ISTITUTO SUPERIORE DI SANITÀ

Seventh international conference

Sino-Italian health days

Istituto Superiore di Sanità
Rome, April 3-4, 2003

Proceedings edited by
Ranieri Guerra and Antonella Marzolini

Ufficio Relazioni Esterne

ISSN 1123-3117

Rapporti ISTISAN
04/17

Istituto Superiore di Sanità
Seventh International Conference. Sino-Italian health days. Istituto Superiore di Sanità. Rome, April 3-4, 2003.
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2004, 63 p. Rapporti ISTISAN 04/17

The main topics addressed by the conference included the Sino-Italian cooperation, applied traditional Chinese medicine, AIDS, cancer, hospital management. Sessions were devoted to present the Italian and Chinese different experiences. The Sino-Italian Health Days were promoted by the Istituto Superiore di Sanità (the Italian National Institute of Health) with the collaboration of Agenda 21, Policlinico Umberto I, Istituto Italo Cinese, Embassy of the People's Republic of China and the Ministry of Foreign Affairs.
Key words: Sino-Italian collaboration, Traditional Chinese Medicine, AIDS, Cancer, Hospital Management

Istituto Superiore di Sanità
Settima conferenza internazionale. Giornate sanitarie italo-cinesi. Istituto Superiore di Sanità. Roma, 3-4 aprile 2003.
Atti a cura di Ranieri Guerra e Antonella Marzolini
2004, 63 p. Rapporti ISTISAN 04/17 (in inglese)

La cooperazione fra Italia e Cina, la medicina tradizionale cinese e la sua applicazione in Italia, l'approccio dei due paesi a tematiche quali l'AIDS, il cancro, il management e la gestione dei grandi ospedali: questi i principali argomenti discussi durante le Giornate Sanitarie Italo-Cinesi organizzate dall'Istituto Superiore di Sanità in collaborazione con l'Agenda 21, il Policlinico Umberto I, l'Istituto Italo Cinese, l'Ambasciata della Repubblica Popolare Cinese e il Ministero degli Affari Esteri.
Parole chiave: Collaborazione Italia-Cina, Medicina tradizionale cinese, AIDS, Cancro, Gestione grandi ospedali

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Presidente dell'Istituto Superiore di Sanità e Direttore responsabile: *Enrico Garaci*
Registro della Stampa - Tribunale di Roma n. 131/88 del 1° marzo 1988

Redazione*: Paola De Castro* e *Sandra Salinetti*
La responsabilità dei dati scientifici e tecnici è dei singoli autori.

INDICE

TUMOR IMMUNOTHERAPY AND PERSPECTIVES FOR THE DEVELOPMENT OF CANCER VACCINES IN ITALY

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Introduction

The impact of cancer in today's society is enormous in terms of human suffering and death as well as in terms of costs and social implications. In developed countries, cancer is second only to hearth diseases as a cause of death and accounts for approximately 20% of all deaths. Because of the profound social and emotional implications of this disease, cancer treatment and prevention deserve a special attention by public authorities responsible for the promotion of public health.

The traditional approach to cancer therapy has been represented by a direct aggression towards the tumor cells by means of physical and chemical injury. Although standard treatments (i.e., surgery, chemotherapy or radiotherapy) still remain essential strategies in cancer therapy, which have been markedly implemented over the last years, some critical issues, such as difficulties in controlling minimal residual disease, drug resistance, metastatic spread and side effects, still represent unsolved problems in cancer management. Thus, the development of novel strategies for cancer treatment capable of inducing long-term antitumor effect without relevant side effects is felt as an urgent need in today's society.

The use of immunological interventions to treat cancer (generally defined as "cancer immunotherapy") has represented an attractive approach characterized by multiple strategies tested with a variable success in clinical settings over the years. The general aim of these anticancer strategies is to trigger an immune response against tumor cells. The three main strategies of cancer immunotherapy tested during the last two decades have been: i) the adoptive transfer of antigen-specific (i.e. CTL) or non-specific (i.e., NK, LAK) immune effector cells; ii) the systemic administration of cytokines (especially IL-2 and IFN-a), with the aim of stimulating host reactive immune cells; iii) the active immunisation by cancer vaccines. Today, an increasing interest is focused on the research on cancer vaccines, as they may represent the most attractive and potentially effective strategy of immune intervention not only for the treatment of human malignancies, but also for the prevention of certain tumors. Moreover, therapeutic cancer vaccines may represent valuable and acceptable interventions as they may induce long-term antitumor effect with negligible toxicity. Therefore, there are a number of scientific, social, economic and medical reasons that justify special attention to the use of vaccination strategies for several neoplastic diseases. The research progress in immunology has opened new opportunities for the immune prevention of pathogen-induced malignancies and for the prevention and treatment of spontaneous tumors. We have now begun to understand how some pathogens can induce the development of human tumors and which pathogen antigens can represent valuable candidates for the development of these types of preventive vaccines. Likewise, it is well known that tumor cells express a variety of antigens recognised by the host's immune system. Table 1 shows the main Tumor-Associated Antigens (TAA), which have been characterized, in human tumors. The research progress in biotechnology and molecular biology has led to multiple modalities for using these TAAs in order to induce an antitumor immune response in cancer patients (1).

In this article, we review the main concepts and perspectives regarding the development of prophylactic and therapeutic vaccines against cancer. We also summarize the results of research projects carried out by our group and review some recent initiatives aimed at the promotion of the research on cancer vaccines in Italy.

Table 1. Main TAA characterized in human tumors

Antigen	Tissue distribution
MAGE	Melanoma, breast, head and neck, bladder, gastric and lung cancer
RAGE	Renal carcinoma, sarcoma, bladder carcinoma, melanoma
HLA-A*201 mut	Renal carcinoma
RAS-D12	Colon and pancreatic carcinoma
K-RAS-D13	Colon carcinoma
P53-mut	Colon and lung carcinoma
HER-2/neu	Breast and ovarian carcinoma
Pml/RARa	Acute Promyelocytic leukemia
Bcr/abl	Chronic myeloid leukemia
MUC-1	Breast, colon and pancreatic carcinoma
Tyrosinase	Melanoma
Gp100	Melanoma
Melan-A/MART-1	Melanoma

Prophylactic vaccines against cancer

There are two main types of prophylactic or preventive vaccines against cancer: i) vaccines directed against pathogens known to induce malignancies in humans; ii) vaccines designed to prevent spontaneous tumors occurring in subjects at genetic risk of cancer development. While the development of some vaccines directed against pathogen-induced tumors is already at the level of clinical experimentation, the generation of preventive vaccines against spontaneous tumors is still at an early stage of development.

Prophylactic vaccines against pathogen-induced malignancies

Pathogen-induced malignancies represent an important portion of human tumors and their prevention may represent a realistic goal of considerable medical importance. According to the data provided by the International Agency for Research on Cancer (IARC), infectious agents are one of the main causes of human cancer (Figure 1) (2), accounting for 9% of cases in the developed world and 23% of cases in developing countries. Indeed, the first cancer vaccine can be considered the anti-hepatitis B vaccine, which is expected to produce a profound reduction in the development of liver cancer in the developed countries where the vaccine is available. Nevertheless, many concerns still remain regarding the immunisation coverage provided by HBV vaccination. In fact, 5-10% of healthy subjects receiving the standard three dose cycle of HBV vaccine fail to respond to vaccination and about 50% of adults participating in vaccination programme fail to receive the third dose, thus remaining still susceptible to infection. Thus, there is an important medical and social need of developing more effective HBV vaccination strategies, capable of conferring a full protection in individuals at risk of infection with a simplified vaccination regimen, which is highly desirable also for developing countries. In this context, we have recently promoted a clinical trial supported by the European Community and based on the preclinical results obtained in both mouse and human models showing a potent

adjuvant activity of type I IFN (3, 4). This study is aimed at evaluating the potential use of IFN-a as an adjuvant of the HBV vaccine. The main objective of the trial is to demonstrate the possibility of reducing the number of vaccine doses necessary to obtain immune correlates of protection using IFN-a as an adjuvant. The demonstration that the IFN-a can act as an effective adjuvant of HBV vaccine in healthy individuals may lead to novel simplified vaccination strategies for HBV infection, opening new perspectives on the use of this cytokine as an adjuvant for other viral vaccines.

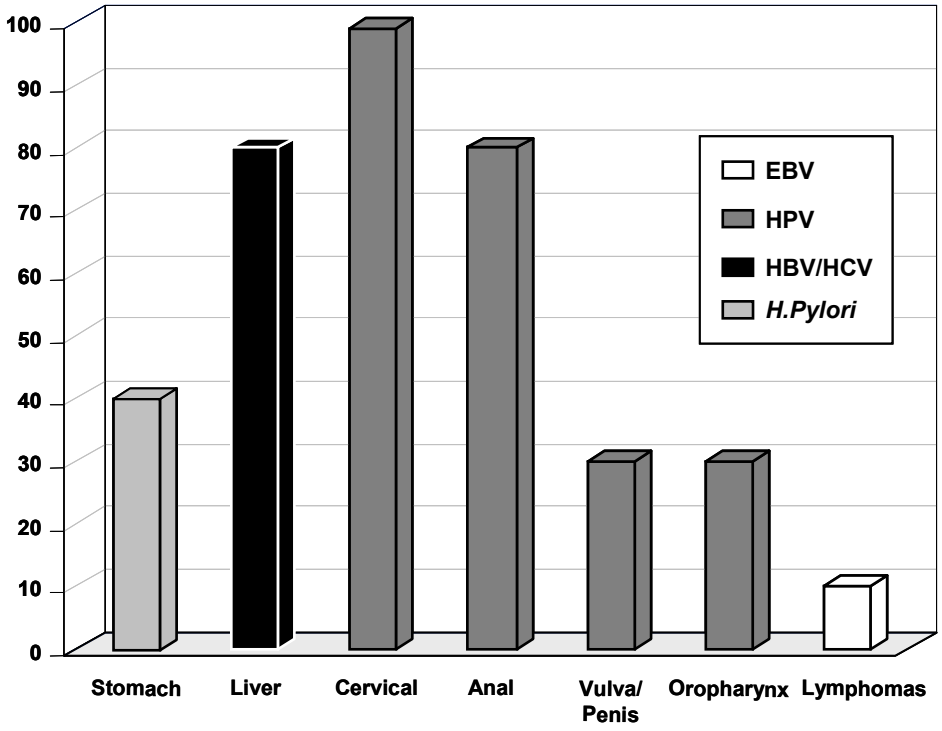

Figure 1. Estimated percentage of cancers caused by chronic infections (for each cancer type the % of infection-associated cases over total cases is reported)

The achievement of other goals relevant for preventing virus-induced human malignancies can be envisaged in the near future. In particular, the development of an anti-papilloma vaccine seems to be a next realistic objective, with a clear-cut benefit for public health, since HPV is involved in the pathogenesis of cervical cancer, which is the second most common cause of cancer deaths in women world-wide (5). Moreover, vaccines directed against other infectious agents associated with human tumors, such as Helicobacter pilori in gastric cancer, are the subject of an intense research effort and there is a realistic perspective of success.

Prophylactic vaccines against spontaneous tumors

In Italy, research projects focused on the development of prophylactic vaccines against spontaneous tumors have been promoted by Guido Forni and colleagues, who used the transgenic model of mice expressing the HER-2/neu oncogene for testing various types of vaccination strategies (6). In this model, the mice develop spontaneous mammary tumors, thus representing an ideal animal system for testing preventive cancer vaccines. By using this model,

Forni and co-workers tested various vaccination strategies and, more recently, found that immunization of the animals with a DNA plamsid coding for a portion of the HER-2/neu oncogene resulted in a strong protection from tumor development (6). These results may have practical implications for the prevention of certain human tumors. The target population for this type of prophylactic vaccines could be represented by individuals with preneoplastic lesions as well as subjects at genetic or environmental risk of developing cancer, that in the future can be detected through genetic screening or by more sophisticated diagnostic tools (6).

Therapeutic vaccines against cancer

The vast majority of clinical studies on cancer vaccines carried out so far have been aimed at the development of therapeutic vaccines. The purpose of any therapeutic vaccination is to control tumor growth or to treat tumors in cancer patients through the activation of effector components of immune system capable of specifically recognising tumor cells (1). Several types of vaccines have been designed and tested during the last decade. One approach has consisted in the use of vaccines capable of inducing antibodies directed against protein as well as non-protein antigens. In this context, recombinant proteins such as MUC-1, CEA and PSA have been tested in association with immunological adjuvants and/or cytokines. Other vaccine strategies have been based on the attempt to induce a cell-mediated immune response against TAAs by using irradiated whole tumor cells. This approach may allow the stimulation of a large variety of CD8+ as well as CD4+ T cells, because of the broad antigenic repertoire of tumor cells. In most cases, autologous tumor cells have been used; in several studies, tumor cells (either autologous or allogeneic) have been genetically modified to express immunostimulatory factors, such as cytokines, in order to enhance the generation of anti-tumor immune responses. Some investigators have used whole tumor cell extracts or Heath Shock Proteins (HSPs), carrying a repertoire of peptides, including TAA-derived peptides. An additional vaccination strategy is based on the use of recombinant TAA or TAA-derived peptides. These types of antigens can be administered either as proteins/peptides or as gene cloned in plasmid vectors or viral vectors genetically engineered for optimal expression in eukaryotic cells. The antigens or the corresponding genes can be administered alone or in combination with certain cytokines capable of orienting the immune response. The scientist who provided the major contributions to the development of clinical studies on therapeutic cancer vaccines in Italy is Giorgio Parmiani. Recent clinical studies of therapeutic vaccination of patients with metastatic melanoma or colorectal carcinoma with autologous tumor-derived HSPs have been performed by Parmiani's group at the National Cancer Institute in Milan (7, 8). The major outcome of these studies is that this type of vaccination can induce a tumor specific immune response in approximately 50% of patients. Notably, a strong direct correlation was observed between the generation of tumor specific T cell-mediated immune reactions and the clinical response, which was detected in approximately 18% of the patients. We have recently promoted a trial of vaccination of stage IV melanoma patients with peptides from two main melanoma TAAs (i.e., MART-1 and gp100) in association with IFN-a as a vaccine adjuvant, in collaboration with the group of G. Parmiani in Milan and of E. Bonmassar in Rome. This study represents the first trial in which IFN-a, a well known cytokine with a long record of clinical use, is utilized as adjuvant of a cancer vaccine. The rationale for this trial stems from an ensemble of preclinical studies carried out in mouse tumor models by our group over the years (9).

Dendritic cells and new perspectives for therapeutic vaccines

Dendritic Cells (DC) are the most potent antigen presenting cells and play a pivotal role in the induction of immune responses, through the activation of naive T cells, B cells and the secretion of cytokines and chemokines. In the last years, DC have been used as natural adjuvants for cancer immunotherapy approaches, in order to trigger immune responses against tumor-derived antigens towards which cancer patients appear to be tolerant. This approach is based on the *in vitro* generation of DC from PBMC of cancer patients, that are loaded ex vivo with tumor antigens (e.g. recombinant tumor antigens, peptides, whole tumor cells or tumor lysates, mRNA from tumor cells) and reinjected into the patients (Fig.2). This procedure can elicit tumor-specific immune responses, possibly associated with a clinical antitumor response. This strategy, together with the identification of several tumor associated antigens, have led to the development of several clinical trials designed to investigate the biological and clinical effects of the use of DC loaded with tumor-derived antigens and administered as a therapeutic vaccine to patients suffering from various types of cancer.

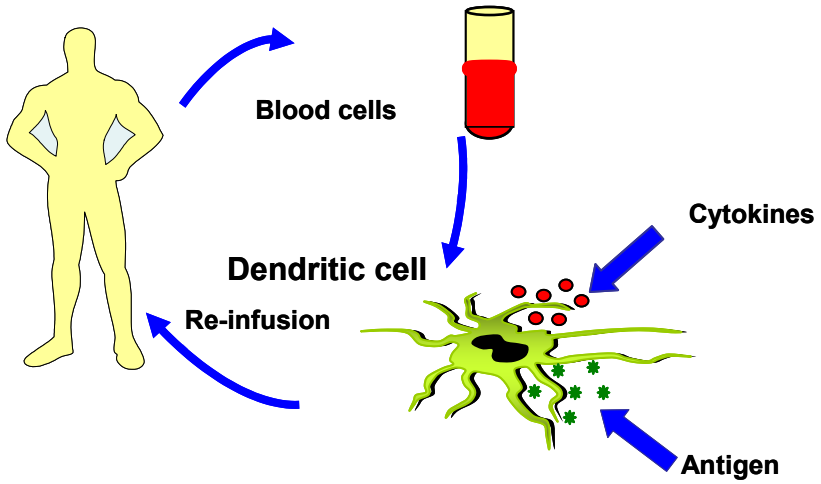

Figure 2. Therapeutic vaccines based on the use of autologous dendritic cells as natural cellular adjuvants

Although, DC-based cancer immunotherapy constitutes today one of the most attractive treatments of cancer, it is not yet clear which DC have the best characteristics, in terms of phenotype and functional activity, for inducing a protective antitumor response. Moreover, the optimal preparation methods for the development of the most effective and reliable DC-based cancer vaccines still need to be determined. One critical need for the development of optimal DC-based therapies is the comparison, in terms of capability to induce a tumor specific immune response, of cell preparations generated by means of different cell culture conditions, in order to determine the best method for a rapid generation of highly active DC. The classical methods for generating DC from monocytes or total PBMC employ GM-CSF (constant factor) and either IL-4 or IL-13. However, these cytokines generate immature DCs requiring a further treatment step to induce DC maturation/activation, which appears to be necessary for obtaining an optimal stimulation of T cell responses. In our laboratory, we have described a new method for the rapid

generation of highly active DC from monocytes. In particular, human DC can be generated in the presence of GM-CSF and IFN-a within 3 days (IFN-DC) and these cells exhibit a strong capability of inducing a primary Th-1 type immune response *in vitro* as well *in vivo* (4, 10). These data strongly support the importance of using new methods, including the use of type I IFN, for generating DCs to be compared with DC generated by standard procedures (11).

A second critical issue for the optimization of DC-based cancer vaccines consists in the choice of tumor antigens and methods for arming the DC for clinical use, by comparing different modalities for DC loading. Trials with DCs have been conducted using cells loaded with tumor antigens from different sources, including whole antigens or peptides, tumour cell lysates, and irradiated tumor cells (12). The definition of optimal antigen loading strategies for DC will lead to an important advance in the development of highly effective DC-based cancer vaccines.

Finally, the optimization of DC administration regimens for the treatment of cancer patients typically implies practical aspects for ensuring maximal reproducibility and standardisation of the cell drugs to be injected. The possibility of DC cryopreservation may represent a crucial improvement for the logistics of the process and would facilitate the application and dissemination of DC-based therapies. Moreover, technical aspects such as PBMC harvesting, the washing of contaminating cells or soluble proteins, simplification of the process and the use of GMP agents, need also to be considered to fully address the issues of DC preparation for clinical use.

Final remarks

There is an increasing interest of the scientific community in the perspectives of the research on cancer vaccines. In this context, institutions responsible for the promotion of biomedical research and public health could play major roles in the promotion and co-ordination of the efforts finalized to the development of prophylactic and therapeutic vaccines against cancer. In particular, public research institutions could play an important “super partes” role in favouring the co-operation with other institutes and private industries, with the specific aim of translating the results of the basic research in tumor immunology into novel clinical applications. With the purpose of promoting and encouraging the research on cancer vaccines in Italy, we have organised two international meetings on Cancer Vaccines, which have been held at the Istituto Superiore di Sanità (Conference on Cancer Vaccines, November 1999 (13); Cytokines as natural adjuvants: perspectives for vaccines development, April 2002) (14). During these meetings, the state of art and the most promising approaches for the development of cancer vaccines have been extensively discussed. One critical issue raised during these meeting was the difficulty felt by several investigators working in public institutions of preparing cells and reagents suitable for clinical use (i.e., prepared under strict GMP conditions). In this context, our institute has recently approved the project for the activation of a “Center of Cellular Immunotherapy” to be dedicated to the preparation of DC-based vaccines. The aim of this initiative is to fill the gap between basic and clinical research in Italy, by promoting well-controlled clinical trials in the field of cancer immunotherapy and cancer vaccines, through the preparation of cells and reagents under GMP condition to be used in clinical trials. All this needs to be viewed in the context of strategies aimed at fostering the international collaboration, which is essential for a global success in this emerging field of biomedical research.

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MOLECULAR ABNORMALITIES OF ACUTE LEUKEMIAS

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Introduction

Hematopoietic development is a complex and carefully orchestrated process that results first in the commitment of pluripotent hemopoietic stem cells into the immature Hemopoietic Progenitor Cells (HPC) and then in the differentiation of HPCs in the bone marrow into differentiated cellular elements of peripheral blood, including Red Blood Cells (RBC), granulocytes, monocytes and platelets. There is a continuous requirement for a careful control of blood cell production related to the large number of circulating cells required for oxygen delivery (RBCs), for protection from infections (granulocytes and monocytes) and for hemostasis (platelets) and to their short half-life. Therefore, it is necessary that the rate of differentiation of HPCs in the bone marrow is finely tuned to meet the requirement for the production of new blood elements that have to replace the senescent blood cells.

Leukemia may be basically defined as a deregulated and disordered hemopoietic cell production deriving from the progressive acquisition of mutations in hemopoietic progenitors that confer a proliferative and/or survival advantage and impair hemopoietic differentiation. Leukemias, as well many other tumors derive from the acquisition of somatic DNA mutations that basically activate proto-oncogenes and inactivate tumor suppressor genes. The genetic instability driving leukemogenesis is fuelled by two types of mechanisms: i) spontaneous DNA mutations and errors made by the DNA machinery; ii) environmental (ionizing radiations and numerous genotoxic chemicals) and products of normal cellular metabolism (i.e., reactive oxygen species and products of lipid peroxidation) that constitute a permanent enemy to DNA integrity.

Acute leukemias are typical examples of neoplasia arising from malignant transformation of hemopoeitic stem cells. Acute leukemias are characterized by premature arrest of myeloid (Acute Myeloid Leukaemia, AML) or lymphoid (acute lymphoid leukaemia) development and the subsequent accumulation of large numbers of non-functional leukemic blasts. Leukemic blasts are usually of clonal origin, thus indicating that the leukemic process originates from a single cell. Although leukemic blasts exhibit relatively homogeneous features, there is clear evidence that the leukemic blast cells present in the bone marrow of each patient are organized in a hierarchical fashion, at a some extent analogous to normal hemopoietic progenitors. Thus, in the leukemic blast cell population there are a minority of cells with features of Leukemic Stem Cells (LSC) in that these cells are capable to propagate leukemic blasts both *in vitro* and *in vivo* in xenogenic mouse models of human leukaemia. The existence of these LSC has fundamental implications for our understanding of the etiology of the leukemic cells, but is also of crucial importance from a clinical point of view in that it implies that to achieve a durable remission is strictly required to ablate LSCs. Several lines of evidence indicate that LSCs exhibit several biological properties different from those displayed by mature leukemic cells and may not be sensitive to standard chemiotherapic treatments: this observation is consistent with the findings commonly observed in clinical studies, where the majority of patients can achieve an apparently complete remission, but in the majority of cases they will relapse.

Current model on the pathogenesis of acute myeloid leukemias

Several lines of experimental evidence suggest that multiple mechanisms are involved in the genesis of human tumors and, particularly, of AMLs (1, 2). Among these different pathogenetic mechanisms the occurrence of chromosomic translocations with consequent generation of fusion genes and corresponding fusion proteins play a major role in the genesis of these diseases. The major contribution of fusion proteins, such as PML/RARα, AML1/ETO, AML1/Evi1 or CBFβ/SMMHC, consists in the capacity to block the differentiation process at a specific stage (reviewed in 3). Studies in transgenic mice harbouring the fusion gene in their genome clearly indicate that the simple expression of this gene in hemopoietic cells greatly contribute to the differentiation block, but per se is not sufficient for the development of a leukemic condition (4). This conclusion is directly supported by several experimental models such as transgenic animals expressing the PML/RARα fusion gene: these animals, in spite a high expression of the transgene, need 1-2 years before the development of leukemia. This time is need for the acquisition in hemopoietic cells of additional mutations, mainly occurring at the level of genes involved in the control of cell proliferation, whose alterations are required for tumor development. Interestingly, similar observations have been also made in M2 leukemias associated with the t(8;21) translocation and the consequent formation of the AML1/ETO fusion protein. Several lines of evidence have indicated that AML1/ETO is insufficient by itself to induce leukemia, but instead alters the self-renewal capacities of hematopoietic stem cells, resulting in the formation of a preleukemic population that lacks a great growth advantage (5). For the development of an overt leukemic condition, AML1/ETO-induced effects must be associated with the effects of secondary mutations that cooperate with the primary mutation and ultimately result in a growth advantage and in a block of cell differentiation (6, 7). In line with these findings a high frequency of N-RAS and c-kit mutations have been observed in AML patients with AML1/ETO$^+$ leukemias.

In the recent years several genes have identified, mutated or abnormally expressed in AML, that confer a proliferative survival advantage to leukemic blasts. These genes are characterized by their involvement in the control of cell proliferation. Thus, the hemopoietic receptor tyrosine kinase Flt3 is constitutively activated in 30-35% of cases of AML, as a consequence of internal tandem-repeat mutations in the juxtamembrane domain, or activating loop mutations (reviewed in 8). Similarly, Flt3 is frequently overexpressed in secondary, therapy-related acute myeloid leukemias (9) and in lymphoblastic leukemia with MLL rearrangements (10). Activating loop mutations have been identified in the hematopoietic receptor tyrosine kinase c-kit in 5% of AML cases (11). Furthermore, activating loop mutations in RAS family members, mainly NRAS and KRAS, have been identified in 15-25% of cases (12).

The concept of multistep, multi-mutational process in the development of acute leukemias is also reinforced by the analysis of leukemias occurring in twins. In these cases both twins usually develop the same type of leukemia, with the same pathogenomic lesion (i.e., with the same chromosomal translocation). The meta-analysis of the natural history of the disease in these cases shows a series of very interesting findings (13). The disease usually has a pre-natal origin and an early role for chromosome translocations is highly conceivable in these conditions. Further, there is a variable period of latency and the need (with the exception for cases of neonatal “congenital” leukemia) for further post-natal exposures to genotoxic agents and/or genetic events to produce clinical disease.

The evidences accumulated in these last years on the genetic basis of human leukemias now provides a very useful framework for attempts to understand etiological mechanisms and to improve the treatment of these diseases.

Interleukin-3 Receptor alpha chain

Acute leukemia cells have usually retained the responsiveness to HGFs stimulation in terms of promotion of cell survival and cell proliferation; however, leukemic cells show little maturation under stimulation with HGFs (reviewed in 14). More particularly, recombinant IL-3 and GM-CSF induce leukemic colonies and activate DNA synthesis in more than 80% of AMLs (15-18). No clear relationship between IL-3 and GM-CSF responses and the French-American-British (FAB) classification of ALs was observed (14). Furthermore, leukemic cells may produce one or more of the principal HGFs, including IL-3 and GM-CSF (19, 20). Thus the concomitant expression of receptors for IL-3 and GM-CSF and production of the respective ligands by leukemic cells, determines the formation of complete autocrine circuits of HGF stimulation. According to these observations it was suggested that autonomous mechanisms of growth may contribute to the clinical biology of leukemia.

IL-3 and GM-CSF exert their biological activities through interaction with cell surface receptors that consist of two subunits, the a subunit specific to each and the β common chain (βc) (21, 22). The α chain (IL-3Rα, GM-CSFRα) binds IL-3 and GM-CSF, respectively, with high specificity, but with low affinity (23). The interaction of a α chain with a β chain leads to the formation of a high-affinity receptor complex which is able to bind the respective ligand in the range of its physiological concentrations and to transduce proliferative, anti-apoptotic and differentiative signals (23-25). The βc expressed alone, in the absence of a specific a chain, confers little binding affinity for either IL-3 or GM-CSF (26-27).

Studies on AML blasts have shown that receptors for IL-3 and GM-CSF are often coexpressed on these cells (28-30). Furthermore, specific IL-3 binding was observed in about 50% of B-ALL (31). Finally, a recent study showed that IL-3Ra chain was overexpressed in $34^+/38^-$ AML blasts as compared to the expression of this receptor chain observed in the corresponding normal counterpart (32).

Other studies have shown that in a significant proportion of both myeloid and lymphoid acute leukemias tranducers of the signal originated from IL-3R/GM-CSFR, such as JAK2 and Stat5, are constitutively activated (33, 34). On the other hand, studies carried out on IL-3 dependent cell lines have shown that overexpression of IL-3Rα chains leads to cell proliferation either in the presence of suboptimal IL-3 concentrations or in the absence of growth factors (35).

According to the ensemble of these observations it seemed of interest to investigate the pattern and the level of IL-3Rα chain expression in acute leukemias, particularly in view of evaluating a possible linkage between the level of this receptor chain and the proliferative status of leukemic blasts.

We have investigated the expression of interleukin-3 receptor α (IL-3Rα) chain in primary blasts from 79 AML, 25 B acute lymphoid leukemia (B-ALL) and 7 T acute lymphoid leukemia (T-ALL) patients to evaluate a linkage between the expression of this receptor chain, blast proliferative status and disease prognosis (36). While in the majority of T-ALL IL-3Rα chain was scarcely expressed, it was overexpressed in 40 and 45% of B-ALL and AML cases, respectively, as compared to the levels observed in normal $CD34^+$ progenitors. The biological and clinical significance of this overexpression pattern was investigated in AMLs. At biological level, the elevated IL-3Rα expression was associated with peculiar properties of leukemic

blasts. Specifically, (i) in all cases the blasts expressing an elevated IL-3Rα level exhibited both higher cycling activity and increased resistance to apoptosis triggered by growth factor deprivation; (ii) Spontaneous Stat5 phosphorylation was observed in 13% of AML patients, all pertaining to the group of patients exhibiting high IL-3Rα expression; (iii) following IL-3 treatment, Stat5 was activated at higher level in blasts with elevated IL-3Rα expression. At the clinical level, (i) a significant correlation was observed between the level of IL-3Rα expression and the number of leukemic blasts at diagnosis; (ii) patients exhibiting elevated IL-3Rα levels had a lower complete remission rate and survival duration, as compared to those showing normal IL-3Rα levels.

These findings suggest that in AML deregulated expression of IL-3Rα may contribute to the proliferative advantage of the leukemic blasts and hence to a poor prognosis.

Our results also suggest that the elevated IL-3Rα expression observed in a significant proportion of AMLs may play a central role in the biology of these leukemias. In fact, the overexpression of IL-3Rα in IL-3-dependent cell lines allowed an increased responsiveness to IL-3 (i.e., the cells proliferate in the presence of suboptimal IL-3 concentrations) and also induced a significant level of mutants exhibiting growth factor independency (i.e., the cells proliferate in the absence of IL-3) (35). Furthermore, overexpression of GM-CSF in mice induces the development of a fatal myeloproliferative disease (37). Third, the lack of expression of JunB in mice induces a marked overexpression of GM-CSFRa, that leads to an increased responsiveness to GM-CSF responsible for the induction of increased levels of the anti-apoptotic molecules Bcl-XL and Bcl-2 and in turn for the development of a chronic myeloproliferative disease (38). In keeping with these observations we suggest that the increased IL-3Rα expression observed in about 40% of AMLs may be in part responsible for the more aggressive leukemic phenotype of these cells and for their reduced response to standard chemotherapy, as observed in the patient population hereby analysed for treatment outcome. Larger clinical trials will allow to definitely assess the prognostic value of IL-3Rα levels in AML.

As above mentioned the mutation of the membrane Flt3 receptor represents the most frequent genetic abnormality observed in AML. It is believed that these mutated receptors are constitutively activated, but it is unknown the mechanism through which they confer a growth advantage to leukemic blasts (AML with Flt3 mutations usually have a high number of blast cells at the diagnosis and have a worse prognosis). Recent studies, however, clearly suggests that the mutated Flt3 leads to the constitutive activation of Stat5 (39, 40) and, therefore, stimulates the growth of leukemic blasts through the same pathway activated by the IL-3R. It is of interest to note that the wt Flt3 receptor appeared mainly to use the MAPK pathway rather than the Stat5 pathway to transmit a proliferative signal, thus indicating that Stat5 activation is a property only of the mutated Ftl3 and therefore must be considered as an aberrant signalling.

Genetic pathways to acute leukemias

The development of animal models to study the events that leads to the formation *in vivo* of some leukemias has allowed to propose a model of cooperating genetic events required for the genesis of leukemia. In this context, particularly interesting was the model of acute promyelocytic leukemia. The initiating event seems to be represented by chromosomal translocation leading to the formation of the fusion protein PML/RAR-alpha that impairs differentiation; however, leukemogenesis requires the activation of additional pathways. The addition of a so-called cooperating mutation that improves the survival of the cells, such as overexpression of an anti-apoptotic protein such as Bcl2 is not sufficient to allow the complete

leukemic transformation. In contrast, mutations that deregulate the control of cell growth, such as constitutively activated cytokine signalling pathways, such as Flt3 or IL3R, are associated with the rapid development of a leukemic condition, associated with the development of additional chromosomic mutations. The pathways activated by Flt3 and IL3R are highly redundant and involve the activation of several target genes mainly represented by c-myc, Bcl-XL and Bcl-2 (41).

According to all these findings a "2-hit" model of leukemogenesis was proposed. Following this model the development of a leukemia requires two types of events. Class I mutations are those that, alone confer a proliferation and survival advantage to the hemopoietic progenitors but do not affect differentiation. Examples of these mutations are represented by Flt3 mutations, c-kit mutations, activating mutations in N-RAS or K-RAS. Class II mutations are exemplified by those leading to the formation of fusion proteins such as PML/RAR-α, AML1/ETO, MLL-related fusion genes, CBFβ/SMMHC are able to impair cell differentiation, but alone are unable to cause the development of an entire leukemic process. The expression of both types of mutations strongly favours the development of a leukemic process. We propose that in addition to class I mutations, there are also events of overexpression of some cytokine receptors such as the IL-3R or Flt3 that may act as class I mutations by eliciting uncontrolled proliferation of leukemic cells.

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DEVELOPMENT AND INTERNATIONAL COOPERATION OF ONCOLOGY IN PEOPLE'S REPUBLIC OF CHINA

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Current situation of oncology in China

The incidence rate of cancer increases to about 129.3/105-305.4/105 of male, and 39.5/105-248.7/105 of female in China.

According to the report of Ministry of Health of People's Republic of China in 2001, cancer has been the top one on mortality rate of all diseases (Table 1).

Table 1. Mortality rate in 2001 of China

Cause of death	Total		Male		Female	
	death rate $1/10^5$	constituent ratio%	death rate $1/10^5$	constituent ratio%	death rate $1/10^5$	constituent ratio%
Malignant tumor	135.59	24.93	163.77	27.64	106.27	21.55
Cerebrovascular disease	111.01	20.41	118.33	19.97	103.39	20.97
Heart disease	95.77	17.61	97.5	16.46	93.98	19.06
Respiratory disease	72.64	13.36	76.42	12.90	68.70	13.93
Injury	31.92	5.87	39.31	6.63	24.24	4.92
Endocrinopathy	17.18	3.16	20.47	3.45	20.50	4.16
Digestive disease	17.06	3.14	13.52	2.36	14.76	2.74
Urinary disease	8.55	1.57	8.9	1.50	8.18	1.66
Psychosis	5.37	0.99	5.94	1.00	5.60	1.13
Neurosis	5.20	0.96	5.15	0.87	4.43	0.90
Total		**92.00**		**92.78**		**91.02**

The top ten of malignant tumor are gastric cancer, hepatocarcinoma, esophageal cancer, colorectal cancer, leukemia, cervical cancer, nasopharyngeal cancer, breast cancer and bladder cancer (Table 2).

In all the inpatients, the constituent ratio of malignant tumor has increased gradually from 2.24% in 1965 to 5.63% in 2001.

There are sixty one cancer hospitals in China, 17,316 sick beds and 22,440 stuff members are in these cancer hospitals.

The number of oncologic doctors including surgeons, physicians and so on, have increased about six times to 12,428.

Table 2. Death rate of top ten malignant tumor ($1/10^5$)

Malignant tumor	Countryside population			City population			Total population		
	male	*female*		*male*	*female*		*male*	*female*	
Total	139.89	83.29	112.57	133.24	79.16	106.91	134.99	80.23	108.39
Gastric cancer	25.23	13.23	19.44	35.62	18.42	27.25	32.89	17.07	25.21
Hepatocarcinoma	28.26	10.11	19.50	29.36	11.62	20.72	29.07	11.23	20.40
Lung cancer	38.08	16.16	27.50	18.94	8.73	13.97	23.97	10.66	17.50
Esophageal cancer	13.11	5.89	9.62	25.35	14.67	20.15	22.13	12.39	17.40
Colorectal cancer	7.13	6.82	6.98	5.29	4.14	4.73	5.77	4.84	5.32
Leukemia	4.03	3.26	3.66	3.93	3.33	3.64	3.96	3.31	3.64
Cervical cancer		3.27	1.58		4.09	1.99		3.88	1.88
Nasopharyngeal cancer	2.61	1.20	1.93	2.27	1.07	1.69	2.36	1.10	1.75
Breast cancer		5.31	2.56		2.92	1.42		3.54	1.72
Bladder cancer	2.16	0.86	1.53	1.22	0.42	0.83	1.46	0.53	1.01

Development of Chinese anti-cancer system

The Chinese Anti-Cancer Association (CACA) was established on 28th April, 1984. It is affiliated to China Association of Science and Technology.

CACA aims its utmost efforts at organizing scientific cooperation and conferences, promoting international non-governmental exchanges, conducting various training courses and seminars, fostering scientific, technological and medical personnel in the field of oncology, compiling and publishing academic periodicals, and mobilizing social forces to take part in popularization and dissemination of the knowledge of tumor prevention.

CACA has set up local associations in almost all provinces with total membership exceeding 30,000 by the end of 2000. CACA is a council unit of the Asia-Pacific Federation of Organizations for Cancer Research and Control (APFOCC).

CACA has 28 professional committees including committee of cancer etiology, tumor markers, cancer epidemiology, cancer biotherapy, cancer chemotherapy, anticancer agent research, pediatric tumors, head and neck tumors surgery, clinical cytology, naopharyngeal cancer, esophageal cancer, lung cancer, breast cancer, stomach cancer, colorectal cancer, sarcoma, liver cancer, gynecological cancer, malignant lymphoma, hematologic malignancies, traditional medicine, rehabilitation, tumor pathology, administration of cancer institute and hospital and clinical oncology cooperative center.

CACA has published seven academic journals, such as Chinese Journals of Cancer Research and so on.

Each year, CACA organizes National Congress on Oncology. And organizes international interchange conference on all aspects of cancer.

The School of Oncology, Peking University (Beijing Cancer Hospital)

It was founded in August 1976. After 20 years of development, it has become a comprehensive center for the prevention, treatment and rehabilitation of cancer, carrying out teaching, research and clinical service at the same time.

It is composed of two divisions, the basic oncology studies and the hospital.

The Division of Basic Oncology Studies comprises eight departments and labs, including Department of Tumor Etiology, Tumor Epidemiology, Biochemistry and Molecular Biology, Tumor Immunology, Cell Biology, Cytogenetics, Experimental Animal Farm, and the Beijing Laboratory of Tumor Cytobiology.

The Beijing Cancer Hospital has 12 departments with a capacity of more than 500 beds, including Department of Tumor Internal Medicine, Tumor Surgery, Radiotherapy, Traditional Chinese Medicine, Pathology, Clinical Test Laboratory, Department of Diagnostic Imaging, Ultrasonic Examination Lab, Out-patient Clinic, Department of Rehabilitation, Preventive Medicine, and Information Center.

International cooperation field

In the recent years, we have developed national and international cooperations.

We carried out the cooperation with NSABP to developed multicenter cooperations on breast cancer, gastric cancer and colorectal cancer among several cancer hospital in China.

We received a research grant from the Sino-US research fund to research the prevention and treatment of gastric cancer in high incident area.

Also we often organized international conferences in China such as the Total Mesorectal Excision, TME workshop, the international conference on cancer rehabilitation and palliative care, the international seminar in chemical and immunological prevention of cancer and so on.

We still want to develop more extensive and intensive international cooperation on every possible aspect. Exchange the cases information, advanced technology, new idea and experience.

EPIDEMIOLOGY OF HIV/AIDS IN CHINA

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HIV/AIDS situation in 2002

A total number of 40,560 HIV infections were identified and reported to the Ministry of Health by the end of 2002 in China.

According to the estimation of the government and UNAIDS, the number might have been reached 1 million.

Nine are the Chinese Provinces with the most serious HIV epidemics by the end of 2001:

1. Yunnan (10,525)
2. Xinjiang (6.036)
3. Guangxi (3.740)
4. Guangdong (2.704)
5. Henan (1.677)
6. Sichuan (1.139)
7. Beijing (911)
8. Anhui (538)
9. Shanghai (529)

Annual reported cases in1985-2002

Currently the indicators for assessing HIV trends in China are 6, collected through Sentinel System:

- HIV prevalence among Intravenous Drug Users (IDU) in selected drug rehabilitation centers (mostly males);
- HIV prevalence among female prostitutes in selected re-education centers;
- HIV prevalence among Sexually Transmitted Diseases (STD) patients in selected sentinel clinics (stratified by sex);
- HIV prevalence among pregnant women in rural areas;
- HIV prevalence among long distance truck drivers;
- HIV prevalence among Men having Sex with Men (MSM).

Risk factors

The HIV/AIDS risk factors in China are:

1. drug use;
2. unsafe sexual behaviours and prostitution;
3. illegal blood collection;
4. Men who have Sex with Men (MSM).

Drug use

Hundred thousands are the drug users registered in the Public Security Systems in China. The total number is constantly increasing.

There are increasing proportion of drug users who are injecting, and proportion of IDU who are sharing needles.

HIV positives rate among the IDU are dramatically increasing year by year.

There are nearly 910,000 drug users registered by Public Security in China; the real number, however, is estimated by the relevant authorities to be several times higher.

Only 1 Province reported HIV cases in IDUs before 1995. 3 Provinces reported HIV cases in IDUs in 1995. 26 Provinces reported HIV cases in IDUs by 2000. All 31 Provinces reported HIV cases in IDUs by 2002.

Table 1. Average HIV positive rate in IDU

	1995	1996	1997	1998	1999	2000
HIV positive rate	0.02	1.7	6.6	10.8	12.1	10.0

Data from Sentinel Surveillance System

Prostitution

The identified prostitutes and their clients was over 600,000 in 2000. The estimated number of prostitutes is 5 to 10 folds of the identified number. Table 2 reports the average HIV positive rate in Commercial Sex Workers (CSW) and Figure 1 the Percentage of condom use among CSW.

Table 2. Average HIV positive rate in CSW

	1995	1996	1997	1998	1999	2000
HIV positive rate	0.02	0.02	0.3	0.4	0.8	1.32

Data from Sentinel Surveillance System

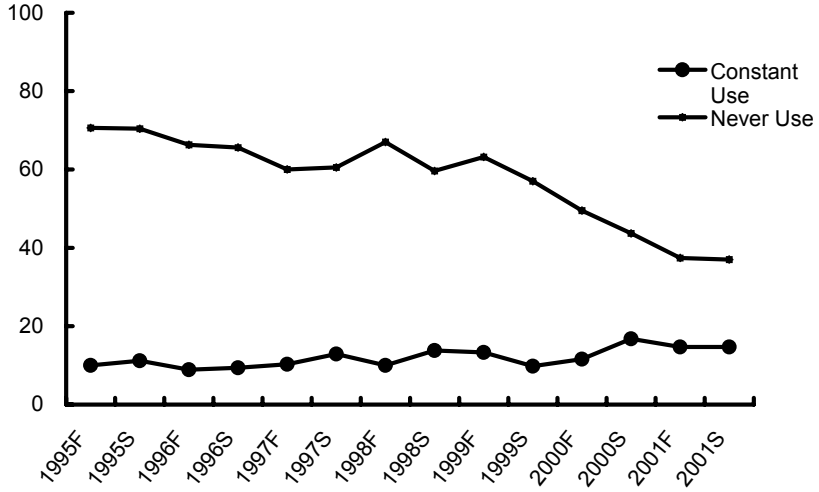

Figure 1. Percentage of condom use among CSW

Summary of epidemic involving plasma sellers

HIV epidemic through illegal plasma selling has been under control. Care and support should be further strengthened to those who had been infected and affected. Table 3 shows the HIV infection among Former Plasma Sellers (FPS).

Table 3. HIV infection among FPS

Population	Number tested	HIV (+)	%
FPS	541	240	44.4
Others	1073	47	4.4
Total	1614	287	17.8

Men having sex with men

The estimated prevalence 1-10%.
The HIV prevalence 10%.
Desensitization of the issue.

Strategies and measures

- China's medium and long-term strategy for HIV/AIDS prevention and control (1998-2010)
- China's Action Plan for Reducing and Preventing the Spread of HIV/AIDS (2001-2005)
- Condom Promotion
- Methadone Treatment/Needle Exchange Program
- Care and Support for People Living With HIV/AIDS (PLWHA)
- Anti-Stigmatization and Discrimination
- Involvement of PLWHA
- Better Functions of NGOs.

STEM CELLS AND CARDIOVASCULAR DISEASES

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Regeneration is an essential function of human body. Recent interest has been focused on the development of stem cells as a therapeutic option to improve tissue regeneration. In all tissues, interruption of blood supply, caused by occlusion of an artery leads quickly to cell death and loss of tissue followed by scar formation. In skeletal muscle the regenerative process is assured by the presence of muscle precursor cells, known as satellite cells, which proliferate, differentiate and fuse to form multinucleated myotubes. In contrast, the heart is less equipped to deal with injury since adult cardiomyocytes are terminally differentiated cells, and, once destroyed, are scarcely replaced. Thus, the identification of factors, which can promote tissue regeneration, represents an important therapeutic goal. Recently it has been demonstrated that, in response to ischemia, the increased production of several growth factors and cytokines, induces mobilization of bone marrow derived precursor cells (EPC) which home and integrate into foci of neovascularization. We demonstrated in fact, that one of these factors, the Vascular Endothelial Growth Factor (VEGF) is produced by ischemic skeletal muscle and play a role, not only in the angiogenic response to ischemia, but also in skeletal muscle regeneration. Satellite cells expresses VEGF receptors, Flk-1 and Flt-1 which are down-regulated during skeletal muscle differentiation. Remarkably, VEGF enhances satellite cells migration and prevents skeletal muscle apotosis following ischemic injury suggesting a novel autocrine role of VEGF in skeletal muscle repair during ischemia.

Current therapeutic approaches to induce cardiac tissue regeneration are limited and include medical therapy. Although promising results have been obtained with transplantation and mobilization of bone marrow cells to the area of the infarction, signals that regulate stem cells homing to areas of tissue injury are not well characterized. Several hypotheses suggest that cell necrosis following an injury such as myocardial infarction may cause the release of signals that circulate and induce mobilization of stem cells. However it is clear that circulating stem cells are not efficiently attracted into the damaged tissues. In our laboratory we analyzed the role of a new cytokine (protein X), in the induction of cardiac tissue regeneration following infarction. Using a mouse model of infarction generated by ligating the coronary artery, we found that protein X injection results in highly efficient myocardial regeneration one week later. This regeneration was characterized by the presence of newly-formed cells in the infracted area, expressing both a-sarcomeric actin, a specific marker of cardiac differentiation and the transcription factor MEF2. Echocardiographic and hemodynamic parameters demonstrated a recovery of cardiac performance in protein X injected hearts compared to hearts injected with a control protein. These results demonstrate that this cytokine promotes cardiomyocytes regeneration in the ischemic area, ameliorating the performance of the infarcted heart.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases are lethal, neurodegenerative disorders of the central nervous system (CNS) affecting animals (e.g. Scrapie in sheep and goats, Bovine Spongiform Encephalopathy in cattle) and humans (e.g. Creutzfeldt-Jakob disease) (1).

MOST COMMON TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

CJD (Creutzfeldt–Jakob disease)
Fatal neurological disease clinically characterised by rapidly progressive dementia, myoclonus, visual or cerebellar signs pyramidal/extrapyramidal signs, and akinetic mutism. There are three different forms: sporadic CJD accounts for 85% of all forms, has a worldwide distribution, with no identifiable cause; iatrogenic CJD occurs accidentally as the result of medical procedures, such as contaminated neurosurgical instruments, cadaveric dura mater graft or therapy with cadaveric pituitary-derived hormones; familial CJD accounts for 10–20% of all cases and is always associated with mutations in the PrP gene (PRNP).

GSS (Gerstmann–Sträussler–Scheinker syndrome)
A very rare inherited autosomal dominant disease, associated with mutations in the PRNP (the most common are at codons 102 and 198). GSS is characterised by chronic progressive ataxia, terminal dementia and a long clinical duration (2–10 years).

FFI (Fatal familial insomnia)
Inherited disease characterised by sleep disturbances, vegetative and focal neurological signs as a result of thalamic lesions. The clinical phenotype depends on the D178N point mutation of the PRNP coupled with a methionine at codon 129.

vCJD (variant Creutzfeldt–Jakob disease)
A newly discovered human TSE that has been related to the consumption of BSE-contaminated food. This form affects almost exclusively young adults and is characterised clinically by a progressive neuropsychiatric disorder leading to ataxia, dementia and involuntary movements without the typical EEG appearance of CJD. Brain magnetic resonance imaging (MRI) shows a bilateral hyperintensity of posterior thalamic nuclei. PrPsc is present in tonsils. Neuropathology shows marked spongiform change and extensive florid plaques throughout the brain.

Kuru
Extinct epidemic disease of Fore people of Papua, New Guinea, related to cannibalistic rituals. No individual born after 1960 has ever developed kuru. Incubation time may be unusually long, exceeding four decades in some cases.

Scrapie
Naturally and worldwide occurring neurological disease of sheep and goats, first described more than two centuries ago.

CWD (Chronic wasting disease)
A fatal brain-wasting disease described in captive and free-ranging cervids in the USA since 1980.

BSE (Bovine spongiform encephalopathy)
First described in 1986 in the UK where more than 180 000 cows have since developed the disease. BSE has also emerged in other European and non-European countries.

They are typically but not exclusively characterised by a triad of pathological lesions within the brain: neuronal loss, glial reaction (i.e. microglial activation, hypertrophy and hyperplasia of astrocytes) and spongiform change (i.e. vacuolation of neurones and neuropil). Amyloid plaque formation is a further prominent feature in some circumstances. Clinical signs of these diseases take months, years or even decades to develop. The infectious agent responsible of these affections is apparently devoid of nucleic acid and the accumulation of an amyloid protein (PrPsc) is the hallmark of the agent replication. According to prion theory, PrPsc is the sole component of the infectious particle but alternative and still valid hypothesis hold that prion protein (PrP) serves as a shuttle substance and/or receptor for an as yet undiscovered infectious agent (2-3). Therefore PrP is present both in the cellular isoform, PrPc, as well as in a misfolded, pathological isoform, PrPsc. PrPc and PrPsc are identical in amino acid sequence but differ only in their conformational structure. Spectroscopic studies reveal that PrPc has a high alpha-helical content whereas PrPsc is rich in beta-sheets (4). PrPc is a host-encoded glycoprotein located at the cell surface, where it is bound by a glycosyl-phosphatylinositol anchor (GPI). It is present in a variety of tissues and is mainly expressed in the CNS. The precise physiological function of PrPc remains elusive: PrPc binds copper and has a superoxide dismutase (SOD) like activity; it may act as a free radical scavenger contributing to the antioxidative capacity of cells (5).

A wealth of experimental data demonstrates its essential role in the susceptibility and in the pathogenesis of TSEs and the conversion of PrPc into the detergent insoluble, partially protease-resistant and infectious isoform PrPsc, is the key event in the pathogenesis of TSEs. The transmission of the infection, the incubation period and the pattern of disease (i.e. phenotype) depend on interactions between the particular strain (or isolate) of infectious agent and the host's genotype. More than 20 scrapie strains have been isolated in mice. Each strain is characterised by a stable and reproducible incubation period in a given host, a typical pattern of histological brain lesions, and a well defined PrPsc glycotype (i.e. glycoform ratios of the protease digested PrPsc) after western blot (6-7). Moreover "Prions" do not induce in the host any apparent immune response probably because they are "self proteins". Development of prion diseases can have an infectious or genetic origin, or can arise spontaneously. Allelic forms of PrPc have been linked to the disease susceptibility in several species including mice, sheep and humans. More than 30 mutations in the human PrP gene (PRNP) have been described and for some of them a strong genetic linkage has been also established (1-8). Aminoacid changes, resulting from these mutations, make likely the protein more susceptible to the conformational change, which is usually associated with acquisition of protease resistance and infectivity. Genetic predisposition is relevant to all types of human TSEs (i.e. inherited, sporadic and iatrogenic).

By far the most important polymorphism of interest is that found at codon 129 of PRNP (i.e. methionine or valine) (9). In the Caucasian population, around 51 per cent are heterozygous (M/V), with around 38 per cent methionine homozygous (M/M) and 11 per cent valine homozygous (V/V). Other polymorphisms of PRNP have been identified, though the full relevance of many of them is as yet not clear. The recent epidemic outbreak of Bovine Spongiform Encephalopathy (BSE) in UK and also the finding that BSE can also be found in many other countries raises anxiety and concerns in public (10). Identification of variant CJD (vCJD) in 1996 was based on novel neuropathological and clinical features in a series of ten patients (11). It seems very probable that vCJD results from the ingestion of BSE infected meat products. In vCJD – differently from the other CJD forms – PrPres accumulation is widespread in lymphoid tissues (i.e. tonsils, spleen, lymph node, and appendix) and showed a uniform and distinct glycotype pattern (12). Until now 142 individuals have been identified with probable or confirmed diagnosis of vCJD (6 in France, 1 in Ireland, 1 in Italy, 1 in Canada, 1 in USA and

132 in UK). All analysed cases of vCJD were M/M at codon 129 of the PRNP. We do not know if cases of vCJD in individuals who are MV or VV will there be in the next future. Continued surveillance is required to further investigate this possibility. So far is not known how many people are incubating vCJD. The potential for vCJD to be transferred among humans via blood, blood-derived products or improperly sterilised surgical instruments is also a matter of concern (13). To prevent the spread of the disease and to determine how many people are infected we need an effective, simple and sensitive, diagnostic test (14). Recently, Zanusso and colleagues demonstrated that PrPsc is deposited in the neuroepithelium of the olfactory mucosa in patients with sporadic CJD, indicating that olfactory biopsy may provide extremely useful diagnostic information in living patients (15).

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DEVELOPMENT AND INTERNATIONAL COOPERATION OF CLINICAL TRIALS OF TRADITIONAL CHINESE MEDICINE

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Management of drug clinical trials in China

In order to improve the scientific and research level of drug trial, thirty-five clinical trial bases was approved by Ministry of Health from 1983 to 1990. In 1998 one hundred and fifteen drug trials bases were authorize once again by Ministry of Health in china including five hundreds and forty-three specialties. At the same time the State Drug Administration (SDA) was formally funded. After that, the SDA has decreed series regulations, such as "Drug Administration Regulations of People's Republic China", "Good Clinical Practice" (GCP), and so on .The China GCP is in accordance with the international recognized principles.

Development of clinical trials of Chinese medicine

Origination

Traditional Chinese Medicine (TCM) was originated from the direct clinical observation, and developed following the clinical practice. Chinese medicine has played very important role in guaranteeing the health care of the Chinese people. On the other hand, it have some shortage needed to been innovated.

Development

Since 1950s, absorbing and introducing scientific research method for reference, clinical trials of TCM has promoted the development of TCM. But meanwhile, some problems are still unresolved as follows: the design is not rigorous, research objects lack comparability, observation index is not clear, and the analysis of therapeutic efficacy is not objective, and so on.

Further development

Since 1990s, the western medicine has been putting more and more concerns on the traditional medicine, and also TCM more extensively assimilated methods of modern medicine. As a result, clinical research concerning the traditional medicine will become the emphasis and focus in the future.

Brief introduction of GCP Center of Guangdong province hospital

Development history

The GCP center of Guangdong province hospital was authorized as Drug Trial Base of Ministry of Health of the People's Republic of China in the early 1992. The hospital carried out a large quantity of clinical research and fulfilled them successfully since then. A program for constructing GCP center for new Chinese medicines was initiated in April 1997, approved by National Ministry of Health and Ministry of Science and Technology. Later the GCP center was verified by the National Ministry of Science and Technology in October 2000 after 3-year implementation. At the same time, the GCP center was authorized as Training Center for Clinical Trial and Base for Clinical Trial of TCM by SDA.

Main task and advantage

The construct of GCP center was based on the WHO instructional standards, so that it will gradually comply with the international GCP standards. During the past ten years our researchers participated training courses and workshops on clinical trials conducted by the WHO cooperated with State Administration of TCM. Some of them went to the USA and Europe for advanced training in the field of GCP. Nearly 100 people took part in the GCP training course held by overseas and domestic experts.

The multi-center clinical trials of 38 herbal products have been implemented, where our hospital acts as the principal investigator unit or cooperator, in the last five years. These trials covered clinical trial phase I to IV. At the same time the training program of clinical trials for Chinese medicine has also been conducted. More than 300 participants from all over the county took part in the training courses. In addition, many research projects related to GCP for TCM, such as systematic reviews on major diseases treated by TCM, standardization of TCM syndromes, quality of life assessment for TCM health care, were carried out in the hospital.

Cooperation field

We have set up cooperation relationship with many drug trial base in China.

We are good at designing, measuring and evaluating the Chinese medicine clinical trials, including other nature drugs. The leader of our GCP center is the member of the conductor committee of traditional medicine of WHO.

We conducted the training program of clinical trials one time for WHO training project, and two times every year for SDA.

We have received 5 research programs listed in the national tenth five-year plan (mainly conduct and carry out the multi-center clinical trials of TCM), undertaken national "863 project" to construct national modern clinical trials technology platform.

In the past years, we start to cooperate with some foreign institution, such as Covance company, Malaysia Clinical Research Center, Singapore Clinical Trials & Epidemiology Research Unit.

HOW WESTERN MEDICINE LOOKS AT THE TRADITIONAL CHINESE MEDICINE

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History

In spite of the fact that recently some researchers hypothesized that a therapeutic form of needles insertion in the skin was used in Europe 5200 years ago (1) it is commonly accepted that Acupuncture (A) as a therapeutic tool was present in China from 1000 BC and its use lasts until our days. The relationship between China and the Western world lasts since many centuries ago. The first information about acupuncture was reported by Jesuits missionaries in XVII and XVIII century (2). According to Joseph Needam's *Clerks and craftsmen in China and the West* (3) we have sufficient reasons to believe that the grown of medical gymnastics in modern Europe has been influenced by information regarding Chinese practices.

Diffusion in western countries

Some western physicians and quacks in XIX century after travelling in the Far East put needles in patient's skin, but the use of acupuncture was extremely rare.

After communist revolution (1948) political conditions made medical exchanges extremely difficult. Only in the '70s, after the visit of U.S. President Richard Nixon there have been an increasingly improvement in cultural exchanges between western and Chinese world, and the practice of acupuncture in treating identifiable pathological conditions grew exponentially and is actually an useful tool for a growing number of physicians (4).

In a survey presented at the *Sino-Italian Health Days* held in Rome in September 2000, the diffusion of acupunture in Italy have been shown to be increased from the 2 millions patients of 1993 to the 3,5 millions of 1999 (+ 75%) (5).

Basic theories

Acupuncture is based on the 'energy balance' between yin and yang: inserting needles in appropriate points of the channels in which energy flows, the practitioner can restore the correct balance, thus fighting illnesses. Some experimental data provided evidence that acupuncture may have biologic effects but we have no data on a direct relationship between acupuncture practice and anatomical and/or biochemical variations.

Western scientific evaluation

Basic research

Some of the most recently published experimental data on animals and men obtained using both traditional needle acupuncture insertion or electro-acupuncture techniques are (6):

- Effects of A on a rabbit have been transferred on another by blood transfusion.
- Animal evidences that A can be a form of stimulation of gene expression for neuropeptides.
- In Spontaneous Hypertensive Rats (SHR) blood pressure significantly lowered by a meridian stimulation.
- In a model of cardiac ischemia on a cat the O_2 request was reduced stimulating the corresponding meridian by needles.
- Naloxone inhibits the effects on sympathetic flow caused by A.
- A significantly attenuates the raise in blood pressure during mental stress in normotensive volunteers.
- It has been documented by Magnetic Resonance that inserting a needle in an A point located on a foot, traditionally correlated with vision, a region of the occipital lobe was activate, as well as it was with the direct light.
- Magnetic Resonance documented a modulation on the limbic region and on grey sub cortical substance caused by A in man.
- Electro acupuncture induces modifications of receptors for cerebral opioids.

Clinical research

There are still some problems about applying the Evidence Based Medicine (EBM) criteria (i.e. randomized clinical trials comparing active treatments versus placebo) because many acupuncturists declare they treat each patient in a different way one from the other. In 1998 have been described some methods to introduce a placebo needle just to permit a correct western approach in acupuncture research (7). Recently more than 500 randomized controlled trials (more than half of which were placebo controlled) that have evaluated the efficacy of acupuncture in a wide pattern of clinical problems have been reviewed (8).

In Table 1 meta-analyses results of trials in principal pain related clinical conditions are summarized. In back pain (but not in lower back pain), acute dental pain, fibromyalgia, headache acupuncture have been shown to be an effective therapeutic tool when compared to placebo and/or conventional pharmacologic treatment. Efficacy remains doubtful in chronic pain.

In Table 2 the most important trials in non pain related illnesses are summarized: in treating COPD and both pregnancy and chemotherapy nausea and vomiting acupuncture showed strong efficacy evidences.

The use of A in treating addictions has no strong evidence of efficacy (Table 3). Some papers in past years suggested a positive role of non conventional medicine in drugs addiction therapy. Recently a rigorous randomized single blind clinical trial on 620 patients published on Jama stated that acupuncture was not more effective than a needle insertion or relaxation control in reducing cocaine use" (9).

Table 1. Meta-analyses of clinical trials in pain related conditions

Condition	Author (year)	RCT	N. Pat	Evidences	Conclusions
Chronic pain	Ezzo *et al.* (2000)	51	2423	24 positive and 27 negative; for A *vs* placebo trials: 15 positive and 17 negative	Inconclusive evidence: efficacy remains doubtful
Back pain	Emst *et al.* (1998)	12	591 (377 pooled)	9 studies pooled: odds ratio of improvement for A *vs* control, 2.30; for placebo trials, 1.37	A superior to various controls, but insufficient evidence to conclude whether superior to placebo
Low back pain	van Tulder (1999)	11	542	No evidence that A better than no treatment; limited evidence that A was not more effective than placebo	Effectiveness remains unclear
Acute dental pain	Emst *et al.* (1998)	16	941	12 trials suggested that A is more effective than controls; 4 trials suggested the contrary	A can alleviate dental pain
Fibromyalgia	Berman *et al.* (1999)	3	149	All positive, including 1 high quality study	A can be effective; more high-quality trials needed
Headache	Melchart *et al.* (1999)	22	1042	Contradictory results in 8 trials that compared A with other treatments; positive trend in 14 trials that compared A. to placebo	Trend in favour of A, but evidence non fully convincing

RCT: Randomized Clinical Trial

Table 2. Meta-analyses of clinical trials in other than pain related conditions

Condition	Author (year)	RCT	N. Pat	Evidences	Conclusions
Pulmonary disease	Jobst (1995)	16	2937	10 positive; when real A was redefined retrospectively, results were positive in14	A is a safe and potentially effective treatment for bronchial asthma and COPD
Stroke	Park *et al.* (2001)	9	538	6 positive and 3 negative; the best trial was negative	No compelling evidence for the effectiveness of A
Emesis	Vickers (1996)	33	3123	27 of 29 performed with no anaesthesia were positive; in a second analysis that was restricted to 12 high quality trials 11 were positive	A point P6 seems to be an effective antiemetic for cancer chemotherapy, pregnancy and surgery
Emesis	Harris (1997)	12	904	Restricted to acupressure trials; acupressure more effective than placebo for nausea during pregnancy, after surgery and for cancer chemotherapy	Acupressure can be used as an antiemetic

to be continued

continues

Condition	Author (year)	RCT	N. Pat	Evidences	Conclusions
Nausea and vomiting of pregnancy	Murphy (1998)	7	686	6 positive and 1 negative; all were acupressure trials	Acupressure benefits many women, but evidence is equivocal
Postoperative nausea and vomiting	Lee *et al.* (1999)	19	1569	Pooled RR similar to antiemetics in preventing early and late vomiting; better than placebo for early nausea and early vomiting	A is similar to antiemetics in preventing early and late vomiting in adults
Nausea and vomiting of pregnancy	Jewell *et al.* (2001)	4		3 positive and 1 negative, the negative trial was the most rigorous	Clear evidence for beneficial effects but results remain equivocal
Tinnitus	Park *et al.* (2000)	6	185	2 unblinded positive trials and 4 blinded negative trials	A demonstrated to be efficacious
Temporo-mandibular junction disorders	Emst (1999)	3	205	All positive: none compared A. with placebo	Studies need confirmation with more rigorous methods

RCT: Randomized Clinical Trial

Table 3. Meta-analyses of clinical trials in addictions

Condition	Author (year)	RCT	N. Pat	Evidences	Conclusions
Addiction	Ter Riet *et al*. (1990)	13	_	Smoking: 11 positive and 1 negative; alcohol: 1 positive	Claims of efficacy are not supported by sound trials
Weight reduction	Emst (1997)	4	270	2 positive and 2 negative	Evidene contradictory and claims of efficacy not based on trial outcomes
Smoking cessation	Law & Tang (1995)	8	2759	Compared with control, 3% (95%CI, -1% to 6%) more of the patients treated with A stopped smoking	A. has little or no effect
Smoking cessation	White *et al*. (1999)	14	3486	Odds ratio: 1.20 (CI 0,98% to 1,48%)	A. no better than placebo

RCT: Randomized Clinical Trial

Adverse events

Published data on acupuncture adverse events are mainly based on retrospective clinical reports. In Table 4 the adverse events occurred in 55291 acupuncture treatments in Japan, reported from a prospective study, are shown (10).

Table 4. Adverse events occurred in 55291 A treatments in Japan

Adverse event	n.
Forgotten needles	16
Transient hypotension	1
Burn injuy	7
Ecchymosis with pain	6
Ecchymosis without pain	5
Malaise	5
Minor haemorrhage	3
Aggravation of symptoms	3
Itching or redness (suspected contact dermatitis)	3
Pain in the puncture region	2
Fall from bed	1

Other complications associated with needle insertion have been described. Among these the most important are the following:

- transmission of infectious disease;
- pneumothorax;
- cadiac tamponade;
- broken needles with remnants migrating to other localizations;
- death (?) (5 cases may have resulted in death, but some authors refute this possibility).

The combined data from both retrospective and prospective studies indicate that "acupuncture is a very safe intervention in the hands of a competent practitioner" (11).

Acupuncture and alternative medicine research

Acupuncture has been first licensed by Nevada, Oregon and Maryland in 1973. It is actually licensed in 42 states and more than 14000 practitioners are licensed in the Unites States (12). In Italy acupuncture has been recognized as "medical act" from a sentence of Corte di Cassazione (the equivalent of Supreme court) and this means that all licensed physicians can perform acupuncture on their patients, without any other credentials as markers of competence. No licence is requested by European regulatory authorities other than national licence.

In 1998 the US Congress established the National Center for Complementary and Alternative Medicine (NCCAM) as a component of the National Institute for Health (NIH) (13). The mission of NCCAM is "to explore complementary and alternative healing practices in the context of rigorous science, educating and training Complementary and Alternative Medicine (CAM) researchers, and disseminating authoritative information to the public and professionals". Current NCCAM projects are investigating Tai Chi exercise, hawthorn, phytoestrogens, biofeedback, Ayurvedic herbals, Acupuncture, qigong, Reiki, meditation, spirituality, special diets and some others. We hope that scientific community very soon will have more scientific papers on there issues.

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MENOPAUSE IN TRADITIONAL CHINESE MEDICINE

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It is necessary to start with some basics of energetical anatomy and physiology, according to the rules of Traditional Chinese Medicine (TCM). The most important energetical channel in Gynaecology is the one called Chong Mai. A "channel" is a tube-like structure which is not yet detectable with present-day technologies. In this structure the so-called "energy" flows and the concept can be compared to that of a blood or lymph vessels. In simple terms, in TCM the mixture between Fire and Water, the basic Elements known also in Mediterranean Hippocratic Medicine, generates "Qi", the "vapour", the energy previously described, which is a source of kinetic energy which nourishes and activates the body. The term "Mai" means channel, often translated as meridian, using geographical language, like the imaginary lines drawn on the globe/human body. The term "Chong" means "assault": it was the shout of attack used by military officers and it was already in use at the time of the Han dynasty. The meaning of the "Channel of Assault" describes the development of energy which, after conception, rises in and builds the anatomical and energetical structure, which will create the human organism within the uterus. In fact, it is also called "the Sea of the 5 Organs and the 6 Viscera" meaning the common origin of the organs, "the Sea of the Blood" because of its close relationship with the whole hematopoietic apparatus and its distribution, "the Sea of the Meridians", the energetical skeleton on which energy circuits unravel.

It starts with the Kidney Lodge, goes towards the perineum (the acupoint Hui Yin, Collecting Yin), bathing the genital region and genitals themselves.

From the perineum the channel divides into several branches.

Abdominal and thoracic branch

It moves to the anterior abdominal region, reaching, at three-fifths of the navel-pubic line, the acupoint Guan Yuan (Ren Mai or Conception Vessel CV 4), which means Gateway for Ancestral/Yuan energy. From here it descends to the pubic symphysis to the acupoint Heng Gu, "Horizontal Bone", Kidney 11, and then follows the Zu Shao yin channel (the Kidney Channel) towards the thorax. Along the track it links up with the Stomach Channel and the acupoints of Stomach and Large Intestine and therefore, with these viscera themselves. On the thoracic track, beside the sternum, it spreads out capillary branches, which connect to the Heart. It reaches the first intercostal gap at the 27th point of the Kidney Channel, Shu Fu, "the Point of the Palace", where it joins with the Lungs. It continues towards the throat, above the ioidal bone, on the median line, then it circles the lips and ends up in nasal fossa and in the upper part of the pharynx.

From the perineum there is also a posterior branch which follows the Du Mai Channel, in the median line of the back part of the body, which rises up along the rachis. Some authors believe it only reaches the dorsal region, others believe it continues as far as the head.

Lower branch

From the acupoint Kidney 11, Heng Gu, it descends along the thigh connecting to Xue Hai, "Sea of Blood", Spleen 10, finally reaching the medial heel bone. A branch runs along the upper part of the foot, where the pedidian pulse can be felt at the 42nd acupoint of Stomach, Chong Yang. From this point start plenty of capillaries that heat the foot (Sun Luo). Another branch descends to the Kidney Channel and, according to some, goes down to the lower heel, Yu Chuan.

Chong Mai relationships

With Ren Mai (Conception Vessel) and with Dai Mai (Belt Channel) which runs from the lumbar region at the gap between L2 and L3 and then comes forward as a belt, reaching the acupoint previously mentioned, Ren Mai 4th. Chong Mai contributes, therefore, to the development of the inner and outer genitals. These are nourished by the Blood, whose production is aided by Chong Mai. By means of Ren Mai, it contributes to the nourishment and metabolism of bones.

Additional functions

It distributes and controls the following: the ancestral Energy Yuan, the trophic Energy, the Defensive Energy Wei, the Blood Xuè and the Organic Liquids, Yin Ye. It balances all these and therefore has an effect on the control of body heat, influencing the balance between Qi/Energy and Xuè/Blood.

It is possible that, since this is the "Sea of Blood", it can be called anatomically the vascular trunk Aorta/Vena Cava. Anyway, it is connected both to the arterial circulation of the lower limbs and to the blood supply of the Uterus. In the thorax it governs the coronary circulation and, in the neck, it controls the carotid arteries.

Flow regulation

It regulates the Energy flow in the meridians, it regulates the menstrual flow, especially in the initial phases of menstruation and in menopause. According to some Authors , there is a counteracting descent of energy from the 16th point of Du Mai (Governor Vessel) situated on the hinge between the Atlas and the Occiput, in the direction of the lumbar region, at the speed of one vertebra per day, over 21 days. Here it reaches the gap between L2 and L3, the 4th acupoint of Du Mai, called Ming Menn, Portal of Light, and thereafter it reaches the Pelvis, and rises again to the 4th acupoint of Ren Mai and continues to rise over the next seven days, until the chin. The cycle of this sub-circuit therefore lasts 28 days, in harmony with moon cycle.

Menopause – Gen Nian Qi

Gen Nian Qi Zong He Zheng is Menopause with its relative symptoms ("All the Symptoms").

Gen means "to change"; Nian means "age" and Qi is "the Phase". In TCM the disappearance of the menstrual cycle usually has no particular significance, since it is considered a normal phase of life and has hardly any symptoms. The psychological aspect is probably more important in the appearance of menopausal symptoms. Many women, who are active and conscious of their "goal in life", have no particular problems while going through this phase.

Physiopathology

The possible causes of symptoms can be found in pathological conditions such as "Lack of Qi" of the Kidney, reduction of Jing and Yuan Qi, lack of Energy in Chong Mai and Ren Mai, Lack of Blood and imbalance between Yin and Yang.

Kidney deficiency explains psychic and neurological symptoms.

Jing deficiency explains osteoporosis and vaginal dryness.

The consumption of Yin/Water allows Yang/Heat to rise (hot-flushes: the so-called Heat/ Deficiency syndrome).

Acupuncture treatment: a suggestion

Open Chong Mai: acupoint Spleen 4th, Gong Sun.

Tonify the Yin: Kidney 4th, Da Zhong, Great Bell, useful also for lumbago, often present, and psychical depression. Alternatively, Kidney 7th, Fu Liu, Return Current, which tonifies both the Yin and the Lidney Yang and, together with Heart 6th, Yin Xi, Lack of Yin, halts perspiration.

Activate Yuan Qi: Conception Vessel 4th, Guan Yuan, which treats Chong Mai and Ren Mai.

Hold Jing: Conception Vessel 7th, Yin Jiao, Crossing of the Yin (of the abdomen), it also nourishes Yin and Xué, especially in the uterus.

Restore the balance Energy/Blood: Urinary Bladder 43rd, Gao Huang Shu, Acupoint of Gao Huang, which is the place between the Heart and the Diaphragm, it dispels Heat in the Blood (it increases Hematocrit, therefore Yin) and influences Jing of the Kidney and the whole body.

Refresh the Blood: Kidney 27th, Shu Fu, Acupoint of the Palace, aiding the Kidney to grasp the Lung energy.

Drug treatment (Zhong Yao)

Although other forms of menopause exist (Yin or mixed) the most common is the one due to Yin Deficiency (Xu Yin).

This disorder appears with signs and symptoms of heat (and therefore, often, dryness), especially in the upper body, as dryness in the mouth, eyes and vagina, lumbago particularly in the standing position, knee pain.

Basic prescription: Liu Wei Di Huang Tang (decoction) or Wan (pills), Decoction of Six Ingredients with Rehmannia glutinosa, made up of:

- SHU DI HUANG, Rehmannia glutinosa, radix praeparata; 20 g.

- SHAN ZHU YU, Cornus officinalis, fructus; 15 g.
- SHAN YAO, Dioscorea opposita, rhizoma; 15 g.
- ZE XIE, Alisma orientalis, rhizoma; 12 g.
- FU LING, Poria cocos, sclerotium; 12 g.
- MU DAN PI, Paeonia suffruticosa, cortex radicis; 10 g.

Materia medica

SHU DI HUANG, Rehmannia glutinosa, radix praeparata, goes towards the channels of Liver, Kidney and Heart; warm, sweet, 20 g in decoction; it tonifies Kidney Yin and Xué/Blood, it benefits the essential energy called Jing.

SHAN ZHU YU, Cornus officinalis, fructus, goes towards the channels of Liver and Kidney; warm, acid; 15 g in decoction; it tonifies the Kidney, nourishes the Liver Yin, holds essential energy, called Jing and strengthens Zheng Qi.

SHAN YAO, Dioscorea opposita, rhizoma, goes towards the channels of Spleen, Lung and Kidney; neutral, sweet; 15 g in decoction; it strengthens the Spleen, consolidates essential energy, called Jing.

ZE XIE, Alisma orientalis, rhizoma, goes towards the channels of Bladder and Kidney; cold, sweet/insipid; 12 g in decoction; it purifies the Heat in the Kidney, aids diuresis and moderates the actions of Shu Di Huang, Rehmannia glutinosa, radix praeparata.

FU LING, Poria cocos, sclerotium, goes towards the channels of Spleen, Lung, Heart and Bladder; neutral, sweet/insipid; 12 g in decoction; it strengthens the Spleen, drains Dampness. It calms the mind and moderates the action of Shan Yao, Dioscorea opposita, rhizoma.

MU DAN PI, Paeonia suffruticosa, cortex radicis, goes towards the channels of Heart, Liver, Kidney and Pericardium (Minister of the Heart); cold, bitter/pungent; 10 g in decoction; it purifies Fire in the Liver, cools the Blood and moderates the warming action of Shan Zhu Yu, Cornus officinalis, fructus.

The action of the herbal medicines is developed along the lines of Taste, Nature, the Target Channel and Direction. The Taste describes the tendency of a drug to reach a certain organ or the associated anatomical structure; the Nature is the intrinsic capacity to increase or to lower the temperature of a certain organ or anatomical region; the Target Channel is the energetic channel towards which the drug goes; the Direction, when specified, aids the deep absorption or bringing to the surface of a drug and its effects.

The previous dosages are the daily dose for decoction, for patients weighing between 60 and 70 kilos. How to do it: in the evening, after a meal, in a non-aluminium pan (steel, earthenware, pirex) pour two cups of cold water. Pour in the contents of a sachet (with the prescribed herbs). Bring to the boil over a gentle heat. Let it boil until it reduces by half. Remove from the heat and filter through filter paper or sieve. Keep the filtered herbs for use the next morning. Allow to cool until warm and then drink, preferably on a full stomach. The next morning, repeat, pouring the filtered herbs into two cups of cold water. Repeat the operation, remove the heat and filter, and discard the herbs. Cool and drink. In the evening repeat the process with a new herb sachet. Usually the treatment lasts 21 days, alternating with a seven day interval, over three or four months, preferably in autumn.

When the menopausal syndrome is complicated by an excess of Heat, add the following:

- Zhi Mu, Anemarrhena asphodeloides, radix, cold, bitter, 6 g per day and Huang Bai, Phellodendron amurense, cortex, cold, bitter, 6 g per day. This modified prescription is called Zhi Bai Di Huang Wan.

If there is extreme dryness of the mouth, slight late afternoon sense of fever and/or dry, unproductive cough, add Mai Men Dong, Ophiopogon japonicus, radix seu tuber,cold, bitter/sweet, g 10 per day, the root can also be chewed if the oral treatment needs to be topical.

Among the Chinese Traditional herbal drugs in use in the western countries, at present for the treatment of the symptoms of menopausal syndrome, Cimicifuga and Soya can also be used:

- ***Cimicifuga***

  Cathegory: pungent, cold drugs which dispel Wind/Heat.
  Chinese pinyin name: SHĒNG MÁ
  Pharmacological name: Rhizoma Cimicifugae
  Botanical classification: Cimicifuga foetida L. or C. dahurica (Turcz); C. heracleifolia Kom. or C. heracleifolia Komar.
  Family: ranunculaceae
  Where cultivated: Shaanxi, Sichuan, Qinghai, Yunnan (C. foetida); Northeastern China (C. dahurica. C. heracleifolia).
  Properties: Sweet, pungent; cold.
  Channels of entry: Large Intestine, Lung, Spleen and Stomach.
  Actions, according to TCM:

  1. it dispels from the surface
  2. it eliminates wind/heat
  3. it aids skin eruptions
  4. it allows yang to rise
  5. it dissolves toxins in the stomach and in the blood
  6. it moves upwards.

  In Western medicine it reduces the hormon LH. It has close affinity with Estrogen Receptors (ER) inhibiting, *in vitro*, proliferation of breast cancer cells. It has an anti-inflammatory and analgesic effect by means of the inhibition of 6-keto-PGF 1α in the macrophages. COX-2.
  Triterpeni have shown a cholesterol reducing activity.
  In menopause: in a randomized clinical trial, RCT, double blind, on 80 women, average age 51,2 y, 20 milligrams of Cimicifuga extract were administered per day, titled as 1 mg. in triterpenic glycosides per tablet, two tablets per day, for twelve weeks, with, at the same time, 0,625 mg, of oestrogens or placebo.
  At the end the symptoms had improved only in the women who were taking Cimicifuga with placebo, with a thickening of the vaginal epitelium.
  Another double blind RCT , showed that the same tablets of the previous RCT, taken for two months, significantly reduced the levels of LH, but without showing significant differences between the drug-treated group and the placebo group.
  Black cohosh was effective in controlling menopausal symptoms, according to a presentation on June 21 at Endo 2002, the 84th annual meeting of The Endocrine Society. In a double-blind study comparing the phytoestrogen to placebo and to conjugated equine estrogen, it had favorable estrogenic effects on bone and lipids but no effect on the uterus.
  "Extracts of the rhizome of black cohosh (Cimicifuga racemosa) are traditionally used to treat climacteric complaints," write Wolfgang Wuttke and colleagues from the University of Goettingen in Germany. "Little is known whether [black cohosh] preparations have effects on other than climacteric complaints such as osteoporosis and lipid metabolism."
  In the study of 97 postmenopausal women, duration of amenorrhea was more than six months and climacteric complaints included having more than three hot flashes per day. In double-blind fashion, the women received daily treatment for three months with commercially available black cohosh (Klimadynona or Menofema, 40 mg), conjugated estrogens (0.6 mg), or placebo. Both black cohosh and estrogen reduced major

climacteric complaints significantly more than did placebo (P<.05). Black cohosh improved blood lipid and bone-specific alkaline phosphatase levels. Although estrogen increased endometrial thickness, black cohosh did not.

"These data, together with animal experimental results suggest that the [black cohosh] preparation contains one or more phytoestrogens with selective estrogen receptor modulator activity with no effect in the uterus but favorable estrogenic activity in the bone and on serum lipids," the authors write.

Animal studies presented by the same group suggested that black cohosh contained phytoestrogens with a mild estrogenic (E2-like) effect on bone, and a stronger effect on fat tissue but not on cholesterol metabolism. In the same animal model, soy extract containing genistein and daidzein was completely ineffective in preventing osteoporosis or improving lipid metabolism (Laurie Barclay, MD. Endo 2002: Abstracts P3-333, P3-317. June 21, 2002)

Over the past two decades, multiple observational studies have suggested that postmenopausal hormone therapy reduces the risks of osteoporotic fractures and coronary heart disease (Deborah Grady. Postmenopausal Hormones - Therapy for Symptoms Only. The New England Journal of Medicine, May, 8th 2003). On the basis of this evidence, hormone therapy was often recommended for women who were at high risk for fractures and coronary disease. But these recommendations were based entirely on observational evidence, which can sometimes be misleading if the groups being compared have different risk patterns and lifestyle. In the early-to-mid-1990s, several large, randomized trials were initiated to provide definitive evidence concerning the risks and benefits of hormone therapy for the prevention of disease.

The largest of these trials, the Women's Health Initiative (WHI), included more than 27,000 older, generally healthy postmenopausal women; those with an intact uterus were randomly assigned to receive estrogen plus progestin or placebo, and those without an intact uterus were randomly assigned to receive estrogen alone or placebo. The estrogen-plusprogestin segment was stopped last summer when results showed that hormone therapy caused small increases in the risks of coronary events, stroke, pulmonary embolism, and breast cancer. There were also small decreases in the risks of hip fracture and colon cancer, but the overall harm outweighed these benefits.

The investigators examined the net effect on these six potentially deadly conditions and reported that hormone therapy results in two such serious adverse events per 1000 women treated for one year. After five years of treatment, the risk was one serious adverse event per 100 women treated. Given that hormone therapy was associated with decreased risks of colon cancer and hip fracture, are there women who are at high risk for these conditions who might have a net benefit from treatment with hormones?

A woman with a family history of colon cancer has a risk of the disease that is approximately twice that of women with no such family history. According to the rates of disease and the relative risks found in the WHI, the estimated harm is lower among such women, but the net effect is still about 1.4 serious adverse events per 1000 women per year. A woman with osteoporosis (defined by a T score for bone mineral density that is lower than 2.5) has approximately double the risk of hip fracture, but the net effect of hormone therapy is still about 1.5 serious adverse events per 1000 women per year.

What about women at very high risk for hip fracture, such as those who have already had a vertebral fracture and have low bone mineral density? Assuming that the risk of hip fracture is increased by about a factor of five among such women, the decreases in the risks of hip fracture and colon cancer will just about balance the increased risks of coronary events, stroke, pulmonary embolism, and breast cancer. Given the availability of

other effective agents, the use of hormone therapy for the treatment or prevention of osteoporosis is not appropriate for most women. The annual increase in the risk of serious adverse events associated with postmenopausal hormone therapy is relatively small, but why should women take any risk? Until recently, it has been argued that many women – even older women who do not have vasomotor or urogenital symptoms – feel better when they take hormones.

This claim has now been laid to rest by new results from the WHI. In this issue of the Journal, Hays *et al.* provide clear evidence that hormone therapy does not result in better quality of life among older women without menopausal symptoms.

- ***Soya (Glycine soja)***

  Family: Leguminosae; Pharmaceutical name: Semen Sojae Praeparatum. DÀN DÒU CHǏ.

  Perennial herbal plant, Glycine max (L.) Merr.; harvested throughout China in summer or early autumn when ripe. Ethymology: Glycine, from the Greek glykys, sweet, because of the taste of the seeds. Soya, from the Japanese Shoyu, is the name of the whole plant. It is sown in Spring, in fertile ground, in rows, like beans.

  Common name: Japanese bean, in Japanese: tantoshi; in English: prepared soybean.

  It was imported from the East at the end of the last century, but now Soya in Italy is only coltivated. All the varieties that are cultivated derive from Glycine usseriensis, which grows wild in Japan and Manchuria. It has a straight hairy stem with side branches and can reach the height of 90 cm.; it has alternating leaves made up of three to five small hairy oval leaves, it has small flowers which can be lilac, white or greenish, single or in clusters (in the Summer); the fruits are in pods, flat and velvety containing big, yellow, greenish, brown or black seeds.

  Parts used: the sprouts and the seeds. Sprouts are harvested in Spring and seeds when ripe at the end of the Summer.

  – *Black Soya*

    Category: legumes/pulses. Sweet and Neutral (Cool if boiled). Goes towards the channels of Kidney, Liver and Spleen.

    Actions: tonifies the Kidney, nourishes Yin, tonifies the Spleen.

    It drains Dampness; it purifies Heat and dispels toxins.

  – *Yellow Soya*

    Category: legumes/pulses. Sweet and Neutral. Goes towards the channels of Spleen, Stomach and Lung..

    Actions: tonifies the Spleen (boiled). It drains Dampness. Nourishes Blood (boiled). Detoxifies. It lowers cholesterol. Difficoult to digest, it can give rise to abdominal bloating.

  – *Soya sprouts*

    Category; sprouts. Sweet and Cold. Goes towards the channels of Stomach and Lung.

    Actions: it purifies Heat. Diuretic.

    Dosage: about g 250 per day.

  – *Soy sauce*

    Sweet, salty and Cold. It goes towards the channels of Spleen, Stomach and Lungs.

Actions: it tonifies the Spleen and the Stomach, it improves digestion, it dispels Heat in the Lung and humidifies. Diuretic.

Active ingredients of Soya

Isoflavones (daidzein, genistein, biocanin and formononetin):Found only in high protein content leguminosae (in the leaves, seeds and roots). E.g. beans, chick-peas, soya, etc. Botanical role: they attract Rhizobium bacteria which fix molecular Nitrogen in the root of the plant, used for aminoacid synthesis, from the air and from the earth. Furthermore, they protect the plant from aggression from parasites and climate by means of their antibacterial, antiparasitic and antioxidant role.

They have close affinity to Estrogen Receptors (ER).

These substancese contained in legumes, fruit and vegetables are only precursors of the so-called phyto-oestrogens, which are activated in the intestine, by means of intestinal flora and carried through the Portal venous system up to the liver.

Absorption of phytooestrogens there is a lot from person to person and even in the same person. Between 10 % and 30 % are eliminated through the urinary tract and very little through the intestine.

The amount of phytooestrogens in food is not constant. It varies according to:

- Place of cultivation
- Climate
- Soil
- Way of extraction
- Preservation

Action mechanism

"Phytoestrogens" work in two basic ways:

- Hormone-like
  They bind with the estrogen receptors triggering off all the cell processes, in particular those regulated by these hormones. They can have different actions: they behave like "weak" estrogens, or antagonists of our hormones. It depend on the type of Estrogen Receptor, alfa (ERα) or beta (ERβ), with which they bind. This action protects from some hormone-dependent tumors, from dismetabolic and cardio-vascular diseases and from osteoporosis.
- Non-hormonal
  They inhibit the differentiation and the proliferation of malignant cells, meaning their ability to multiply and invade the organism. Other antitumoral biological effects include the prevention of neoangiogenesis, the basic requirement for the development of the tumour. They have (especially isoflavones) antioxidant action (they reduce free-radicals), an anti-inflammatory and anti-hypertensive action.

Isoflavones are effective on hot flushes, they reduce FSH and LH blood levels, they increase the mineral content in bones and reduce the risk of heart disease.

ACUPUNCTURE AND HEADACHE

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The headache is a pathology known since the antiquity which currently interests a great part of the western population. In the 1988 the International Headache Society has defined a classification that comprises three main forms of headache so called "primitive" (the western medicine don't recognize any etiological cause), which are hemicrania, the tensive headache and the headache to cluster. The actual classification of headache in China speaks about forms of:

- attack of wind-cold
- attack of wind-heat
- attack of wind-damp
- empty of blood
- empty of energy
- empty of energy and blood
- empty of kidney
- stasis of energy and blood
- excess of Yang of liver
- persistence of catarrh.

The Chinese traditional medicine defines the headache:

- tou tòng (pain to the head)
- tou feng (wind to the head)

The wind causes the clogging of the energetic circulation and provokes the headache.

To comprise this affirmation it is perhaps necessary to make some premises of energetic physiology.

All things possess a bipolar nature (dualism yin-yang) and from the dialectic yin-yang are born the energetic rhythms of the universe, of which the man endure the effects, being himself a product of them.

The energy circulates according determinate ways that in the man we define "meridians". All the meridians of polarity Yang coincide in the zone of skull. The energy of opposite polarity (yin) arrives to the head thanks to the meridian of the heart and its vase low, that penetrates in the eyes, and to the meridian of the liver that arrives until the top of the skull.(du 20) (Figure 1).

The MTC classifies the headache according to:

- etiology
- topography (meridians)
- symptomatic situation (type of pain)
- clinical course.

The pathogenetic factors are considered the constitutional state, the emotional stress, the job, the sex, the nourishment, trauma and climatic factors.

In the classification according to the etiology we find:

- headache from Cosmo pathogenic disorders
- headache from psychical disorders
- headache from pathogenic energy of alimentary origin
- headache from break of the equilibrium yin-yang.

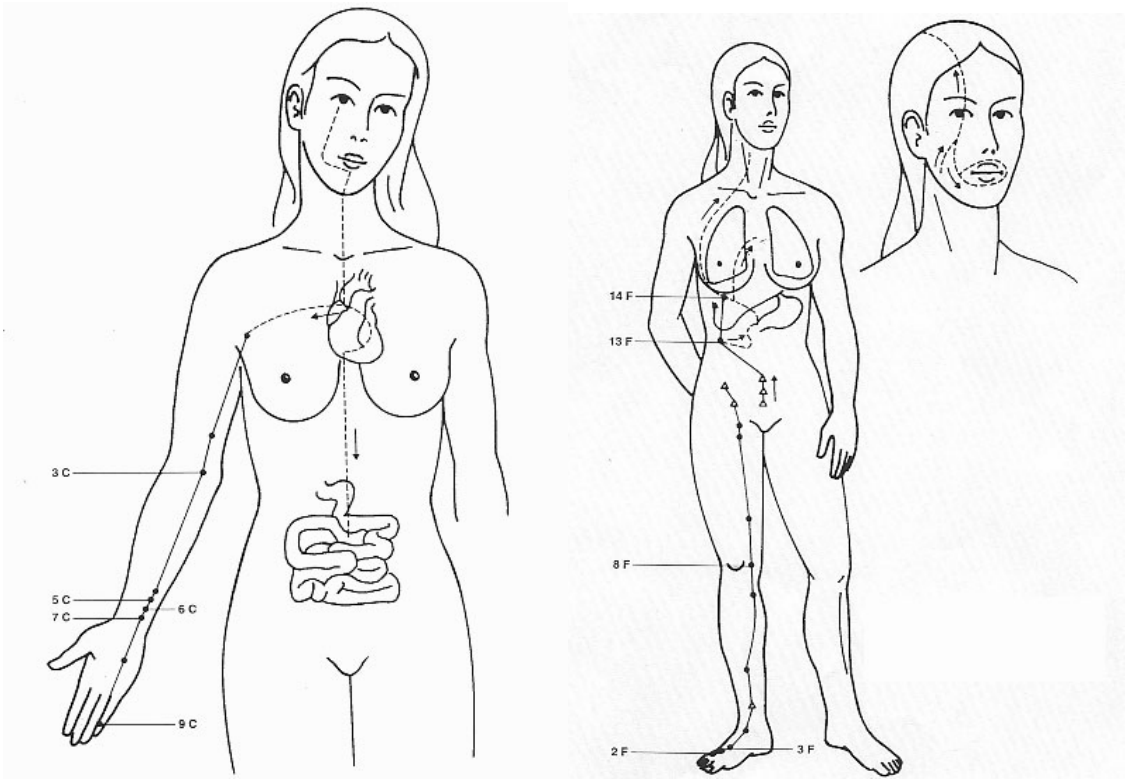

Figure 1. Meridians of the heart and liver

Headache from cosmopathogenic disorders

The penetration of "perverse energy" (EP) in superficial fascia of the body causes a "fullness" in the meridians with appearance of the pain. How evolves the "perverse energy"? There is a starting phase in which the PE penetrates in the secondary vessels of the head causing "fullness" in surface and deficit in dept (inner-outside) with alteration of the energetic circulation and vasoconstriction.

It follows therefore the painful phase with advance of EP in the main meridians (depth) causing its fullness and vasodilatation. Finally arrives the phase of the continuous headache when the energetic block stops the yin (blood) with the concentration of the liquids and painful edema.

Headache from pathogenic energy of alimentary origin

A nourishment badly balanced and/or too much rich damages the organism. The alimentary energy (five tastes) feeds the corresponding organ, in case of excess it will be fullness with energetic disorders and pain (ex. too much acid damages the liver with come back of the full to the head along the corresponding meridian, Figure 2).

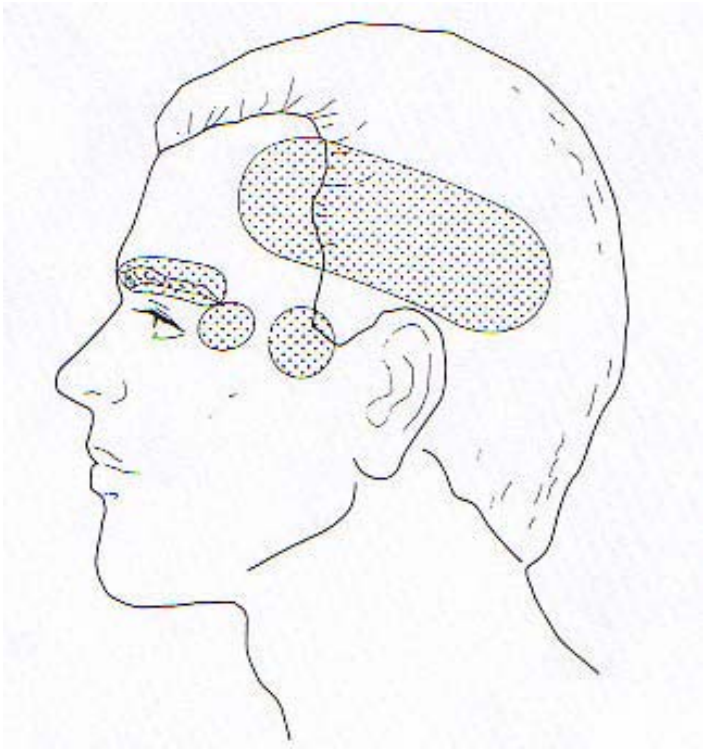

Figure 2. Headache areas from the Yang meridian of the liver

Headache from psychical disorders

Repeated and/or too much strong emotions damage the corresponding organ weakening its energy yin. The yang no more withheld goes to the head again.(ex. an excessive joy alters the energy yin of the heart whose yang goes back to the head causing pain).

Headache from break of the equilibrium yin-yang

These are headache caused by energetic reductions of the yin –yang root of the kidneys. We can have two situations:

1. Insufficiency of the kidney yang and consequent headache with edema.
2. Insufficiency of the kidney yin and headache with hipertonia.

In the classification according to the topography we find the forms:

1. Headache Tay Yang
2. Headache Yang Ming
3. Headache Shao Yang
4. Headache Tue Yin
5. Headache Shao Yin (Figure 3).

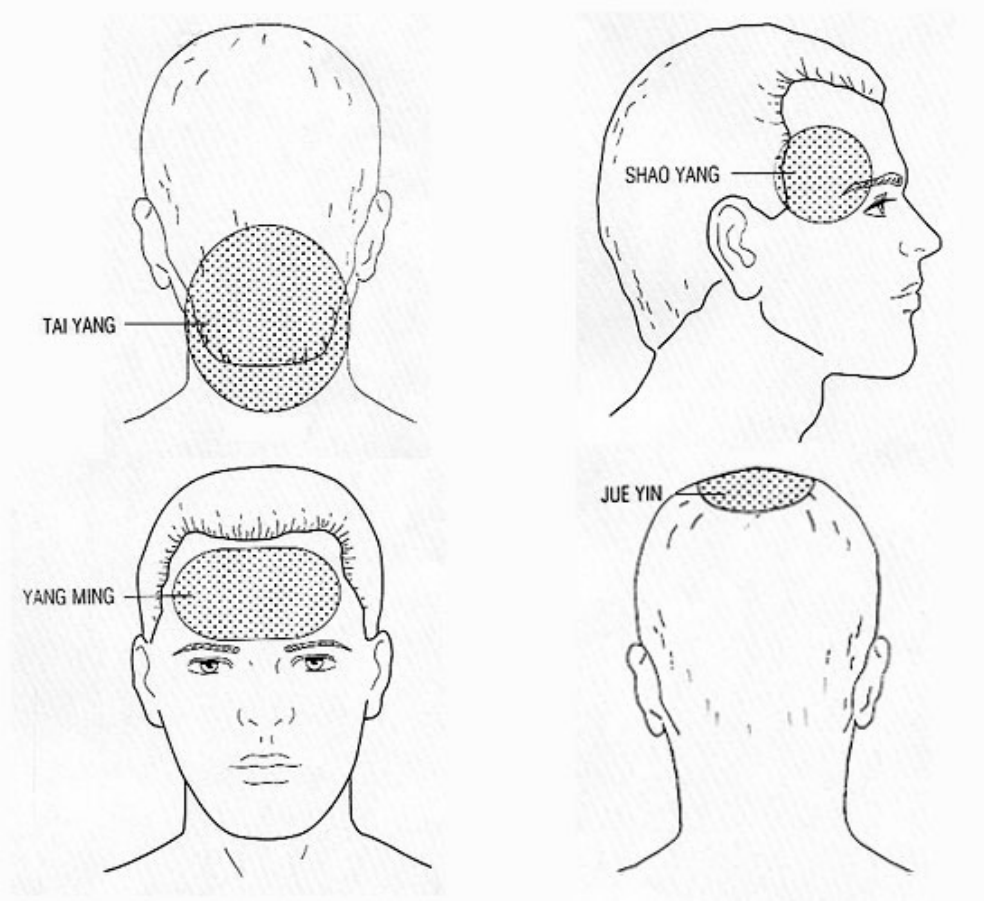

Figure 3. Headache classification according to the topography

Headache Tai Yang

- Meridian Tai Yang (blister-intestine tenuous)
- Acute forms → wind-cold
- Chronic forms → empty of kidneys
- Tensive form
- Fronto-nuchal, occipital and intense pain
- Marked muscular rigidity.
- Greatest painful on the points 2BL,10BL
- Needle-active points: 1BL, 2BL,10BL,16GV,20GV,24GV,3SI,4LI.

Headache Yang Ming

- Meridian Yang Ming (stomach- large intestine)
- Empty of the stomach → dull pain
- Heat of the stomach → acute pain
- Damp → feeling of gravity and stun
- Flegma → gravity and stun + vertigos and dimmed sight

- Frontal and face pain (atypical facial neuralgia)
- Irradiation to the dental arch
- Sensitive point 8ST
- Needle-active points: 8ST, 36ST, 44ST,4LI, 11LI, 4GB, 20GV.

Headache Shao Yang

- Meridian Shao Yang (vesicle biliary - triple heating)
- Climb of the yang of liver, the fire of the liver, the wind of the liver.
- Acute parieto-temporal pulsating pain
- Irradiation to the sides of the neck
- Vascular form? Hemicrania?
- Sensitive points:14GB, 20GB
- Needle-active points: 14GB, 20GB, 1GB, 41 GB, 16GV, 20GV, 4LI, 5TE

Headache Jue Yin

- Meridian Jue Yin (liver-pericardium)
- Escape of Yang of liver
- Empty of Qi-Xue
- Empty of liver blood
- Empty of heart blood
- Pain to the apex
- Sensitive point : 20GV.
- Needle-active points: 3LR, 14LR, 20GV, 20 GB, 6PC, 4LI.

Headache Shao Yin

- Dull, relaxing pain
- Localization nape-apex
- Entire head
- From energetic deficit-empty of the Jong renal →chronic forms
- Unsuccessful nutriment to the brain

In the classification according to the symptomatic table the type of pain is considered:

Dull → condition of empty
Acute → condition of full
Gravity sense → damp or flegma
Sense of swelling and/or pulsation → liver escape yang
Rigidity → wind-cold
Sense of ripping → external acute forms
→ inner wind of liver

Acute and sharp pain → stasis of blood
Sense of empty head → empty of kidney
In the classification according to the clinical state we analyze:

1. *Beginning*
   Acute (unexpected) → headache of external type
   Chronic (gradual) → headache of internal type

2. *Time of the day*
   Day-time → empty of Qi or of Yang
   → damp
   Night → empty of blood and/or of Yin
3. *Influence of state of the atmosphere*
   Aversion to the wind and to the cold → external cause
   Worse from cold → empty of Yang
   → syndrome from cold
   worse of heat → syndrome of heat
   → Yang escape of liver and/or raise of fire of liver
   worse of damp → damp or flegma
4. *Influence of activity-quiet*
   Worse from activity and → empty of Qi or of blood improved from clinostatismo
   Worse from clinostatismo → damp or flegma
   Improved from light exercise→ escape Yang of liver
   → flegma
5. *Emotional stress*
   Anger → Yang of liver or fire of liver
   Relax (week end) → escape of Yang of liver
   Worsening after unexpected excitement → escape of Yang of liver
6. *Sexual activity: → empty of kidneys*
7. *Food*
   Alimentary allergy
   Warm food → retention of food-heat to the stomach
   Damp food → damp-flegma
   Bitter food → Yang escape of liver
   Headache that improves with food → escape of Qi or blood
8. *Menstruation*
   Headache beginning cycle → stasis of Qi or blood
   → Escape of Yang of liver
   During → fire of liver or stasis of blood
   End cycle → empty of blood
9. *Local palpation*
   If worses → condition of full
   If improves → condition of empty

Physiopathology foundation of treatment

Every headache has to be analysed according to the eight diagnostics rules and particularly according to the standard “empty-full”:

– Headache from full
  external causes: wind – cold
  wind – heat
  wind – damp

internal causes: Yang of liver
Fire of liver
Wind of liver
Stasis of Qi of liver
Accumulation of cold in the liver meridian
Damp
Cloudy flegma
Cloudy flegma – wind
Retention of food
Stasis of blood
Heat of stomach

- Headache for empty
  Empty of Qi
  Empty of blood
  Empty of kidney
- Headache for full of external cause
  1. Treating local fullness of PE → prick aching point
  2. Freeing the external → dispersion of cosmopathogenic element
  3. Treating the empty of essential energy → stimulate the medium and superior heater
  4. Treating the full of "superior region" → particular points HO
  5. Treating the distinct meridians → points Jing distal to opposite
- Headache from full of inside cause
  1. Dispersing the full for head
  2. Freeing energetical obstruction
  3. Removing the stasis of blood
- Headache from empty
  1. The Qi doesn't gains the head to purify the orifices → strengthen and raise the Qi (energy)
  2. The blood doesn't feed the brain → feed the blood, strengthen and circulate the Qi
  3. Empty of kidney → strengthen kidney and feed the marrow.

General principles of treatment

Selection of points:
1. Locals:
   - On the basis of meridian interested
     (ex. Headache on the meridian VB--- Acupuncte GB6
   - On the basis of localisation of headache
     (ex. frontal headache → DU25, GB14
     of vertex headache → DU20, DU21
     occipital headache → BL10, DU19
     temporal headache → GB8, TAI YANG)
2. Distals:
   - On the basis of meridian interested
   - On the basis of energetic etiology
     (ex. Headache from "escape of Yang of liver" that interests the meridian VB)
     LR3 (for the Yang of liver) + GB43 (for the meridian)

PROTOCOL FOR THE TREATMENT OF CLIMACTERIC SYMPTOMS WITH ACUPUNCTURE

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Introduction

Menopause is a critical turning point in a woman's lifetime, as it entails leaving behind a former equilibrium and adapting to new psychological, social and physical conditions.

Hormone replacement therapy, which serves to control vasomotor and genitourinary symptoms – i.e. those most closely related to an oestrogen deficiency – and to improve, though to a lesser degree, a woman's psycho-emotional balance, represents the treatment of choice.

One of the non-conventional therapies that have been proposed to control hot flushes in the climacteric syndrome is acupuncture.

Aims of the study

To assess the effectiveness of acupuncture versus veralipride in reducing the number of hot flushes and in improving the score of Greene's climacteric scale, the SCL score (which measures anxiety, depression and insomnia) and the quality of life as rated with the WHOQOL-BREF questionnaire.

Study design

The women meeting the inclusion criteria are handed randomisation envelopes by which they are assigned to the 2 crossover study stages:

- STAGE A = veralipride therapy for 3 months (one 100 mg tablet per day for 20 days each month);
- STAGE B = a weekly acupuncture session for 3 months (12 sessions).

The design provides for a 3-month period of no treatment (wash-out) between stages A and B or vice versa.

A 3-month follow-up is planned after stage B.

Inclusion criteria

Women of at least 50 years of age who have had amenorrhea for at least 12 months and no longer than 48 months.

Exclusion criteria

1. Women undergoing hormone replacement therapy, either at present or within the last 6 months.
2. Women in surgical menopause (hysteroalpingo-oophorectomy).
3. Women who have undergone chemotherapy and or radiotherapy.
4. Women who are undergoing endocrinotherapy for previous mammary neoplasia.
5. Obese women (Body Mass Index > 33).
6. Women who are undergoing therapy for hypertension.
7. Women with hyperprolactinemia.
8. Women with galactorrhea.
9. Women with breast pathologies (ongoing or suspected mammary dysplasia or neoplasia).
10. Women confirmed or presumed to have pheochromocytoma.
11. Women with acute porphyria.
12. Women who are undergoing treatment for thyroid disease.
13. Women with psychiatric illnesses.
14. Women who are being treated with psychotherapeutic drugs (except for benzodiazepine).
15. Women with an endometrial thickness equal to or > 5 mm.
16. Women with FSH and estradiol values outside the menopausal range of the laboratory of reference.
17. Women with TSH outside the normal range of the laboratory of reference.
18. Women who are taking phytooestrogens or homeopathic drugs for climacteric symptoms.

Exams requested prior to randomisation

Before any randomisation procedure is started, all the patients who meet the inclusion criteria and agree to participate in the study will be asked to give written informed consent.

Prior to randomisation, patients must:

- have a transvaginal ultrasound scan (performed within the previous 6 months);
- have a mammogram (performed within the previous 12 months);
- undergo hormonal assays: FSH, TSH, estradiol, prolactin (performed within the previous 3 months);
- fill out 2 forms relative to Greene's climacteric scale (2 wks. before randomisation);
- fill out WHOQOL and SCL-90 quality-of-life questionnaires.

Patient recruitment and method of conducting the study

Patients will be recruited at the menopause centre of each participating unit.

After obtaining the patient's consent, the appointed gynaecologist will ask her to fill out 2 forms pertaining to Greene's climacteric scale in order to determine the number of hot flushes and any other symptoms of the climacteric syndrome that may have occurred during the 2 weeks prior to randomisation. The patient will be given an appointment, after 2 weeks, for a meeting with the appointed acupuncturist and randomisation to one of the two treatments.

The acupuncturist who receives the patient will open the sealed, opaque randomisation envelope and assign the patient to stage A or B.

The patient will then be asked to complete the WHOQOL and SCL-90 questionnaires. The questionnaires will be checked to make sure that all questions have been answered.

Before patients undergo the first acupuncture session or are administered the 1st tablet of Veralipride, it will be verified whether they have correctly answered all the questions contained in Greene's climacteric scale.

All randomised patients will be seen weekly during stages A and B of the study and will be invited to fill out, on a daily basis, a specific diary for evaluating Greene's climacteric scale.

The patients assigned to stage A (pharmacological treatment) must take 1 tablet of Veralipride per day, 20 days a month for 3 months. Once a week they must hand in the aforementioned diary to the appointed acupuncturist.

The 1st package of Veralipride will be given to the patient at the time of randomisation and the subsequent ones at the start of each month of treatment. In addition, the patient must return the used package each month so that adequate records may be kept of the drugs taken.

The patients assigned to stage B (acupuncture treatment) will undergo one treatment session per week. At each visit, like their counterparts in stage A, they must turn in the climacteric scale evaluation diary. The duration of treatment is identical, i.e. 3 months.

Every week the appointed acupuncturist will note down any side effects of treatments A or B on the data collection sheet and record any concomitant pharmacological therapies.

The first stage will be followed by a 3-month wash-out period (during which the patients will not undergo any treatment for the disorder in question). After this interval has elapsed, the treatment planned for the next stage will begin. For example, after the 3-month wash-out, the patients who were randomised to begin with treatment stage A will then be crossed over to treatment stage B and vice versa for the patients starting from stage B. A follow-up period lasting 3 months will start on completion of the second stage. At the end of this period patients will be seen for the last time and will exit the study.

Patients will also be asked to complete quality-of-life questionnaires (WHOQOL and SCL-90) 3, 6, 9 and 12 months after the start of the study.

If patients no longer complain of any symptoms and report a condition of complete well-being prior to the end of treatment (either with drugs or acupuncture), the treatment will nonetheless be continued as per protocol.

The patients undergoing treatment with acupuncture will be considered valid subjects of evaluation if they complete at least 6 out of the 12 sessions prescribed and do not miss more than 3 consecutive sessions.

The patients undergoing pharmacological treatment will likewise be considered subject to evaluation if they take more than two thirds of the total number tablets prescribed (at least 40 tablets out of the 60 prescribed) and do not skip taking the drug for more than 5 days in a row.

Rating the effectiveness of the therapy

The patients in both groups are instructed to fill out, on a daily basis, a specific diary (Greene's climacteric scale) that takes account of somatic symptoms, vasomotor symptoms and psychological aspects. There are 21 questions and the severity of the symptoms is rated with a score from 0 to 3. A 22nd question on the daily number of hot flushes has been added to the form to be given to patients.

At the start of therapy and after 3, 6, 9 and 12 months, patients answer the SCL-90 questionnaire, which comprises several subscales: depression, anxiety, insomnia, somatization, obsessive-compulsive behaviour, interpersonal sensitivity, aggressiveness-hostility, phobia, psychoticism, paranoid delusions. Either the total or partial score of individual subscales may be used for statistical purposes.

At the start of therapy and after 3, 6, 9 and 12 months, patients answer the WHOQOL - BREF questionnaire, recommended by the World Health Organization (WHO) and available in 30 different languages, including Italian. The WHOQOL can be used jointly with other rating methods and allows an assessment to be made of the changes in Quality of Life occurring during the treatment itself. The questionnaire takes four areas into consideration: physical and psychological well-being, social relations and environment. Each area has a corresponding score.

Therapeutic protocol

Pharmacological treatment (Stage A)

Veralipride 1 tablet per day 20 days per month for 3 months.

Acupuncture treatment (Stage B)

In clinical experiments with acupuncture, it is always necessary to reconcile two opposite requirements, i.e. to standardise the therapy and simultaneously adapt it to the clinical reality of each patient. Climacteric symptoms vary greatly from one woman to the next; therefore, the specific "acupoints" used for hormonal deficiencies in each subject will be associated with others according to the symptoms reported by each patient.

Chinese medicine identifies four "syndromes" in climacteric women. Each syndrome has precise symptoms, which are associated with specific acupoints.

Prior to being treated with acupuncture, the patients are interviewed in order to determine the prevalent "syndrome" among those listed, i.e. the one associated with the most frequently occurring symptoms. The acupoints corresponding to this syndrome will thus be used in combination with the standard acupoints and the treatment will remain unchanged throughout the first 6 sessions (6 weeks). Before the 7th session, the patient will again be interviewed to assess whether the acupoints need to be changed (as is possible in Chinese medicine) in response to changes in symptoms, which may result in another syndrome prevailing over the initial one. The newly chosen acupoints must remain unchanged until the end of treatment (12th week).

The nomenclature and numbering proposed by the WHO committee in 1986 were used for the purpose of identifying points.

Randomisation procedures

Adoption of sequences of numbers randomised in blocks of 24 or 30.

The secretary or nurse is in possession of sealed opaque envelopes, each containing a number. An envelope is drawn for every patient included in the study, who is thereby assigned to group A or B.

Sample calculation

The sample is calculated on the basis of the differences in the number of hot flushes at 1 and 3 months in patients undergoing treatment with veralipride and acupuncture. In a multi-centre study able to gather data about approximately 150 cases over a 3-year period, significant differences may be observed in favour of acupuncture, such differences amounting to 10-14 hot flushes per week.

As an alternative to a programmed experiment of a fixed size (150 patients), a sequential experiment may be planned, in which the results are evaluated as cases are accumulated. It is well known that the expected sample size in a sequential experiment is smaller than in a fixed experiment.

Statistical assessment

The comparison between stages A and B will regard the number of hot flushes and the weekly score of Greene's climacteric scale during the 3 months of the first stage, after the wash-out period, during the 3 months of the second stage and during the 3-month follow-up for a total of 12 months. The other comparison will be based on the total score of the SCL-90 scale and the WHOQOL questionnaire at the time of enrolment and after 3, 6, 9 and 12 months. A between-group comparison will be made in the various stages using the Student's t-test for paired data or Wilcoxon's test, according to the measurement used.

An assessment will be made of the side effects (number and type) and the number of patients who withdraw from the study.

Reporting of serious adverse events

An adverse event is defined as any undesirable medical occurrence (symptom or disease, including abnormal laboratory values) that may be correlated to the use of the drug undergoing examination or the treatment used.

A serious adverse reaction is defined as any unfavourable clinical manifestation that:

- is fatal;
- places the individual's life at risk;
- requires or prolongs hospitalisation;
- causes a permanent or major invalidity / disability.

All serious adverse events must be reported to the study coordinator. The first notification must be e-mailed or faxed within 24 hours; a complete report must be submitted as soon as possible.

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EVIDENCE BASED MEDICINE AND EVIDENCE BASED ACUPUNCTURE

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At present, the definition of EBM refers to "cautiously, accurately and wisely adopting the best currently available study criteria (evidence), combined with personal professional skills and many years' clinical experience, considering the value and wish of the patient: in summary, it perfectly integrates these three aspects aiming to work out the optimal therapeutic measures for the patients"(1).

The key element of the whole concept is not really to elaborate a therapeutic strategy in the clinical practice according to the evidence provided by scientific objective data, but rather it has to do with the elaboration of an organic combination between the clinician's professional skill and clinical experience on one hand and the most updated optimal results obtained by the systematic study (evidence) on the other hand. Within the evidence research process, the primary goal is in fact represented by the patient and therefore, a thorough evaluation and complete analysis of the specific condition of the patient is necessary in order to adopt the best evidence in the treatment.

To summarise, when considering the elaboration of a therapeutic strategy, the physician will have to take into account three key factors:

1) the clinical evidence;
2) the professional skills acquired in years of practice combined with clinical experience;
3) the values and desires of the patient.

None of these three factors should be neglected.

Specifically, we have to take into consideration: 1) the specific circumstances of that single peculiar clinical case; 2) the specific moment and appropriate modalities for that precise clinical case with regards to the selected evidence; 3) requests and system of values that the specific clinical case expresses (1).

When confronting a clinical problem, the physician should provide a first answer on the basis of his/her own professional skill and experience acquired in years. In fact, if the professional skills and the clinical experience, i.e. the "ability of the doctor", are neglected, there is the risk that the clinical practice is restricted to the evidence that comes from outside. This means that the external evidence valid for a specific case could be inappropriate or even inapplicable for that particular patient. Goals, protocols and results of the study must therefore be analysed and evaluated in a dialectic manner. Hence, in front of a concrete case, efficient, reasonable, practical and economically acceptable evidence for that particular circumstance and that particular patient will be adopted.

The research of the best current evidence should be combined with personal skill and best clinical experience. All these elements have equal importance. In fact, if the evidence that comes from different studies is considered the most valid and updated acquisition, but is not used in an appropriate and timely manner, the clinical practice could turn out to be inadequate, disadvantages would be higher than benefits and the patient could be affected negatively.

Finally, a qualified clinician has the obligation to be at the service of the patient with sincerity and sense of abnegation. Therefore, whenever a therapeutic decision is taken, all the

requests and the system of values of the patient must be taken into account to elaborate the optimal plan of action according to the dictates of EBM.

In an important editorial titled "Evidence Based Medicine: what it is and what it is not", David Sackett, one of the pioneers of the new movement for the practice of the EBM, and his colleagues outline that "the practice of EBM resides in the integration of individual clinical skill with the best available clinical evidence derived from systematic researches" (2). The individual skill is the experience acquired through clinical practice: this means that, when treating a single patient, the clinician does not simply and blindly follow the rules derived from other sources. It is given for granted that the physician knows the personal history of his patient, his/her family and social context, life style and working situation, and therefore is aware of his/her specific needs, more than what can be gathered from medical literature, whose aim is to suggest general solutions that are applicable to all patients with certain distinctive factors.

This outlook allows us to underline the concept that the EBM uses both RCT and the personal professional skill and clinical experience to the benefit of the patient.

Therefore studies and researches on acupuncture-moxibustion, conducted in a correct manner, make clear that many elements that are inborn in the nature itself of acupuncture-moxibustion shape this discipline as a true expression of the EBM (3).

In fact the analysis of the peculiar aspects of acupuncture highlights the following contents:

1) Acupuncture-moxibustion lays stress on the outcomes that are obtained at the end of a therapeutic process and that show to be really beneficial to the patient, and not the ones that can be observed at the beginning of treatment. For example, after an acupuncture-moxibustion treatment for indigestion an initial worsening of symptomathology may occur accompanied by nausea and vomit, followed by a real deep improvement of the clinical picture, or, after a cold stroke acupuncture-moxibustion may facilitate the onset of a high but short-term fever, which helps the patient to recover immediately and completely. What counts for much in treatment is the final outcome.
2) Acupuncture-moxibustion attaches special importance to the patient and therefore outcomes obtained are not surrogate outcomes. The state of the patient, given by the sum of his/her single functions and by the subjective condition, represents the endpoint. In many chronic pathologies laboratory or instrumental tests are surrogate outcomes. Sometimes, with the same lab data or instrumental tests, two patients may have a completely different clinical result: i.e. in the carpal tunnel or in the paraesthesia and in functional limitations of a cervical brachialgia due to a cervical spondyloarthrosis, it is possible that functionality proves to be very different, despite equal neurographic and radiological tests.
3) Acupuncture-moxibustion attaches importance not only to the partial results of laboratory or instrument tests, but also to the stability of the final result obtained by the patients, in fact in acupuncture-moxibustion improvements tend to be durable.
4) Acupuncture moxibustion takes great care of the patients' wishes in the selection of treatment tools. Acupuncture-moxibustion has a wide range of tools to make the treatment well accepted, facilitating the patient's best answer to therapy. For example, if the patient is afraid of needles the acupuncturist can use the complementary methods of acupuncture-moxibustion (i.e. auriculotherapy, plum blossom needle, etc.) which do not require the insertion of needle nor necessarily apply sharp instruments.
5) Acupuncture-moxibustion diversifies treatments according to the individual needs of the patients. The syndrome differentiation is a method elaborated with the aim of applying a treatment that is respectful of the pathological individuality and adequate to the patient's needs, even if in the ambit of a certain level of standardization.

These remarks allow us to put forward a legitimate hypothesis for the establishment of an Evidence Based Acupuncture-moxibustion which, starting from the conception of EBM, enriches it with those individualized procedures of acupuncture-moxibustion itself that can originate a medicine really close to the patient and addressed to his/her benefit.

We should recall that evidences in EBM are generally divided into 6 grades on the basis of quality and reliability (4):

Grade I Systematic assessment or meta-analysis conducted after the collection of all qualified reliable trials and randomized controls in relation to a specific therapy for a given disease.

Grade II Experimental results with duly-dimensioned single samples in randomized control trials (RCT)

Grade III A study having a control group without randomized grouping.

Grade IV A series of case clinical observations with no control group

Grade V Descriptive study based on the opinion of a well-known specialist or on clinical evidences or on the report of a specialists' committee.

Grade VI Experiences acquired from outside sources.

Reliability is very high in the first two grades, while it decreases progressively in the successive grades.

In the last decades activity of clinical research in acupuncture-moxibustion has developed to a certain degree. The majority of literature is published in specialized periodicals and is subject to criticism in reference to the conduct of RCT studies, which are not always considered methodologically complete and correct (5).

Here below are listed the remarks made on the majority of studies:

1. the randomized case grouping is not sufficiently clear or is inaccurate,
2. blindness involves only a 15% of the trials,
3. the samples size is generally too small,
4. the selection of control group is not rational,
5. few are the evaluation criteria of the therapeutic effect selected in conformity with international standards, which consider the patient as the core and as the endpoint index,
6. studies are not sufficiently addressed to the long term,
7. the term of follow-ups is too short,
8. drop-out and compliance data are uncertain,
9. the statistical analysis and the explanation of findings are inaccurate,
10. the efficacy indexes are rarely reported in detail, the transcription of basic data (age, sex, etc.) and of possible adverse effects is poor.

The conclusion is that the evidences gathered from RCT studies in acupuncture-moxibustion often have a low-degree reliability. This points out the necessity to find funds and qualified personnel to conduct adeguate acupuncture RCTs.

These aspects have been catched also by Chinese researchers, who have clearly focused the importance of EBM and the need to produce acupuncture-moxibustion studies according to its standards.

Wu Bin and Li Ning of the EBM Center affiliated to the Huaxi Medical University in Chengdu, China, assert that EBM is a sort of new field of medicine arisen from clinical practice, through absolutely innovative concepts it is able to promote the development of medicine as well as of the entire science of life (1). They have also stressed the fact that even if EBM comes from classic medicine it also differs from it. The differences mainly lie in the following five aspects:

1. *Source and collection of the evidences*
   Classic medicine lays stress on animal experiments, laboratory tests, individual clinical studies and knowledge from literature, but the information collected is not interpreted with a systemic holistic view, while EBM stresses the results of clinical studies on men and the collection of systemic global information.
2. *Assessment of evidence*
   Classic medicine is lacking in it, while EBM stresses its importance.
3. *Methodology*
   Classic medicine attaches importance to the disease and considers doctors as the center, while EBM considers the patients as the center.
4. *Selection of therapies and evaluation of therapeutic effect*
   Classic medicine attaches importance to results of laboratory tests, animal experiments and personal clinical experience, in the evaluation of the therapeutic effect it pays great attention to changes of lab indexes or radiographic tests, while EBM highlights the best available clinical evidence, and takes the final result achieved by the patient as the final evaluating index of effectiveness.
5. *Clinical decision*
   Classic medicine relies on works in literature, and the patient is not involved in selecting of therapies, while EBM pays attention to selection of therapies to the benefit of the patient.

The analysis of the above five aspects leads to the need of further investigating the contents of EBM because, if correctly interpreted, it can give great impulse to the growth of acupuncture-moxibustion scientific dignity.

This hypothesis challenges acupuncturists and entails the need of acting in the following two areas to allow a definitive achievement of acupuncture-moxibustion (6):

- *A. Clinical evidence of EBM*
  It is necessary to produce a higher number of evidences on the basis of the important guidelines issued by WHO. Those evidences must be correct also from the point of view of the acupuncture-moxibustion requirements according to a very well defined specific methodology (RCT in acupuncture-moxibustion). To this respect we have to open several questions, such as:
  i. How to integrate the TCM syndrome complex with the nosological pictures of modern medicine? Is it sufficient to choose one single accredited scheme of syndrome differentiation as a reference for a specific disease?
  ii. How can the various needle manipulation techniques be quantified in an acupuncture-moxibustion treatment inside an RCT so as to rely on unified standards?
- *B. Clinical experience*
  We should work to demonstrate the value of the clinical experience accumulated in thousand years of acupuncture-moxibustion practice, which necessarily cannot be suggested by evidences, being in strict relationship with what can be grasped from the physician's skills and experience and from the single clinical needs of that particular patient. In particular the acupuncture-moxibustion clinical experience, if suitably systematized, can be classified and objectivated and become helpful for being confronted and diffused: the procedure of differentiated diagnosis and the consequent differentiation of syndromes are in fact a method created to make the acupuncture-moxibustion

treatment focused on the patient's comprehensive needs and it offers, even when this procedure is schematized, a certain level of objectivity in the process of individualizing the patient's condition as required by the acupuncture practice.

Well, I believe that, if in this Symposium we shall be able to build up the foundation necessary to find the way to coordinate these two aspects, we will go towards a real concrete advance which not only will allow acupuncture-moxibustion to definitely become part of the medical science but also to enrich it, thanks to its specific potentialities that at present have made its success in the clinical field: simple execution, absence of side effects, low cost, ability to provide individual and therapeutic solutions that tend to be definitive.

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SITUATION OF ACUPUNCTURE IN CHINA AND IN THE WORLD

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It might seem inappropriate that an Italian doctor talks about the status of acupuncture in China, since there are Chinese colleagues attending this event. Likewise, being this congress hold by such a prestigious institute as the ISS, which has institutional relations with the most important countries world-wide, it might seem as well inappropriate that the status of acupuncture in the world, including countries such as American countries, is presented by a doctor who is not inside any institution, though believing in the role of health institutions and having an intense collaboration with them. Yet I think it useful that the description of the state of acupuncture in China and in the world is covered also from the point of view of the acupuncturist. After all, acupuncture is a topical matter in Western countries thanks to the activity of those acupuncture doctors that from the '70s have worked hard to establish relations with the most important Chinese institutions of acupuncture and Traditional Chinese Medicine (TCM), and even more thanks to the people that have addressed acupuncturists for receiving therapy while being granted no institutional support.

Available data on the state of TCM and acupuncture in China show that they represent a reference reality, not only because they originated in that country and are widely spread there, but also because in China education in these disciplines is developed at a university level, clinical and research activities have impressive scope and, most of all, the national legislation favours the development of TCM and acupuncture, since it acknowledges equal dignity to both Western academic medicine and TCM and acupuncture.

The World Federation of Acupuncture Societies (WFAS), constituted in Beijing in 1987, which I represent as vice-president, has since then acted world-wide with the continuous growing support of the Chinese Government. Since 1997 it has become an Ngo of WHO.

It seems to me that, even if with the necessary caution, China is developing the science's ecumenical concept that can be synthesised in the words "One medicine, several medical systems".

In the International Symposium on Acupuncture Research Methodology held last November in Rome in the seat of CNR (Italian National Council of Researches), Dr. Shen Zhixiang, director-general of the Department for the International Co-operation of the Chinese State Administration for TCM, and secretary-general of the WFAS, presented a study on the range of pathologies that can be treated with acupuncture. Then he related the pathological ambits where a higher efficacy of acupuncture is evident. Higher efficacy of acupuncture refers to the cases where the treatment of a specific disease obtains the best results with the exclusive use of acupuncture or with acupuncture used as the main therapy. In conclusion of his speech, he highlighted the need to widen the range of the therapeutic employment of acupuncture (1).

In Italy all health professionals that for thirty years have worked for the development of acupuncture through the constitution of TCM and acupuncture scientific societies, obviously feel that the Chinese reality properly represent them.

Since five years also the United States intend to be a reference point for NCMs, including TCM and acupuncture. As an example we recall the well known Consensus Conference that acknowledged therapeutic value to acupuncture, not only as a pain therapy (2).

In fact, after the declaration on the efficacy of acupuncture issued on November 5, 1997 by the Consensus Development Program of the National Institutes of Health, the National Centre for Complementary and Alternative Medicine (NCCAM) was established and since then has continued to operate in the States. Objective of the NCCAM is the fulfilment of a program of studies on medicines they define as "alternative and complementary", conducted with a rigorous scientific methodology, which includes also the training of researchers that can work specifically in the field of acupuncture and TCM. A particular activity of this body is the constant spreading of information addressed to the public and professionals. The NCCAM has constituted in February 2001 the internal Office of International Health Research (OIHR), which promotes and plans national and international research programs, also in acupuncture and TCM. The OIHR has four objectives: 1) promote research on acupuncture and TCM, 2) promote training for research activity in the field of acupuncture and TCM, 3) widespread information on research, 4) favour integration between conventional and non conventional medicines through the use of integrated models.

How do Italian acupuncturists watch the American work?

Favourably for what concerns the set up of activities addressed to reorganise this field especially from the regulative point of view; with some perplexity for having placed acupuncture also within the competence of non MD health professionals, with no proper differentiation; with reserve as to the persistent adoption of the term "Complementary and Alternative Medicine" also for TCM and acupuncture. Such term insistently underlines that Non Conventional Medicines (NCMs) and in our case TCM and acupuncture are simply a complement of Academic Medicine. This idea is not acceptable. In fact in front of different single clinical cases, each medical system can prove to be fundamental or more or less complementary. For instance, in the case of a heart and circulatory emergency, the pharmacological medicine of the Academic Medical System will be the priority-therapy and acupuncture complementary. Yet in the case of a recurrent chronic disorder where it is impossible to trace any cause (for instance in some sine causa pseudo-allergic diseases), the acupuncture branch of the Traditional Chinese Medical System will be the priority-therapy and Academic Medicine complementary.

Again, we don't agree with the term "alternative", since it expresses a confliction that nobody wishes, since the present direction is towards the integration of medicine.

Let's turn to Europe now.

We spoke before about WFAS, such organisation was settled to promote the development of acupuncture and the scientific exchange among professionals throughout the world. After 14 years of activity, WFAS counts 75 scientific societies from 40 countries world-wide with a total of about 70,000 acupuncturists. Out of these, the European region counts 30 societies from 17 countries with a total of 13,966 acupuncturists (data 2001) (3).

In Europe, professional training in acupuncture and TCM has generally no official acknowledgement. For instance, in the field of acupuncture, the leader among NCMs, the situation of education has not substantially changed from the outline drawn by the "European Workshop for the harmonisation of acupuncture education" assigned to the Paracelso Institute in Rome by the Council of Europe in 1990 (4, 5). Education is still carried out mainly by private scientific acupuncture societies and organisations, whose standards express unequal levels of qualifications. An interesting case is Finland, where the five universities that have a School of Medicine include a few-hour theoretical course on acupuncture, after which students may decide to attend optional acupuncture courses, both theoretical and practical. France has introduced an inter-university diploma of acupuncture, co-ordinated by 6 universities. In other countries, such as Spain, Italy and UK, there are post-graduate acupuncture courses, more

informative than formative, which usually last 1 or 2 years. Yet it seems that UK is going to establish a really formative university course.

The limit of the educational activities present in Europe is represented by programs with a very low amount of hours (150-1500), if compared with countries where NCMs are made official or are recognised. Moreover, with very few exceptions, the educational courses are penalised by the absence of adequate structures for practical training.

In fact, if we again refer to acupuncture, we can observe a big gap between the European courses, which at most reach an amount of 1500 hours, and the university courses in China or in the private colleges of some of the United States, where training hours raise to over 5000. Even in the case of courses established inside universities, such as the acupuncture courses, these are not organically integrated in the curricula of medical studies, nor do they originate a professional health *infra lauream* certificate, and they do not formally represent a "specialisation".

The Council of Europe had issued a recommendation for a post-graduate 4-year educational program on acupuncture, prepared by the Paracelso Institute inside the aforementioned European Acupuncture Workshop. After this recommendation, university educational courses have been given a better and better structure, such as the Master on acupuncture techniques that has been recently introduced, thanks to the new legislation on Masters, in the Institute of Forensic Medicine at the Rome University "La Sapienza", through a convention with the Paracelso Institute (6).

Also, legislation on the subject hardly exists, despite resolutions and acts issued by the Council of Europe and the European Parliament. Regulation in single countries is limited to mainly administrative acts or views expressed by advisory organisms and in some of its aspects is not homogeneous. For instance, as concerns the definition of the professional figure qualified for the practice of acupuncture in the UE, there are three different addresses:

1. countries where the medical degree is sufficient to practice acupuncture under the doctor's total professional responsibility (France, Italy, Finland).
2. countries where not all graduate doctors are legitimated to practice acupuncture, but only those that have specific scientific competence and professional training (Austria, Sweden).
3. countries where the practice of acupuncture is not limited to medical doctors (Belgium, Holland, UK, Germany, Spain, Norway) (4, 5).

As concerns the situation in Italy, after the position assumed by the National Medical Association (FNOMCeO) towards NCMs, including acupuncture and TCM, it is imminent the issue of an outline law on NCMs that will establish some general rules, but will still be far from covering all the aspects relevant to a correct practice of acupuncture and NCMs in general (7).

We then have to signal that the Italian scenery of acupuncture and TCM has recently witnessed the introduction of a growing number of universities, institutional subjects and prestigious bodies that can give a significant contribution to the development of acupuncture in our country.

The Italian scientific acupuncture societies will feel their task lightened if such contribution will be harmonious and will respect the experience and heritage that has been accumulated up to now.

Acupuncture needs to be cultivated with devotion and disinterestedness as an instrument to support the fundamental requirements of the sick. So it has been for thousands of years, so the Italian scientific acupuncture societies that have promoted the success and development of acupuncture up to now, want it to be in the present and in the future.

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ESTABLISHMENT OF NATIONAL NEW DRUG RESEARCH AND DEVELOPMENT SYSTEM IN CHINA

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Establishment of national new drug research and development system is to elevate the quality and quantity of new drugs research and development, and promote pharmaceutical industry in China. To achieve this goal, several subsystems have to be established along the pipeline of research and development.

1. establishment of new drug screening subsystem;
2. establishment of preclinical pharmacodynamic evaluation subsystem for new drugs;
3. establishment of preclinical pharmacokinetic research subsystem for new drugs;
4. establishment of preclinical safety evaluation subsystem with international glp standard for new drugs;
5. establishment of clinical trial subsystem with international GCP standard new drugs.

Under the leadership of China Ministry of Science and Technology, The administrative center for China's Agenda 21 is responsible for the coordination and management of this system's establishment.

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Roma, settembre 2004 (n. 3) 5° Suppl.