming cells of the bone marrow, it leads to low blood cell counts and subsequently can lead to increased chance of infections, easy bruising or bleeding and fatigue.

Along with these, some side effects are specific to certain medicines, for example:

- **Hand-foot syndrome** can occur during treatment with capecitabine or 5-FU (when given as an infusion). This starts out as redness in the hands and feet, which can then progress to pain and sensitivity in the palms and soles.
- **Neuropathy** (painful nerve damage) is a common side effect of oxaliplatin. Symptoms include numbness, tingling, and even pain in the hands and feet. It can also cause patients to have intense sensitivity to hot and cold in the throat and esophagus, causing swallowing problems.
- **Diarrhoea** is a common side effect with many of these drugs, but can be particularly worse with irinotecan.

Most side effects are short-term and tend to disappear after treatment is finished. Some, such as hand and foot numbness, may persist for a long-time.

Regarding to CRCs, the most common side related to the chemotherapeutic agents used are resumed in the table below.

| **Regimens** | **XELOX** (capecitabina + oxaliplatin) | **FOLFOX** (5-FU/LV/oxaliplatin) | **FOLFIRI** (5-FU/LV/irinotecan) |
|---|---|---|---|
| **Side effects** | • Nausea and vomiting<br>• Diarrhoea<br>• Hand-Foot syndrome<br>• Neuropathy | • Risk of infection<br>• Diarrhoea<br>• Mouth sores<br>• Han-foot syndrome<br>• Numbness or tingling<br>• Plantar-palmar syndrome | • Nausea and vomiting<br>• Risk of infection<br>• Mouth sores<br>• Anemia |
| **Type** | Neoadjuvant treatment | Adjuvant treatment | Palliative treatment |

**Table 5. Most common regimens associated side effects in the management of CRCs**

It is well recognized that chemotherapies induce imunosuppression, depleting selectively a regulatory immune cell population at some doses – potential immunomodulation of chemotherapies.[79] The local or systemic immunosuppression can prevent the formation, recruitment or action of cognate immune effectors, thus precipitating therapeutic failure and sealing the patient's inexorable fate.[80]

## 4. CRC Immunity

The development and progression of CRC may be influenced by different factors, such as microsatellite instability (MS) or inflammatory mediators. Inflammation is considered a risk factor for tumour progression and metastasis formation. According to the immunoediting theory, the innate and adaptive immune system can protect against tumour development. Moreover, as stated before, the tumour stroma influence growth, survival and invasiveness of cancer cells contributing to the tumour microenvironment, together with immune cells.

Innate immunity, which is responsible for early detection and elimination of malignant cells, may be inefficient in patients who develop malignancy[81]. Several innate immune cells are implicated in CRC development and progression, macrophages, lower numbers and high density of mast cells, neutrophils, NK cells and DCs.[82]

Studies suggest that tumour-associated macrophages (TAMs) enhance the proliferation of CRC cells. This pro-tumorigenic activity is exert by the macrophages through mainly: (1) the release of IL-1β, which, subsequently, inhibit the tumour necrosis factor related apoptosis inducing ligand (TRAIL); and (2) the release of cytokines, growth factors and inflammatory mediators that may directly influence and stimulate the growth and migration of tumour cells; indeed, the macrophages' release of IL6 and IL10 are associated with CRC progression and prognosis. A low number and high density of MCs, an elevated neutrophil/lymphocyte ratio, as well as the decreased number of NK cells and DCs are associated with worse prognosis, poorer survival time and higher rate of recurrence in CRC. [82]

It has been proposed that once CRC become clinically detectable, the adaptive immune response plays a role in preventing tumour recurrence and metastasis. The adaptive immune system seems to have a double-faced role, being the balance between immune-surveillance (CD8+ and CD4+ T cells) and tumour-promoting inflammation (diverse types of T lymphocytes) to change over time, and eventually dictating disease progression.

CD8+ T lymphocytes constitute one of the leading effector of antitumor immunity by the release of lytic components via cell-cell interaction, the perforin. This cytolitic protein, is found in the granules of CD8+ and NK cells determines cell death by disruption of the cell membrane and the secreted enzymatic proteases leads to activation of the apoptotic pathway. The CD4+ lymphocytes are distinguished in Th1 lymphocytes, that secrete IFNγ, TNF$\alpha$ and IL-2, and in Th2 Lymphocytes that secrete IL-10, IL-4 and IL-5.

The CD4+ lymphocytes have a regulatory action on CD8+ cells: IL-2 is essential for CD8+ T cells proliferation and TH2 cells limit CD8+ T cells proliferation.

The role of B lymphocytes in cancer immunology remains complex and somewhat controversial. However they seem to play a complementary role in the antitumoral immunity: additionally to the antibody production, B lymphocytes mediate several other functions fundamental for immune homeastasis, namely the antigen-presenting role in the initiation of T-cell immune responses. Depending on their state of activation, B lymphocytes (in resting state) can suppress T-Cell mediated antitumour immunity by acting on CD4+ and CD8+ T cells and, when activated, can enhance the ability to generate tumour-infiltrating lymphocytes in vitro involving anti-CD3 and Il-2. [82]

In despite of undefined exact mechanisms in the regulation of tumour immunology is the role of regulatory T cells (Treg cells), a T-cell population that functionally suppresses an immune response by influencing the activity of another cell type. It was showed that increased levels of Treg cells are associated with a favourable prognosis in CRC and their role appears to depend on tumour pathogenesis. [83]

Human epithelial tumour cells of colorectal origin frequently produce the soluble carcinoembryonic antigen (sCEA) molecule, the soluble form of a tumour-associated antigen. sCEA has been evaluated as a phenotypic marker in relation to the presence of various cancer diseases. Identified as a member of the Ig superfamily, it has been shown to act as a homotypic and heterotypic cell-adhesion molecule. This production is directly related to the degree of differentiation, and it has been studied for its use as a prognostic marker for tumour progression and patient survival. It is known that sCEA can interfere in NK cell/ tumour-cell interaction and suppresses T- and B-cell responses. [84] In fact, the authors found that the increase in sCEA levels in patients with colon cancer is correlated with the rise in sIL-2R, IL-4 and IFN-γ serum levels, suggesting suppressive immune conditions, as IL-4 (a Th2 cytokine) suppresses IFNγ, inhibiting Th1 and NK cells. Berghella et al, also highlights the fact that the impaired NK cells functions can be re-established using anti-CEA antibody in an *in vitro* model, suggesting that a correction of the host's immune response could represent a new means of treatment for CRC. [84]

Resuming, despite the host immune system can initiate an immune response against CRC, tumour cells may utilize different ways to escape these defence mechanisms, such as the loss or downregulation of HLA class I antigens, the lack of co-stimulation defective death receptor signalling, apoptosis of activated T cells, immunosuppressive cytokines and activation of suppressor T cells.

The immune system may promote, in cases of chronic inflammation, progression of CRC, but also can induce tumour regression through the innate immune system with tumour specific activation of CTLs and effector CD4+ T cells. The high incidence and death rate of CRC suggest that the immune response is ineffective in many cases.

### 4.1 Role of NK cells in CRC prognosis

Prognosis of CRC patients is dependent on the local immunological tumour microenvironment. In addition to tumour stage, infiltration of NK cells in the tumour margin is independent prognostic factors in CRC patients. The infiltration of these cells into tumour is inversely correlated with the presence of a BM-like structure around the tumour cell nests and the presence of certain HLA-I loci. [85]

The results obtained from a study that analysed the number and activity of NK cells in the peripheral blood 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. [86]

Halama *et al* [87] found that NK cells are scarce in CRC tissue despite high levels of chemokines and cytokines, and the NK cell migration into CRC tissue is impaired early during tumour development by mechanisms that do not affect T-cell infiltration. In fact, they found that 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.

# CHAPTER III – TRADITIONAL CHINESE MEDICINE

**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.

In the 1950s, these principles were modernized in order to integrate many anatomical and pathological notions with modern scientific medicine. Leibniz developed the binary numbering system out the *I Ging* ("The Book of Changes", the oldest book of mankind) which enables to describe circular processes, the monad, bigram and trigram. The *yin* and *yang* signs as presented on this book may be considered as a mathematical expression of numbers (monad). 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.[88]

Chinese Medicine may be defined as "a system of sensations and clinical signs and findings designed to define the regulatory state of the body" [89] For many thousand years is rooted on the approach that human being as whole and in interaction with its environment, thus embedded in the rhythm of the Universe.[90] 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. Another major characteristic of 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. The organization of the findings according to a system of correspondence allows identifying these functional systems (*Zang Fu*). [88]

The most fundamental element in Chinese medicine is the diagnosis[89], and encompasses four major basis steps: a) eight diagnostic principles; b) recognition of the pathogenic factor; c) identification of *orbs* [1]affected and d) constitution. According with Porkert[88], Chinese medicine is primarily concerned with the function, movement and vital manifestations that characterize every pathological abnormality. "Agents" are defined as factors that elicit a functional deviation and these can be exogenous (climatic or social), endogenous (emotional or constitutional factors favouring a deviation from the normal function), and neutral agents. A pathological response ("heteropathy") is a deviation or impairment from de straight or correct direction of function ("orthopathy") and is determined by agents of disease. According to the ancient Chinese medicine the

[1] *Orbs* refer to a group of diagnostically significant signs and findings that are grouped and named after organs or the region where some of the symptoms take place.

predisposition of each individual reacts and copes with diverse events in life is associated with the five elements (wood, fire, earth, metal and water), and that is reflected in the physical appearance as in emotional state/ behaviour.

The "Heidelberg model" of TCM is a new scientific theory of Chinese Medicine that decodes the major postulations of Chinese medicine to western medical concepts and reveals the role of unbalanced states as major determinants of illness.[91]

## 1. Heidelberg Model of Chinese Medicine: TCM as System Biology

According to Greten,[91] the integration of Chinese Medicine in Western Healthcare systems requires three preconditions: 1) Chinese Medicine should be rationally accessible; 2) Scientific evidence of the underlying mechanisms, clinical efficacy and general safety has to be raised and, 3) Quality control measures have to be put up on the basis of the developing knowledge of this medical system.

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 supported by the Leibniz' analysis of the *I Ging* ("The Book of Changes").

In the Heidelberg Model,[91] which is a scientific model to allow a rational access to Chinese medicine and stresses the value of the internal logic of the Chinese Medicine, the central postulates of Chinese Medicine are recompiled and explained as a logical model of system biology based on a mathematical language.[91] Leibniz developed the binary numbering system out the *I Ging*, which enables to describe circular processes, the monad, bigram and trigram. The *yin* and *yang* signs as presented on this book may be considered as a mathematical expression of numbers (monad). In classical Chinese natural sciences and philosophy binary numbers were used to describe circular movement, the quarter sections of a circular movement. An example of such a movement is the circular process of the seasons - spring, summer, autumn and winter. The Trigrams, representing $1/8^{th}$ of the process allows to distinguish half-seasons or half-daytimes or shorter sections of the human behaviour which rhythmically occur during the day. The Taiji sign means that *Yin* and *yang* form a binary language of numbers that are able to describe circular functions such as regulation.

The biological network of regulation in humans is polygenic and therefore not linear. In TCM four main descriptive models (guiding criteria - *Bagang*) have evolved to organize the complex relationships of body regulation, at four different physiological

levels: the neurovegetative level, humorovegetative level, the neuroimmunologic level, and the cellular level (figure 9). Current understanding of these criteria is that they consist of an extension of the vegetative regulatory curve on processes such as microcirculation ("heat/cold"), defence mechanisms (theory of six stages of the *Shan Han Lun*) and the relation of the amount of the cell population and the respective regulatory processes (the *yin*, "substance").[92]

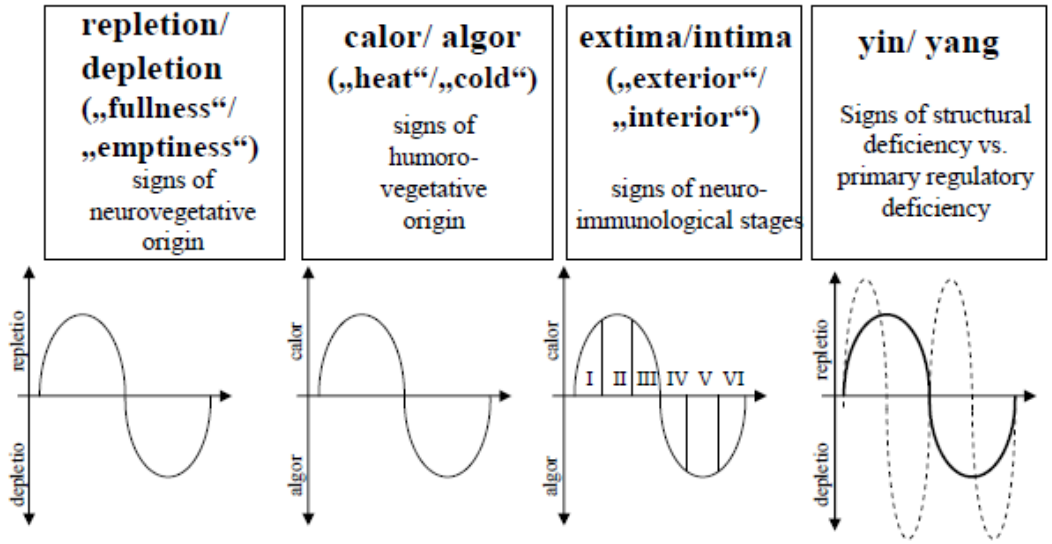



**Figure 10. The parallelism between TCM concepts of disease and physiological process at four levels of regulation, according to Heidelberg Model of TCM.**

The guiding-criteria, as directional standard conventions of physiology, permit the interpretation of the actual symptoms that are a manifestation of the overall body regulation.

The first GC is repletion/depletion ("excess/emptiness"), and refers to clinical signs that have a primary neurovegetative origin. Repletion is analogous to relative over-excitation of neurovegetative activating mechanisms, while depletion is a lack of respective activation or excess of de-activation.

The second GC is "*calor*/*algor*" (heat/cold) and evaluates clinical signs predominantly originated from the humorovegetative system in western medicine terms. These signs include: (1) the effect of microcirculation within the disease on a systemic and regional level (local interdependent mechanisms of the plasma, blood cells, endothelium, and functional tissue); (2) the activation of body fluids, evoking vegetative and systemic responses in the context of fluid distribution, fluid supply and circulation. Thus, "*calor*" includes signs of over-activation of *xue* and "*algor*" signs of a lack of functional microcirculation.

The third GC is "*extima*/*intima*" ("exterior/interior") and evaluates signs that apparently originate from the effects of a pathogenic factor (agent) invading the body from the exterior (The model of the six stages - *Shang Han Lun*; "*algor laedens* theory"). According to Prof. Porkert, the process of the agent "*algor* damaging the body" or "*algor laedens* theory" (ALT), have a parallel on clinical signs induced by neuroimmunological mechanisms, on western perspective. According to this theory, when "*algor*" affects the body system, it causes a regional lack of microcirculation through defence reflexes to cold, through viruses (adhesion molecules, complement system, coagulation) in face of which and a counter-reaction ("*reactive calor*") take place, resulting on a general increase in microcirculation, inflammation, fever and sepsis.

The fourth GC is *yin*/*yang* and according to TCM, consists on the evaluation of signs that distinguish between primary deregulation (*yang*) and secondary deregulation due to structural deficiency (*yin*). In cases of deficient functional tissue, it prompts an up-regulation with increasing of tissue function that cannot be kept up and consequently resulting in functional deficiencies. In western terms, this can be explained by a deficient cell population that can be vegetatively overstimulated and cause vegetative clinical signs similar to repletion, followed by a phase of almost functional break-down with signs similar to depletion. As such, in *yin*-diseases, symptoms are due to deficiency of the functional tissue ("body substance", *yin*). As the term "*yin*" refers also to *xue*[2], body fluids[3] and *Jing*[4], the *yin*-diseases may also be due to lack of *xue* (lack of microcirculation within the tissue), lack of body fluids (lack of *milieu*-factors), lack of *jing* (impaired functions of the cell nucleus, or genetic deficits). *Yang*-diseases symptoms are due primarily to deregulation described by the first three guiding-criteria, while *Yin*-diseases by these last.

Are described four kinds of mechanisms that may cause disease according to TCM: (1) excess of an agent; (2) transitional problems from one "evolutive phase[5]" to the next; (3) imbalance of antagonist phases; (4) *yin* deficiency. [89]

In summary, Chinese Medicine developed as a systematic rational on clinical signs which allow the definition of the regulatory status of the individual. As it has is foundations largely on reflexology and on a rational theory of vegetative (autonomic) nervous system activation patterns, Chinese Medicine can be considered a vegetative medicine, as postulated by the Heidelberg Model.[89,91]

[2] *Xue* refers to a form of functional capacity bound to body fluids with functions such as warming, moisturizing, creating qi and nutrifying a tissue.
[3] Body fluids involve the concept of the hydratation and homeostasis of the tissue within the intersticial space.
[4] *Jing* refers to the structive potential and includes the functional repertoire of a cell via the DNA anda t the same time reproductive functions
[5] *Phase*, in terms of Heidelberg model of TCM, refers to vegetative functional tendencies and it's manifestations are called orbs.

**Diagnosis according to the Heidelberg Model of TCM**

The Diagnosis is one of the most important elements in TCM.[89] Based on Heidelberg Model; functional diagnosis is composed of four main steps (Figure 10):

- The constitution: tendency of an individual to express predominantly an "*orb* pattern".
- 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
- The guiding criteria, 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.

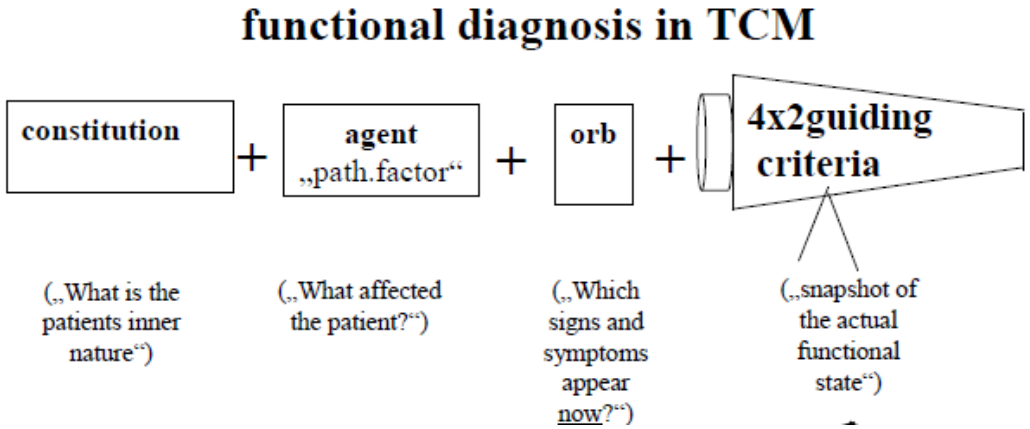



**Figure 11. The four components of functional diagnosis.**

**2.1 The *Algor Laedens* ("harmful cold disease") Theory – ALT**

According to Prof. Porkert, the ALT is the most common pathophysiological model that explains the process of "cold-invading" diseases in TCM, which have a parallel on clinical signs induced by neuroimmunological mechanisms on a western perspective (Figure 11). 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* and *xue*, and constitute a specific approach of interpretation of the guiding-criteria *extima*/*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 fibromyalgia, rheumatic disorders, or chronic inflammation.

The Three *Yang*/ *Extimal* Stages

*Yang* stands for *extima*, for activity in general and within the meaning of the phases it stands for *reactive calor*. This is why among the first three stages, Stage II with the highest *calor* in the clinic is characterised very often by too much *calor*, being this reactive *calor* the responsible for the most of the symptoms. This over-riding, over-extensive *calor* is in Chinese language called *yangming*, whose Latin transcription is *splendor Yang* (Stage II). In comparison to this *splendor yang* stage, the two other *extimal* stages can be divided into the *yang* that rises, *Taiyang* (Stage I) or in Latin the *Yang major*; and the *lesser yang*, *Shaoyang, Yang minor* (Stage III).

The Three *Yin*/ *Intimal* Stages

Within the *yin*, the *intimal* phases, the *reactive calor* is deficient resulting in internal *algor*; which could be defined as a *yin* state or a state of deficient functions that are under the target value of the production of warmth and heat. In these context, the target value states for balanced temperature behaviour of the body. Besides the hypofunctional state, the term *yin* also refers to the body substance, *yin*. 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* – Satge 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*.

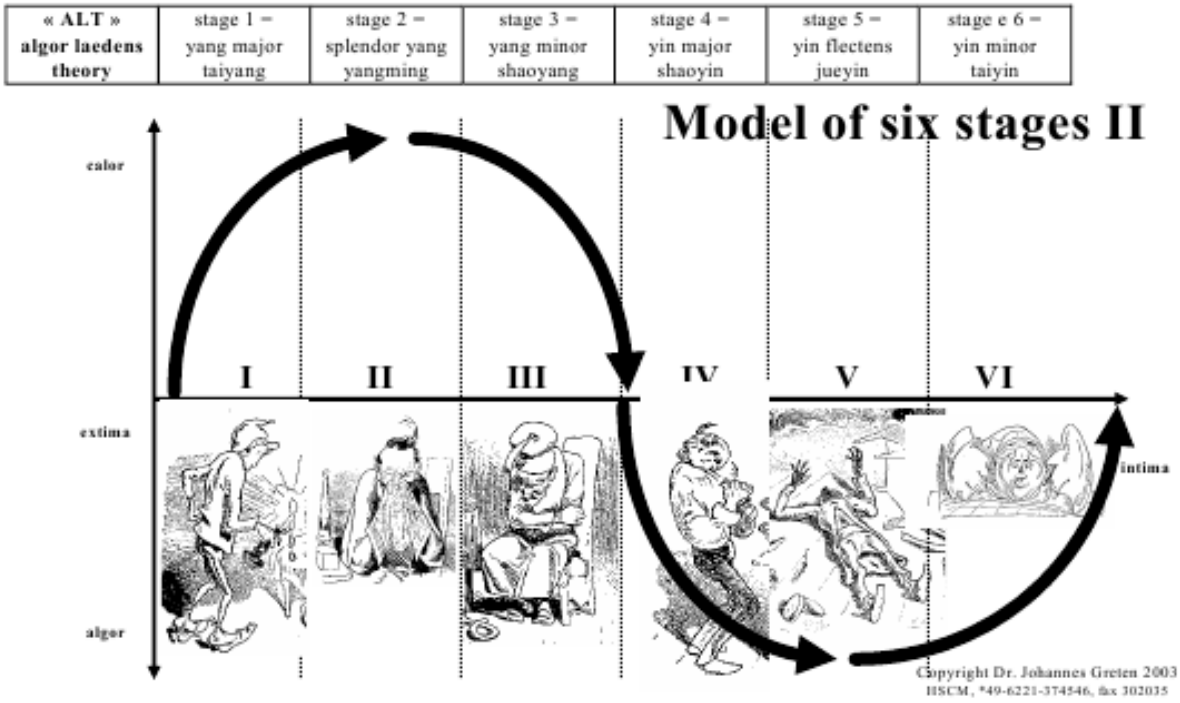



**Figure 12. Schematic model of *Algor Laedens Theory.***

Complementary, to the theory of the six levels another TCM theory prevails and states for the existence of six layers of functional powers within the body, six major defence mechanisms, which the agents have to overcome when invading the body – the six stages of defence (Figure 12). The *extimal* stages of defence encompass the defensive *qi*[6] as first layer, followed by the *qi* of the conduits [7]and, finally, the *xue* of the

[6] *Qi* refers to vegetative capacity to function of a tissue or organ which may cause the sensation of pressure, tearing or flow.
[7] 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.*

conduits; and the *intimal* stages includes the *qi* of the body island, followed by the *xue* of the body island and finally the *yin*.

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 of the cells, the substrate out of which functions (*yang*) develop.

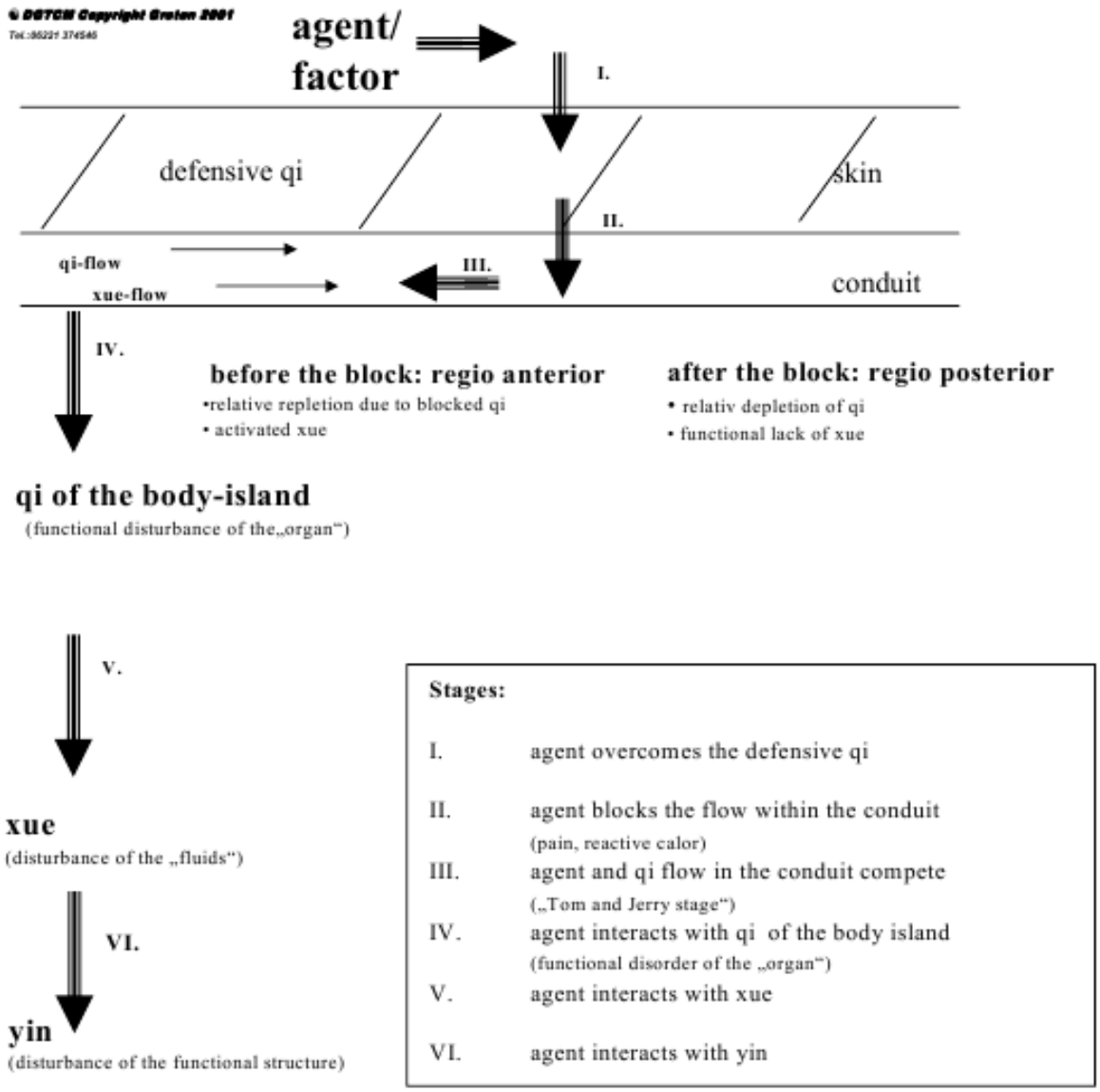



**Figure 13. The six layers of energy.**

Therefore, it's possible to distinguish six strategic scenarios (Table VI) 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.

| ALT STAGES | ENERGY LAYERS | MECHANISMS | KEY SYMPTOMS |
|---|---|---|---|
| I<br>*Yang major* | Defensive *qi* | Regional lack of microcirculation | Cold shivering, pale skin or goose bumps |
| II<br>*Splendor Yang* | Conduit *Qi* | Regional block of flow of *qi* or *xue* | *Algor*-related pain (stabbing pain) and reactive *calor* (fever) |
| III<br>*Yang minor* | Conduit *xue* | Reverse flow of *xue* into the interior | Hot/ cold sensations |
| IV<br>*Yin major* | Body island *qi* | Lack of microcirculation | Mucus, swellings |
| V<br>*Yin flectens* | Body island *xue* | Lack of *xue*<br>(blood, body fluids) | Cold limbs, extreme fatigue |
| VI<br>*Yin minor* | *Yin* | Lack of *yin* (structural deficiencies) | Bony appearance, stiffness of joints, prolonged regeneration or convalescence |

**Table 6. The six stages of *Algor-laedens* Theory; Energy layers, mechanisms of action and key symptoms.**

### *Algor*-induced signs and *orb*-signs evoked – ALT description

When the agent *algor* invades the skin, it affects the defensive *qi* and may induce a localised de-activation of *xue* or in western terms a regional lack of microcirculation. As the body is unable to warmth that specific region, it produces generalised *calor*, reactive *calor*, to warmth the conduits and *extima* in order to expel the invading agent.

*Algor* is a *yin* agent that diminishes the defensive *qi* as well as all other functional powers of the respective tissue, and in western concepts causes lack of microcirculation or de-activation of *xue*. Thus, *algor* may affect primarily the conduits that contain more *xue* than *qi*, the *vesical* and *tenuintestinal* conduits – ALT Stage I. If this attempt to expel *algor* fails, it may proceed the invasion and leading to a regional block of flow of *qi* and *xue*, thus causing pain. The flow of *qi* is blocked more easily than the *xue*, as "*qi* moves the *xue*", so the phases and orbs more prone to this affection are the ones more dependent on *qi*, the *Stomachal* (S) and *Crass Intestinal (CI)* conduits – ALT Stage II. When the agent *algor* overcomes the flow of *xue* within the conduits, it may lead to a reverse flow of

*xue* into the interior, and if there is a relative lack of reactive heat, the *algor* may further invade causing sensations of cold in the interior. Frequently, *xue* from the inside will be mobilized against the agent causing sensation of internal heat again or, if the *algor* is driven out, even temporary heat of the exterior (skin). It may be said that the agent *algor* and the flow of *xue* within the conduit play "Tom and Jerry", driving each other in and out repeatedly -- ALT Stage III. As this stage occurs still on the *extima* and the mobilization of internal heat is a feature of the phase wood, it affects the *felleal (F)* conduit and as this scenario causes symptoms and signs of "imbalanced distribution" of energies it also affects the *tricaloric* (TK) conduit.

On the **Stage IV**, the *calor* is overwhelmed and the *algor* invade the interior as the interior is then a lack of microcirculation: the guiding-criteria *algor* is present. When the *algor* affects the *qi* of the body islands, the orbs affect are those more sensitive to diminished activity of *qi*, the *lineal (L)* and *pulmonary* orbs.

When *algor* affects the *xue* within the interior, *orb* functions depending on *xue* are more easily affected, the *hepatic* and *pericardial orbs* – **Stage V**. When *algor* affect the body island *xue*, it represents the lowest point of energy in which reactivity has totally vanished, and is called the "flat-down" phase, a breakdown of orthopathy. Stage V is decisive for the survival of the individual. If, apart of *yin*, *xue* is sufficient then is possible to warm up slowly towards stage VI.

The *yin* is the structural condition for the development of *yang*. Accordingly, also *yin* (functional tissue) is a technical form of energy. When this energy is affected by *algor*, signs of a lack of *yin* and a persistence of *algor* are seen. As *yang* arises from the *yin* (in the language of the 4$^{th}$ guiding-criteria), an analogue process in the technical terms of the 1$^{st}$ guiding criteria is the development of *yang qi* upwards which is in a rapport with the cardiac *qi*. This connection is also expressed by the term of a cardio-renal axis, the *yin* pole being the renal *orb* and the *yang* pole the *cardiac orb* (Figure 13).

**Stage-hopping**

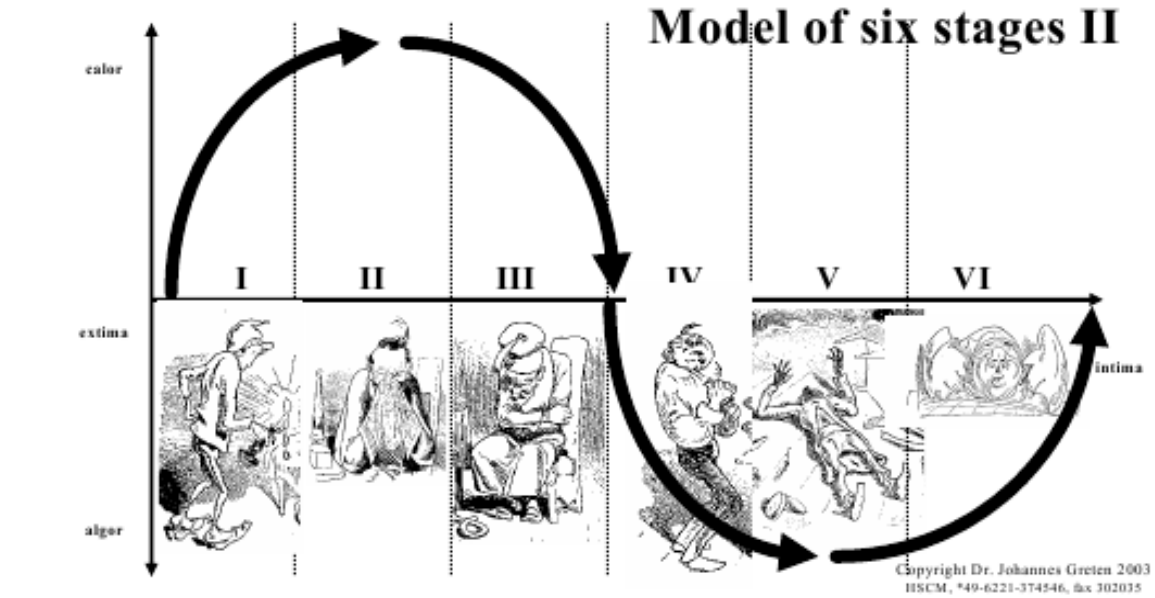
The six layers of defence consist of functional powers. They are gradually overwhelmed by the agent. If one of these functional powers is weak, overwhelming is a quick process. So in the case that the defensive *qi* is lacking, the result may be that the disease directly begins in Stage II. If the *qi* of the conduits is low, the disease will start directly with hot-cold sensations (Stage III). Lack of *xue* in the conduits results in cold *extima*, thus *algor* is able to invade the interior and symptoms of bronchitis are recurring (Stage IV). If the body island is deficient on *qi*, so *xue* is affected more easily, as in cases

of chronic burnout or hepatitis in mononucleosis (Stage V). If *xue* is lacking in the interior, stage VI is very probable. It's frequently seen in old patients, especially in elderly women (Figure 13).

**Outside-Inside hopping**

Consists of an alternative pathway to agent' invasion. This pathway is based on the fact, that all conduits are coupled with another conduit within the same phase (*extimal-intimal* connections). Thus, the agent may pass from an *extimal* conduit to an internal conduit. So, stage I can transform directly into Stage VI, Stage II can transform into Stage IV and Stage III into Stage V (Figure 13).

| « ALT » algor laedens theory | stage 1 = yang major taiyang | stage 2 = splendor yang yangming | stage 3 = yang minor shaoyang | stage 4 = yin major shaoyin | stage 5 = yin flectens jueyin | stage e 6 = yin minor taiyin |
|---|---|---|---|---|---|---|




| Symptoms of phases „catchword“ | „Sciatic and ear pain“ | „Acute sinusitis“ | „Hot-cold-nausea-phase“ | „Mucus and bronchitis“ | „Flat-down-phase“ („pneumonia“) | convalescence |
|---|---|---|---|---|---|---|
| **Involved conduits and orbs** | V - IT | IC - S | F - TK | P - L | H - PC | R - C |
| **Linked conduits and orbs** | R - C ($2^{nd}$ circuit) | P - L ($1^{st}$ circuit) | H - PC ($3^{rd}$ circuit) | IC - S ($1^{st}$ circuit) | F - TK ($3^{rd}$ circuit) | V - IT ($2^{nd}$ circuit) |
| **Exemplary decoction** | Dec. Ephedrae, Dec. Ram. Cassiae | Dec. Ancillae Yue | Dec. Bupleuri | see text | see text | see text |
| **Variation I: energetic factors determining the course** | Lack of xue and defensive-qi: more prone to phase 1 | Additional ventus: even acuter calor | Weakness of orthopathy (qi): faster transition into phase 4 | Lack of qi worsens the course, as P und L are qi-dependent | Mare xue: part of hepatic yin, therefore worse, if xue deficient | Yin (body substance) is decisive |
| **Variation II: „outside-inside hopping“ or „inside-outside-hopping“** | If yin is extremely weak: direct transition into stage 6 possible | If lack of qi and depletion of the centre: direct transition into stage 4 | If lack of qi and xue: direct transition into stage 5 | “sinu-bronchitic syndrome” (transition from stage 4 to stage 2) | stage 5 may go over into stage 3, i.e. as a form of recovery in pneumonia in the elderls | In stage of recovery: i.e.still aching limbs of algor kind (1. stage) |
| **Variation III: add. ventus** | All phases tend to show more signs of yang, repletion and especially more CALOR!! | | | | | |

**Figure 14. ALT related *orbs* and cross-linked orbs.**
Energetic factors determine the course. Variations in the course: outside-inside and inside-outside hopping. Gretten 2010.

## 2.2 Cancer and Immunity according to TCM

According to TCM, cancer occurs is due to an impairment of the smooth flow of *Qi* and/or an imbalance between *Yin* and *Yang*, manifests as a group of syndromes that are generally characterized through a disharmony in the body-mind-environment network.[93]

A study performed [94] to determine the clinical diagnosis distribution in six common cancers and the expectation to uncover a theoretical basis for TCM diagnosis as a clinical cancer treatment, revealed that in colon cancer there are six main syndromes, such as *pituita* stagnation, *Qi* and *xue* deficiency, R and L deficiency, R and H *yin* deficiency, stagnation of blood and toxins and cold-dampness that affects the L. And among them the most prevalent described by the authors is *pituita* [8]stagnation (*Shi-Re-Yin-Jie* syndrome; Figure 14). *Pituita* stagnation is caused by a malfunction of the lineal and stomach due to the retention of dampness and heat in the body and is characterized by epigastric or abdominal oppression, lack of appetite, heavy body weight, and thirst with little or no desire to drink, abdominal pain, loose stools, nausea, vomiting, fever, headache, red tongue body with a yellow sticky coat and/or slippery rapid pulse. This syndrome has been associated with many diseases, especially those involving the gastrointestinal tract as well as a broad range of inflammatory conditions (eczema, psoriases, cystitis, urethritis, gastroenteritis, vaginitis, cervicitis, meningitis, conjunctivitis, rheumatoid arthritis and allergic reactions). In fact, is has been reported that the symptoms characteristic of *pituita* stagnation are common among gastrointestinal cancer patients, including cancers of duodenum, colon, liver, pancreas and gallbladder.

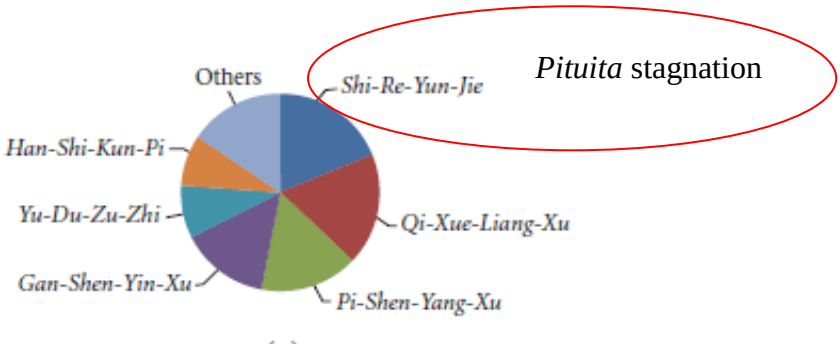



**Figure 15. Most common cancer syndromes, according to TCM.**
Extracted and modified from Chen *et al.* [94]

Also, it has been described an correlation between this syndrome and alterations on the expression of inflammatory cytokines such as TNF-α and IL13, as well as others cytokines involved in cancer-associated myofibroblast proliferative activities and in

[8] *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.

tumour-associated macrophage infiltration. Thus, suggesting that there is an association between *pituita* stagnation and changes in tumour microenvironment.[94]

The terms related to immune functions in TCM are *zheng qi, yuan qi, yang qi*, *wei qi*, *qi* of *zang fu*[9], including lung (pulmonal) *qi*, spleen (lineal) *qi* and kidney (renal) *qi*, along with the related materials and function such as *jing*, *qi* and *shen*[10]. [95]

*"If zheng qi remains strong, xie qi cannot invade the body, zheng qi must be weak when invasion of xie qi takes place."*

*Zheng qi* consists of *yuan qi* (primary or congenital *qi*), *zong qi* (pectoral *qi*), *yin*g *qi* (nutrient *qi*), *wei qi* (defensive *qi*), and *qi* of all the *zang fu* organs. *Zheng qi* consists in the physiological functions of *zang fu* organs, meridian channels and tissues. It also represents the adaptability and accommodation of the body to the environment and the resistance of the body to diseases. Since *zheng qi* represents such a broad category, it is clear that *zheng qi* is involved in the immune system. From a western point a view, z*heng qi* appears to represent the nerve-endocrine-immune network.

*Wei qi* (defensive *qi*)*,* also called *wei yang*, is derived from the fierce *qi* of food essence. It is part of the *yang qi* that circulates outside the blood vessels and protects the surface of the body, such as skin and muscle, from exogenous pathogenic factors, controls the opening and closing of pores, moistens the skin and hair, regulates body temperature and warms up the *zang fu* organs. From a western point of view, some functions of *wei qi* correspond to those of the autonomic nervous system and the central nervous system. These facts indicate that *wei qi* is related to the defense function, but it is very limited, being far from enough to explain the function of the immune system. [95]

*Qi* of *zang fu*. In the study of *zang fu*, researchers try to find the relationship between the micro-index in immunology and symptoms in traditional Chinese medicine. For example, the *heart governs blood and the vessels*. It has been reported an association between heart *qi* deficiency and deficient cell-mediated immunity in patients with coronary disease; but not with the humoral immunity. [95]

[9] *Zang Fu* means organs and visceras.
[10] *Shen*, according to Heidelberg model of TCM, refers to functional capacity to put order into mental associativity and emotions, thus creating "mental presence".

The *lung dominates qi*, controls respiration and connects with skin and hair and also regulates water passages. Deficient lung *qi* appears to be associated with low transformation rate of lymphocytes, IgG and IgM. [95]

The *spleen controls digestion, assimilation and distribution of nutrients and water throughout the body*. Hence, the spleen and stomach are viewed as the foundation of the latter heaven-acquired constitution. [95]

*"If the Spleen qi is strong during all four seasons, the body will not be attacked by evil qi.";*
*"All diseases start with Spleen and Stomach deficiency."*

The spleen deficiency was found to be mainly a result of the dysfunction of the digestive system and it involves the nerve-endocrine-immune network. In which, the ability to produce antibodies is low, humoral immunity is deficient, Th-cell (helper T-lymphocytes) count is low, suppressor T-cells is high and cell-mediated immunity is deficient. [95]

The liver stores blood, maintains the free flow of *qi* and stores the soul. It has been shown that stagnation of liver *qi* causes reduction of lymphocytes E-rosette, namely the ability of releasing $H_2O_2$ from monocytes and mixed lymphocyte reaction; thus liver stagnation is implied in deficiency of the immune function. [95]

The kidney stores vital essence, dominates water metabolism, development and reproduction. It is in charge of bone and manufacturing bone marrow. Kidney deficiency was shown to be associated with low levels of T helper lymphocytes, serum hemolysin and plaque-forming cell assays and high levels of suppressor T-lymphocytes. These facts suggest that the ability to produce antibodies is low in the kidney-deficient patients. Kidney *yang* deficiency is associated with low levels of serum IgG while in kidney *yin* deficiency, serum IgM is elevated, urine IgG and IgA are elevated. [95]

In modern medicine, the pituitary-adrenal cortex-lymphocyte corticoid receptor is the important part of nerve-endocrine-immune network. The lymphocyte corticoid receptor is the peripheral connector of the nerve-endocrine system with the immune system. The kidney, in traditional Chinese medical terms, has a very close relationship with the nerve-endocrine-immune network.

Resuming, each organ in TCM corresponds to several organs and various physiological systems in Western medicine, especially the nervous, endocrine and immune systems. In all of the organ deficiency-type symptoms, there is a certain reduction of the immune function. On the other hand, the nervous-endocrine-immune network runs through every organ in the body. The immune system has its special structure and function. It is not the same as *zhang fu* organs. The structural difference is clearly

demonstrated by the anatomy which should be distinguished from the unique function of the immune system, for example spleen deficiency in the common situation is different from the spleen deficiency in AIDS.

In recognizing immune-related diseases, it is necessary to point out that the major pathological changes are in the immune system. Using *zang fu qi* to represent the immune function cannot reveal the special nature of the immune system and its important role in the development of diseases. [95]

### 2.3 Cancer and Immunity - Heidelberg Model of TCM

As discussed previously, *defensive qi* constitute the foremost defence line of an individual. This kind of active energy that resides within the *extima*, outside the conduits and gives warmth to the flesh, its normal tonus to the skin, regulating the opening and closing of the pores and all other functions by which this tegument responds to outward stimuli. "*Qi*" designates the active aspect of all energies constituting the vital functions of an individual. Therefore, an affection of the *Qi* implies that a disturbance has penetrated deeper and spread further than if it affects only the defensive energy. Since the defensive energy is essentially deployed in the skin, the *pulmonal orb* (P *orb*) has an eminent role within the defence system of each individual. The specific function of P *orb* is giving the rhythmic cue for the deployment of energy at all levels of personality.[88]

*Qi construtivum* is the term designating the aspect of that structive energy (*xue*) which flows within the conduits, and totally represents the circulation of energy between all orbs, thus contributing to and maintaining the material development and sustenance of the body. When this kind of energy is affected gives rise to symptoms such as impairment distribution of blood, restlessness and apprehension, etc. *Qi Constructivum* and *xue* constitute different technical aspects of ultimately the same structural energy. In Chinese Medicine *xue* refers not only to blood, but also to the basic form and concept of all liquids, fluids and secretions. Thus, affections of the *xue* contribute to a serious and even critical state of illness.[88] The hepatic *orb* (H *orb*) on one hand is responsible for the mobility of the individual, for the production and employment of locomotive force, for the active projection of the personality in general; and on the other hand, is responsible for the storage and dosage of individually specific structive energy (*xue*). The *hepatic orb* is mostly affected by repletion rather than depletion. In cases of repletion of the H *orb* there are two possible types of agents: (1) emotions that impair the normal outflow of energy from the H *orb*,

producing congestions eventually leading to *ardor*[11]. As a consequence, the storage and control of active energies may be impaired producing symptoms of *ventus* [12]*internus*; (2) *algor externus* retained in the conduits of H *orb*, producing congestive symptoms, encroachments or contravections of the *qi hepaticum*, general lack of force and drive, pains, congelations, and indirectly hematomas and congestions, spastic tensions, tympanites, constipation and finally concretions (tumours).[88]

In summary, according to **Heidelberg Model of TCM**, cancer may result from an imbalance on the **Metal-Wood axis**. These represents an imbalance of *Qi defensivum* and *Qi construtivum*, 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 construtivum* 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.

**Chemotherapy – TCM Heidelberg Model Perspective**

Chemotherapeutic agents 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.

Primarily, there are two aspects that need to be considered: the first is that an hypofunctional state (*yang* deficiency) leads to ice coldness, weakness and extreme sensitivity to external cold, and the second is that chemotherapy, considered as *toxic algor*, 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

[11] *Ardor* resembles to the feeling of a piece of glowing charcoal touching the skin or other organs, producing intense pain with reddishness of pulsating nature, just as seen in inflammation conditions.
[12] *Ventus* refers, in western terms, to a neurovegetative reaction "as if in a draught of air", with two mechanisms: archaic turning movement (autochthonic muscules) and substance P-mast cell reflex.

on this stage, the *algor* affects the *Stomach orb* and patient develops a *splendour yang* syndrome with accumulation of *pituita*, which affects the face becoming swollen. Also affects the CI, 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. When *toxic algor*, affects the *qi* of P and L *orbs* leads to more vulnerability to airways infections and feelings of dullness (accumulation of *humor*[13]), respectively – Stage IV. In Stage V, the *xue* of H and PC 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. On Stage VI, the extreme *yin* deficiency (cachexia) is due to (1) tumour itself, but also enhanced by chemotherapy; (2) affection of structure and function – the R *orb* is affected occurring symptoms such as pain in the bones, stiffness in all joints, myalgic and weak muscles; (3) affections of the urinary tract (frequent urination); and (4) affection of the brain, more precisely short-memory loss.

In summary, and according to the theory of phases, cancer reflects **impairment on Metal-Wood axis**, represented by the P *orb* and H *orb*. According to ALT, these orbs (P and H) correspond to stage IV and V, and are coupled to L and PC orbs, respectively. Regarding to chemotherapy effects, it may be considered that "toxic *algor*" is the agent that directly affects all ALT stages. In addition, one may extrapolate that cancer patients undergoing for chemotherapy are in a hypofunctional state, between stage IV (P and L orbs) and V (H and PC orbs), and most probably symptoms are sensitivity to cold, weakness of the limbs, tiredness, feelings of dullness and vulnerability to airways infections. However, as described before through the *intimal-extimal* connections of the conduits, Stage II and Stage III symptoms may be seen, such as nausea, loss of appetite and hot-cold sensations.

## 3. Acupuncture - overview

Acupuncture has been employed as a health care modality for over 3000 years. Over the last few decades research has been carried out seeking to explain how acupuncture works, and what it can and cannot treat.

The 1997 National Institute of Health (NIH) Consensus of Acupuncture reports that "studies have demonstrated that acupuncture can cause multiple biological responses, mediated mainly by sensory neurons to many structures within the central nervous

[13] *Humor* refers to a group signs originated from pre-oedema and oedema, such as swollen limbs and tissues, feeling of heaviness, dyspnoea and generalised pain.

system. This can lead to activation of pathways affecting various physiological systems in the brain as well as in the periphery".

The NIH Consensus also suggests that acupuncture "may activate the hypothalamus and pituitary gland, resulting in a broad spectrum of systemic effects. Alteration in secretion of neurotransmitters and neurohormones and changes in the regulation of blood flow, both centrally and peripherally have been documented. There is also evidence of alterations in immune functions produced by acupuncture."

It has been proposed different theories on the mechanism of acupuncture.[96,97,98]

a) **Neurotransmitter Theory** – acupuncture effects higher brain areas, stimulating the secretion of β-endorphins and encephalin in the brain and spinal cord, which influences the immune system and the antinociceptive system.

b) **Autonomic Nervous System Theory** – acupuncture stimulates the release of norepinephrine, acetylcholine and several types of opioids, affecting changes in their turnover rate, normalizing the autonomic nervous system and reducing pain.

c) **Gate Control Theory** – acupuncture activates nonnociceptive receptors that inhibit the transmission of nociceptive signals in the dorsal horn, "gating out" painful stimuli.

d) **Vascular-interstitial Theory** – acupuncture manipulates the electrical system of the body by creating or enhancing closed circuit transport in tissues. This facilitates healing by allowing the transfer of material and electrical energy between normal and injured tissues.

e) **Blood Chemistry Theory** – acupuncture affects the blood concentrations of triglycerides, cholesterol and phospholipids suggesting that acupuncture can both rise and diminish peripheral blood components, thereby regulating the body toward homeostasis.

## 4. Acupuncture and Immunomodulation

Mark Bovey in a review of the literature[99] verified that there are many publications from around the world that demonstrate that acupuncture can affect different aspects of the immune system. However, an investigation into the specific area of chemotherapy-induced neutropenia/ leukopenia is almost entirely dependent on Chinese research, most of it written in Chinese and published locally. Although, the best information comes from the review and meta-analysis of Lu *et al*, [100] which reported a significant effect of acupuncture in comparison to usual care but pointed out that there were major deficiencies in the quality and reliability of the reports on which this result was based.

Subsequently workers from the same US cancer institute have published data from their own small pilot trial,[101] which largely confirms the Chinese results, suggesting that acupuncture treatment could protect against myelosuppressive chemotherapy.

Bovey conclude that there is enough indicative evidence of effect, together with enough information on how to deliver the acupuncture treatment, to consider a) trying to replicate the US results, either with the same type of cancer or another, b) a pilot study comparing acupuncture to normal medical care, rather than 'sham' acupuncture, and/or c) running a larger trial. [99]

Acupuncture seems to operate through multiple mechanisms in its effect on leukopenia, promoting the release of WBC from bone marrow cells into the peripheral blood; prolongs the life of WBC; increasing the activity of CSF; promoting the proliferation of haematopoietic stem cells; diminishing the damage to done to haematopoietic stem cells by chemotherapy; improving the microcirculation.[99] In concordance to these studies, Lu *et al*[100] reported also that acupuncture could increase serum CSF and promote the maturation of granulocytes. They also cited evidence of an effect on non-marrow factors, hence again suggesting multiple mechanisms.

Humoral and, especially, cellular immune function have been reported as being boosted by acupuncture and moxibustion in cancer patients with significant increases in several T lymphocyte subsets. Immune system modulation has been noted also for various other conditions, such as asthma, [102] autoimmune and inflammatory diseases[103].

Takahashi *et al*,[104] reviewed the ascribed immunomodulation of acupuncture and conclude that acupuncture treatment appears to be able to modulate immunosuppressed or immunoactivated conditions through various functions, including the activities of macrophages, neutrophils, NK cells and lymphocytes, immunoglobulin production and complement systems. In fact, it was previously reported two possible mechanisms involved: NK-related gene expression in the spleen [105] and the sympathetic nervous system[106].

A recent review [107] about the mechanisms of acupuncture in modulating various immune responses and the relationship between acupuncture mediated immune regulation and neurological involvement, indicates an immunomodulating role of acupuncture. The authors verified that acupuncture enhances the NK cells activity and its subsets [108]; modulates the balance between Th1 and Th2 cells and acupuncture treatment may affect somewhat different neural-immune signalling pathway depending on the condition (e.g. normal vs. Th1-dominant vs. Th2-dominant conditions).

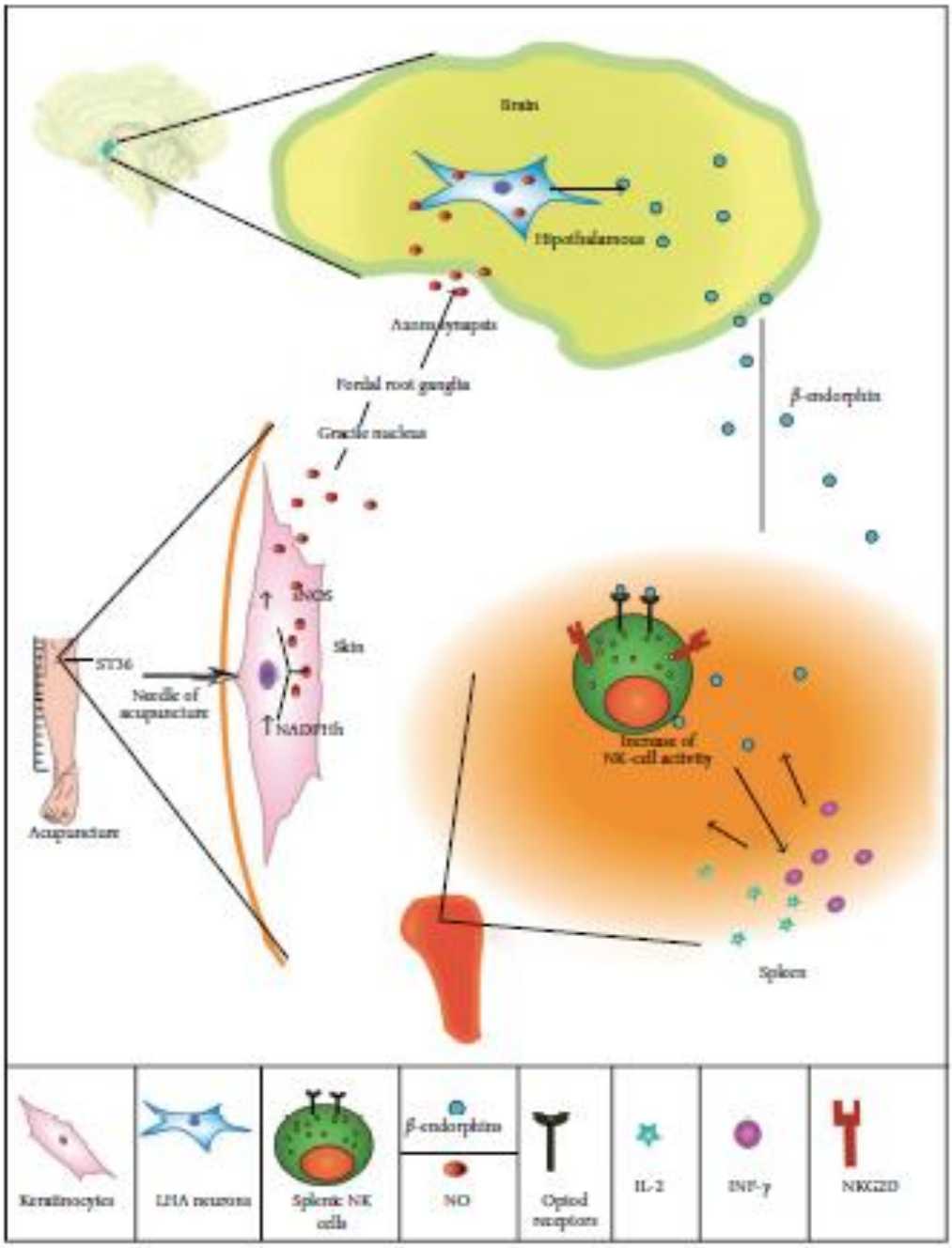



**Figure 16. Hypothetic model of the mechanisms how acupuncture stimulates the immune system.** Acupuncture stimulation of ST36 acupoint induces release of nitric oxide (NO). NO, a neurotransmiter, stimulates via the sensory nerves, spinal cord and medulla oblongata, gracile nucleus the lateral hypothalamic area (LHA), where it promotes secretion of opiod peptides such as *β*-endorphin. *β*-endorphin travels via blood circulation to the spleen and other body locations containing immune cells where it binds to opiod receptors expressed on the surface of NK cells and stimulates NK cells to amplify their expression of cytotoxic molecules and consequently tumoricidal activity, and production of IFN-*γ*. This cytokine induces the expression of NK cell receptors and cytokine receptors on NK cells and perhaps cytokine secretion by other immune cells, thereby orchestrating and further amplifying anticancer immune functions. Extracted from Johnston *et al* 2011[109]

Moreover, Johnston *et al*,[109] have combined heterogeneous research findings with cellular immunological and molecular biological theory producing the "acupuncture immunoenhancement hypothesis": acupuncture stimulation increases the cytotoxicity of NK cells by promoting cross talk between the neurotransmitter network and immune system that is [1] orchestrated by nitric oxide, *β*-endorphin and cytokines and [2] anchored by opioid and NK cell receptors (Figure 15).

Silvério-Lopes and Gonçalves-Mota[110] review the literature about the role of acupuncture in modulation of immunity. The authors concluded that the principal immunomodulatory effects of acupuncture include: a) decrease of cellular apoptosis; b) increase cytotoxic activity; c) biochemical synergism between electro-acupuncture and neurotrophyn; d) mobilization of corticosterone, endorphine and ACTH; and e) pro-inflammatory and anti-inflammatory effects. Moreover they verified that in all studies about immune response, the NK cells were used as markers and there was an increase of these cells after acupuncture and electroacupuncture treatment. No study revealed a reduction of NK cells expression.

In respect to studies on CRC, we found the reports of only two Chinese studies. One study focus in the effect of acupuncture on the activity of the peripheral blood T lymphocyte subsets and NK cells,[111] showed that the value of T lymphocyte subsets such as CD3, CD4, and CD8 , as well as NK cells were obviously increased after treatment, and there were significant differences between them before and after treatment. The other study has focus in the effects of moxibustion on the improvement of gastrointestinal and immune function in patients with postoperation of CRC[112] .The authors concluded warming needle moxibustion constitutes a good therapeutic effect and is able to regulate bidirectionally peripheral blood lymphocytes and neutrophil granulocyte and improve T lymphocyte subgroup and NK cells, promoting the recovery of immune function of these patients.

## 5. The importance of moxibustion in the immune modulation

Moxibustion is an important part of the science of TCM, being documented in the Han dynasty silk books. These books have been discovered in 1973 and there was no mention of acupuncture therapy leading to presume that the origin of moxibustion is previous to that of acupuncture.

Moxibustion is a part of acupuncture practice for 1000 years and entails burning the moxa herb *Artemisia vulgaris* to warm points in order to alleviate symptoms.

According to TCM, moxa is warm in property and fragrant in flavour and have the functions of smoothing and facilitating circulation in the 12 meridians, regulating the *qi* and blood, expelling coldness and dampness. According to modern research, moxibustion can promote metabolism, improve immune system functions and regulate the physiological function of internal organs.

Moxibustion can be administered by different ways: a) through a burning moxa stick directly over the skin at an acupoint – direct moxibustion; and b) indirect moxibustion, that consists in placing insulating materials such as sliced ginger or garlic between the body and a burning moxa cone.

It has been postulated that the mechanism of moxibustion effect is associated with the pharmacological action of the materials used, the thermal action and the infrared radiation. However, to some researchers the thermal effect of moxibustion is the key to its therapeutic effect, because it stimulates three different types of sensory receptors - cold receptors, warmth receptors and pain receptors – that enable the perception of gradations of thermal sensation. So, these authors attribute to moxibustion the capacity to activate and modify the nervous system by stimulating receptors under the skin. The exact effect of the afferent information on thermal sensitive neurons and the involvement of the sympathetic nerve system remain unknown but are the important links of moxibustion therapy.[113]

Freire *et al*[114] in order to assess the physical characteristics underlying the action of moxibustion in specific acupoints, verified that the therapeutic effect is also dependent on the temperature and the material used for moxibustion.

As stated previously Zhang *et al*[112] reported that warming needle moxibustion has a good therapeutic effect on gastrointestinal function and can regulate bidirectionally peripheral blood lymphocyte and neutrophile granulocyte and improve the T lymphocyte subgroup and NK cells so as to promote the recovery of immune function in patients with CRC after operation.

# PART TWO – STUDY METHODOLOGY

## 1. OBJECTIVES

The objectives of this study are:

- evaluate the effect of AcuMoxa protocol, according to Heidelberg Model of TCM, on NK cells in CRC cancer patients undergoing for chemotherapy;
- evaluate the effect of increased NK cells on the patients emotional state;
- evaluate the effect of increased NK cells on the patients quality of life

evaluate the increased NK cells on the patients.

## 2. Sampling and Recruitment Procedures

### Selection of patients

This study was approved by the ethics comission of Centro Hospitalar S.João and Centrp Hospitalar Vila Nova de Gaia/ Espinho. Written informed consent was obtained from all patients before study enrollment. Patients were eligible for inclusion as follows: newly diagnosed or recurrent colorectal cancer, regardless of stage; receiving myelosuppressive chemotherapy; no regular use of acupuncture within 120 days prior to enrollment; ability to give informed consent; and 18 years of age. Exclusion criteria were the following: absolute neutrophil count (ANC) less than 500/μL; (b) platelet count less than 25,000/μL; (c) altered mental state; (d) clinically significant cardiac arrhythmias; and (e) other unstable medical condition.[115]

### Study design

All patients enrolled were evaluated at baseline. Patients were randomized into one of two groups: active acupuncture (AcuMoxa) or non-acupuncture (control group). All of the study patients, were blinded to randomization assignments.

Patients in the experimental group received 6 sessions of acupuncture, twice a week, beginning 1 week prior to cycle of chemotherapy and ending at the beginning of the following cycle of chemotherapy (Fig. 17). At the conclusion of the intervention, patients in the study arm continued their remaining chemotherapy cycles without any acupuncture treatment. Patients in the control arm were offered the active acupuncture protocol immediately after they completed the four weeks of blood sampling as a courtesy.

### Acupuncture protocol

All acupuncture treatments were performed at Centro Hospitalar S. João and Centro Hospitalar de Vila Nova de Gaia/Espinho. The acupuncture treatments were administered only by one acupuncturist (the researcher). A standardized acupuncture protocol was developed, which was based on Heidelberg Model of TCM.

The experimental group (AcuMoxa). Acupuncture points and their anatomical locations were as follows: lower extremity (LR3, ST36, SP9, VB39); upper extremity (LI4, PC5, Tk5, P7) and lower abdomen (Rs 6). Disposable acupuncture needles with a size of 36G, 0,20x25mm (Tewa®). The depth of needling was at approximately 10mm. A de qi

sensation was required. The moxa treatament was used in the following points: IT6, TK5, S32. Each session was 45 minutes.

The control group. The individuals collect the blood sample as the experimental group, during 4 weeks. And then it was administered acupuncture treatment to the individuals.

Clinical and laboratory evaluation

Complete blood counts were collected at baseline, and then once every 7 days at 4 time points during the study period (Fig.17).

Lymphocyte populations and NK cells (total counts and subsets counts) were analysed by flow cytometry (CD45-FITC/CD56-PE/CD19-ECD/CD3-PC; Beckman Coulter Portugal), at department of Immunology of Hospital Santo António, Porto.

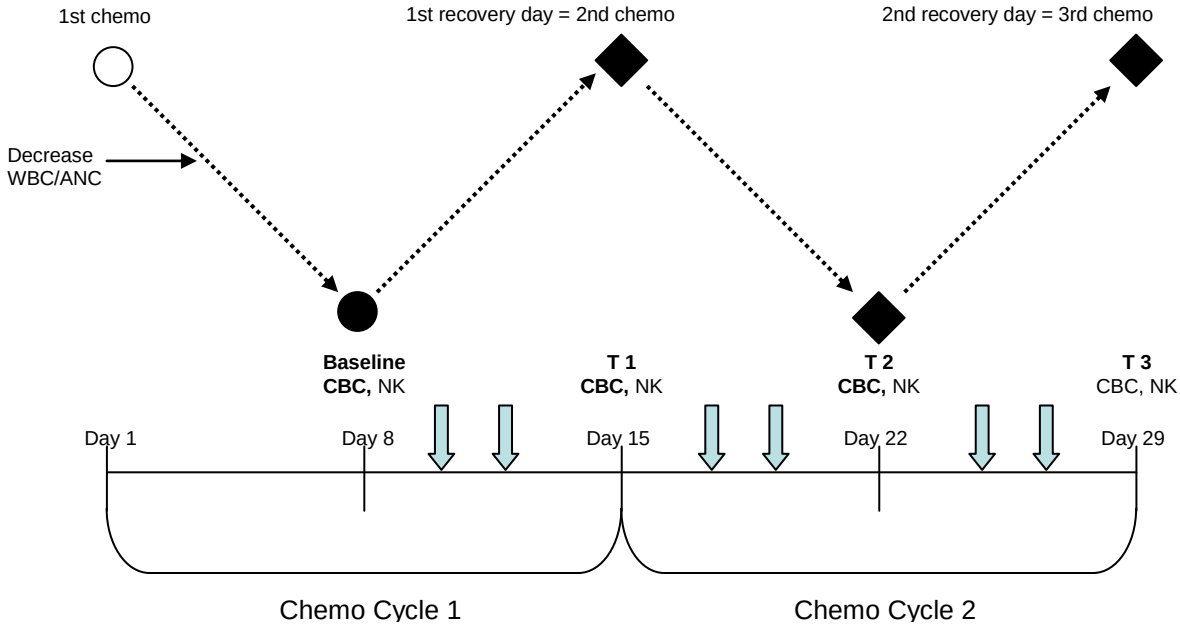



**Figure 17:** Study flow chart. Black solid dots: time points of outcome measurements. Open circle: first chemotherapy day. Black diamonds: the primary endpoints of the study. Dashed lines: the expected changes, during chemotherapy, of white blood cells (WBC) and absolute neutrophil counts (ANC). Short, blue down arrows: acupuncture treatments. CBC, complete blood counts; NK, NK cells and subsets.

**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 acupuncture on NK cells in patients with CRC.

Nonparametric Wilcoxon–Mann–Whitney tests were used to assess between-group comparisons as unadjusted analyses. Pearson analysis of correlation was used to examine variables correlations.

The analysis was performed in accordance with the intention-to-treat principle. Missing data were handled based on available data approach. SPSS for Windows was used for statistical computation. All p value $<0.05$ was considered statistically significant.

# PART THREE – RESULTS AND DISCUSSION

## 1. RESULTS

Baseline characteristics - Sample characterization

At baseline, the patients and the controls shared similar demographic and clinical characteristics (Table 7).

The majority of patients (88,9%) have a colon cancer, with stage II (16.7%) and Stage III (55.6%); 66,7% of the patients are submitted to the Folfox chemotherapy regimen and 16.7% of the patients undergoes with the Xelox chemotherapy regimen.

| Experimental Group | Characteristics | Control Group |
|---|---|---|
| 62.2<br>38-74 | Age<br>Mean<br>Range | 56.6<br>43-74 |
| 8 (88.9%)<br>1 (11.1%) | Cancer Type<br>Colon<br>rectum | 9 (100%)<br>0 |
| 1 (11.1%)<br>7 (77.8)<br>1 (11.1%) | Tumor Staging<br>II<br>III<br>IV | 2 (22.2%)<br>3 (33.3%)<br>4 (44.4%) |
| 6 (66.7%)<br>1 (11.1%)<br>1 (11.1%)<br>1 (11.1%) | Type of chemotherapy<br>Folfox<br>Xelox<br>Folfiri<br>Capecitabine | 6 (66.7%)<br>2 (22.2%)<br>0<br>1 (11.1%) |

**Table 7: Socio-demographic and clinical characteristics of patients.**

Comparison of blood analyses at baseline showed no statically differences among groups (Table 8).

| Experimental Group (n=9) | Baseline Blood analyses (Mean; s.d.; p value) | Control Group (n=9) |
|---|---|---|
| 5.842; 0.897 | **WBC** (AbsV.); p=0.400 | 5.916; 1.288 |
| 49.01; 11.74 | **ANC** (%); p=0.479 | 53.19; 12.68 |
| 27.54; 9.00 | Total **Lymphocyte** (%); p=0.456 | 31.88; 14.45 |
| 10.17; 2.66 | **B cells** (%); p=0.369 | 7.88; 6.9 |
| 78.33; 6.99 | **T cells** (%); p=0.099 | 85.38; 9.84 |
| 11.48; 5.53 | **NK cells** (%); p= 0.07 | 6.66; 5.00 |
| 10.67; 4.77 | **NK $^{bright}$ cells** (%); P=0.06 | 6.17; 4.58 |
| 0.80; 0.89 | **NK $^{dim}$ cells** (%); p=0.408 | 0.48; 0.69 |

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

A total of 18 (100%) patients completed the questionnaires of QOL-CR29 and HADS at the beginning and at the end of the study. Comparison of QOL and Anxiety and depression scores did not reveal any statistical differences among groups (Table 9); however the experimental group reveals high scores of depression.

| Experimental Group (n=9) (Mean; s.d.) | QOL-Cr 29 & HADS scores (Mean; s.d.)(p value) | Control Group (n=9) (Mean; s.d.) |
|---|---|---|
| 14.78 | Anxiety (p=0.722) | 15.67 |
| 16.11 | Depression (p=0.432) | 14.33 |
| 7.67 | Urological Symptoms (p=0.632) | 7.89 |
| 7.67 | Gastrointestinal Symptoms (p=0.609) | 8.44 |
| 6.22 | Defecation symptoms (p=0.78) | 10.67 |
| 2.89 | Stoma-related symptoms (p=0.66) | --a |
| 8.33 | Chemo side effects (p=0.96) | 6.67 |
| 3 | Male sexual function (p=0.239) | 1.56 |
| 0.78 | Female sexual function (p=0.148 | 1.78 |
| 2.44 | Weight concerns (p=0.857) | 2.56 |
| 4.56 | Body image (p=0.819) | 4.78 |
| 2.89 | Future concerns (p=0.398) | 3.33 |

**Table 9. QOL-CR 29 and HADS Scores. Comparison between groups at baseline.**

[a]. no patient in the control group with stoma.

**Effect of acupuncture on WBC and ANC**

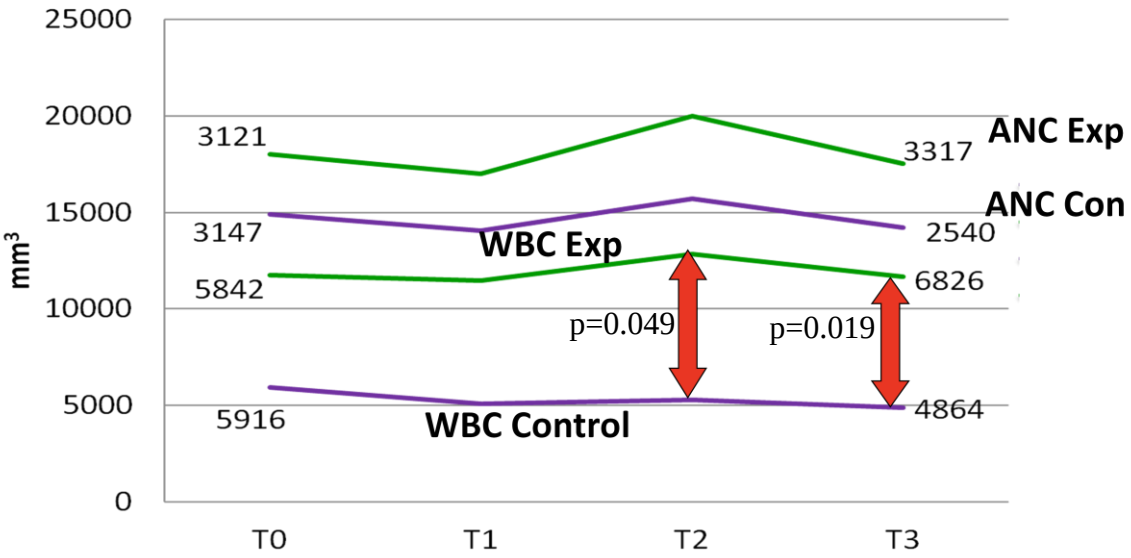
The effects of acupuncture treatment on WBC counts and ANC in study patients are shown in Table 10.

| Groups | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| Control | **(n=9)** | **(n=9)** | **(n=9)** | **(n=8)** |
| WBC (AbsV. Mean; s.d.) | 5.916; 1.288 | 5.087; 1.03 | 5.300; 1.264 | 4.863; 0.806 |
| ANC (% Mean; s.d.) | 53.19; 12.68 | 51.04; 8.89 | 53.58; 14.57 | 52.22; 9.74 |
| | 3147 | 2596 | 2839 | 2539 |
| Experimental | **(n=9)** | **(n=9)** | **(n=9)** | **(n=8)** |
| WBC (AbsV. Mean; s.d.) | 5.842; 0.897 | 6.368;1.922 | 7.563; 2.930 | 6.826; 1.971 |
| ANC (% Mean; s.d.) | 49.01; 11.74 | 50.31; 11.23 | 56.77; 10.1 | 54.46; 15.28 |
| | 2863 | 3204 | 4294 | 3717 |

**Table 10: WBC (absolute value(AbsV) means and standard deviation) and ANC (percentage means and standard deviation), in both groups at different time points.**

The difference in WBC counts between the two groups became statistically significant after patients received 4 sessions of acupuncture (p=0.049) and remain higher at time point T3 (p=0.019).

Differences in ANC values between AcuMoxa and control groups showed a pattern similar to that of WBC counts but were not statistically significant. At the end of the study, average ANC values in the AcuMoxa group were greater than in the control group.



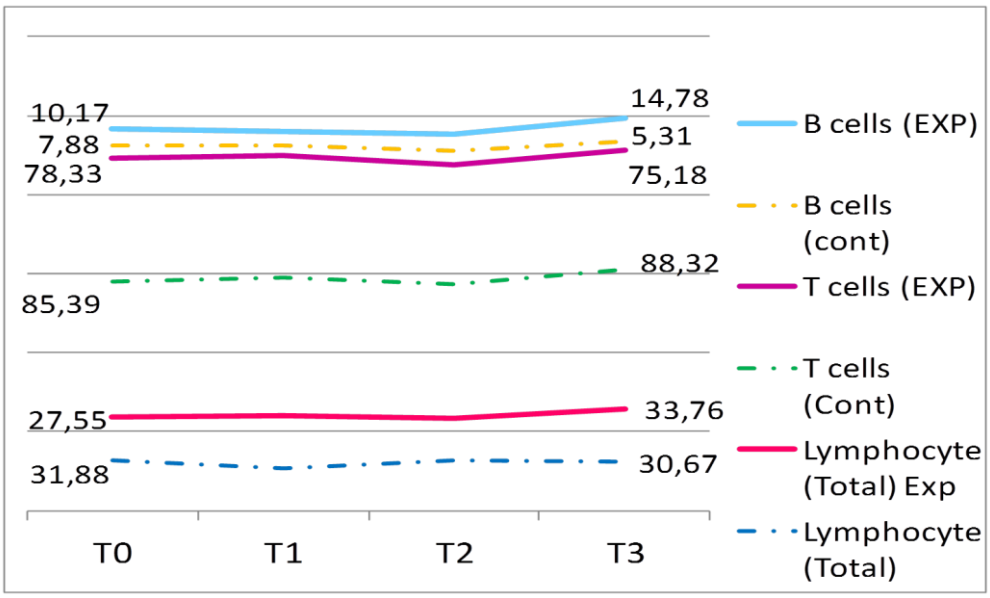

**Graphic 1: WBC and ANC counts.**

### Effect of acupuncture on lymphocyte populations

The average of total lymphocyte populations were not statistically different among groups (Table 11 and Graphic 2), either on the lymphocyte population B. However, the percentage of total lymphocytes are higher in the experimental group and the B cells have a increasing tendency, being significantly higher in the experimental group. Among T cells, the control group exhibits statistically significant higher levels of T cells, since time point T1.

| | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| **Total Lymphocyte** (% Mean; s.d.) | **(n=9)** | **(n=9)** | **(n=9)** | **(n=8)** |
| **Control** | **31.88; 14.45** | **26.55; 9.29** | **32.21; 11.24** | **30.67; 7.67** |
| **Experimental** | 27.55; 9.00 | 33.46; 7.45 | 26.47; 10.97 | 33.76; 15.87 |
| | (p=0.456) | (p=0.101) | (p=0.289) | (p=0.625) |
| **B cells (% Mean; s.d.)** | | | | |
| **Control** | **7.88; 6.9;** | **6.33; 3.63** | **8.8; 5.18** | **5.31; 2.63** |
| **Experimental** | 10.17; 2.66 | 8.87; 3.01 | 10.39; 2.55 | 14.78; 14.32 |
| | (p=0.369) | (p=0.127) | (p=0.421) | (p=0.106) |
| **T cells (% Mean; s.d.)** | | | | |
| **Control** | **85.39; 9.84** | **87.09; 6.00** | **84.85; 8.92** | **88.32; 7.00** |
| **Experimental** | 78.33; 6.99 | 77.97; 7.21 | 75.52; 9.24 | 75.18; 14.6 |
| | (p=0.099) | (p=0.01) | (p=0.05) | (p=0.038) |

**Table 11. Lymphocyte populations: Total lymphocytes, B cells and T cells. Comparison between groups at different time points (p<0.05, statistically significant).**



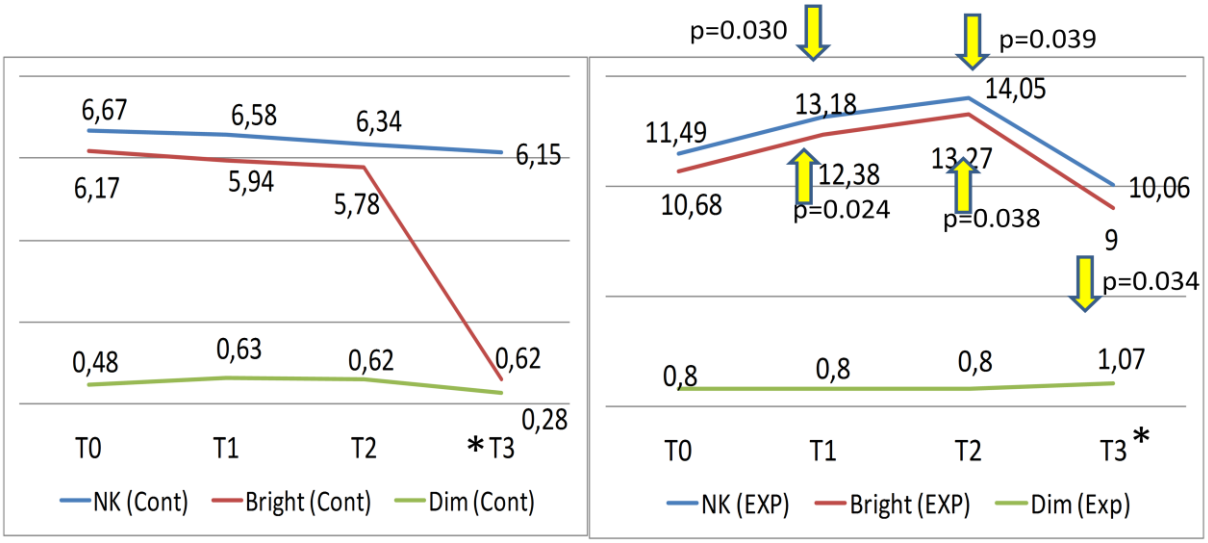

**Graphic 2: Lymphocyte populations. Comparison between groups and across time.**

**Effect of acupuncture on NK cells**

Statistically differences among groups on total NK cells and NK bright cells values were revealed after 2 sessions of AcuMoxa treatments (p=0.030, p= 0.024, respectively) and remained until T2 (p=0.039 and p= 0.038, respectively). At T3, it was verified a decrease in total NK cells. This may be due to the fact that two patients have mouth infections and received antibiotics treatment, and two other patients (one on control group and other on the experimental group) blood samples were coagulated impossibilitating the analyses of these patients in this time point.

NK Dim subset become statistically different between the two groups after 6 sessions of AcuMoxa treatment (p=0.034).

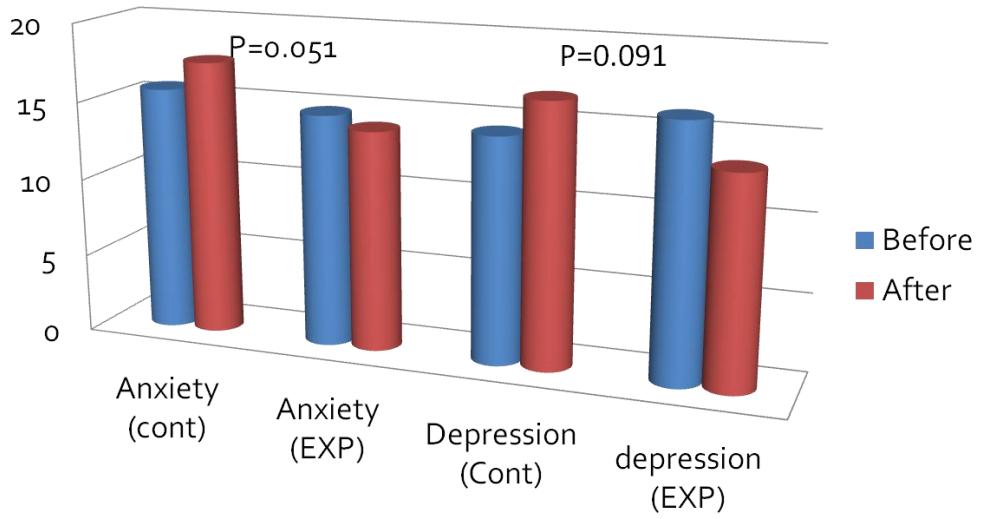
Also, it is clear that experimental group reveals a increasing tendency, which is opposite to the observed in the control group: a decreasing tendency (Graphic 3).




**Graphic 3: NK cells and subsets. *at T3, n=7.**

### Effect of acupuncture on Anxiety and Depression Levels

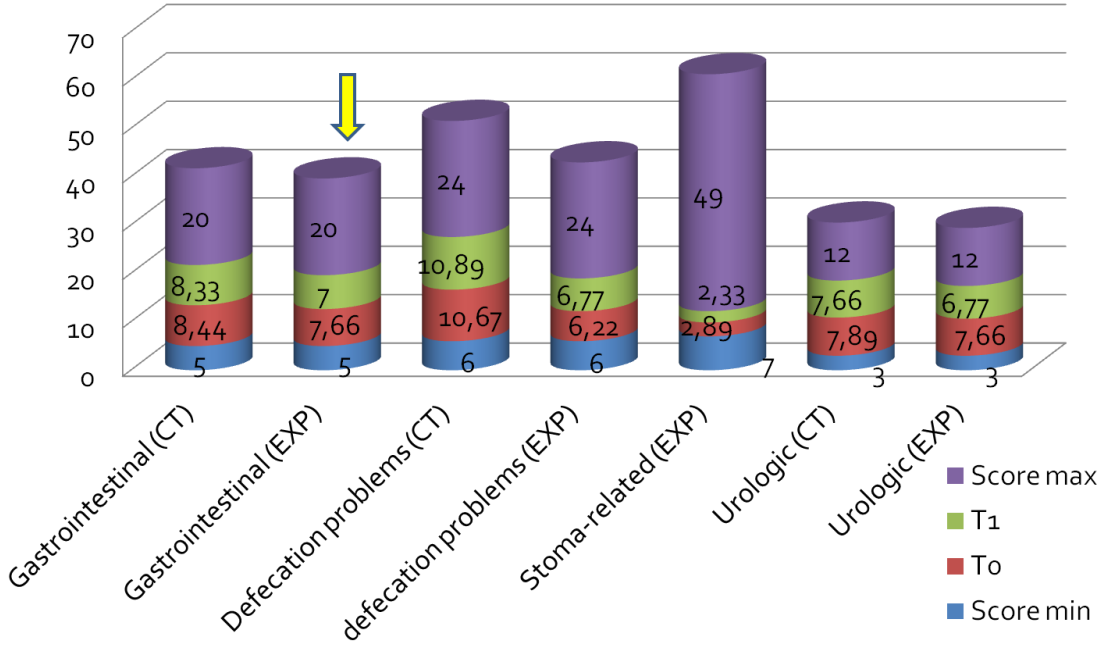
The comparison of the two groups at the begining of the study shows an worse emotional status of patients on the experimental (Graphic 4). After intervention differences among the groups were verified: the AcuMoxa group have a lower score of anxiety and depression when compared with the control group; the control group reveals worse scores at the end of the study than in the begining. Moreover, a negative correlation between depression and anxiety scores and NK cells was revealed (p=0.015 and p=0.003, respectively).



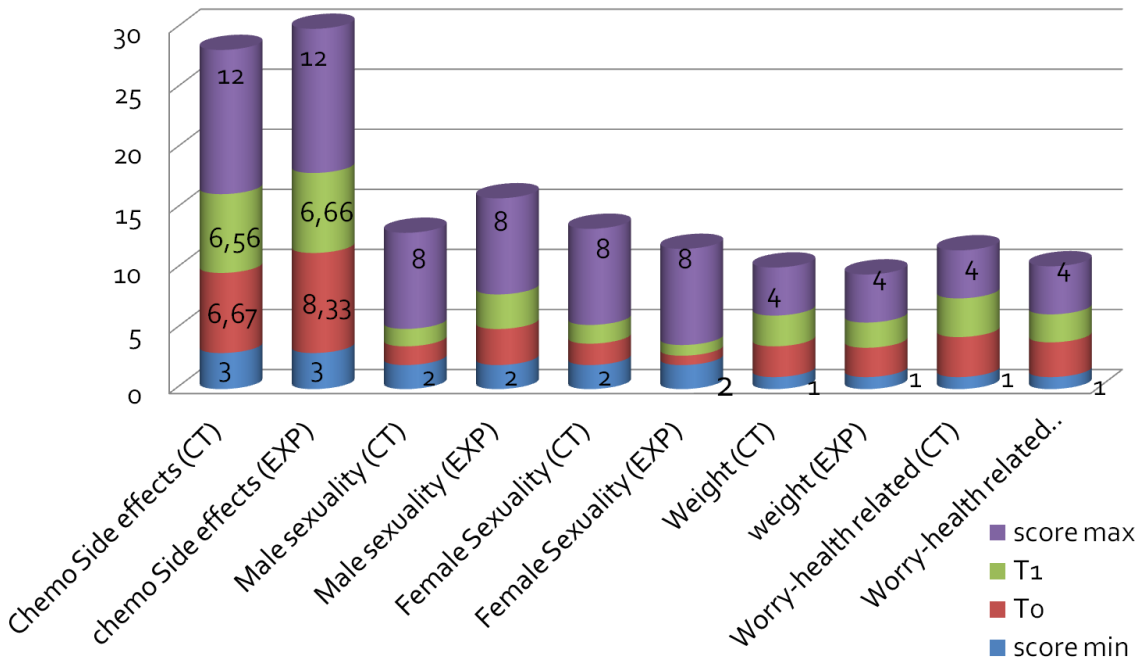

**Graphic 4: Comparison of Anxiety and Depression scores among groups, at begining and at the end of the study.**

**Effect of Acupuncture on patients QOL**

It was not identified differences among groups most probably due to the fact that was a short period of intervention and follow-up of patients (3 weeks of AcuMoxa treatments). However, the data collected shows a tendency of reduction of several symptoms such as gastrointestinal symptoms, urological symptoms, stoma-related symptoms, chemotherapy side effects, sexual function (male) and also about concerns on weight and future perspectives.




**Graphic 5: QOL assessment through questionnaire CR29 (I).**




**Graphic 6: QOL assessment through questionnaire CR29 (II).**

### Adverse events during the study period

No adverse events occurred during the study period.

## 2. Discussion

CRC is one of the most common cancers and a major cause of death due to cancer worldwide. 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.[23]

Studies on CRC tumourigenesis and prognosis[86, 87] 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.[5]

However, some West trials revealed that acupuncture modulates NK cell number and function in diverse clinical situations: in women with severe anxiety[125] and in pain syndromes[126] and also healthy volunteers[108].

There is a lack of data regarding the effect of acupuncture and moxibustion on CRC patients. In fact, only two eastern studies have addressed modulation of NK cells subset activity in CRC patients[105,106] revealing that acupuncture and moxibustion increased T lymphocyte subsets as well as NK cells.

To our knowledge, this is the first controlled clinical trial on acupuncture and moxibustion NK cells modulation in CRC conducted in the West. Although our results must be interpreted cautiously, due to small samples sizes, we found consistent trends of higher WBC, NK cells and its subsets in AcuMoxa group relative to control group of patients with colorectal cancer undergoing chemotherapy.

Our results reveal that AcuMoxa protocol increases WBC counts and ANC resulting in approximately a 1,5 reduction in leukopenia and neutropenia rates compared to the control group, suggesting a myeloprotective effect of acupuncture and consequently a reduction of treatment delays, chemotherapy regimens modifications (cycle delay, dose reduction), as well as reduction of supportive care costs. These observations must be confirmed in future larger studies. However, our results are consistent with the results of other clinical trials referred in a meta-analysis[100] and with a clinical trial on patients with ovarian cancer undergoing chemotherapy[101].

Regarding to NK cells counts, our results revealed an increase of total NK cells and $NK^{Bright}$ cells after one week of AcuMoxa treatment pointing to an immediate immunoregulatory effect of AcuMoxa treatment and a later cytotoxic effect: increase of $NK^{dim}$ cells after two weeks of AcuMoxa treatments. Our results are congruent with the literature[15,18] that stresses that $NK^{Bright}$ cells are immature precursors of $NK^{dim}$ cells that produces several cytokines, such as IFNγ, triggering the generation of tumour-specific TH1 T cells and CTL. The activation of CTL induces the antitumor activity of NK cells. In fact, IFN has been associated with antigen presentation stimulation through upregulation of MHC class I and II molecules on many cell types, modulation of leukocyte interactions with the endothelium, cell proliferation control and sensitivity to apoptosis, stimulation of the bactericidal activity of phagocytes and with the block of angiogenesis.

Moreover, Yu *et al*[17] found that during the NK cells maturation process occurs a successive and significant decrease in cytokine-mediated proliferation and IFN production, an increase in granzyme B and perforin 1 expression, and CD94-mediated redirected killing.

As described previously, psychological and emotional stress has negative implications for increased symptom perception and poor quality of life, and is significantly associated with decreased lymphocyte percentage, and more specifically with decreased NK cells numbers and activity.[24] In fact, some studies showed an association between stress reduction in cancer trajectory and improvement of immune responses in women with breast cancer. [24]

Our results are consistent with the literature showing that anxiety and depression levels decrease with increasing NK cells values and indicating a negative correlation between depression and anxiety levels and NK cells.

Moreover, Arranz et al reported that acupuncture improve impaired immune functions on anxious women (chemotaxis, phagocytosis, lymphoproliferation and NK activity) and that acupuncture reversed the lowering of IL-2 levels and elevating of TNF-alpha and cortisol seen in anxious women.

In respect to quality of life, our study do not express any statistically significant results. This is probably due to the short period of treatment (only three weeks; 6 AcuMoxa treatments). However, the results shows a tendency of decreasing of certain symptoms, such as gastrointestinal symptoms, urological symptoms and chemotherapy side effects as well as the improvement of sexual function in men. Also, shows a tendency

of improvement about the patients owns future perspectives, a loss of concern about weight and body image.

It is know that the higher frequency and severity of clinical symptoms are associated with significant levels of depression and anxiety in cancer patients. From our preliminary results we can conclude that the improvement of the immune system induces a decrease of anxiety and depression levels and consequently improves the QOL of CRC patients (Fig. 18).

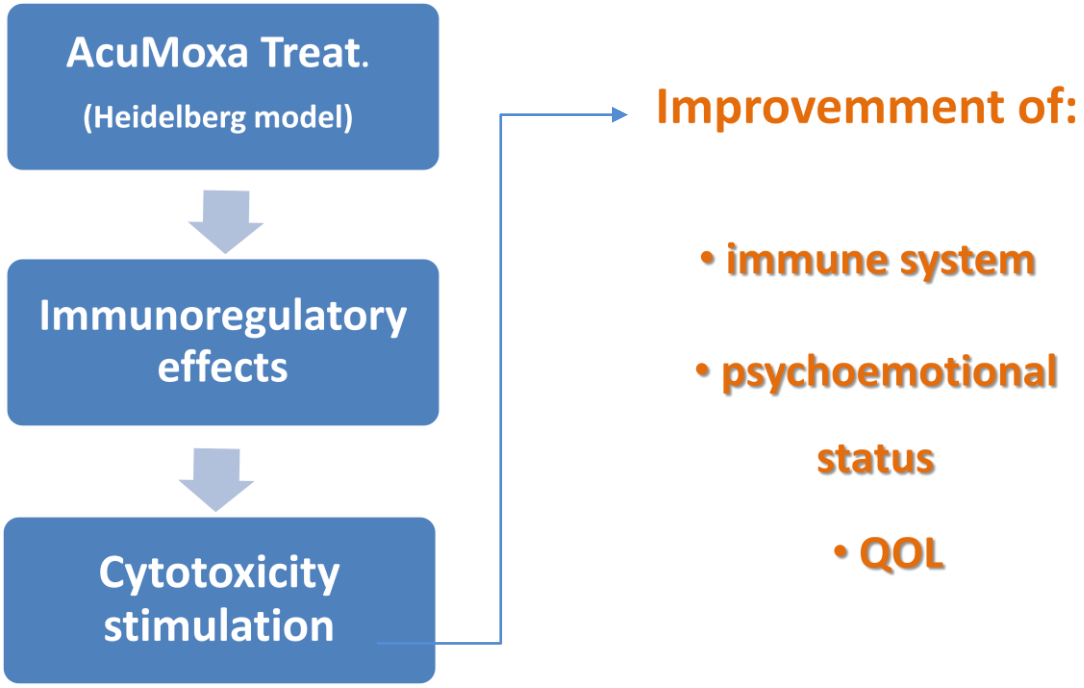



**Figure 18: Effects of AcuMoxa protocol in immune system, according to Heidelberg Model of TCM.**

Regarding to the mechanism through which acupuncture acts on the immune system is generally accepted that an increase on the release of endogen opiod peptid is the key pathway. (Han *et al*., 1999)

Takahashi *et al*,[104] reviewed the ascribed immunomodulation of acupuncture and conclude that acupuncture treatment appears to be able to modulate immunosuppressed or immunoactivated conditions through various functions. In general, immunomodulatory effects of acupuncture might be expressed under three categories: local, neuronal and neurohumoral immunomodulation.

As previously described (chapter 3, section 4), Johnston hypothesized that acupuncture may promote secretion of opioid peptides, induced by the action of nitric oxide (NO) into several central pathways. *β*-endorphin may then influence immune cells

by binding to opiod receptors on the surface of NK cells. Consequently, it would promote tumoricidal activity through the expression of cytotoxic molecules and production of IFNγ. This cytokine would further increase the expression of NK cell receptors and possibly cytokine secretion by other immune cells, thereby amplifying anticancer immune functions.

On the other hand, two more mechanisms were addressed to acupuncture immunomodulation effects: the regulation of the NK-related gene expression in the spleen[105] and the regulation of sympathetic nervous system (SNS).

In fact, the activation of HPA axis and SNS, that occurs in cancer, results in high levels of catecolamines and glucocorticoids, which increases sympathetic outflow and decreases NK activity in the periphery: citotoxicity diminishes in 1 hour and NK counts diminishes in 20 min. Catecolamines affect predominantly NK cells and can suppress them indirectly, by their selective suppression of Th1 response and inhibition of IL12 and IFNγ, and directly due to the high amounts of β2-adrenoreceptors (corticotropine-release hormone-SNS axis).

Thus, we can foresee that acupuncture acts on the SNS and the HPA axis, diminishing the levels of catecolamines and consequently reduce the suppressive effects of catecolamines on NK cells, allowing the maturation of $NK^{bright}$ to $NK^{dim}$ cells and the balancing Th1 and Th2 responses.

In summary, our study protocol provide evidence that acupuncture and moxibustion may stimulate anticancer immunity via activation and subsequent differentiation of NK cells, through regulation of the SNS and the HPA axis. As consequence it is achieved the improvement of the immune system status, the reduction of chemotherapy side effects, as well as the improvement the quality of life of CRC patients.

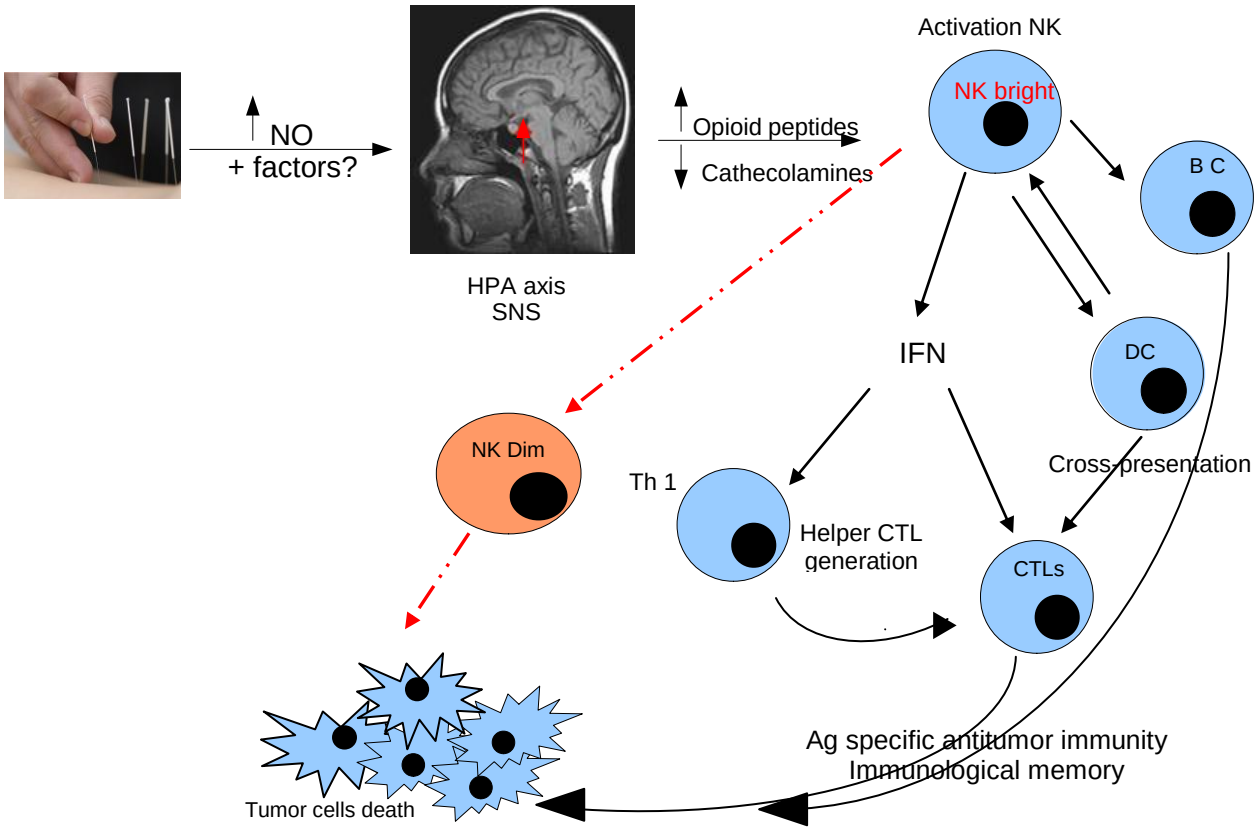



**Figure 19.** Simplified scheme of acupuncture mechanisms of action on the NK cells tumoricidal activity.

# PART FOUR – CONCLUSIONS AND FUTURE PERSPECTIVES

## CONCLUSIONS

Current literature data supports a plausible effect of acupuncture immunomodulation via activation of NK cells. These cells act both on innate and adaptive immunity.

Our clinical research protocol revealed that acupuncture/moxa treatment according to the Heidelberg Model of Traditional Chinese Medicine promote NK cells activity, initially stimulates the immunoregulatory activity and later the cytotoxic activity, resulting on the improvement of the immune system, of the psychoemotional status, and of the QOL of CRC patients, as well as in the reduction of chemotherapy side effects in CRC patients.

In the future, this complementary treatment might have a positive impact in the prognosis and QOL of CRC patients and may constitute an adjuvant strategy on the conventional treatment of CRC as well as on the treatment of other cancers.

In fact, NK cells are being feasible targets of immunotherapeutic approaches like antibody-based strategies. However, there is some limitations regarding the toxicity of these therapies using interleucine. Acupuncture treatment appears to be a possible and efficient strategy that overcomes such limitations.

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## REFERENCES


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# APPENDIXES

## Appendix 1. Approval statement of the Ethics Committee

Direcção Clínica
9.9.13

17.5.13

**Exmo. Senhor**
**Presidente do Conselho de Administração do**
**Centro Hospitalar de S. João – EPE**

AUTORIZADO
CONSELHO DE ADMINISTRAÇÃO REUNIÃO DE 24 SET 2013

**Assunto:** Pedido de autorização para realização de estudo/projecto de investigação

**Nome do Investigador Principal:** Irene Maria Teixeira Pais

**Título do projecto de investigação:** EFEITO DA ACUPUNTURA NA FUNÇÃO IMUNOLÓGICA DE DOENTES COM CANCRO COLO-RETAL SUBMETIDOS A QUIMIOTERAPIA - ESTUDO

Pretendendo realizar no(s) Serviço(s) de Oncologia Médica do Centro Hospitalar de S. João – EPE o estudo/projecto de investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efectivação.

Para o efeito, anexa toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de S. João respeitante a estudos/projectos de investigação, à qual endereçou pedido de apreciação e parecer.

Com os melhores cumprimentos.

Porto, 29 / Maio / 2013

O INVESTIGADOR/PROMOTOR

Irene Pais

Comissão de Ética para a Saúde do Centro Hospitalar de S. João – EPE
Modelo CES 01

**Comissão de Ética para a Saúde – Centro Hospitalar São João**

**Parecer**

**Título do Projecto**: Efeito da acupuntura na função imunológica de doentes com cancro colo-rectal submetidos a quimioterapia – Estudo prospectivo randomizado controlado.

**Nome do Investigador Principal**: Irene Maria Teixeira Pais

**Local onde sera realizado o estudo**: Serviço de Oncologia Médica e Patologia Clínica do Centro Hospitalar de São João, havendo autorização dos respectivos Directores de Serviço. Apresenta, ainda, como Elo de Ligação ao Serviço de Oncologia Médica, a Dra Isabel Pimentel.

**Objectivo do estudo**:
Avaliar os efeitos da acupuntura no sistema imunológico em doentes com cancro colo-rectal submetidos a quimioterapia.

**Período previsto de conclusão**: Setembro 2013

**Benefício**: Estão referidos como benefícios, uma possível melhoria da qualidade de vida dos doentes, melhor analgesia e controlo de sintomas associados a doença oncológica e laterais à quimioterapia.

**Riscos:** Estão referidos como incomodo, ligeira dor e equimose no local associada a acupuntura.

**Respeito pela liberdade e autonomia do sujeito do ensaio**: Está previsto a obtenção de um consentimento informado, bem como um exemplar, de uma informação escrita para o participante, sobre o estudo.

**Confidencialidade dos dados**: está garantida a confidencialidade dos dados e esta informação será restrita à investigadora principal.

Está previsto a realização de um questionário aos participantes.

A Investigadora Principal dispõe de competência técnica e científica para a realização do estudo.

**Custos**: O estudo prevê custos acrescidos para a instituição (exames complementares de patologia clínica) estando referido, por partes dos investigadores, que serão suportados pelo Serviço de Patologia Clínica – autorização por parte do respectivo Director de Serviço.

**Parecer**: Em face da análise do protocolo de estudo, proponho a sua aprovação pela CES do CHSJ.

Porto, CHSJ, 08 de agosto de 2013

O Relator

Dr. John Preto

CES

COMISSÃO DE ÉTICA PARA A SAÚDE

**7. SEGURO**

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)

NÃO ☐

NÃO APLICÁVEL ☑

**8. TERMO DE RESPONSABILIDADE**

Eu, Irene Maria Teixeira Pais,

abaixo-assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo no decurso do actual internamento ou da mesma consulta.

Porto, 29 / Maio / 2013

A Comissão de Ética para a Saúde tendo aprovado o parecer do Relator, aguarda que o Investigador/Promotor esclareça as questões nele enunciadas para que possa emitir parecer definitivo.

2013.06.28

Irene Maria Teixeira Pais

O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO

emitido na reunião plenária da CES de ___/___/___

Reunião que forma os esclarecimentos prestados pela investigação,

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

2013.08.29

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Autorizado
Dr. Jorge F. Santos
27.9.2013

U.PORTO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO
Rua de Jorge Viterbo Ferreira n.º 228,
4050-313 PORTO

Porto, 1 de Março, 2013

**Exmo. Senhor**
**Presidente Conselho de Administração**
**Centro Hospitalar de Vila Nova de Gaia/Espinho (CHVNG/E)**
**Doutor Álvaro Ferreira Cunha Monteiro**

***Assunto**: Pedido de autorização para realização de estudo científico de Tese de Mestrado.*

Na qualidade de membro coordenador do curso de Mestrado de Medicina Tradicional Chinesa (MTC), a decorrer no ICBAS, Universidade do Porto, venho solicitar a colaboração do Serviço de Oncologia do vosso Hospital, com o objectivo de levar a cabo um estudo científico para a conclusão de uma tese de mestrado em MTC.

O trabalho proposto a estudo visa avaliar os efeitos da Acupunctura no sistema imunitário de doentes com patologia colorrectal sujeitos a tratamento de quimioterapia e será realizado pela mestranda em MTC, Irene Maria Teixeira Pais, sendo apoiada na sua orientação pelo Prof. Henry Greten (director do curso de MTC-ICBAS) e com a co-orientação de Dr. Moreira Pinto do CHVNG/E. Segue em anexo a proposta com os objectivos e metodologia de trabalho para que V.Exa. possa melhor ponderar sobre a exequibilidade do mesmo.

Agradecemos que nos possa responder com a brevidade possivel, dado que o periodo lectivo, para a elaboração da tese de mestrado, termina em meados de Agosto do corrente ano.

Com os melhores cumprimentos

CHVNG/E, EPE p/ Dr. Moreira Pinto
Director do Serviço Oncologia
Nº Mecanográfico: 5780
7/2013

Secretariado do C.A.
Entrada nº 21960
1ª Entrada 17/7/13
2ª Entrada / /
3ª Entrada / /

P/a Comissão Coordenadora do MTC

Prof. Doutor Jorge P. Machado
Lab. Fisiologia Aplicada
**CHVNG/E, EPE**
Nº 305/2013
Data 4/7/2013
Tipo de documento: Ofício
Serviço de Formação, Ensino e Investigação

Contacto: mail: irene.mtc@gmail.com
Tlm: 917993644

## Appendix 2. Informed consent

# DECLARAÇÃO DE CONSENTIMENTO

***Considerando a "Declaração de Helsínquia" da Associação Médica Mundial***
***(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)***

***Designação do Estudo (em português):***

EFEITO DA ACUPUNTURA NA FUNÇÃO IMUNOLÓGICA DE DOENTES COM CANCRO COLO-RETAL SUBMETIDOS A QUIMIOTERAPIA - ESTUDO PROSPECTIVO, RANDOMIZADO, CONTROLADO

**Eu, abaixo-assinado, (nome completo do doente ou voluntário são)**

declaro não ter participado em nenhum outro projecto de investigação durante este internamento, tendo compreendido a explicação que me foi fornecida acerca do meu caso clínico e da investigação que se tenciona realizar. Foi-me ainda dada oportunidade de fazer as perguntas que julguei necessárias, e de todas obtive resposta satisfatória.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação ou explicação que me foi prestada versou os objectivos, os métodos, os benefícios previstos, os riscos potenciais e o eventual desconforto. Além disso, foi-me afirmado que tenho o direito de recusar a todo o tempo a minha participação no estudo, sem que isso possa ter como efeito qualquer prejuízo na assistência que me é prestada.

Por isso, consinto que me seja aplicado o método, o tratamento ou o inquérito proposto pelo investigador.

Data: ____ / _____ / 201___

***Assinatura do doente ou voluntário são:*** _____

O Investigador responsável:

***Nome:*** Irene Maria Teixeira Pais

***Assinatura:***

Comissão de Ética do Centro Hospitalar de S. João – Modelo CE 01

## INFORMAÇÃO AO PARTICIPANTE

Caro Participante,

Este estudo designa-se "**O efeito da acupuntura na função imunológica de doentes com cancro colo-retal submetidos a quimioterapia**". O investigador principal é a **dra. Irene Pais** que poderá contactar sempre que necessário para **telemóvel 917993644.**

Pretende-se com este estudo avaliar os efeitos da acupuntura no sistema imunitário em doentes com tumor colo-retal submetidos a quimioterapia. A participação neste estudo é de **carácter voluntário** e dela não resultará qualquer interferência com o seu tratamento médico habitual. Terá o tempo que entender necessário para decidir livremente sobre a sua participação este estudo. A qualquer momento poderá retirar-se do curso, sem qualquer comprometimento da sua relação com os médicos ou dos seus direitos assistenciais.

Este estudo será conduzido no Serviço de Oncologia e em colaboração com o Serviço de Patologia Clínica do Centro Hospitalar de São João. Serão convidados a participar neste estudo doentes com cancro colo-retal submetidos a tratamento de quimioterapia. Para tal é necessário que assine o documento de consentimento informado.

Esta avaliação implicará a comparação de um tratamento "verdadeiro" de acupunctura com um tratamento "falso" de acupuntura. Através de um sorteio, cada participante poderá ser incluído no grupo "verdadeiro" (acupuntura/moxabustão verdadeira) ou no grupo "falso" (grupo sem tramento de acupunctura). Serão usadas agulhas de acupuntura descartáveis de 36G, 0,20x25mm, de aço inoxidável e revestidas de silicone (Tewa®). A profundidade da agulha será de aproximadamente 10 mm. Na terapia de moxabustão serão usados cones ocos auto-adesivos de moxa (*Artemisia vulgaris*) sobre os pontos de acupuntura. O tratamento terá a duração de aproximadamente 30 minutos. Sendo a acupuntura uma técnica de baixo custo e com raros efeitos laterais quando executada por profissionais de formação acreditada, o impacto desta técnica terapêutica é sempre mínimo.

No caso de se obterem resultados considerados benéficos com o tratamento verdadeiro, os participantes do grupo "falso" serão convidados para realizarem um tratamento verdadeiro.

Todos os dados serão tratados com privacidade e confidencialidade.

Será necessário estar disponível para 2 a 3 tratamentos de acupuntura durante a semana e para 3 colheitas de sangue venoso periférico.

Este estudo foi aprovado pela Comissão de Ética para a Saúde do Centro Hospitalar de São João.

A investigadora principal

Dra Irene Pais

## Appendix 3. Socio-demographic, QOL and HADS questionnaires

### I - QUESTIONÁRIO SÓCIO-DEMOGRÁFICO

Por favor, responda às seguintes questões gerais.

**1.** Idade: _____anos **2.** Data de Nascimento: _____/_____/_____

**3.** Género: 0. ____ Feminino 1. ____ Masculino

**4.** Estado Civil: 0___Casado(a) ou união de facto 1.___ Solteiro(a)

2.___Divorciado(a) ou separado(a) 3.___Viúvo(a)

**5.** Habilitações Literárias: 0.___ 1º Ciclo; 1.___ 2º Ciclo

2.___ Secundário 3.___Ensino Superior

**6.** Profissão: _____

**7.** Situação Profissional: 0.__Estudante 1.___Empregado(a) 2.___ Desempregado 3.___ Baixa médica 5.___ Reformado(a)

**8.** Concelho de residência: _____

**9.** Religião: 0.____Católico 1.___Agnóstico(a)

2.___Evangélico(a) 3.___Testemunha de Jeová

**10.** Tempo que passou desde o diagnóstico: _____

**11.** Com quem mora: 0.___Sozinho 1.___Companheiro(a) e Filho(s)

2.___Pais 3.___Companheiro(a) e Filho(s)

4.___Outros

## II A - EORTC QLQ - CR29

Por favor, indique o grau de ocorrência dos seguintes sintomas durante a semana passada. Por favor, responda assinalando com um círculo no número que melhor descreve a sua saúde.

| **NA ÚLTIMA SEMANA:** | **NÃO** | **POUCO** | **MODERADO** | **MUITO** |
|---|---|---|---|---|
| 31. Urinou frequentemente durante o dia? | 1 | 2 | 3 | 4 |
| 32. Urinou frequentemente durante a noite? | 1 | 2 | 3 | 4 |
| 33. Teve algum episódio de incontinência urinária? | 1 | 2 | 3 | 4 |
| 34. Sentiu dor durante a micção? | 1 | 2 | 3 | 4 |
| 35. Sentiu dor abdominal? | 1 | 2 | 3 | 4 |
| 36. Sentiu dor no ânus ou reto? | 1 | 2 | 3 | 4 |
| 37. Teve sensação de inchaço abdominal? | 1 | 2 | 3 | 4 |
| 38. Verificou a presença de sangue nas fezes? | 1 | 2 | 3 | 4 |
| 39. Verificou a presença de muco nas fezes? | 1 | 2 | 3 | 4 |
| 40. Sentiu a boca seca? | 1 | 2 | 3 | 4 |
| 41. Teve queda de cabelo? | 1 | 2 | 3 | 4 |
| 42. Teve perda de paladar? | 1 | 2 | 3 | 4 |
| 43. Esteve preocupado(a) com a sua saúde no futuro? | 1 | 2 | 3 | 4 |
| 44. Esteve preocupado(a) com o seu peso? | 1 | 2 | 3 | 4 |
| 45. Sentiu-se menos atrativo(a) fisicamente? | 1 | 2 | 3 | 4 |
| 46. Sentiu-se menos feminina/masculino? | 1 | 2 | 3 | 4 |
| 47. Sentiu-se insatisfeito(a) com o seu corpo? | 1 | 2 | 3 | 4 |
| 48. Possui um saco de colostomia? | Não | | | Sim |

RESPONDA ÀS SEGUINTES QUESTÕES
**APENAS SE USAR UM SACO DE COLOSTOMIA**

| **NA ÚLTIMA SEMANA** | **NÃO** | **POUCO** | **MODERADO** | **MUITO** |
|---|---|---|---|---|
| 49. Teve episódios de libertação intencional de flatulência do saco? | 1 | 2 | 3 | 4 |
| 50. Teve perda de fezes através do saco? | 1 | 2 | 3 | 4 |
| 51. Possui a pele dorida em volta do estoma? | 1 | 2 | 3 | 4 |
| 52. Efetua frequentemente mudanças de saco durante o dia? | 1 | 2 | 3 | 4 |
| 53. Efetua frequentemente mudanças de saco durante a noite? | 1 | 2 | 3 | 4 |
| 54. Sente-se envergonhado devido ao uso de saco? | 1 | 2 | 3 | 4 |
| 55. Tem problemas em transportar o saco? | Não | | | Sim |

RESPONDA ÀS SEGUINTES QUESTÕES **APENAS SE NÃO USAR UM SACO DE COLOSTOMIA**

| **NA ÚLTIMA SEMANA** | **NÃO** | **POUCO** | **MODERADO** | **MUITO** |
|---|---|---|---|---|
| 49.Teve episódios de libertação intencional de flatulência? | 1 | 2 | 3 | 4 |
| 50. Teve perda de fezes? | 1 | 2 | 3 | 4 |
| 51. Possui a pele dorida em volta do ânus? | 1 | 2 | 3 | 4 |
| 52. Ocorrem movimentos intestinais frequentes durante o dia? | 1 | 2 | 3 | 4 |
| 53. Ocorrem movimentos intestinais frequentes durante a noite? | 1 | 2 | 3 | 4 |
| 54. Sente-se envergonhado devido aos movimentos intestinais? | 1 | 2 | 3 | 4 |

| **NAS ÚLTIMAS 4 SEMANAS** | **NÃO** | **POUCO** | **MODERADO** | **MUITO** |
|---|---|---|---|---|
| **Para os Homens apenas** | | | | |
| 56. Teve interesse sexual? | 1 | 2 | 3 | 4 |
| 57. Teve dificuldade em ter ou manter uma ereção? | 1 | 2 | 3 | 4 |
| **Para Mulheres apenas** | | | | |
| 58. Teve interesse sexual? | 1 | 2 | 3 | 4 |
| 59. Sentiu dor ou desconforto durante a relação sexual? | 1 | 2 | 3 | 4 |

## II B – QUESTIONÁRIO EORTC QLQ – LMC21

Por favor, indique o grau de ocorrência dos seguintes sintomas durante a semana passada. Por favor, responda assinalando com um círculo no número que melhor descreve a sua saúde.

| NA ÚLTIMA SEMANA: | NÃO | POUCO | MODERADO | MUITO |
|---|---|---|---|---|
| 31. Teve falta de apetite? | 1 | 2 | 3 | 4 |
| 32. Teve sensação de saciedade logo após início da refeição? | 1 | 2 | 3 | 4 |
| 33. Sentiu perda ou alteração de paladar? | 1 | 2 | 3 | 4 |
| 34. Sentiu dor durante a micção? | 1 | 2 | 3 | 4 |
| 35. Sentiu a boca seca? | 1 | 2 | 3 | 4 |
| 36. Tem feridas na boca ou na língua? | 1 | 2 | 3 | 4 |
| 37. Tem estado menos ativo do que desejaria? | 1 | 2 | 3 | 4 |
| 38. Sentiu formigueiro nas mãos ou pés? | 1 | 2 | 3 | 4 |
| 39. Sentiu dor no estômago? | 1 | 2 | 3 | 4 |
| 40. Sentiu desconforto no estômago? | 1 | 2 | 3 | 4 |
| 41. Teve a pele ou os olhos amarelos? | 1 | 2 | 3 | 4 |
| 42. Sentiu dor nas costas? | 1 | 2 | 3 | 4 |
| 43. Sentiu-se mais lento(a)? | 1 | 2 | 3 | 4 |
| 44. Sentiu falta de energia? | 1 | 2 | 3 | 4 |
| 45. Sentiu dificuldades em conviver com amigos? | 1 | 2 | 3 | 4 |
| 46. Teve dificuldades em falar sobre os seus sentimentos com família ou amigos? | 1 | 2 | 3 | 4 |
| 47. Sentiu-se sobre stress? | 1 | 2 | 3 | 4 |
| 48. Sentiu-se menos capaz em se divertir? | 1 | 2 | 3 | 4 |
| 49. Preocupou-se com o seu futuro estado de saúde? | 1 | 2 | 3 | 4 |
| 50. Preocupou-se com a sua família no futuro? | 1 | 2 | 3 | 4 |
| **Nas últimas 4 semanas:** | | | | |
| 51. A doença ou tratamento afetou (para pior) a sua vida sexual? | 1 | 2 | 3 | 4 |

## III - ESCALA DE ANSIEDADE E DEPRESSÃO HOSPITALAR (versão Portuguesa-Pais Ribeiro)

Este questionário foi construído para ajudar a saber como se sente. Pedimos-lhe que leia cada uma das perguntas e faça uma cruz (X) no espaço anterior à resposta que melhor descreve a forma como se tem sentido na última semana.

1. Sinto-me tenso/a ou nervoso/a:

( ) Quase sempre ( )Muitas vezes ( ) Por vezes ( ) Nunca

2. Ainda sinto prazer nas coisas de que costumava gostar:

( ) Tanto como antes ( ) Não tanto agora ( ) Só um pouco ( ) Quase nada

3. Tenho uma sensação de medo, como se algo terrível estivesse para acontecer:

( ) Sim e muito forte ( ) Sim, mas não muito forte

( ) Um pouco, mas não me aflige ( ) De modo algum

4. Sou capaz de rir e ver o lado divertido das coisas:

( ) Tanto como antes ( ) Não tanto como antes ( ) Muito menos agora

( ) Nunca

5. Tenho a cabeça cheia de preocupações:

( ) A maior parte do tempo ( ) Muitas vezes ( ) Por vezes

( ) Quase nunca

6. Sinto-me animado/a:

( ) Nunca ( ) Poucas vezes ( ) De vez em quando ( ) Quase sempre

7. Sou capaz de estar descontraidamente sentado/a e sentir-me relaxado/a:

( ) Quase sempre ( )Muitas vezes ( ) Por vezes ( ) Nunca

8. Sinto-me mais lento/a, como se fizesse as coisas mais devagar:

( ) Quase sempre ( ) Muitas vezes ( ) Por vezes ( ) Nunca

9. Fico de tal forma apreensivo/a (com medo), que até sinto um aperto no estômago:

( ) Nunca ( ) Por vezes ( )Muitas vezes ( ) Quase sempre

10. Perdi o interesse em cuidar do meu aspeto físico:

( ) Completamente ( ) Não dou a atenção que devia

( ) Talvez cuide menos que antes

( ) Tenho o mesmo interesse de sempre

11. Sinto-me de tal forma inquieto/a que não consigo estar parado/a:

( )Muito ( ) Bastante ( ) Não muito ( ) Nada

12. Penso com prazer nas coisas que podem acontecer no futuro:

( ) Tanto como antes ( ) Não tanto como antes ( ) Bastante menos agora

( ) Quase nunca

13. De repente, tenho sensações de pânico:

( ) Muitas vezes ( ) Bastantes vezes ( ) Por vezes ( ) Nunca

14. Sou capaz de apreciar um bom livro ou um programa de rádio ou televisão:

( ) Muitas vezes ( ) De vez em quando ( ) Poucas vezes

( ) Quase nunca