Addendum

Errata:

Chapter 6

In Table 2 (page 100) and Table 3 (page 102) "Column 4" consisting of the main term Condition and the interaction term Condition by Baseline Value has to be "Column 3". Note both the main term Condition and the interaction term Condition by Baseline Value are entered *simultaneously* in the regression equation. "Column 3" consisting of the interaction term Condition by Baseline Value only has to be "Column 4".

Chapter 7

Note "Column 3" in Table 2 (page 118) consists of both the main term Condition and the interaction term Condition by Baseline Value. Both these terms are entered *simultaneously* in the regression equation. In Table 2 the main term Condition (Column 2) and the interaction term Condition by Baseline Value (Column 4) are entered *separately* in the regression equation.

Chapter 8

In case of a second order interaction calculation of the change scores of the immune and endocrine outcome measures (page 131) is as follows.

$$\Delta POST_{(T3\text{-}T2)} - \Delta PRE_{(T3\text{-}T2)}$$

N.B.:

Comments on the thesis "Mind-body interactions in breast cancer. Neuroendocrine and immune aspects of acute psychological stress and psychosocial intervention in breast cancer patients":

Gieta van der Pompe
University of Groningen
Experimental & Work Psychology
Grote Kruisstraat 2/I
9712 TS Groningen
Telephone + 31 50 363 63 76
Telefax + 31 50 363 63 04
e-Mail: G.van.der.Pompe@PPSW.RUG.NL

Mind-body interactions in breast cancer

Cover Erik Tack
Printed by Febodruk b.v. Groningen
ISBN 90-9010348-1

The research described in this thesis was carried out at the Helen Dowling Institute for Biopsychosocial Medine, Rotterdam, The Netherlands.

The study was supported by the VSB Foundation, Utrecht and The Josephine Nefkens Foundation, Rotterdam, The Netherlands.

bART Internet Services B.V., Rotterdam, The Netherlands contributed to the printing of this thesis.

Mind-body interactions in breast cancer

Neuroendocrine and immune aspects of acute psychological stress and psychosocial intervention in breast cancer patients

Lichaam-geest interacties bij borstkanker

De neuroendocriene en immunologische aspecten van acute psychologische stress en psychosociale begeleiding van borstkankerpatiënten

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE ERASMUS UNIVERSITEIT ROTTERDAM OP GEZAG VAN DE RECTOR MAGNIFICUS

PROF. DR P.W.C. AKKERMANS M.A.

EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 12 MAART 1997 OM 13.45

DOOR

GRIETJE VAN DER POMPE
GEBOREN TE LEIDEN 16 MAART 1959

PROMOTIECOMMISSIE
PROMOTOR : Prof. Dr M.J. de Vries

OVERIGE LEDEN: Dr J.J. Heijnen
Prof. Dr J.W. Oosterhuis
Prof. Dr S.W.J. Lamberts
Prof. Dr P.M.G. Emmelkamp

Voor de vrouwen die hebben deelgenomen aan dit onderzoek.

Contents

The following chapters of this thesis are in print, in press, accepted or submitted for publication.

Chapter 1:
Pompe G van der, Antoni MH, Mulder CL, Heijnen CJ, Goodkin K, Graeff A de, Garssen B, Vries MJ de (1994) Psychoneuroimmunology and the course of breast cancer: an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psycho-oncology 3:271-288

Chapter 2:
Pompe G van der, Antoni MH, Visser A, Heijnen CJ Immune and cardiovascular responsivity to a standardized laboratory challenge in breast cancer patients and healthy women, submitted for publication

Chapter 3:
Pompe G van der, Antoni MH, Heijnen CJ (1996) Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. Psychoneuroendocrinology 21:361-374

Chapter 4:
Pompe G van der, Antoni MH, Duivenvoorden HJ, Heijnen CJ The relations of ACTH and cortisol levels with the distribution and function of peripheral blood cells in response to a behavioral challenge in breast cancer patients. Int J Behav Med, accepted for publication

Chapter 5:
Pompe G van der, Antoni MH, Visser A, Garssen B (1996) Adjustment to breast cancer: The psychobiological effects of psychosocial interventions. Pat Edu Coun 28:209-219

Chapter 6:
Pompe G van der, Antoni MH, Duivenvoorden HJ, Vries-Kragt K de, Pelgrim F, Vries MJ de. Prediction of psychological adjustment to breast cancer after participation in a group psychotherapy program. Br J Clin Psych, (prov) accepted for publication

Chapter 7:
Pompe G van der, Duivenvoorden JH, Antoni MH, Visser A, Heijnen CJ. The effect of a group psychotherapy program on endocrine and immune function in breast cancer patients. J Psychosom Res, in press

Chapter 8:
Pompe G van der, Antoni MH, Duivenvoorden HJ, Graeff A de, Simonis RFA, Vegt SGL van der, Heijnen CJ. The effect of a group psychotherapy program on the reactivity to acute stress on the cardiovascular, endocrine and immune system in breast cancer patients, submitted for publication

Preface

The proposition that stress plays a role in the progression of breast cancer has been a source of inspiration for researchers to study the associations between psychological, neuroendocrine and immune parameters. Until now a number of studies attempted to provide insight in the link between stress and breast cancer by correlating psychological stressors with baseline endocrine and immune values. There is a growing literature supporting the notion that by using a reactivity model more reliable information can be obtained about the organizational level of the neuroendocrine system as well as the sensitivity of the immune system to the endocrine signals than by determining baseline values. However, apart from studies that examined the alterations in neuroendocrine system and distribution and function of peripheral blood cells in breast cancer patients in rest, data from reactivity studies are scarce.

The series of studies described in the first part of the present thesis were designed to investigate changes in cardiovascular, endocrine and immunological values in response to an acute stressor in breast cancer patients and to compare their responses with those of age-matched healthy women.

Before the results of these studies are presented, this thesis will start with a review of studies that examined the relations between stressors, endocrine and immune processes, and progression of breast cancer (Chapter 1). This will enable the reader to place the data regarding the acute stress-induced endocrine and immunological changes in the broad context of previously established findings regarding the effect of stress on immune function of breast cancer patients. In chapter 2 the results of a study on the immune and cardiovascular responses to an acute stressor (speech task) in breast cancer patients in two different disease stages (i.e., either with positive axillary lymph nodes or with distant metastases) and healthy women are described. Chapter 3 focus on the effect of this challenge test on changes in adreno-corticotropin releasing hormone (ACTH), prolactin and cortisol in the same group of breast cancer patients and healthy women. Chapter 4 deals with the question whether changes in distribution and function of peripheral blood cells can be predicted by ACTH and cortisol levels and by the health of the donor.

The results of several studies indicate that psychosocial interventions have not only a positive effect on psychological adjustment of breast cancer patients but may also result in changes in immune function. The second part of this thesis was designed to investigate the efficacy of an existential-experiental group psychotherapy (EEGP) program on psychological adjustment to breast cancer diagnosis and medical treatment and on the function of the neuroendocrine and immune function of breast cancer patients. The research described in this part of thesis forms part of the psychooncology line of the HDI, which was iniated in 1987 at the Faculty of the Medicine, Erasmus University, in Rotterdam. Chapter 5 will summarize studies that focussed on the specific psychological responses of breast cancer patients across different stages and studies investigating the effect of psychosocial intervention programs on psychological, endocrine and immune function in cancer patients. Chapter 6 focus on the effect of the EEGP program on psychological distress, coping and social support in breast cancer patients. In order to determine whether this program will be equally useful to all breast cancer patients, special attention will be given to the question whether the effect of EEGP on psychological distress, coping and social support can be predicted by their psychological profile at baseline. Chapter 7 deals with an exploration of the effect of the

EEGP program on endocrine and immunological measures in breast cancer patients. Because breast cancer patients across different stages of their disease show variability in endocrine and immune parameters, special attention will be given to the question whether the effect of EEGP on endocrine and immune function is related to patients' respective baseline values. As stated earlier, a reactivity model can be a useful tool in the search for information about the neuroendocrine and immune system interactions. Chapter 8 elaborates on the effect of EEGP on the reactivity to acute stress of the cardiovascular, endocrine and immune system in breast cancer patients. Conclusions regarding the acute stress-induced cardiovascular, endocrine and immune changes and the EEGP-effect, and directions for future research will be described in chapter 9.

Chapter 1

Psychoneuroimmunology and the course of breast cancer: an overview

The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms

G. van der Pompe[1], M.H. Antoni[2], C.L. Mulder[1], C.J. Heijnen[3], K. Goodkin[2], A. de Graeff[4], B. Garssen[1], M.J. de Vries[1]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Department of Health Psychology and Psychiatry, Miami

[3] University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', Utrecht

[4] Academic Hospital Utrecht, Utrecht

Abstract

This review focuses upon possible mediators of the oft noted associations between psychosocial factors and progression of breast cancer. First, host-related endocrine and immunological processes that may play an important role in the carcinogenesis and progression of breast cancer are summarized. Second, the effects of several endocrines on different components of the immune system that have been shown to be related to the course of breast cancer are addressed. Third, studies examining the effect of psychosocial factors on immune function conducted with breast cancer patients and healthy individuals are described. Based on this review, it can be concluded that causal relations between psychosocial stressors, endocrine and immune processes, and breast cancer progression remain to be elucidated. It can be hypothesized that psychosocial stressors influence the course of breast cancer by modulating endocrine processes which are directly related to tumor growth or indirectly related by decreasing immunologic control over tumor development and metastases. Finally, methodological pitfalls that plague this line of research are summarized and recommendations for future psychoneuroimmunologic research in breast cancer are provided.

Introduction

In the past twenty years several studies have shown, although not conclusive, that negative life experiences are associated with progression of breast cancer (reviewed by Mulder, Van der Pompe, Spiegel, Antoni, De Vries, 1992). To provide insight in the psychooncological associations the focus is now on the psychobiological processes that may mediate the effect of chronic life stress on disease outcome. In the field of psychoneuroimmunology (PNI) a vast number of studies have been published which describe endocrine and immunological changes in response to different types of stressors. The majority of these studies described endocrine and immune responses to naturalistic occurring life stressors and chronic psychiatric disorders in healthy individuals. Inspired by these findings it has been hypothesized that the influence of chronic life stress on mammary tumor progression can be explained by interindividual differences in endocrine and cellular immune responses. This may be likely because endocrine and immunological processes have been suggested to play an important role in the onset and progression of human breast cancer. This chapter is an attempt to integrate the large body of information as it relates to chronic life stress and progression of breast cancer.

Endocrine regulation of mammary tumor growth

The growth of the mammary gland has been shown to be regulated by estrogen, progesterone, prolactin, growth hormone, adrenal corticoids and triiodothyronine (Forsyth, 1991). There exists both experimental animal findings and clinical evidence that some of these hormones may play a role in the initiation and growth of breast cancer. Most attention has been focused on the role of estrogens, progestens and prolactin. This is a very complex field and it is impossible to do justice to the subject in the present review. The interested reader is referred to reviews that cover this vast literature (Sutherland, 1987; Bulbrook et al., 1988). We will address briefly the hormone dependance and endocrine responsiveness of breast cancer and implications for treatment strategies.

Animal work strongly implicates a prime role for the estrogens and prolactin in the etiology of breast cancer (Bulbrook et al., 1988). The effects of the steroid hormones on mammary tumor growth seem to be mediated through their association with receptors, especially estrogen and progesterone receptors. In mice, early ovariectomy results in a delay in the appearance of mouse mammary tumor virus (MMTV)-induced tumors. Conversely, castration and the administration of estrogen can encourage mammary tumorigenesis in males. The development of these tumors can also be facilitated by the administration of progesterone or prolactin (Jordan, 1991). In rats, administration of carcinogens may induce mammary tumors. Most of these tumors have estrogen receptors and regress after removal of the ovary or pituitary (Sutherland, 1987).

Although these animal models have clarified the relationship between hormone action and the induction of cancer, their clinical relevance for human disease remains uncertain. However, there are some arguments that steroid hormones may influence the development and course of disease in human breast cancer patients (Henderson & Bernstein, 1991). First, epidemiologic evidence suggests that the major risk factors for human breast cancer include early age at menarche, late age at first full-term pregnancy,

late age at menopause and obesity (Henderson & Bernstein, 1991). This pattern may be explained as a result of overexposure to estrogens (Bulbrook et al., 1988). However, the data relating endogenous estrogen exposure and breast cancer is still unconvincing (Toniolo et al., 1991). Second, we know that the presence of estrogen or progesterone receptors in primary breast cancer tissue is correlated with a better prognosis. This may be due to different proliferation rates in receptor positive and negative breast carcinoma (Aaltomaa et al., 1991) or, alternatively, to a greater responsiveness to endocrine treatment in the receptor-positive cases (Neifeld, 1989). However, it should be kept in mind that receptor positivity is still a much weaker prognostic factor than are axillary lymph node status and tumor size (Chevallier et al., 1988; Carter et al., 1989; Rosen et al., 1989; Aaltomaa et al., 1991; Neifeld, 1989). It is known that estrogen and, to a lesser degree, progesterone receptor status, seem to be related to patterns of metastatic spread. In a study of Koenders et al. (1991) patients with estrogen receptor positive tumors had bone metastases three times more often than patients with estrogen receptor negative tumors. Patients with receptor negative tumors on the other hand, had a 50% higher incidence of visceral metastases. And last, endocrine treatment (e.g., antiestrogens, aromatase inhibitors) is effective in patients with breast cancer (especially in those with estrogen receptor positive tumors), both in the adjuvant setting and in metastatic disease. Antiestrogens (e.g., toremifine or tamoxifen) are known to inhibit the stimulatory effects of estrogens on breast cancer growth (Dickson & Lippman, 1987), though the mechanisms by which they trigger regression are not completely understood.

In conclusion, there is a substantial body of experimental and epidemiological evidence that suggests that hormones play a role in the etiology and development of breast cancer. In addition, endocrine manipulation appears to be capable of influencing the course of this disease. The mechanism of influence of endocrine processes on the initiation and development of human breast cancer has not been elucidated.

The immune system and breast cancer

Following the early theories of Ehrlich, McFarlane Burnet (1970) formulated the immune surveillance theory. He proposed that most malignantly transformed cells are immunogenic and stimulate the host's immune defenses and are probably destroyed by an effective host defense. Accordingly, only those cells escaping destruction may give rise to a manifest tumor. This theory gained support by many observations in immunocompromised humans and animals and by studies of tumor antigenicity in different animal systems (Krueger, 1989). However, this theory could not explain why only certain tumors such as lymphomas and leukemias have a tendency to grow, nor could it clarify differential findings regarding the level of immunogenicity across virus-induced and chemically or physically-induced murine tumors (Gleichmann & Gleichmann, 1973).

Although, the immune surveillance theory is controversial and not specific to different carcinomas, it has been useful for developing the concepts of immunogenicity and antigenicity in oncogenesis and for stimulating research examining the different ways malignant cells can evade the protective potential of the immune system.

Immunologic defense processes

T-cell mediated immunity appears to be primarily responsible for the control of immunogenic tumor growth (Gorelik & Herberman, 1989; Melief & Kast, 1990; 1991). In virally-infected cells, virus-associated genes encode the expression of specific antigens which can serve as a target for activated CD4+ T helper (Th) and CD8+ cytotoxic T lymphocytes (CTL), which can only recognize an antigen in the context of major histocompatibility (MHC) molecules on the cell surface (Ljunggren & Kärre, 1990; Melief, 1992). In general, T cell-mediated immunity against virus-induced tumors or other immunogenic tumors follows the rules of antiviral immunity. For a tumor to be immunogenic, it needs to present processed antigen as peptide bound to MHC class I or class II molecules (for review see Melief, 1992). Studies with animal models have shown that virus-induced (e.g., Epstein Barr Virus [EBV], human papillomavirus [HPV]) tumors are most likely to be immunogenic (Melief, 1992). Other tumors which are chemically or UV-light induced appear to be low- to non-immunogenic. However, human breast carcinoma does not appear to be virally, chemically, or UV-light-induced and accordingly may be relatively non-immunogenic. Yet, there is some evidence that in a significant fraction (20%) of human breast carcinomas, tumor growth restriction is mediated by the highly effective CTLs. Indeed, the human gene MAGE-1, which appears to direct expression of a potential tumor rejection antigen on a human melanoma cell line (Van der Bruggen et al., 1991), has been reported to be expressed by some breast tumors (Brasseur et al., 1992).

An increasing body of experimental data indicates that, in addition to T cell-mediated immunity, non-specific natural killer (NK) cells and macrophages could participate in the control of tumor growth and, especially, in the development of metastases (Herberman, 1984; Gorelik & Herberman, 1989). Through a hitherto unindentified mechanism, NK cells are apparently capable of recognizing and eliminating moderate numbers of tumor cells that fail to express self MHC class I molecules (Ljunggren & Kärre, 1990). Therefore, it has been suggested that most human tumors, including human breast carcinomas, which express insufficient levels of antigenic determinants, may be surveyed by NK cells (Herberman, 1984). Activated macrophages have also been shown in vitro to exert a high level of cytotoxic and cytostatic activity against a variety of tumor cells including mammary adenocarcinoma (Alexander, 1976; Hibbs et al., 1978; Nayar & Fidler, 1989). In addition, in vivo eradication of tumor tissue involves CD4+ T-cell mediated induction of tumoricidal acitivity in macrophages that is initiated by interferon-gamma and other cytokines secreted by CD4 cells (Greenberg et al., 1988).

To summarize, some tumors, including human melanoma and a fraction of human breast carcinomas appear to express sufficient levels of cell surface antigens to activate the highly specific and effective CTLs. Growth restriction of less immunogenic breast tumors may be more dependent on the prompt action of NK cells and macrophages.

Immunologic escape

Most cancer patients, including breast cancer patients have an intact immune system at the time of the initial diagnosis. Thus assuming that the variability in the clinical course of breast cancer is attributable, in part, to the efficiency of immune control mechanisms, tumor cells must have ways to escape eradication by the immune system. Only some of these mechanisms have been identified. First, much experimental data clearly indicate that a

tumor is a heterogeneous population of malignant cells, composed of a series of related but genotypically distinct individual clones (Gorelik & Herberman, 1989). It has been suggested that specific host defense mechanisms can select in favor of less immunogenic cell populations. This selection of tumor antigen-negative variants, occuring under the pressure of tumor-specific T cells, has been documented in a variety of immunogenic tumor systems (for review see Melief, 1992). Second, loss of cell surface antigen is a frequently observed phenomenon that may give tumor cells the ability to evade several immune system barriers (Ljunggren & Kärre, 1990). Third, many animal models using chemically and virally-induced tumors, have demonstrated that the cytolytic action of CD8+ cells can be suppressed by soluble immune complexes and "suppressor cells" that are usually signalled by cytokines produced by CD4+ cells (Awwad & North, 1990). Fourth, tumor cells themselves have the ability to exert local effects that prevent T cells from displaying full efficacy, allowing escape from immune surveillance (Krueger, 1989; Melief, 1992). This effect is mediated by transforming growth factor (TGF-) beta. Malignantly transformed cells secrete the cytokine TGF-beta, which is known to inhibit IL-2 dependent proliferation of T lymphocytes and the induction of CTLs in mixed lymphocyte cultures (Wahl et al., 1989). Conceivably, TGF-beta secreted by tumor cells might similarly suppress T lymphocyte proliferation and CTL generation, at least locally (Brunson & Goldfarb, 1989).

Since the scope of this review only allows a brief overview of immune system influences in tumor growth control and escape, it is inevitable that not all possible immune system components have been discussed. The interested reader is referred to several compendiums that cover the vast literature (Herberman et al., 1987; Herberman, 1989; Melief, 1992).

Immunologic changes occuring during progression of breast carcinoma

Studies in women with breast cancer show that both normal and decreased levels of T lymphocyte counts and proliferative responses occur, dependent on the stage of the disease. In stage I patients, the number and proliferative responsivity of T lymphocytes to phytohemagglutinin (PHA) are hardly different from normal controls. However, as the disease progresses (to stages II to IV), T lymphocyte number and function become significantly reduced (Contreras Ortiz & Stoliar, 1988; Hacene et al., 1986; Burford-Mason et al., 1989; Mohanty et al., 1991). When measured prior to surgery, both total T lymphocyte counts and proliferative responses to PHA are identified as prognostic factors for overall survival: breast cancer patients with lower than normal levels of T lymphocyte counts and reactivity to PHA have a significantly higher risk of recurrent disease than those who have normal levels of T lymphocytes and reactivity (Ownby et al., 1983; Hacene et al., 1986). In addition, several studies suggest that NK cell activity (NKCA) in peripheral blood of breast cancer patients is decreased compared to healthy women. As with T cell function this decrement is progressive and related to the clinical stage, the least impairment being seen in stage I and the most in stage IV (Spuzic & Konjevic, 1990; Konjevic & Spuzic, 1991; Kindzel'skii et al., 1990). The prognostic value of NKCA is still being disputed. On the one hand, a study using stage I and II breast cancer patients concluded that NKCA is a strong predictor of disease outcome (Levy et al., 1991). On the other hand, another study of stage I to IV breast cancer patients demonstrated that the percentage of NK cells among perip-

heral blood mononuclear cells (PBMC's), and their activity, are independent of the clinical and pathological stage of this disease (Bonilla et al., 1990).

Local immunity to breast carcinoma

Lymphocytic infiltration of breast cancer tissue has often been associated with a favorable prognosis (Griffith et al., 1990). However, neither the density of lymphocytic infiltration by particular lymphocyte subsets, nor the ratio of T helper-inducer (CD4) to T suppressor-cytotoxic (CD8) cells (CD4/CD8 ratio) was related to improved short-term survival or recurrence (Griffith et al., 1990; Stewart, 1991; Wintzer et al., 1991). Thus local cell-mediated immunity may be ineffective or irrelevant in preventing metastatic spread from the primary breast tumor. In addition to T cells, a large number of macrophages are present in the cellular infiltrates of several types of carcinomas (e.g., breast, melanoma, kidney, lung) (Van Ravenswaay Claasen et al., 1992). However, the relationship between the presence or absence of macrophages in cellular infiltrates and the course of breast cancer is still unknown.

Methodological issues

One controversy in the field of tumor immunology concerns the relative clinical relevance and predictive power of site-specific vs. peripheral immune responses. To address this issue, studies have to be initiated in which both local and systemic immunity are evaluated. So far, several studies have defined changes in various immune measures across stages of disease progression in breast cancer. This relationship has been characterized by a gradual decline in both enumerative and functional immune measures. From the standpoint of the PNI research agenda, this knowledge is particurlarly important in differentiating tumor-related immune changes from host defense alterations caused by co-morbidity (e.g., viral infections), medical treatment (e.g., chemo- and radiotherapy), behavioral (e.g., tobacco and alcohol use) and psychosocial factors (e.g., socioeconomic status, chronic stressors, distress states, social support, coping), which may heighten the risk for metastatic spread of the tumor (Holland, 1990). These potential confounding variables may be controlled by sampling decisions and by statistical means. The immune impairments seen in breast cancer patients may be secondary to endocrine changes that are more primary to the disease process. We now review some of these endocrine system alterations and the evidence to date for endocrine-immune associations.

Endocrine-immune interactions in breast cancer

A relatively recent view is that the central nervous system (CNS) and immune system are linked (Ader et al., 1991). Specific receptors for several endocrines are found on different lymphoid cells providing a means for hormonal as well as direct, neuronal communications with lymphoid tissues (Blalock, 1989; Khansari et al., 1990). Moverover, in addition to the effects of estrogens, progestens and prolactin (PRL) on the mammary epithelium described previously, there is evidence that these substances may also act as regulators of lymphoid cells. As such, it is possible that endocrine processes may influence mammary tumor growth indirectly by modulating immune function. While the LHPA-related hormones (corticotropin-releasing hormone [CRH], adrenocorticotropin hormone [ACTH], beta-

endorphin and cortisol) and SAM-related hormones (adrenaline and nor-adrenaline) have been shown to play a limited role in the regulation of mammary epithelium, they can produce changes in phenotypic and functional measures of the immune system, which in turn, may be related to progression of breast cancer by way of impaired cellular surveillance.

Estrogens

Animal and human studies suggest that estrogens may suppress the immune response, for exmaple NKCA (Styrt & Sugarman, 1991). The results of one experimental animal study indicate that estradiol and testosterone administration over a 2-wk period cause a substantial reduction of NKCA (Hou & Zheng, 1988). However, in another experimental animal study, estradiol administration for 2 days resulted in a significant increase in the number of uterine eosinophils, CD4+ T lymphocytes and macrophages but failed to elicit a significant increase in the number of CD8+ T lymphocytes and NK cells (Zheng et al., 1988).

In one human study the NKCA in female volunteers was measured throughout their menstrual cycle. During the peri-ovulatory period there was a significant fall in NKCA compared to normal male volunteers, suggesting that estrogen rises are negatively related to NKCA (Sulke et al., 1985). It has also been found that estrogen analogues (such as diethylstillbestrol - DES) may be more likely to suppress NKCA than estradiol-17-beta itself (Uksila, 1985). Systemic alterations of the maternal immune system and changes in the inflammatory process also appear during pregnancy. In one study, increasing levels of estrogen hormones (beta-estradiol, estriol and estrone) were negatively related to NKCA in pregnant women (Pope, 1990). In a separate study, beta-estradiol levels were negatively correlated with NKCA in each trimester, suggesting that high doses of this hormone could contribute to pregnancy-associated NKCA suppression (Gabrilovac et al., 1988). However, it has also been found that female sex steroid hormones and pregnancy do not inhibit normal human NK-cells in vitro (Uksila, 1985). The inconsistencies across these studies may be due to the fact that many other factors are changing during gestation and menstruation. The fact that the bulk of these studies used correlational designs precludes any causal interpretation.

To summarize, although some inconsistency exists in the literature, animal and human studies suggest the possibility that estrogens can modulate cell-mediated immunity, including suppression of NKCA.

Progestens

Progesterone may suppress NKCA in healthy women (Feinberg et al., 1991). Interestingly, an additive suppression of cytotoxicity was observed when this hormone was combined with estriol, estradiol and estrone (Feinberg et al., 1991). Progesterone and estradiol were demonstrated to downregulate the release of gamma interferon (IFN) -a key cytokine for optimal NKCA. It is important to note that at physiological concentrations both of these hormones also have the capacity to modify the proliferation of PHA-stimulated human lymphocytes (Herrera et al., 1992). On the other hand, among postmenopausal patients with rheumatoid arthritis and age-matched controls, progesterone does not appear to influence anti CD3 or PHA-stimulated lymphocyte proliferation (Van den Brink et al., 1992).

Prolactin

Both animal and human studies underline the stimulatory role of PRL on several immune functions. Administration of PRL induces IL-2 receptor expression, IL-2 production, proliferation of thymic and splenic lymphocytes, and lymphocyte growth in ovariectomized female rats and hypophysectomized rats of both sexes, respectively (Viselli et al., 1991; Berczi et al., 1991). Bromocriptine, a dopaminergic agonist and a suppressor of PRL, appears to suppress the immune system as evidenced by an elevation of anti-DNA antibodies and serum IgG levels (McMurray et al., 1991).

Further, recent animal studies suggest a relationship between PRL levels, tumor development and lymphocyte function. Daily administration of bromocriptine increased the latency period between injection of tumor cells and the development of a tumor, and decreased the incidence of tumor development in Hyperplastic Alveolar Nodule (HAN)-bearing mice (Tsai et al., 1992). In the same study responsiveness to mitogens and NKCA of HAN infiltrating lymphocytes was reduced by bromocriptine (Tsai et al., 1992). In a study with normal and tumor-bearing mice, glucocorticoids suppressed concanavalin-A (Con-A)-induced lymphocyte proliferation; PRL reversed the inhibitory effect of glucocorticoids in the normal mice, but failed to abrogate inhibition of the proliferative response in tumor-bearing hosts (Biswas & Chattopadhyay, 1992). Because the serum PRL level was similar in these normal and tumor-bearing mice, the altered responsiveness was not a function of circulating PRL. What this work suggests is that PRL may act differently upon glucocorticoid-induced immune effects during the cancer process.

Human studies have shown that PRL is necessary for the proliferation of T lymphocytes in response to IL-2 (Clevenger et al., 1991). Further, it has been demonstrated that PRL enhances recovery of the receptors for sheep red blood cells, thereby enhancing their capacity to form E-rosettes. This hormone also appears to stimulate the metabolic activity of peripheral blood neutrophils (Rovensky et al., 1991) Finally, women with chronically raised PRL levels display a normal T cell phenotype, but a significant reduction of leu-7 NK-cells and NKCA (Nicoletti et al., 1989). To summarize, a moderate level of PRL is essential for optimal immune functioning, but chronic elevated levels may be immunosuppressive. Additionally, the influence of PRL upon glucocorticoid-immune interactions may change with the development or progression of cancer. The biological significance of this three-way communication between PRL, corticosteroids and lymphocytes is unclear at present but may be a mechanism that is critical for the regulation of normal cell-mediated immune function during periods of chronic stress. Because the PRL effect appears dampened in tumor-bearing hosts, it may be these individuals who are particularly vulnerable to extended stress-induced and hormonally-mediated impairments in immune function. Extensive human studies are needed, however, before we are able to draw such a conclusion.

Beta-endorphin

Recently, much attention has been focussed on the immunologic effects of endogenous opioid peptides. Endogenous opioids exert a variety of CNS functions, as well as modulating some aspects of human lymphocyte functions (Kay et al., 1990) though the direction of this modulation is controversial in humans. For instance, the activity of beta-endorphin and methionine-enkephalin may result in either enhancement (Mathews et al.,

1983; Puente et al., 1992; Yeager et al., 1992) or suppression of NKCA (Kastin et al., 1991; Levy et al., 1991a; Chiapelli et al., 1991). Another research group did not obtain any effect of beta-endorphin on NKCA of human donors (Flores et al., 1990). Beyond methodologic variations, these differential effects can be partly explained by a dose-related effect on the direction of the modulation (Williamson et al., 1987). This pattern also holds for the differential effect of beta-endorphin on mitogen-induced proliferative responses. Beta-endorphin can have positive, negative or neutral effects on the mitogen-stimulated proliferation of lymphocytes, dependent on dosage (Heijnen et al., 1987; Kavelaars et al., 1990; Millar et al., 1990).

While differences in applied methodology and in dosage can explain some of the apparent contradictions just noted, the occurrence of human subjects with and without significant immune effects who are exposed to the same dose of beta-endorphin suggests that the problem is more complex than initially appreciated (Millar et al., 1990). For example, it has been shown that the ability of beta-endorphin to influence the function of T lymphocytes depends on the activation state of the T cell (Kavelaars et al., 1990); its major influence is observed in the early phases of the T cell response (Fattorossi et al., 1991). One study found that continuous presence of opioid peptides during culturing of T cells does not affect proliferation in adult male F344 rats (Van den Bergh et al., 1991). Finally, it has been revealed in a study with healthy laboratory personnel that opioid peptides (e.g., beta-endorphin), which are secreted together with ACTH, can abrogate cortisol-induced inhibition of proliferative responses (Millar et al., 1990).

CRH, ACTH and cortisol

Animal studies show that CRH can exert influences on immune functions such as NKCA (Irwin et al., 1989). CRH administered intracerebroventricularly in rats produced both a rapid, greater than 50% reduction in NKCA and a prolonged elevation in plasma corticosterone levels (Irwin et al., 1989). ACTH is known to induce a cortisol response in the adrenal gland which could subsequently influence several immune functions, e.g., suppression of NKCA, T lymphocyte proliferation and cytokine production (Van den Brink et al., 1992). Besides this indirect effect, ACTH is also able to modulate immune function directly. For instance, the proliferative activity of lymphocytes can be enhanced or decreased depending on the concentration of ACTH though this relationship may also be donor-dependent (Heijnen et al., 1987). ACTH has also been shown with in vitro paradigms to suppress antibody production, macrophage activation and to enhance B-cell proliferation (Bost et al., 1987; Johnson et al., 1984; Koff & Dunegan, 1985; Alvarez-Mon et al., 1985).

Catecholamines

The immunological decrements observed after sympathectomy have led to the notion that catecholamines are essential to immune function (Blalock et al., 1989). Results have shown that the influences of catecholamines on cellular immunity are bidirectional resulting in both increments (e.g., NK cells, monocytes and eosinophils and NKCA) and decrements (e.g., responsiveness of peripheral blood T lymphocytes) in immune measures (Gabriel et al., 1992; Felsner et al., 1992). In addition, recent work with healthy individuals indicates that NKCA rises and lymphocyte proliferation decreases in response to a beta-andrenergic behavioral challenge (Manuck et al., 1991; Naliboff et al., 1991). It may be that

sympathetic nervous system-mediated increases in catecholamine outflow and production from the adrenals is important in selectively mobilizing the immunologic response during periods of acute stress. The degree to which these associations hold up in breast cancer patients is unknown at present. Such information may be critical in understanding the role that stressors and stress-related hormonal changes play in immunosurveillance in this disease.

In summary, both mammary tumor-related (estrogens, progestens and PRL) and "stress-related" hormones (CRH, ACTH, beta-endorphin, cortisol and catecholamines) have differential influences on the immune response. In addition, sex steroids in general potentiate T and B cell function while estrogens tend to suppress NKCA. In most studies PRL has been shown to have a stimulatory effect on immune function. However, when chronically elevated, PRL can have a negative effect on NKCA. Cortisol also tends to exert immunosuppressive effects on cell-mediated immune functions, though these effects may be attenuated (or reversed) by PRL, a phenomenon that may vary with tumor development and progression. As mentioned before, the reproductive hormones are directly related to carcinogenesis and mammary tumor growth. The immune system, especially in early stage breast cancer when the host defense mechanisms are mostly intact, plays a potentially important role in the destruction of micrometastases and control over metastatic spread. It is well-known, however, that during cancer progression tumor cells acquire the ability to resist and escape immunosurveillance. Moreover, it is also known that psychosocial stressors and psychiatric conditions can modulate the function of both the endocrine and immune systems. Thus, besides host-related biological processes observed during tumor growth, it could be that psychosocial factors can contribute to tumor progression via endocrine dysregulation and/or immune suppression. This would appear to provide justification for PNI research in this disease while at the same time exemplifying the wide variety of methodologic issues that one faces in such lines of investigation.

Psychoneuroimmunology and breast cancer

Psychoneuroimmunologic studies with breast cancer patients

What do we know about the possible influence of psychosocial stressors and related phenomena upon the course of this disease? As mentioned previously, psychosocial stressors, social support and the way of responding to a diagnosis of breast cancer are associated with the course of the disease. On the other hand, research directed at examining stressor-induced immunological changes in breast cancer patients is very sparse (for review see Bovbjerg, 1990). Up to this point very few psychooncology research groups have integrated immunologic and endocrinologic variables in their study designs. The inclusion of such variables would aid in the understanding of the possible consequences of stressor-induced endocrine changes and other effects mediated by the CNS on targets that could be linked to cancer initiation or progression (Goodkin et al., 1993).

One study of stage I and II breast cancer patients examined the association between perceived social support and NKCA. Levy and her colleagues found that the patient's perception of emotional support from their spouse or intimate other, perceived social support from the patients' physician, and seeking social support as a coping strategy were related to greater NKCA (Levy et al., 1990). This suggests that emotional support may

buffer the influence of stressors on NKCA in this patient group (Berkman & Syme, 1979). However, NKCA was also associated with ER- negative status and with having an excisional biopsy as surgical treatment (Levy et al., 1990). This is rather puzzling since we know that patients with ER-negative tumors have a poorer prognosis. It has been suggested that the higher NK cytotoxicity reflected an attempt of the immune system to compensate for the greater threat posed by an aggressive tumor in order to control the spread of the disease. Importantly, higher NKCA in the follow-up period after the adjuvant treatment predicted a greater disease-free interval after the conclusion of the study. Elevated distress in the follow-up period predicted shorter time to recurrence for those showing disease progression (Levy et al., 1991b).

On the basis of these sparse data we cannot draw any firm conclusions that PNI mechanisms are linked to mammary tumor progression. We have to confine our-selves to the statement that in breast cancer patients several psychosocial factors are associated with immunologic measures, which, in turn, have been related to the progression of breast cancer. In healthy subjects numerous psychosocial stressors such as marital discord and bereavement, and psychological distress states such as depression, which occur in a large percentage of breast cancer patients, have effects on phenotypic and functional immune measures. Therefore, we now summarize the results of some PNI studies that may be useful for guiding future PNI research in breast carcinoma. These studies focus specifically on the immunologic correlates of psychosocial phenomena that reflect disruptions in social ties and affective disturbances such as depression.

Psychoneuroimmunologic studies in physically healthy donors

It has been shown that men and women who appraise their relationship with their spouse as low in quality or who are separated are more depressed and lonely and have decreased proliferative response to mitogens compared to age-matched controls (Kiecolt-Glaser et al., 1987; 1988). Bereavement as the extreme point on the continuum of separation has been associated with alterations in immune function by a number of investigators. In a prospective study Bartrop and colleagues (1977) documented a significant decrement in lymphocyte responsivity at six (but not two) weeks after death of a partner. This finding was replicated and extended to pokeweed mitogen responsivity by Schleifer and colleagues (1983) who found that proliferative responses returned to pre-bereavement baselines sometime between 4 and 14 months after the loss. This period parallels the time course of increased morbidity and mortality from a variety of illnesses in the year post-bereavement (Parkes & Weiss, 1983).

In both cross-sectional and longitudinal studies, Irwin and colleagues (1987a, 1987b) found that bereaved individuals displayed lower NKCA than non-bereaved matched controls. This alteration was related to depressed mood, and not to anticipation of stress or other stressful life events. This latter study suggests that the ways in which the individual deals with the stressful event or burden may play a role in determining immune status.

Psychoneuroimmunologic studies in populations with affective disorders and dysphoric mood states

The impact of psychological distress and psychiatric disorders on neurohormonal and immune function comprises a growing literature based upon studies conducted over the past

several decades. Since psychological distress and depression occur in a great percentage of breast cancer patients some highlights of this literature are noteworthy. In general, some cases of major depressive disorders are associated with HPA-hyperactivity resulting in increased secretion of ACTH and cortisol; higher levels of these substances may appear in plasma, cerebrospinal fluid and urine (Brambilla et al., 1986; Von Bardeleben & Holsboer, 1988; Kitamura et al., 1989: Rubin et al., 1989). Major depressive disorder-related changes in other pituitary-derived endocrines have also been reported. For example, it was found that plasma beta-endorphin and also beta-lipotropin were significantly higher in depressed men compared to healthy controls (Genazzani et al., 1986).

The role of PRL in depression is rather controversial. Some studies find abnormalities in plasma concentrations (Linkowski et al., 1989; Rubin, 1989), whereas others fail to find such changes (Kitamura et al., 1989). In contrast to LHPA axis abnormalities, hypothalamic-pituitary-gonadal (HPG) axis function in depressives is relatively normal (Rubin et al., 1989). The findings concerning relations between depression and functional immune measures, namely, lymphocyte proliferation and NKCA, are numerous (Stein et al., 1991; Weisse, 1992). On the basis of a recently conducted meta-analysis Herbert and Cohen (1993) conclude that clinical depression is associated with several alterations in cellular immunity including lowered proliferative responsivity of T lymphocytes and NKCA and these associations are greater in both older and hospitalized samples (Herbert & Cohen, 1993). In addition, there was evidence of a linear relation between intensity of depressive affect and indicators of cellular immunity (Herbert & Cohen, 1993). With respect to diurnal variation in NKCA, Petitto et al. (1992) also found that diurnal variations in NKCA are absent in major depressive disorder. Recently, Evans et al. (1992) found that depression-related alterations in NK cell count and functional capacity are gender-related. Men with major depression showed reductions in immune function while depressed women did not differ from normal control women. This of course, can have implications for PNI studies in breast cancer. However, what is required at this point is a meta-analysis of extant PNI studies of depression that evaluate the influence of gender upon NKCA assocations.

It is important to note that information about social support and coping styles is often lacking in the depression-PNI literature (Goodkin et al., 1993). In many of these studies, depressive moods and high levels of anxiety are assumed to reflect lack of social support and maladaptive coping behavior. As mentioned previously social support has been found to buffer the deleterious effects of life stressors on subsequent onset of physical illness in healthy individuals (Sarason et al., 1985). Besides an indirect effect of social support, for example, by providing the opportunity for active coping, social support may also show direct effects. For instance, epidemiological studies suggest that adequate social networks are associated with lower cancer incidence (Berkman & Syme, 1979). As noted previously, social support was also associated with longer survival time in breast cancer patients (Waxler-Morrison et al., 1991).

Baron et al. (1990) found that lower social support provisions were associated with decreased NKCA among spouses of cancer patients who had a terminal diagnosis. Adaptive coping has been suggested to be related to "fight or flight" preparation responses, which might result in secretion of SAM-related hormones (e.g., catecholamines) (McCabe & Schneiderman, 1985). As noted previously, elevations in these hormones may in turn, stimulate immune function, namely NKCA. Maladaptive coping patterns are associated

with passivity, helplessness, and withdrawal and are accompanied by activation of the HPA axis (McCabe & Schneiderman, 1985), which tends to suppress several aspects of immune function.

To summarize, a few studies have established a relationship between psychosocial factors and mammary tumor-relevant immune measures in breast cancer patients. More compelling evidence for PNI associations, though, comes from observations of stressor-related decrements in lymphocyte proliferation and NKCA among healthy subjects. However, the extent to which these associations generalize to breast cancer patients has not been put to the test.

Conclusions

Based on this review, the following conclusions can be drawn. First, host-related endocrine and immunological processes may play an important role in the carcinogenesis and progression of breast cancer. Second, the effects of several endocrines on the different components of the immune system have been shown to be related to the course of breast cancer. Third, PNI research has taught us that different psychosocial stressors influence both endocrine and immune function. Chronically stressed individuals display the same pattern of immunological changes, namely decrements in T lymphocyte function and NKCA, as are observed during breast cancer progression. Based on these observations, it can be hypothesized that psychosocial stressors can influence mammary tumor growth either directly by modulation of endocrine processes and (or) immunologic function, or indirectly via immunologic control of tumor growth. So far, only a handful of studies has examined the relations between psychosocial stressors on the one hand and immunological changes and tumor progression on the other. Overall, it can be concluded that the causal relationships between stressors, immune function and breast tumor progression still remains to be elucidated.

References

Aaltomaa S, Lipponen P, Eskelinen M, Kosma V, Marin S, Alhava E, Syrjanen K (1991) Hormone receptors as prognostic factors in female breast cancer. Ann Med 23:643-648

Ader R, Felten D, Cohen N (1991) Central neural circuits in neural-immune interactions. In Psychoneuroimmunology (R Ader, D Felten, N Cohen eds).New York:Academic Press

Alexander P (1976) The functions of the macrophage in malignant disease. Ann Rev Med 27:207-219

Alvarez-Mon A, Kehrl J, Fauci S (1985) A potential role for adrenocoricotropin in regulating human B lympocyte function. J Immunol 135:3823-3826

Antoni M (1987) Neuroendocrine influences in psychoimmunology and neoplasia: a review. Psych Health 1:3-24

Awwad M, North R (1990) Radiosensitive barrier to T cell-mediated adoptive immunotherapy of established tumors. Cancer Res 50:2228-2233

Bardeleben U von, Holsboer F (1988) Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. Prog Neuro Psychopharmacol Biol Psych 12:S165-S187

Baron R, Cutrona C, Hicklin D, Russell D, Lubaroff D (1990) Social support and immune function among spouses of cancer patients. J Pers Soc Psychol 59:344-352

Bartrop R, Luckhorst E, Lazarus L, Kiloch L, Penny R (1977) Depressed lymphocyte function after bereavement. Lancet 83:834-836

Berczi I, Nagy E, de Toledo S, Matusik R, Friesen H (1991) Pituitary hormones regulate c-myc and DNA synthesis in lymphoid tissue. J Immunol 146:2201-2206

Bergh P van den, Rozing J, Nagelkerken L (1991) Two opposing modes of action of beta-endorphin on lymphocyte function. Immunology 72:537-543

Berkman L, Syme S (1979) Social networks, host resistance, and mortality: a nine year follow-up study of Alameda County residents. Am J Epidemiology 2:186-204

Biswas R, Chattopadhyay U (1992) Altered prolactin response of the lymphocytes of tumor-bearing mice. Int J Cancer 50:93-98.

Blalock J (1989) A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Phys Rev 69:1

Bonilla F, Alvarez-Mon M, Merino F, Giron J, Menendez J, Espana P, Durantez A (1990) Natural killer activity in patients with breast cancer. Eur J Gynaecol Oncol 11: 103-109

Bost K, Smith E, Wear B, Blalock J (1987) Presence of ACTH and its receptor on a B lymphocytic cell line: a possible autocrine function for a neuroendocrine hormone. J Biol Regul Homeostatic Agents 1:23-27

Bovbjerg D (1990) Psychoneuroimmunology and cancer. In Handbook of Psychooncology (J Holland & J Rowland eds). New York:Oxford University Press

Brambilla F, Petraglia F, Facchinetti F, Genazzani A (1986) Abnormal beta-endorphin and beta-lipotropin responses to TRH and LRH administration in primary and secondary affective disorders. Acta Endocrinologica 112:481-486

Herberman R (1989) Influence of the host on tumor development. Dordrecht:Kluwer Academic Publishers

Herbert T, Cohen S (1993) Depression and immunity: a meta-analytic review. Psychol Bull 113:472-486

Herrera L, Montero R, Leon-Cazares J, Rojas E, Gonsebatt M (1992) Effects of progesterone and estradiol on the proliferation of phytohemagglutinin-stimulated human lymphocytes. Mutat Res 270:211-218

Heijnen C, Zijlstra J, Kavelaars A., Ballieux R (1987) Modulation of the immune response by POMC-derived peptides.I. Influence on proliferation of human lymphocytes. Brain Behav Immun 1:284-291

Hibbs J, Chapman H, Weinberg J (1978) The macrophage as an antineoplastic surveillance cell: Biological perspectives. J Reticuloendothel Soc 24:549-570

Holland J (1990) Behavioral and psychosocial risk factors in cancer: Human studies. In Handbook of Psychooncology (J Holland & J Rowland eds). New York:Oxford University Press

Hou J, Zheng W (1988) Effects of sex hormones on NK and ADCC activity in mice. Int J Immunopharmacol 10:15-22

Irwin M, Daniels M, Smith T, Bloom E, Weiner H (1987) Impaired natural killer cell activity during bereavement. Brain Behav Immun 1:98-104

Irwin M, Daniels M, Bloom E, Smith T, Weiner H (1987) Life events, depressive symptoms, and immune function. Am J Psych 144:437-441

Irwin M, Jones L, Britton K, Hauger R (1989) Central corticotropin realising factor reduces natural cytotoxicity. Time course of action. Neuropsychopharmacology 2: 281-184

Johnson H, Torres B, Smith E, Dion L, Blalock J (1984) Regulation of lymphokine production by corticotropin. J Immunol 132:246-250

Jordan V (1991) Animal models of hormone-dependent breast cancer. In Breast diseases (J Harris, S Hellman, I Henderson, D Kinne eds). Philadelphia:J.B. Lippincott Company

Kastin A, Seligson J, Strimas J, Chi D (1991) Failure of met-enkephalin to enhance natural killer cell activity. Immunobiology 183:55-68

Kavelaars A, Ballieux R, Heijnen C (1990) Secretion of beta-endorphin by lymphocytes after mitogenic or antigenic stimulation. Brain Behav Immun 4:171-179

Kay N, Morley J, Allen J (1990) Interaction between endogenous opioids and IL-2 on PHA-stimulated human lymphocytes. Immunology 70:485-491

Khansari D, Murgo A, Faith R (1990) Effects of stress on the immune system. Immunol Today 11:170-175

Kiecolt-Glaser J, Fisher L, Ogrocki P, Stout J, Speicher C, Glaser R (1987) Marital quality, marital disruption and immune function. Psychosom Med 49:13-34

Kiecolt-Glaser J, Kennedy S, Malhoff S, Fisher L, Speicher C, Glaser R (1988) Marital discord and immunity in males. Psychosom Med 50:213-229

Kindzel'skii L, Zlochevskaia L, Tsyganok T, Shabaeva M, Zhukova M, Rudaia O (1990) The count of the large granulecontaining lymphocytes of the peripheral blood and the natural killer activity in patients with malignant tumors. Vrach-Delo 8:31-32

Kitamura T, Kano S, Shima S, Asai M (1989) Hormonal changes in major depressive disorder. Keio J Med 38:40-52

Koenders P, Beex L, Langens R, Kloppenborg P, Smals A, Benraad Th (1991) Steroid hormone receptor activity of primary human breast cancer and pattern of first metastasis. Br Can Res Treat 18:27-32

Koff W, Dunegan M (1985) Modulation of macrophage-mediated tumoricidal activity by neuropeptides and neurohormones. J Immunol 135:350-354

Konjevic G, Spuzic I (1991) The effect of serum on NK cell activity. Glas Srp Akad Nauka Med 41:113-120

Krueger G (1989) Abnormal variation of the immune system as related to cancer. In Influence of the host on tumor development (R Herberman ed). Dordrecht:Kluwer Academic Publishers

Levy S, Herberman R, Whiteside T, Sanzo K, Lee J, Kirkwood J (1990) Perceived social support and tumor estrogen/progesterone receptor status as predictors of natural killer cell activity in breast cancer patients. Psychosom Med 52: 73-85

Levy S, Fernstrom J, Herberman R, Whiteside T, Lee J, Ward M., Massoudi M (1991) Persistently low natural killer cell activity and circulating levels of plasma beta-endorphin risk factors for infectious disease. Life Sci 48:107-116

Levy S, Herberman R, Lippman M, D'Angelo T, Lee J (1991) Immunological and psychosocial predictors of disease recurrence in patients with early-stage breast cancer. Behav Med:67-75

Linkowski P, Cauter EL van, Hermit-Baleriaux M, Kerkhofs M, Hubain P, Hermite M, Mendlewicz J (1989) The 24-hour profile of plasma prolactin in men with major endogenous depressive illness. Arch Gen Psychiatry 46:813-819

Ljunggren H, Kärre K (1990) In search of the 'missing self': MHC molecules and NK cell recognition. Immun Today 11:237-244

Manuck S, Cohen S, Rabin B (1991) Individual differences in cellular immune responses to stress. Psych Sci 2:111-115

Mathews P, Froelich C, Sibbit W, Bankhurst A (1983) Enhancement of natural cytotoxicity by B-endorphin. J Immunology 130:1658-1662

McCabe P, Schneiderman N (1985) Psychophysiologic reactions to stress. In Behavioral Medicine: The biopsychosocial approach (N Schneiderman & J Tapp eds). Hillsdale:Lawrence Erlbaum

McMurray R, Keisler D, Kanuckel K, Izui S, Walker S (1991) Prolactin influences auto-immune disease activity in the female B/W mouse. J Immunol 147:3780-3787

Melief C, Kast W (1990) Efficacy of cytotoxic T lymphocytes against virus-induced tumors. Cancer Cells 2:116-120

Melief C, Kast W (1991) Cytotoxic T lymphocyte therapy of cancer and tumor escape mechanisms. Cancer Biol 2:347-353

Melief C (1992) Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv Cancer Res 58:143-175

Millar D, Hough C, Denise L, Mazorow D, Gootenberg J (1990) Beta-endorphin's modulation of lymphocyte proliferation is dose, donor and time dependent. Brain Behav Immun 4:232-242

Mohanty I, Nayak M, Nanda B (1991) Cell mediated immune status in breast carcinoma. Indian J Pathol Microbiol 34:1-6

Mulder C, Pompe G van der, Spiegel D, Antoni M, Vries M de (1992) Do psychosocial factors influence the course of breast cancer? Psychooncology 1:55-167

Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom E, Makinodan T, Gilmore S (1991) Immunological change in young and old adults during brief laboratory stress. Psychosom Med 3:121-132

Nayar R, Fidler I (1989) Role of macrophages in recognition and destruction of metastatic cells In Fundamental aspects of cancer (R Goldfarb ed). Dordrecht:Kluwer Academic Publishers

Neifeld J (1989) The potential of hormone receptors in the treatment of various cancers. Oncology 3:57-62

Nicolleti T, Gerli R, Orlandi S, Migliorati G, Rambotti P, Riccardi C (1989) Defective natural killer cell activity in puerperal hyperprolactinemia. J Reprod Immunol 15:113-121

Ownby H, Roi L, Isenberg R, Brennan M (1983) Peripheral lymphocyte and eosinophil counts as indicators of prognosis in primary breast cancer. Cancer 52:126-130

Parkes C., Weiss R., (1983) Recovery from bereavement. New York:BasicBooks

Petitto J, Folds J, Ozer H, Quade D, Evens D (1992) Abnormal diurnal variation in circulating natural killer cell phenotypes and cytotoxic activity in major depression. Am J Psych 149:694-696

Pope R (1990) Immunoregulatory mechanisms present in the maternal circulation during pregnancy. Baillieres Clin Rheumatol 4:33-52

Puente J, Maturana P, Miranda D, Navarro C, Wolf M, Mosnaim A (1992) Enhancement of human killer cell activity by opioid peptides: similar response to methionine-enkephalin and beta-endorphin. Brain Behav Immun 6:32-39

Ravenswaay Claasen H van, Kluin P, Fleuren G (1992) Tumor infiltrating cells in human cancer on the possible role of CD16+ macrophages in antitumor cytotoxicity. Lab Invest 67:166-174

Rosen P, Groshen S, Saigo P, Kinne D, Hellmans (1989) Pathological prognostic factors in stage I and stage II breast carcinoma: a study of 644 patients with median follow-up of 18 years. J Clin Oncol 7:1239-1251

Rovensky J, Vigas M, Marek J, Blazickova S, Korcakova L, Vyletelkova L, Takac A (1991) Evidence for immunomodulatory properties of prolactin in selected in vitro and in vivo situations. Int J Immunopharmacol 13:267-272

Rubin R, Poland R, Lesser I (1989) Neuroendocrine aspects of primary endogenous depressionVIII.Pituitary-gonadal axis activity in male patients and matched control subjects. Psychoneuroendocrinology 14:217-229

Rubin R (1989) Pharmaendocrinology of major depression. Eur Arch Psychiatry Neurol Sci 238:259-267

Sarason I, Potter E, Sarason B, Antoni M (1985) Life events, social support and physical illness. Psychosom Med 47:156-163

Schleifer S, Keller S, Camerino M, Thorton J, Stein M (1983) Suppression of lymphocyte stimulation following bereavement. JAMA,250:374-377

Spuzic I, Konjevic G (1990) The significance of determination of NK cell activity in malignant diseases. Glas Srp Akad Nauka Med 39:153-160

Stein M, Miller A, Trestman R (1991) Depression, the immune system, and health and

illness. Arch Gen Psychiatry 48:171-177
Stewart H (1991) Density of lymphocyte infiltration of primary breast cancer does not affect short-term disease-free interval or survival. J R Coll Surg Edinb 36:142
Styrt B, Sugarman B (1991) Estrogens and infection. Rev Infect Dis 13:1139-1150
Sulke A, Jones D, Wood P (1985) Variation in natural killer activity in peripheral blood during the menstrual cycle. Br Med J Clin Res Ed 290:884-886
Sutherland D (1987) Hormones and cancer. In The basic science of oncology (I Tannock, R Hill eds). Toronto:Pergamon Press
Toniolo P, Pasternack B, Shore R, Sonnenschein E, Koenig K, Rosenberg C, Strax P, Strax S (1991) Endogenous hormones and breast cancer: a prospective cohort study. Breast Cancer Res Treat 18:s23-s26
Tsai S, Loeffler D, Heppner G (1992) Associated effects of bromocriptine on neoplastic progression of mouse mammary preneoplastic hyperplastic alveolar nodule line C4 and on hyperplastic alveolar nodule-infiltrating and splenic lymphocyte function. Cancer Res 52:2209-2215
Uksila J (1985) Human NK cell activity is not inhibited by pregnancy and cord serum factors and female steroid hormones in vitro. J Reprod Immunol 7:111-120
Viselli S, Stanek E, Mukherjee P, Hymer W, Mastro A (1991) Prolactin-induced mitogenesis of lymphocytes from ovariectomized rats. Endocrinology 129:983-990
Wahl S, McCartney-Francis N, Mergenhagen S (1989) Inflammatory and immunomodulatory roles of TGF-beta. Immunol Today 10:258-261
Weisse C (1992) Depression and immunocompetence: a review of the literature. Psychol Bull 111:475-489
Williamson S, Knight R, Lightman S, Hobbs J (1987) Differential effects of B-endorphin fragments on human natural killing. Brain Behav Immun 1:329-335
Wintzer H, Bohle W, Von Kleist S (1991) Study of the relationship between immunohistologically demonstrated lymphocytes infiltrating human breast carcinomas and patients' survival. J Cancer Res Clin Oncol 117:163-167
Yeager M, Yu C, Campbell A, Moschella M, Guyre P (1992) Effect of morphine and beta-endorphin on human Fc receptor-dependent and natural killer cell functions. Clin Immunol Immunopathol 62:336-343
Zheng Y, Zhou Z, Lyttle C, Teuscher C (1988) Immunohistochemical characterization of the estrogen-stimulated leukocyte influx in the immature rat uterus. J Leukoc Biol 44:27-32

Chapter 2

Immune and cardiovascular responsivity to a standardized laboratory challenge in breast cancer patients and healthy women

Gieta van der Pompe[1], Michael H. Antoni[2], A. Visser[1] & Cobi J. Heijnen[3]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Department of Psychology and Psychiatry, Miami

[3] University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', Utrecht

Abstract

The present study examined changes in sympathetic nervous system activation and immune responses to a standardized laboratory challenge (speech task) in 31 node-positive and metastatic breast cancer patients. As a control group 15 age-matched healthy women were included in the study. The results show that before task exposure breast cancer patients differ only from healthy controls in their basal heart rate. Additionally, we found differences in the baseline immunological values in breast cancer patients in comparison with age-matched healthy women. Progressive and clinically stage-related decrements were observed in some lymphocyte subsets and proliferation to T lymphocytes and natural killer cell activity (NKCA). The pattern of changes induced by the challenge with regard to phenotype and function of peripheral blood cells confirm earlier findings derived from healthy samples. However, in the present study we describe that there are differences in the pattern of distribution of CD4, CD8 and function of NK cells in response to the task between breast cancer patients with distant metastases and healthy controls. These findings are discussed from the standpoint of clinical relevance as well as psychoneuroimmunologic mechanisms associated with tumor growth.

Introduction

An increasing body of experimental data indicates that the host's natural and acquired immune defenses participate in the inhibition of tumor growth and the development of metastases (Herberman, 1984; Herberman, Wiltrout & Gorelik, 1987; Herberman, 1989; Gorelik & Herberman, 1989). An effective antitumor response is determined by (a) the extent that the tumor expresses surface antigens which can serve as a target for activated cytotoxic T lymphocytes, and (b) the sensitivity of the tumor cell to lysis by non-specific effector mechanisms such as natural killer (NK) cells and macrophages (Ljunggren & Kärre, 1990;Melief, 1992). In breast cancer patients several critical aspects of the host's acquired and natural immunity have been observed to be altered including decrements in the number and function of T lymphocytes and NK cells (Hacene, Desplaces, Brunet, Lidereau, Bourguignat & Oglobine, 1986; Contreras Ortiz & Stoliar, 1988;Burford-Mason, Gyte & Watkins, 1989; Spuzic & Konjevic, 1990; Kindzel'skii & Zlochevskaia, Tsyganok, Shabaeva, Zhukova & Rudaia, 1990;Konjevic & Spuzic, 1991; Mohanty, Nayak & Nanda, 1991). These decrements appeared to be progressive and related to the clinical stage.

Psychological stress has been observed to modulate several aspects of the individual's natural and acquired immune response (Van der Pompe, Antoni, Mulder, Heijnen, De Graeff, Garssen & De Vries, 1994). Moreover, it has been shown that sympathetic adrenomedullary (SAM)-related or hypothalamic pituitary adrenal (HPA)-related neuroendocrine substances modulate the activity of lymphoid cells (Van der Pompe et al., 1994). Because sympathetic arousal is an important component of the response to stress, the focus of the present study is on investigating changes in sympathetic nervous system activation (SNS) and immune responses to a ß-adrenergic challenge (speech task) in breast cancer patients at different disease stages. Recent studies with healthy individuals have shown that NK cell activity (NKCA) increases and lymphocyte proliferation responses decrease in response to tasks that are primarily ß-adrenergic behavioral challenges (Naliboff, Benton, Solomon, Morley, Fahey & Bloom, 1991;Bachen, Manuck, Marsland, Cohen, Malkoff, Muldoon & Rabin, 1992; Zakowski, McAllister, Deal & Baum, 1992; Herbert, Cohen, Marsland, Bachen, Rabin, Muldoon & Manuck, 1994). As these studies were conducted with healthy subjects the degree to which these changes hold up in breast cancer patients is unknown at present. Such information may be critical in understanding the role psychological stressors play in modulating immunosurveillance of this disease.

The present study was designed to investigate changes in cardiovascular and immune function at baseline and in response to the speech task in breast cancer patients and healthy women, and to compare the acute stress-induced cardiovascular and immune responses of node-positive breast cancer patients and metastatic breast cancer patients with those of healthy women. As a model for an acute stressor we used a standardized speech task, which has been shown previously to elicit reliably significant cardiovascular changes consistent with SNS activation (Saab, Llabre, Hurwitz, Frame, Reineke, Fins, McCalla, Cieply & Schneiderman, 1992).

Methods

Subjects

Breast cancer patients who had been treated with a curative intention (surgery) for primary breast cancer and were diagnosed with either positive axilliary lymph nodes ($T_{1-3}N_{1-3}M_0$) or supraclavicular lymph nodes, skin or distant metastases ($T_{1-4}N_{1-3}M_1$) were included in the study. Patients diagnosed with positive axillary lymph nodes were entered 4 months after surgery. This period has shown to be sufficient to overcome the detrimental effects of surgery on immune function (Van der Pompe, Antoni & Heijnen, in press). The patients with distant metastases were entered in the study after notification of the recurrence. All patients received tamoxifen, either as adjuvant treatment or as first-line endocrine treatment for distant metastatic disease. No other type of treatment was administered throughout the course of this study. Excluded were breast cancer patients with brain metastases or other malignancies.

Several hospitals in the western region of the Netherlands participated in this study. Eligible patients were invited by their clinicians. If a patient was motivated to participate she was scheduled for a session in which a more elaborate description of the study was provided including issues of confidentiality, right to refuse participation at any time without loss of optimal treatment, and the time commitments. After this procedure patients were asked to sign an informed consent form. Only patients having signed informed consent were included in the study. This procedure resulted in a cohort of 31 breast cancer patients aged 50 to 70 years (mean=58.8, SD=8.0). The group consisted of 23 curatively treated breast cancer patients with positive lymph nodes, but without distant metastases (mean age = 60.50, SD=6.1) and 8 patients diagnosed with breast cancer and distant metastases (mean age=54.7, SD=10.8).

We also recruited 15 age-matched healthy women (mean age=55.7; SD=4.3). These healthy subjects were recruited through a newspaper advertisement asking for volunteers for the study "Stress, Immunity and Health in Women aged 50 to 65". Eligibility for participation was determined by a standard telephone screening procedure by a medical doctor. Volunteers who reported chronic health problems (e.g., malignancy, diabetes) were excluded from the study.

Experimental manipulation

Patients were asked to fill out psychological questionnaires and were medically examined including blood pressure, height and weight. Subjects were scheduled for the reactivity session one week later and were asked to follow a low monoamine and caffeine-free diet the day before the session.

Reactivity session. Subjects were invited at the laboratory at 9 AM. Blood sampling was done with the Dakmed Ambulatory Withdrawal Pump (model ML 6-5S3R). A thromboresistent butterfly needle (model no. NT5-19) was inserted into the antecubital vein of the arm opposite to the surgical site to prevent oedema and connected it to the withdrawalpump via a sterile heparinized tubing set. The experiment started with a 30 min

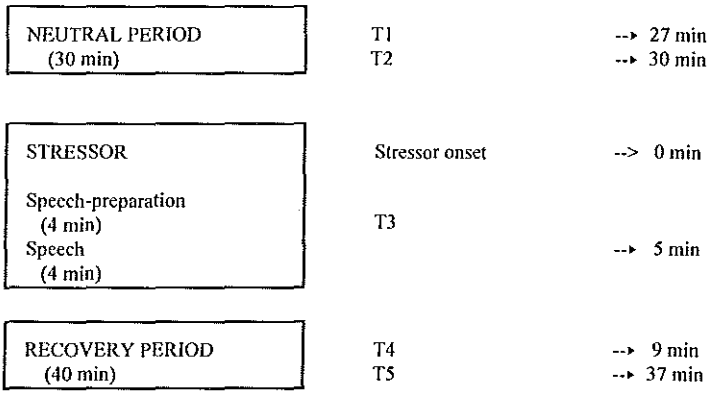

Figure 1. Schedule of the reactivity session.

pre-task rest period following venipuncture during which subjects watched non-stressfull movie. Subsequently, they were asked to prepare a story (for 4 min) about a threatening situation ("How would you react if you were accused of stealing a coat in a shop?") and then recount it aloud (for 4 min) while being videotaped. The experiment ended with a 32 min post task period during which subjects again watched a non-stressfull movie. This task was modified from well-standardized challenge previously shown to reliably elicit cardiovascular changes consistent with sympathetic activation (Saab et al., 1992). The schedule of the reactivity session is presented in Figure 1. A total of 5 blood samples for immune measures were collected during the pre-task baseline period (27 and 30 min post-venipuncture), task performance (5 min post-task onset) and the post-task period (9 and 37 min post-task onset).

Demographic and biobehavioral measures

At the start of the study demographic characteristics of the patients were assessed including age, partnership status, number of children, education, and employment status. In addition, a set of biobehavioral factors (i.e., caffeine and alcohol intake, cigarette smoking, hours of sleep, frequency of physical exercise, and the use of pain medication or tranquillizers) were assessed wich are known to modulate immune function.

Psychosocial measures

Affective state measures included (1) the Beck Depression Inventory (BDI) consisting of 13 items, (2) the State-Trait Anxiety Inventory (STAI), and the Profile of Mood States (POMS) consisting of five dimensions: depression, anger fatigue, vigor and tension. The total mood disturbance (TMD) score can be computed by adding the negative scales and substracting "vigor" (Beck, Ward, Mendelson, Moch & Erbaugh, 1961; Spielberger, 1980;McNair, 1971). All these scales have been translated and validated for the Dutch

population (Bouman, Luteijn, Abersnagel, Van der Ploeg, 1985;Van der Ploeg, Defares & Spielberger, 1980;Wald & Mellenberg, 1990).

Cardiovascular measures

Changes in heart rate, diastolic blood pressure and systolic blood pressure were measured during the experiment by Finapress (no. 4, Toegepast Natuurwetenschappelijk Onderzoek [TNO]). Heart rate (or "interbeat interval"), systolic and diastolic blood pressure were sampled at each heart beat during the neutral period, exposure to the task and post-task period. Data were recorded on magnetic tape for later analysis. Mean values per 30 seconds were determined for heart rate, and systolic and diastolic blood pressure. The last fourteen 30-second blocks of the rest period, all blocks recorded during the speech task and the first 2 blocks directly after the task (first post-task time point) and the last 8 blocks (second post-task time point).

Immune measures

Subsets of peripheral blood cells

Lymphocytes and granulocytes/ml were determined in whole blood using an automated closed tube sampler (Technikon H1 system). Lymphocyte subset analysis was performed by incubating 100 ul of whole heparinized blood with one of the following combinations of conjugated monoclonal antibodies (Becton and Dickinson, USA): CD4, CD8, CD3/HLA-DR, CD16/56 and CD19. Subsequently, red blood cells were lysed (lysing solution Becton and Dickinson) and washed with PBS containing 0.1% sodium-azide. Cells were analyzed using a Flow cytometer (FACSscan, Becton and Dickinson).

Natural Killer cell activity

Natural killer cell activity (NKCA) was determined in heparinized whole blood with some modifications to the original protocols (Ottenhof, Morales & Baines, 1981; Baron, Klimas, Fischl & Fletcher, 1985). K562 was used as a target cell line: cells were labelled with $Na^{51}CrO_4$ (3.7 MBq; Amaersham UK) for one hour at 37°C, 5% CO_2. After labelling cells were washed twice and resuspended in RPMI-1640 (Gibco USA). Undiluted blood (100ul) and serial dilutions in medium were dispensed into a 96 well round-bottom tissue culture plate. A fixed number of labelled K562 cells was added to each well (100ul; 10,000 cells per well). The plates were centrifuged for 5 min (100 x g), and incubated at 37$_o$C. After 4 hours, plates were centrifuged again and supernates (100 ul) of triplicate samples were counted in a gammacounter for 4 minutes Maximum ^{51}Cr release and spontaneous release were determined in wells containing 1% Triton-X100 or medium respectively. Specific ^{51}Cr release was calculated as follows: % release = (ER - SR)/(TR-SR) x 100, where ER = mean cpm experimental release, SR = mean cpm spontaneous release, TR = total release.

Proliferative responses

Lymphocyte proliferative responses were tested according to the method as described Bloemena, Roos, Van Heijst, Vossen & Schellekens (1989). Heparinized blood was diluted 10 x with RPMI-1640 supplemented with penicillin (100 IU per ml), streptomycin (100 ug/ml). L-glutamine (2mM). 100 ul of the diluted blood was incubated with 50 ul of Phytohaemagglutinin (PHA, Wellcome Diagnostics, UK) in various dilutions or with 50 ul of Pokeweed Mitogen (PWM, Gibco USA) in 96 well round-bottom plates. Proliferative responses were determined after 4 days of culture (5% CO_2, 37°C) by measuring incorporation of ^{3}H-thymidine (37 KBq, Amersham UK), added 16-18 hours before harvesting the cultures.

Statistical analyses

Correlations were calculated between the two baseline cardiovascular and immune measurements (T1 and T2). Since all cardiovascular and immune measures showed high correlations between the two sampling points ($r > .70$), the 2nd time point was used as a baseline value for data analysis.

In order to compare the baseline cardiovascular and immunological values of metastatic and node-positive breast cancer patients with those of healthy women, univariate analysis of variance (ANOVA) for each dependent variable were performed using Group as the between groups factor. Where the univariate F-test was significant, Tukey's HSD procedure for post-hoc comparisons was performed to determine which pairs of groups were significantly different ($p \leq .05$).

The effect of the speech task on cardiovascular and immunological variables at the different time points (baseline T2, speech T3 and two subsequent post-task time points T4 and T5) was tested by using multivariate analyses of variance (MANOVA) for repeated measures with Task as the within group factor and Group (i.e., metastatic and node-positive breast cancer, vs. healthy women) as the between-group factor, and the cardiovascular and immunologic values as the dependent variables. If a variable had a non-normal distribution an appropriate transformation was applied prior to conducting the analyses.

Differences between the groups with regard to demographic, biobehavioral and psychosocial variables were tested with univariate analyses of variance (ANOVA) for continuous data and the Kruskal-Wallis test for ordinal data.

Results

Demographic and biobehavioral variables

Except for the fact that node-positive breast cancer patients had a lower level of education (Kruskal-Wallis test, $p \leq .01$), metastatic, node-positive breast cancer patients and healthy women did not differ significantly on age, number of children, marital status or employment status.

A set of biobehavioral factors were assessed which are known to modulate immune function. Metastatic breast cancer patients consumed more alcohol (M=7.38 consumptions a wk) than node-positive breast cancer patients (M=1.18 consumptions a wk), $F(2,41)=3.61$, $p \leq .05$; node-positive breast cancer patients did not differ from healthy controls. The three groups showed no significant differences in either caffeine intake or cigarette smoking, hours of sleep, frequency of physical exercise, nor in their use of pain medication or tranquilizers.

Psychosocial variables

In our sample, the scores on the Beck Depression Inventory (BDI) of metastatic patients and controls were within the mild to moderate range (range=9-14) (see Table 1), while those of node-positive breast cancer patients were only within the mild range (Bouman et al., 1985). Likewise, the scores on the State-Trait Anxiety Inventory (STAI-DY) were within symptomatic ranges of psychiatric symptoms (M=40.8 and 45.8 for state and trait anxiety, respectively) for metastatic breast cancer patients and healthy women while those for node-positive breast cancer patients tended to be somewhat lower with values close to norms for women aged 51-60 years (M=37.0 and 38.7 for state and trait anxiety, respectively) (Van der Ploeg et al., 1980). The scores on the POMS-TMD for all three groups were actually higher than those observed in women with severe sleep disturbances (Wald & Mellenberg, 1990). There were no group differences, however, in POMS-TMD, STAI, or BDI scores.

Table 1: Means (and sd) for psychosocial variables among breast cancer patients with distant metastases, axillary lymphnode metastases and controls

PSYCHOSOCIAL VARS	Distant metastases		Postive lymph nodes		Controls		F
	Mean	SD	Mean	SD	Mean	SD	
Beck Depression Inventory							
Total score	10.8	4.4	7.3	6.7	10.9	5.8	1.61, p=.21, df=2,38
State-Anxiety Inventory							
State anxiety	45.4	13.8	38.5	12.2	41.5	12.6	.87, p=.42, df=2,38
Trait anxiety	41.8	8.6	39.8	11.6	45.1	11.5	.97, p=.38, df=2,38
Profile-of-Mood States							
Total mood disturbance (TMD)	49.6	12.1	46.8	18.2	47.9	19.8	.08, p=.92, df=2,43
Depression	13.2	3.4	12.2	6.2	12.0	5.4	.21, p=.81, df=2,43
Anger	11.0	5.1	10.0	4.2	11.3	5.6	.35, p=.71, df=2,43
Fatigue	11.0	4.0	12.4	7.0	12.9	7.2	.27, p=.76, df=2,43
Vigor	14.8	4.7	14.7	4.8	14.7	4.3	.00, p=.99, df=2,43
Tension	14.1	4.3	12.2	4.7	11.8	4.7	.71, p=.49, df=2,43

Baseline cardiovascular and immune system measures

The pre-stress heart rate was significantly different for metastatic, node-positive breast cancer patients and healthy women, $F(2,38)=3.83$, $p \leq .05$. Metastatic breast cancer patients had a higher basal heart rate than healthy women, $p \leq .05$. There were no significant differences in the baseline systolic and diastolic blood pressure values between the three groups. With respect to distribution of peripheral blood cells at baseline, there were significant group differences in percentages of CD3 and CD4 cells, $F(2,42) =6.00$, $p \leq .01$ and $F(2,42)=6.97$, $p \leq .01$, respectively). Metastatic breast cancer patients had lower percentages of CD3 cells than healthy women. Both metastatic and node-positive breast cancer patients had lower percentages of CD4 cells than healthy women, all p's $\leq .05$. Moreover, percentages of NK cells were significantly different for the three groups ($F[2,42]=3.49$, $p \leq .05$), with node-positive breast cancer patients having higher percentages of NK cells than metastatic breast cancer patients and healthy controls. There were no differences in percentages of total lymphocytes or in CD8 and CD19 percentages between breast cancer patients and healthy women.

With respect to the functional indices, there were group differences in proliferative response to high dose of PHA (200 µg/ml) ($F[2,44]=4.67$, $p \leq .05$) and to pokeweed (PWM) at both low dose (25 µg/ml) ($F(2,44)=7.87$, $p \leq .01$) and high dose (100 µg/ml), $F(2,44)=6.17$, $p \leq .01$. At a low dose of PHA (25 µg/ml) no group differences emerged. These results indicate that metastatic breast cancer patients had significantly lower PHA (200 µg/ml) and PWM (25 and 100 µg/ml) responses than age-matched healthy women. The three groups did not differ in NKCA.

Table 2: Mean (and SD) diastolic, systolic blood pressure and heart rate values during the reactivity session among healthy women (N=15), node-positive and metastatic breast cancer patients (N=31).

Cardio-vascular	Baseline T2		Speech T3		Recovery I T4		Recovery II T5		Task		Task x Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	p	F	p
Diastolic BP												
controls	71.0	7.4	84.7	12.3	77.2	10.7	68.1	5.5	27.48	.00[c]	.47	.83
node-positive	77.6	10.3	92.6	13.6	84.6	13.6	75.7	11.8				
metastatic	79.0	13.7	97.1	11.2	86.3	13.6	77.9	14.0				
Systolic BP												
controls	126.8	19.5	156.4	30.7	144.7	26.7	131.4	16.3	37.14	.00[c]	.95	.46
node-positive	125.4	19.8	162.2	31.7	143.1	34.5	131.2	18.6				
metastatic	140.9	21.2	192.3	34.0	166.7	42.5	146.9	28.8				
Heart Rate												
controls	62.1	8.1	77.2	13.3	72.2	11.2	65.2	8.0	40.62	.00[c]	.63	.70
node-positive	66.5	11.7	85.0	16.4	75.6	15.3	70.3	12.0				
metastatic	75.7	8.9	95.0	10.2	84.7	14.2	77.0	11.0				

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

Table 3: Mean (and sd) lymphocyte and mononuclear cell phenotype percentages during speech task and among healthy women (N=15), node-positive and metastatic breast cancer patients (N=31).

Immune	Baseline T2		Speech T3		Recovery I T4		Recovery II T5		Task	TaskxGroup
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F p	F p
Lymphocytes										
controls	30.1	8.5	32.3	8.7	34.5	8.2	30.8	8.4	10.69 .00[c]	2.52 .03[a]
node-positive	34.3	13.4	37.0	13.6	36.1	13.0	32.2	12.2		
metastatic	28.0	15.0	31.4	17.0	35.2	22.1	29.3	16.9		
CD4										
controls	45.4	11.0	40.9	10.5	39.7	11.0	47.9	8.5	31.96 .00[c]	2.53 .03[a]
node-positive	37.0	9.7	34.0	9.0	31.4	8.6	37.0	10.1		
metastatic	28.4	10.2	23.2	8.3	23.3	7.1	28.9	10.7		
CD8										
controls	24.1	9.4	27.6	9.8	29.0	10.4	22.7	7.5	22.96 .00[c]	2.17 .05[a]
node-positive	25.8	9.7	29.5	10.4	29.9	10.3	25.3	8.8		
metastatic	19.3	13.6	21.0	16.1	21.0	16.6	21.0	14.3		
CD3										
controls	65.1	6.9	59.8	8.1	60.6	8.1	65.9	5.6	11.98 .00[c]	.69 .66
node-positive	55.7	11.1	51.5	12.8	50.8	11.5	54.8	11.3		
metastatic	45.0	19.2	39.9	17.6	41.2	16.2	46.7	19.9		
CD16/56										
controls	7.9	3.8	13.6	6.5	15.2	7.1	7.5	3.2	31.36 .00[c]	.57 .75
node-positive	11.1	4.8	16.3	7.0	17.2	7.0	10.8	4.1		
metastatic	9.5	7.4	16.6	11.9	17.0	12.7	9.9	5.9		
CD19										
controls	12.8	4.7	10.8	4.5	10.2	4.2	12.8	4.3	23.85 .00[c]	1.09 .38
node-positive	14.1	8.6	12.6	8.2	11.4	7.8	14.2	8.0		
metastatic	17.0	15.7	12.8	12.6	12.8	11.5	14.9	14.1		

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

Cardiovascular and immune system changes to the speech task

In our group of breast cancer patients and healthy controls, systolic and diastolic blood pressure and heart rate changed significantly over time in response to the task, all p's $\leq .001$ (see Table 2). Specifically, significant task-induced increases were observed in diastolic blood pressure, systolic blood pressure and heart rate. There were no significant Task x Group interactions. Overall, diastolic and systolic blood pressure and heart rate reached their peak values during execution of the speech task (T3) and showed average changes of 20%, 29% and 26%, respectively relative to their baseline levels.

In our entire sample of breast cancer patients and healthy controls, all immune measures were changed significantly over time in response to the challenge (p's varied between .05 and .001) with the exception of proliferative responses to PWM (25 µg/ml) and PHA (12.5 and 200 µg/ml) (see Tables 3 and 4). Specifically, significant task-induced increases were observed in percentages of lymphocytes, CD8 cells, CD16/56 cells and NKCA, while decreases were seen in percentages of CD3, CD4 and CD19 cells and proliferative responses to PWM (100 µg/ml).

Table 4: Mean (and sd) natural killer cell activity (NKCA) and proliverative responses to PHA and PWM during speech task among healthy women (N=15), node-positive and metastatic breast cancer patients (N=31).

Immune	Baseline T2		Speech T3		Recovery I T4		Recovery II T5		Task		Task x Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	p	F	p
NKCA												
controls	40.6	17.2	56.0	16.7	54.7	14.6	37.9	12.9	34.66	.00[c]	3.22	.01[a]
node-pos.	36.5	12.8	47.7	13.4	50.3	13.0	42.2	13.5				
metastatic	26.5	9.1	39.7	17.5	44.2	17.2	27.4	8.8				
Prol. PWM 25 µg/ml												
controls	2496	1462	2275	2073	1950	1564	1979	1464	1.53	.22	.49	.81
node-pos.	1470	1320	1262	1219	1174	1076	1653	1994				
metastatic	210	370	244	384	143	133	253	276				
Prol. PWM 100 µg/ml												
controls	5354	2801	5172	3308	5279	3100	5809	3482	3.11	.04[a]	.44	.85
node-pos.	3552	2867	3703	3304	3359	2675	4438	3471				
metastatic	1268	1319	843	629	709	496	1720	1856				
Prol. PHA 12.5 µg/ml												
controls	16125	11515	15445	11774	13646	10384	21107	17248	2.58	.07	.59	.74
node-pos.	14705	13475	15768	13885	15017	13273	18094	14886				
metastatic	8751	6550	7872	5228	8242	4300	13185	5964				
Prol. PHA 200 µg/ml												
controls	30707	14413	29052	14275	29224	16033	30969	12697	.42	.74	1.11	.36
node-pos.	20034	12483	20758	10784	20440	12327	22415	13605				
metastatic	15676	8314	17324	8113	17641	6237	16083	7651				

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

Interestingly, there was a significant Task x Group interaction for percentages of lymphocytes ($p \leq .05$). This might indicate that the changes in the distribution of this cell type induced by the task were significantly different for metastatic and node-positive breast cancer patients, and healthy controls (Table 3). Figure 2 presents changes in distribution of percentages of lymphocytes from baseline levels in response to the task. As evident in this figure, metastatic breast cancer patients had more profound increases (12% and 26%) in percentages of lymphocytes during execution of the task (T3) and the first post-task time point (T4) than node-positive breast cancer patients and healthy controls showing increases of 8% and 9%, and 7% and 13%, respectively.

In addition, a significant Task x Group interaction emerged for percentages of CD4 cells, $p \leq .05$ (Table 3). As can be seen in Figure 3 metastatic breast cancer patients showed profound decreases of 18% during the task (T3) and the first post-task time point (T4), whereas CD4 cell percentages of node-positive breast cancer patients had decreased

by 8% and 15% and those of healthy controls by 10% and 13% at these time points. The interaction Task x Group was (marginally) significant for percentages of CD8 cells, $p \leq .05$. Contrary to the relatively large changes in percentages of lymphocytes and CD4 cells observed in metastatic breast cancer patients, the increases in percentages of CD8 cells during task exposure (T3) were only marginally (9%) and remained at this level at the following two post-task time points (T4 and T5) (Figure 4). Percentages of CD8 cells of both node-positive breast cancer patients and healthy women reached the highest level at 9 min post-task onset (T4) and showed increases of 19% and 16%, respectively. Finally, there was a significant Task by Group interaction for NKCA, $p \leq .01$ (Table 4). As evident in Figure 5 metastatic breast cancer patients reached their peak value at the first post-task time point (T4) and showed large increases from baseline of 67% as compared to 38% increase seen in both healthy women and node-positive breast cancer patients at that time point.

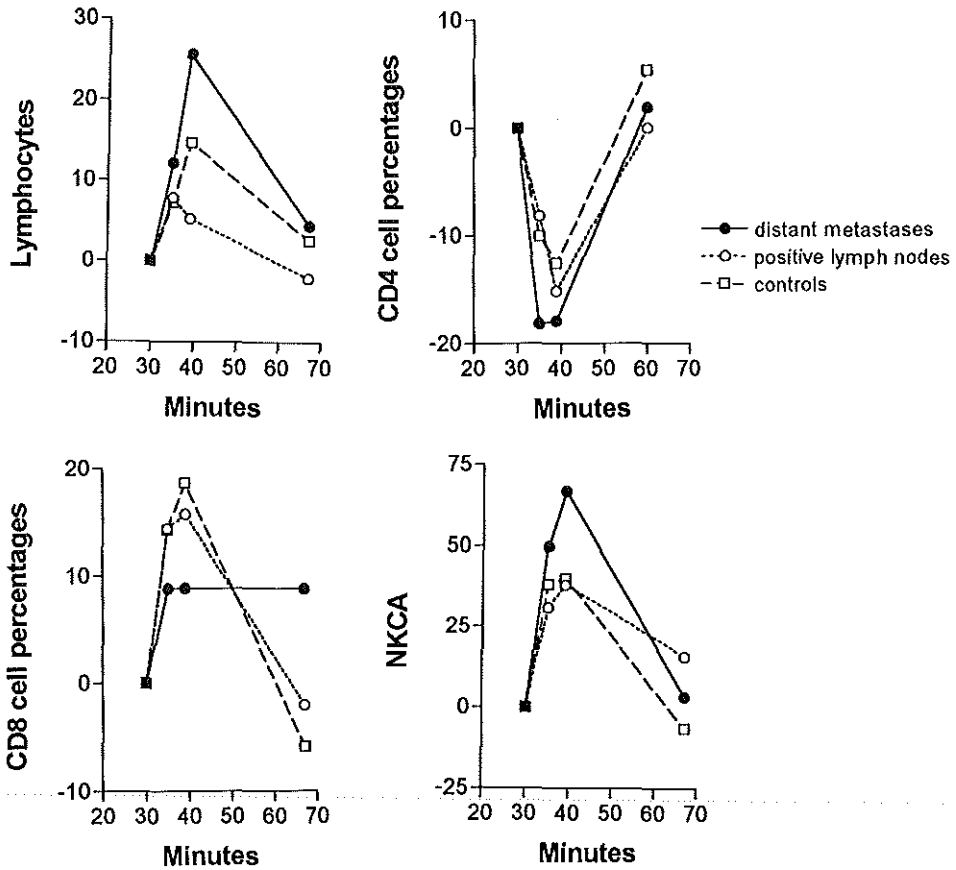

Figure 2-5. Changes in total lymphocyte count, CD4 cells, CD8 cells andNKCA in response to the speech task in metastatic and node-positive breast cancer patients, and age-matched healthy women. Values represent percentage of change from baseline in response to acute stress.

Healthy women, on the other hand, appear to reach their peak value during task exposure (T3) and also appear to show a faster return to baseline in comparison with both metastatic and node-positive breast cancer patients.

To summarize, the task-induced changes in cell subtypes and NKCA of metastatic breast cancer are in contrast with those observed in healthy controls. In contrast, node-positive breast cancer patients showed task-induced immune changes much more reflective of healthy controls.

Discussion

Baseline cardiovascular and immune function in breast cancer

The present study was designed to compare the baseline cardiovascular and immune values of breast cancer patients with those of healthy women. Metastatic breast cancer patients demonstrated, in addition to a marginally higher diastolic and systolic blood pressure, a significant higher heart rate than healthy women at baseline. The baseline heart rate values of node-positive breast cancer patients were also elevated, though not statistically significant. Several studies have shown that the psychological distress levels are precursors of elevated blood pressure and heart rate (Schnall, Landsbergis, Pieper, Schwartz, Dietz, Gerin, Schlussel, Warren & Pickering, 1992). Because the psychological distress levels of metastatic breast cancer patients were essentially similar to those of healthy women, it is unlikely that the psychological stress of being medically treated for metastatic disease accounted for the elevated heart rate levels. We consider it more likely that these differences are associated with the physiological stress of a chronic-progressive illness. Moreover, elevation of blood pressure is one of the frequently observed main side-effects of hormonal therapy (e.g., tamoxifen, megestrol acetate) in addition to weight gain and vaginal bleeding (Roberts, Bates, Bozzino, Brock, Clarke, Durrant, Evans & Tobias, 1990;Brufman, Isacson, Haim, Gez & Sulkes, 1990) and has therefore been considered as a potential risk factor for cardiovascular disease in post-menopausal women (Love, Wiebe, Newcomb, Cameron, Leventhal, Jordan & Feyzi, 1991).

In addition, we found alterations in the baseline immune values of breast cancer patients in comparison with healthy controls. Progressive and clinical stage-related decrements were observed in some lymphocyte subsets and functional measures. Metastatic breast cancer had smaller percentages of CD3 cells and CD4 and impaired proliferation of T lymphocytes to PHA (200 µg/ml) and PWM (25 and 100 µg/ml) than controls at baseline. In contrast, node-positive breast cancer patients showed values much more reflective of healthy women with the exception of percentages CD4 cells, which was significantly decreased. These findings are consistent with the breast cancer-related immune alterations documented by other research groups (Hacene et al., 1986;Contreras Ortiz et al., 1988;Burford-Mason et al., 1989;Spuzic et al., 1990;Kindzel'skii et al., 1990;Konjevic et al., 1991; Mohanty et al., 1991). These studies also reported a gradual decline in both enumerative and functional immune measures along different disease stages (Hacene et al., 1986;Burford-Mason et al., 1989;Spuzic et al., 1990;Kindzel'skii et al., 1990;Konjevic et

al., 1991;Mohanty et al., 1991) or in comparison with normal controls (Contreras Ortiz et al., 1988). Contrary to what might be expected on the basis of studies that reported immunological alterations in human breast cancer, percentages of NK cells appeared to be higher in node-positve and metastatic breast cancer patients as compared to healthy women, while NKCA of both groups of breast cancer patients was (not significantly) decreased compared to the healthy group. There exists some evidence that most human (spontaneous) tumors including breast carcinomas are susceptible to NK cell lysis in vivo (Herberman, 1984; Herberman et al., 1987;Herberman, 1989;Gorelik et al., 1989). Since decrements in NKCA are accompanied by elevated numbers of NK cells, it may be hypothesized that the lytic potential of these cells in the peripheral circulation is reduced in breast cancer.

Task-induced cardiovascular and immune changes

There were no statistically significantly differences in the task-induced cardiovascular changes between metastatic and node-positive breast cancer patients, and healthy women. However, metastatic breast cancer patients had (statistically non-signficant) higher blood pressure and heart rate than node-positive breast cancer patients and healthy women. This might indicate that the speech task had elicited equivalent degrees of activation in breast cancer patients and age-matched healthy women.

In our cohort of breast cancer patients and healthy women the task induced significant changes in various enumerative and functional measures. Specifically, reliable task-induced increases emerged in percentages of CD8 and NK cells as well as in NKCA, and decreases in percentages of CD3, CD4, and CD19 cells, and proliferation of peripheral blood leukocytes to PWM (100 µg/ml). This pattern of immunological changes in phenotype and function of peripheral blood cells confirm earlier findings derived from healthy samples (Naliboff et al., 1991;Bachen et al., 1992;Zakowski et al., 1992;Herbert et al., 1994;Ottaway & Husban, 1992).

It is an interesting phenomenon that, although, the task-induced changes in percentages of NK cells were similar to controls, metastatic breast cancer patients showed a more profound increase in NK cell function than controls. It could be that NK cells of metastatic breast cancer patients recruited from the spleen are already in an activated state and may therefore be more active on a cell per cell basis. Future research is needed to further clarify this phenomenon. In contrast, the task-induced changes in NK cell function of node-positive breast cancer patients were similar to those of healthy women.

Benschop, Nieuwenhuis, Tromp, Godaert, Ballieux, and Van Doornen (1994) have shown that the changes in phenotype and functional indices induced by acute psychological stressors are mediated by the sympathetic nervous system (SNS). These authors showed that the task-induced changes in the immune parameters are affected by catecholamines that trigger $ß_2$-adrenergic receptors on leukocytes. The pattern of changes in the present study induced by the task with regard to phenotype and function of peripheral blood cells of breast cancer patients and of healthy women confirm these findings. In the present study we also showed that acute stress leads to a increase of CD8 cells in peripheral blood cells of healthy women. However, in metastatic breast cancer patients we observed no change in percentages of CD8 cells in response to the task. Moreover, a profound decrease in CD4 cells as a result of the task was evident in peripheral blood of metastatic breast cancer

patients. These results are in contrast with responses of healthy individuals to acute psychological stressors. With respect to the results found on the CD8 cell distribution we suggest the possibility that $ß_2$-adrenergic receptor function in CD8 cells of metastatic breast cancer patients is decreased.

In addition, brief physiological increases in plasma levels of catecholamines have also been reported to decrease T cell proliferation to various mitogens (Bachen et al., 1992;Zakowski et al., 1992;Herbert et al., 1994). In metastatic breast cancer patients we observed an (non significant) increase in proliferation to PHA (200 µg/ml) in response to the task (Table 3). This increase in proliferation to PHA may also point to an impaired $ß_2$-adrenergic receptor function. Triggering of the T cells after acute stress may not result in an increase in cAMP and subsequent inhibition of proliferation which is normally observed in healthy individuals (Heijnen & Kavelaars, 1991).

In addition to detrimental influences of stress on the host's defense functions, it has been hypothesized that circulating catecholamines may participate in the process of growth and differentiation of the mammary gland through triggering of $ß_2$-adrenergic receptors (Draoui, Vandewalle, Hornez, Revillion & Lefebvre, 1991). Since carcinoma-derived mammary epithelial cells express $ß_2$-adrenergic receptors (Draoui et al., 1991), it may be likely that they are subject to direct receptor-mediated adrenergic modulation. However, the pathophysiological significance of $ß_2$-adrenergic receptors in carcinoma-derived mammary epithelial cells remains to be established. Brenner, Felten, Felten, and Moynihan (1992) found that chemical axotomy of mice prior to, but not after, injection of alveolar carcinoma cells caused an increase in the number of pulmonary metastases. The authors concluded that the state of the peripheral SNS at the time of, or immediately prior to, injection of tumor is critical for the increase in pulmonary metastases. Moreover, Kinnard, Chelmicka-Schorr, Checinski, Jones, and Arnason (1986) found that decreased adrenergic stimulation leads to increased in vitro proliferation of C-6 glioma cell line which expresses ß-adrenergic receptors. Thus, it may be likely that an increased activity of the sympathetic nervous system (i.e. higher heart rate and blood pressure levels at baseline as well as in response to the stressor) as observed in metastatic breast cancer patients in the present study not only desensitize $ß_2$-receptors on lymphoid cells, but also modulate those on tumor cells, which finally may contribute to the process of tumor growth.

Conclusions

We want to emphasize that due to small sample size the nature of this study is exploratory and does not allow us to draw firm conclusions. Nevertheless, the results seem to indicate that there are differences in the baseline and the task-induced immune responses between breast cancer patients and healthy women. The alterations in distribution and function of peripheral blood cells were most significant in patients with metastatic disease. The clinical relevance of these findings has yet to be established. In the future large scaled studies have to be initiated to examine the role of the acute stress-induced immune changes in disease progression. In this line of research it will be advisable to pay attention to the effect of catecholamines on $ß_2$-adrenergic receptor function in this disease.

References

Bachen EA, Manuck SB, Marsland AL, Cohen S, Malkoff SB, Muldoon MF, Rabin BS (1992) Lymphocyte subset and cellular immune responses to a brief experimental stressor. Psychosom Med 54:673-679

Baron GC, Klimas NG, Fischl MA, Fletcher MA (1985) Decreased natural cell-mediated cytotoxicity per effector cell in acquired immunodeficiency syndrome. Diagn mmunol 3:197-204

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561-571

Benschop RJ, Nieuwenhuis EES, Tromp EAM, Godaert GLR, Ballieux RE, Van Doornen LJP (1994) Effects of ß-adrenergic blockade on immunologic and cardiovascular changes induced by mental stress. Circulation 89:762-769

Berry J, Green B, Matheson D. (1987) Modulation of natural killer cell activity by tamoxifen in stage 1 post-menopausal breast cancer. Eur J Cancer Clin Oncol 23:517-520

Bloemena E, Roos, MTL, Van Heijst JLAM, Vossen JMJJ, Schellekens PTA (1989) Whole-blood lymphocyte cultures. J Immunol Methods 122:161-167

Bouman TK, Luteijn F, Abersnagel FA, Ploeg FAE van der (1985) Enige ervaringen met de Beck Depression Inventory (BDI). Gedrag 13:13-24

Brenner B, Margolese R (1991) The relationship of chemotherapeutic and endocrine intervention on natural killer cell activity in human breast cancer. Cancer 68:482-488

Brenner GJ, Felten SY, Felten DL, Moynihan JA (1992) Sympathetic nervous system modulation of tumor metastases and host defense mechanisms. J Neuroimmunol 37: 191-202

Brufman G, Isacson R, Haim N, Gez E, Sulkes A (1994) Megestrol acetate in advanced breast carcinoma after failure to tamoxifen and/or aminoglutethimide. Oncology 51: 258-261

Burford-Mason A, Gyte G, Watkins S (1989) Phytohaemagglutinin responsiveness of peripherial lymphocytes and survival in patients with primary breast cancer. Breast Cancer Res Treat 13:243-250

Contreras Ortiz O, Stoliar A (1988) Immunological changes in human breast cancer. Eur J Gyneacol Oncol 6:502-514

Draoui A, Vandewalle B, Hornez L, Revillion F, Lefebvre J (1991) Beta-adrenergic receptors in human breast cancer: identification, characterization and correlation with progesterone and estradiol receptors. Anticancer Res 11:677-680

Gorelik E, Herberman R (1989) Immunological control of tumor metastases. In: (R Goldfarb ed) *Fundamental aspects of cancer.* Dordrecht:Kluwer Academic Publishers

Hacene K, Desplaces A, Brunet M, Lidereau R, Bourguignat A, Oglobine J (1986) Competitive prognostic value of clinicopathology and bioimmunologic factors in primary breast cancer. Cancer 57:245-250

Herberman R (1984) Possible role of natural killer cells and other effector cells in immune surveillance against cancer. J Invest Dermatol 83 (Sppl.1):137s-140s

Herberman R, Wiltrout R, Gorelik E (1987) *Immune responses to metastases.* Florida: CRC Press, Boca Rotan,

Herberman R (1989) *Influence of the host on tumor development.* Dordrecht:Kluwer Academic Publishers

Herbert TB, Cohen S, Marsland AL, Bachen EA, Rabin BS, Muldoon MF, Manuck SB (1994) Cardiovascular reactivity and the course of immune response to an acute psychological stressor. Psychosom Med 56:337-344

Heijnen CJ, Kavelaars A (1991) The contribution of neuroendocrine substances to the immune response. Neth J Med 39:281-294

Kindzel'skii L, Zlochevskaia L, Tsyganok T, Shabaeva M, Zhukova M, Rudaia O (1990) The count of the large granulecontaining lymphocytes of the peripheral blood and the natural killer activity in patients with malignant tumors. Vrach Delo 8:31-32

Kinnard RL, Chelmicka-Schorr E, Checinski M E, Jones KH and Arnason BG (1986) Sympathetic nervous system and glioma growth. Eur J Cancer Clin Oncol 22: 501-503

Konjevic G and Spuzic I (1991) The effect of serum on NK cell activity. Glas Srp Akad Nauka Med 41:113-120

Little RJ (1988) Missing-data adjustments in large surveys. J Buss Econ Stat 6:287-295

Ljunggren H, Kärre K (1990) In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 11:237-244

Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feyzi J (1991) Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. Ann Intern Med 115:860-864

McNair DM, Lorr M, Droppleman LF (1971) *Profile of Mood States.* San Diego:Educational and industrial testing service.

Melief C (1992) Tumor eradication by adoptive transfer of cytotoxic T Lymphocytes. Adv Cancer Res 58:143-175

Mohanty I, Nayak M and Nanda B (1991) Cell mediated immune status in breast carcinoma. Indian J Pathol Microbiol 34:1-6

Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom E et al (1991) Immunological changes in young and old adults during brief laboratory stress. Psychosom Med 53:121-132

Ottaway CA, Husband AJ (1992) Central nervous system influences on lymphocyte migration. Brain Behav Immun 6:97-116

Ottenhof PC, Morales A, Baines MG (1981) Quantitation of a whole blood assay for human natural killer cell activity. J Immunol Methods 425:305-318

Ploeg HM van der, Defares FD, Spielberger CD (1980) *Handleiding bij Zelfbeoordelingsvragenlijst (ZBV).* Lisse: Swets & Zeitlinger, The Netherlands

Pompe G van der, Antoni MH, Mulder CL, Heijnen CJ, De Graeff A, Garssen B, De Vries MJ (1994) Psychoneuroimmunology and the course of breast cancer: an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psychooncology 3:271-288

Pompe G van der, Antoni MH, Heijnen CJ. The effects of surgical stress and psychological stress on the immune function of operative cancer patients. Psych Health, in press.

Roberts JT, Bates T, Bozzino JM, Brock JE, Clarke DG, Durrant KR, Evans RG, Tobias JS (1990) Treatment of carcinoma of the breast with high dose oral medroxy-proges-

terone acetate: does increased bioavailability improve the therapeutic ratio? Clin Oncol (R Coll Radiol) 2:324-327

Saab PG, Liabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, McCalla J, Cieply LK, Schneiderman N (1992) Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white americans. Psychophysiology 29:384-397

Schnall PL, Landsbergis PA, Pieper CF, Schwartz J, Dietz D, Gerin W, Schlussel Y, Warren K, Pickering TG (1992) The impact of anticipation of job loss on pschylogical distress and worksite blood pressure. Am J Ind Med 21:417-432

Spielberger CD (1980) *Test manual for the State-Trait Anxiety Inventory -Stai form Y.* Palo Alto:Consulting Psychologist Press

Spuzic I and Konjevic G (1990) The significance of determination of NKcell activity in malignant diseases. Glas Srp Akad Nauka Med 39:153-160

Tichatschek E, Zielinski C, Müller CH, Sevelda P, Kubista E, Czerwenka K, Spona J, Wolf H, Eibl M (1988) Long-term influence of adjuvant therapy on natural killer cell activity in breast cancer. Cancer Immunol Immunother 27:278-282

Wald FDM, Mellenberg GJ (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). Ned Tijdschr Psychol 45:80-99.

Zakowski SG, McAllister CG, Deal M, Baum A (1992) Stress, reactivity and immune function in healthy men. Health Psychol 11:223-232

Chapter 3

Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer

Gieta van der Pompe[1], Michael H. Antoni[2] & Cobi J. Heijnen[3]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Department of Psychology and Psychiatry, Miami

[3] University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', Utrecht

Abstract

Hormones of the hypothalamic-pituitary-adrenal system were studied in 31 patients with node-positive breast cancer and patients with metastatic breast cancer. Both groups received tamoxifen as first-line treatment. As a control group 15 age-matched healthy women participated in the study. The results showed that breast cancer patients had significant elevations in baseline cortisol levels compared to controls. Metastatic breast cancer patients had higher cortisol levels than node-positive breast cancer patients. No significant differences between breast cancer patients and controls were found in baseline plasma ACTH and prolactin levels. These data provide evidence that breast cancer is associated with a hyperactive adrenal gland, which may be due to the physiologic stress associated with the presence of (micro)metastases or tumor cells in the circulation, in combination with administration of tamoxifen.

In response to a behavioral challenge increases were observed in plasma ACTH and prolactin. Metastatic breast cancer patients had a faster prolactin response to acute stress than healthy women. However, metastatic breast cancer patients showed a blunted ACTH response compared to healthy women. Stress-induced ACTH responses and baseline cortisol levels were negatively correlated in the metastatic group only. Thus, the blunted ACTH response to the behavioral challenge might be related to hypercortisolemia suggesting that the pituitary corticotroph cell in metastatic cancer is appropriately restrained possibly by the negative feedback effects of chronic cortisol elevations. Interestingly, the behavioral challenge induced decreases in cortisol levels in all three groups. However, metastatic breast cancer patients had a faster cortisol decline compared to healthy women. We hypothesize that this is caused by increased metabolic clearance of cortisol due to increased utilization of metabolic substrates often observed in the presence of tumor.

Introduction

The onset and progression of human breast cancer is likely to be sensitive to numerous hormonal factors (Forsyth, 1992). In addition to estrogens and progestens, animal work suggests an important role for prolactin in the etiology and growth of mammary tumor (Moore et al., 1986). However, the evidence linking prolactin to the development of human breast cancer is inconclusive (Ingram et al., 1990). It has also been hypothesized that endocrine mediators secreted by organs of the hypothalamic-pituitary-adrenal (HPA) system may also participate in the process of growth and differentiation of human breast cancer (Labrie et al., 1990). Studies examining the *in vitro* action of glucocorticoids on growth of breast tumor cells have generated discrepancies, including descriptions of inhibitory and stimulatory effects (Labrie et al., 1990, 1992;Lopaz-Boada et al., 1994). The inter-relationship between human breast cancer and hormones is complex and inconclusive.

The HPA system is known to play a key role in the adaptation of the body to both physical and psychological stress. Evidence of increased secretion of cortisol is found in a number of studies on diseases including depression, anorexia nervosa, hypothalamic amenorrhea and multiple sclerosis (Biller et al., 1990;Gold et al., 1986, 1987;Hotta et al., 1986;Michelson et al., 1994). Despite this increasing body of evidence pointing to the importance of HPA function in systemic illnesses and its potential involvement in neoplastic disease, HPA system activity has not been well studied in breast cancer. Some evidence exists that women with breast cancer display elevated levels of plasma cortisol (Hays & O'Brian, 1989).

To examine the HPA system, we measured baseline plasma ACTH and cortisol levels in a group of patients with node-positive breast cancer and patients with metastatic breast cancer and in age-matched healthy women. We also measured plasma ACTH and cortisol responses to a behavioral challenge, which has previously been shown to induce significant changes in blood pressure and heart rate and changes in the distribution and function of peripheral blood cells in the same group of breast cancer patients and age-matched healthy women (Saab et al., 1992; Van der Pompe et al., unpublished data). Moreover, because prolactin seems to play an important role in the development of mammary carcinoma, baseline levels and responses to a behavioral challenge have also been evaluated.

Methods

Subjects

Subjects were breast cancer patients who were diagnosed with either positive axillary lymph nodes ($T_{1-3}N_{1-3}M_0$) or supraclavicular lymph nodes, skin or distant metastases ($T_{1-4}N_{1-3}M_1$) were included in the study. During the study period all patients had received first-line endocrine treatment (tamoxifen) only. The group consisted of 23 breast patients with axillary lymph node metastases, but without distant metastases (mean age=60.50, SD=6.11) and 8 patients diagnosed with breast cancer and distant metastases (mean age=54.67,

SD=10.76). We also recruited 15 age-matched healthy women (mean age=55.69; SD=4.27). These healthy subjects were recruited through a newspaper advertisement asking for volunteers for the study "Stress, Immunity and Health in Women aged 50 to 65". For details about recruitment see Chapter 2.

Experimental manipulation

After obtaining informed consent, a psychological assessment and medical assessment, including blood pressure, height and weight was conducted. In addition, a blood sample was taken for the hormonal baseline profile. Subjects were scheduled for the reactivity session one week later. Subjects were asked to follow a low monoamine and caffeine-free diet the day before the session.

Reactivity session. As a model for an acute stressor we used a standardized speech task, which has been shown previously to elicit reliably significant cardiovascular changes consistent with sympathethic nervous system (SNS) activation (Saab, Lliabre, Hurwitz, Frame, Reineke, Fins, McCalla, Cieply & Schneiderman, 1992). For details about the reactivity model see Chapter 2.

Endocrine measures

Plasma was obtained by centrifugation of EDTA blood (2000 *g*, 10 minutes at 4°C) and was frozen and stored at -20°C. ACTH was determined with a radioinmunoassay: antiserum was obtained from IgG Corporation USA, and ^{125}I-labeled ACTH from CIS Bioindustries, France. The intra- and interassay coefficients were <10% and 11%, respectively. Cortisol levels were determined using a fluorescence polarisation assay on a TDx analyzer (Abbott, USA). The intra- and interassay coefficients of variation were both 6%. Prolactin was measured with an immunoenzymetric system on an ES-600 analyzer (Boehringer Mannheim, Germany). The intra- and interassay coefficients were 4% and 6%, respectively.

Psychosocial measures

Affective state measures included (1) the Beck Depression Inventory consisting of 13 items, (2) the State-Trait Anxiety Inventory (STAI), and (3) the Profile of Mood States (POMS) consisting of five dimensions: depression, anger, fatigue, vigor and tension. The total mood disturbance (TMD) score can be computed by adding the negative scales and substracting 'vigor' (Beck et al., 1961;McNair et al., 1971;Spielberger, 1980). For details about the questionnaire see Chapter 2.

Statistical analysis

Correlations were calculated between the two baseline endocrine measurements (T1 and T2). Since all endocrine measures showed high correlations between baseline sampling points ($r > .70$), the 2nd time-point (T2) was used as the baseline value for data analysis.

In order to test for baseline differences between groups on endocrine parameters,

we performed univariate analysis of variance (ANOVA) for each dependent variable using Group (healthy, node-positive or metastatic breast cancer) as the between groups factor. Where the univariate F-test was significant, Tukey's HSD procedure for post-hoc comparisons was performed to determine which pairs of groups were significantly different ($p \leq .05$).

The effect of the speech task on dependent variables at the different time-points (baseline, speech and two subsequent recovery time-points) was tested by using multivariate analyses of variance (MANOVA) for repeated measures with Task as the within group factor, Group as the between-group factor, and endocrine measures as the dependent variables.

In addition, change scores were computed for each endocrine parameter by subtracting the baseline value (T2) from the measurement at T3, T4 and T5. We performed separate between-group univariate analyses using these change scores as dependent variables. Significant ANOVAs were followed up with Tukey tests to determine which group pairs were significantly different.

Differences between the groups with regard to demographic, biobehavioral and psychosocial variables were tested with univariate analyses of variance (ANOVA) for contious data and Kruskal-Wallis test for ordinal data.

Results

Demographic and biobehavioral variables

Except for the fact that node-positive breast cancer patients had a lower level of education (Kruskal-Wallis test, $p \leq .01$), metastatic, node-positive breast cancer patients and healthy controls did not differ significantly on age, number of children, marital status or employment status. Concerning the biobehavioral factors, metastatic breast cancer patients consumed more alcohol (M=7.38 consumptions a wk) than node-positive breast cancer patients (M=1.18 consumptions a wk), $F(2,41)=3.61, p \leq .05$ However, node-positive breast cancer patients did not differ from healthy controls. The three groups showed no significant differences on either caffeine intake or cigarette smoking, hours of sleep, frequency of physical exercise, nor on their use of pain medication or tranquilizers.

Psychosocial variables

There were no group differences, however, in POMS-TMD, STAI, or BDI scores. The psychological responses of breast cancer patients and healthy women are described in detail in Chapter 2.

Baseline endocrine measures

The pre-stress plasma cortisol values were significantly different for the three health status groups ($F(2,42)=19.72, p \leq .001$) (see Table 1, column "baseline"). Breast cancer patients with distant metastases had significantly higher baseline cortisol values than node-positive

breast cancer patients, and both groups of breast cancer patients showed higher cortisol levels than healthy women ($p \leq .05$). There were no significant group differences in either baseline plasma ACTH or prolactin levels.

Endocrine changes to the speech task

In the combined groups of breast cancer patients and healthy controls, ACTH, prolactin and cortisol levels changed significantly over time in response to behavioral challenge as shown by significant MANOVA'S (Table 1). Specifically, significant task-induced increases were observed in ACTH and prolactin, while decreases were observed in cortisol values. Importantly, there was a significant Task by Group interaction for cortisol and marginally significant interaction for prolactin indicating that the changes in these endocrine values in response to the task are significantly different for metastatic, node-positve breast cancer patients and healthy women (Table 1). The interaction Task by Group for ACTH was not statistically significant.

Figures 1, 2 and 3 present ACTH, cortisol and prolactin changes from baseline levels in response to the task. It can be seen (see Figure 1) that the ACTH values of node-positive breast cancer patients increased about 9% at 5 minutes (T3) and 9% at 9 minutes after task onset (T4), whereas healthy women showed elevations of 4% and 15% at similar time points. No appreciable change was demonstrated in the ACTH response of metastatic breast cancer patients (-2% and 1%).

Table 1: Means (and sd) adrenocorticotropin hormone (ACTH), cortisol and prolactinvalues during the reactivity session among metastatic breast cancer patients, node-positive breast cancer patients (N=31) and age-matched healthy controls (N=15).

Hormones	Baseline T2		Speech T3		RecoveryI T4		RecoveryII T5		Task		Task x Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	p	F	p
ACTH												
metastatic	32.7	5.3	31.9	3.9	33.1	4.8	31.9	4.8	3.58	.023[a]	1.47	.200
node-positive	37.4	10.6	40.7	12.1	40.8	12.4	37.0	11.9				
controls	30.7	7.7	31.8	10.0	35.3	14.0	29.3	6.6				
Cortisol												
metastatic	.70	.20	.64	.19	.66	.18	.54	.17	12.81	.000[c]	2.24	.048[a]
node-positive	.49	.17	.47	.16	.47	.16	.43	.21				
controls	.29	.08	.27	.06	.27	.06	.25	.07				
Prolactin												
metastatic	.24	.32	.25	.31	.23	.31	.12	.04	3.87	.017[a]	2.19	.053[a]
node-positive	.11	.03	.11	.03	.11	.03	.10	.03				
controls	.15	.10	.15	.10	.16	.11	.13	.08				

Reference values:
ACTH < 52 ng/l between 8-10 A.M
Prolactin < 0.70 IU/l
Cortisol < 0.61 µmol/l between 8-10 A.M.
[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

The change in ACTH was significantly different for the groups at 5 minutes after stressor onset (T3), $F(2,38)=3.81$, $p \leq .05$, with metastatic breast cancer patients showing significantly smaller change than node-positive breast cancer patients (Tukey test, $p \leq .05$).

Figure 2 illustrates the significant interaction Task by Group for cortisol values. As evident in this figure, the mean cortisol values of metastatic breast cancer patients showed a faster decrease (9%) at 5 minutes after task onset (T3) than those of node-positive breast cancer patients and healthy women (4% and 7% respectively). Moreover, at the first post-task time point (T4) metastatic breast cancer patients showed a larger increase (5%) than node-positive breast cancer patients (2%). Overall, metastatic breast cancer patients showed a larger decrease (23%) as compared to the node-positive breast cancer patients (12%) and healthy women (17%). Cortisol responses of node-positive breast cancer patients remained within the range of the healthy women with the exception of a slight increase (2%) at the first-task time-point (T4), whereas cortisol values of healthy women remained stable at this point. The change in plasma cortisol levels between baseline levels and subsequent reactivity time points was significantly different for the groups at the 37 minutes post-task time point (T5), $F(2,42)=3.87$, $p \leq .05$, with metastatic breast cancer patients showing significant larger declines than node-positive breast cancer patients and controls (Tukey tests, $p \leq .05$).

The significant interaction Task by Group for prolactin values is illustrated in Figure 3. In metastatic breast cancer patients plasma prolactin responses had already occurred at 5 minutes after task onset (T3) (increase of 8% over baseline), while in node-positive breast cancer patients and healthy women this response did not occur until 9 minutes after task onset (T4) (increases of 9% and 7%, respectively). Thus, metastatic breast cancer patients showed a faster change in prolactin levels than node-positive breast cancer patients and healthy controls. At the first post-task measurement (T4) prolactin values of metastatic breast cancer patients had already returned to baseline values and, surprisingly, showed an additional decrease of 50% at the final post-task time point (T5) when compared with the baseline levels. The prolactin levels of node-positive breast cancer patients and healthy women returned to baseline values at the last time-point (T5). Thus, metastatic breast cancer patients showed not only a faster response to the task but also a faster return which was considerably below their baseline values. Group comparisons of the mean change of plasma prolactin at different time points were not statistically significant.

To summarize, the task-induced changes in hormones of metastatic breast cancer patients differ from those observed in healthy controls. In contrast, node-positive breast cancer patients showed task-induced endocrine changes more reflective of healthy controls.

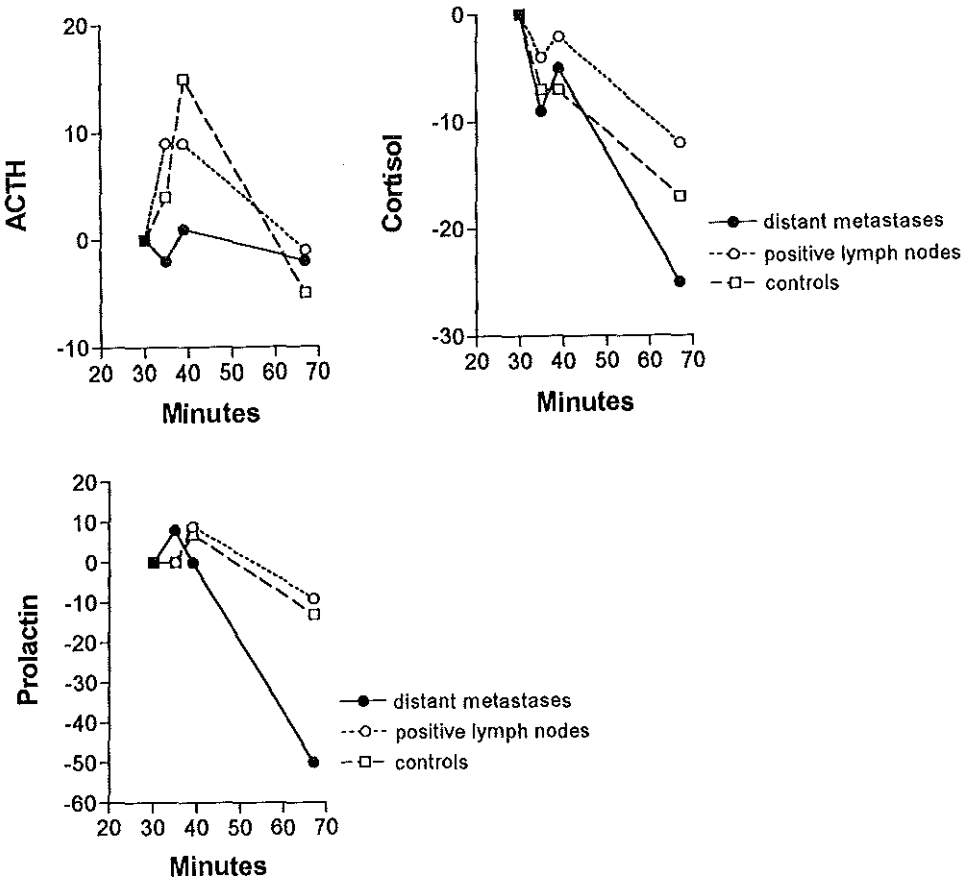

Figure 1-3. Plasma ACTH, cortisol and prolactin. Values represent percentage of change from baseline in response to acute stress in metastatic, node-positive ($n = 31$) and healthy women ($n = 15$).

Discussion

Baseline HPA system function in breast cancer

The current study was designed to explore the function of HPA system in breast cancer patients. Compared to controls, breast cancer patients showed significant elevations in resting blood cortisol levels, while their baseline ACTH values remained within the normal range. Elevations in baseline cortisol levels appeared to be related to clinical stage; the lowest levels were seen in node-positive breast cancer patients and the highest levels in metastatic breast cancer patients. This finding confirms earlier work showing

that an illness such as breast cancer is associated with increased levels of plasma cortisol (Hays & O'Brian, 1989). No significant differences were found in baseline prolactin between breast cancer patients as a group and controls, though metastatic breast cancer patients did reveal higher baseline prolactin levels than node-positive breast cancer patients and controls, both of which were within the normal range.

Several studies have shown that psychiatric disorders (e.g., major depression, anorexia nervosa) are associated with an increased secretion of ACTH and cortisol (Brambilla et al., 1986; Kitamura et al., 1989; Rubin, 1989;Von Bardeleben & Holsboer, 1988). Because the psychological distress levels of metastatic breast cancer patients were similar to those of healthy women, it is unlikely that psychological stress of an acute worsening illness accounted for elevated baseline cortisol levels. The mechanisms involved in activation of the HPA system in cancer still remain to be established. It could be that the presence of tumor cells in the circulatory system or a solid tumor may result in an acute and/or chronic activation of the immune system, which subsequently leads to the production of cytokines which may influence neuroendocrine mechanisms (e.g., the HPA system and the SNS).

The use of tamoxifen may also be a possible cause for the elevated plasma cortisol levels in breast cancer patients in this study. It has been shown that plasma concentrations of cortisol can be markedly increased in women who are pregnant or are using low-estrogen oral contraceptives (Gaspard et al., 1992). The effects of tamoxifen are analogous to those of endogenous estrogens and may therefore result in increased levels of estrogen, which in turn, may result in higher levels of cortisol. Tamoxifen use may have contributed to the increased baseline cortisol levels in patients with node-positive breast cancer and patients with metastatic breast cancer, but cannot explain the observation that metastatic breast cancer patients had significantly higher levels than their node-positive breast cancer counterparts.

To summarize, these data seem to indicate that breast cancer is associated with a hyperactive adrenal gland resulting in chronically increased plasma cortisol levels. These increased levels may be due to the stress related to this chronic-progressive illness in combination with the administration of tamoxifen.

Task-induced hormonal changes

It is an interesting phenomenon that in our cohort of breast cancer patients and healthy women the plasma cortisol levels decreased in response to the task. However, the cortisol response in metastatic breast cancer patients declined faster than in node-positive breast cancer patients and healthy women. So far, cortisol changes in response to various experimental stressors (e.g., arithmetic, cold pressor test) have generated discrepancies; both inhibitory and enhancing effects have been described (Berger et al., 1987; Pettingale et al., 1989). The results reported here may be attributable to several factors. First, it may be that the task was not 'stressfull' enough to elicit an HPA system response. However, a modest increase in plasma ACTH levels in response to stress was observed in the node-positive breast cancer patients and healthy women. More likely is that the HPA system was in a temporary state of low responsiveness following the effects of venipuncture

(Kirschbaum & Hellhammer, 1989; Pettingale et al., 1989). Time-lag between venipuncture and sampling have been found to vary between 10 and 25 minutes (Harris et al., 1988; Hubert & Nieschlag, 1988). Thus, it could be that the pre-stress period was not sufficient for cortisol levels to overcome the homeostatic dysregulation caused by insertion of the catheter. Thirdly, it may also be possible that the level of 'anticipatory' stress may have yielded a level of arousal (Kirschbaum & Hellhammer, 1989), that precluded us from observing additional reactivity of the HPA system to subsequent stimuli. These findings may shed some light on the diminished cortisol response in both breast cancer patients and healthy women, but it remains a point to be established why metastatic breast cancer patients demonstrated a faster cortisol decline to acute stress.

In healthy subjects, an insolvable puzzle was presented by a decreased cortisol response; in subjects reporting a high incidence of daily hassles these cortisol levels declined more rapidly than in those reporting fewer hassles (Benschop et al., 1994). Since it has also been shown that increased levels of daily stress are associated with elevated levels of both plasma and urinary measures of cortisol (Brantley et al., 1988), it could be that persistent increased levels of cortisol, coincide with a reversed reaction of the HPA system to experimental stress. Consistent with this assumption, we found that breast cancer patients and healthy women who displayed low BDI-depression scores were more likely to show increases in plasma cortisol in response to the speech task than those with high BDI-depression scores (For details about the correlations between BDI-depression scores in both breast cancer patients and healthy women and the change in plasma cortisol in response to speech task and between BDI-depression scores and the change in plasma cortisol in response to physical stress in healthy women see Appendix I). In rats with evidence of a hyperactive pituitary-adrenal system, the repeated exposure to stress resulted in decreased corticosterone levels (De Souza & Van Loon, 1982). The fact that the psychological distress levels of breast cancer patients as well as those of healthy women were in the symptomatic range might explain why the cortisol levels of all three groups decreased in response to acute stress. Interestingly, wild silver foxes also have decreased cortisol levels in reaction to acute stress in winter, while they showed increased levels in summer (Oskina & Tinnikov, 1992). Since glucocorticoids are known to regulate metabolic activity, the authors suggest that the more intensive metabolic processes in these animals in winter than in summer result in a maximum activity of the HPA system which, in turn, result in a reversed reaction to acute stress. In healthy subjects, the half-life of cortisol is approximately 73 minutes (Iranmanesh et al., 1990). It is known, however, that the presence of a tumor results in an increased utilization of metabolic substrates (Mulligan & Tisdale, 1991). Thus, the faster decline of cortisol observed in our metastatic breast cancer patients compared to healthy women, may possibly be caused by increased metabolic clearance of cortisol.

Clinical studies with oCRH and hCRH in depressed patients have demonstrated that a hypercortisolemic condition is associated with both normal and increased cortisol responses (Dored et al., 1990;Gold et al., 1987;Lesch et al., 1989;Rupprecht et al., 1989). However, a blunted cortisol and ACTH response after oCRH administration has been found in overtrained runners (Luger et al., 1987). In addition, compared to healthy subjects, patients with psychiatric disorders show a significantly blunted ACTH response

to oCRH (Dored et al., 1990;Gold et al., 1987;Lesch et al., 1989;Rupprecht et al., 1989). These data have been interpreted to suggest that the pituitary corticotroph in patients with these disorders is intact and appropriately restrained in its response to oCRH by the high plasma levels of cortisol (Gold et al., 1987). This hypothesis was underlined by a significant negative correlation between baseline cortisol levels and the ACTH response to CRH (Gold et al., 1987). In our sample, metastatic breast cancer patients also showed a blunted ACTH response to acute stress. We also found negative, but non-significant correlations between baseline cortisol levels and ACTH responses of our metastatic breast cancer patients at 5 and 9 minutes after task onset (r=-.43 and r=-.38 respectively), while these correlations were marginally positive in healthy women (r=.35 and r=.10). No correlations between either of these measures could be obtained in node-positive breast cancer patients. These findings suggest that, similar to what has been found in depressed patients, the pituitary corticotroph cell in metastatic breast cancer patients is appropriately restrained by the negative feedback effects of cortisol elevations, and also suggest that cancer patients may have a hypersecretion of CRH. These findings have to be interpreted with caution, because differences may occur in ACTH and cortisol responses to administration of CRH and to the speech task used in our design. The assumption of hypersecretion of CRH is underlined by the findings of Berger et al. (1987), who reported only a marginal positive correlation (r=.30) between cortisol responses to CRH and responses to different stress tests in healthy subjects (e.g., cold pressor test, arithmetic test, bicycle ergometric test).

To summarize, metastatic breast cancer patients had high baseline cortisol levels compared to controls in concert with altered ACTH and cortisol responses to acute stress when compared to healthy women. These responses were compatible with those found in overtrained subjects. Although the endocrine responses of metastatic breast cancer patients reported here share some common features displayed by depressed patients (hypercortisolism and blunted ACTH response), the decreased levels of cortisol post-stress suggest a different mechanism in this group.

Prolactin release is regulated at the hypothalamic-pituitary level. Among the various neuropeptides, thyrotropin-releasing hormone and vasoactive intestinal peptide are suggested to be stimuli for prolactin release (McLeod et al., 1985). Compared to healthy controls and node-positive breast cancer patients, metastatic breast cancer patients demonstrated in addition to marginally increased baseline levels a faster prolactin response to acute stress followed by a faster decline. Although a fully satisfactory explanation is lacking at present, the alterations in the prolactin responses may be associated with changes in dopaminergic inhibition at the level of the pituitary (Delahunt et al., 1987). The current findings may be important, because animal work suggests an important role for prolactin in the etiology and growth of the mammary tumor (Moore et al., 1986). It could be that abnormal prolactin responses to psychological stressors or distress may unfavorably influence disease progression.

There is evidence that the above mentioned endocrine mediators may also act as modulators of lymphoid cells (Van der Pompe et al., 1994). Cortisol is known to suppress immune function, including natural killer cell activity and T cell proliferation to phytohaemagglutinin and pokeweed mitogen (Van den Brink et al., 1992). A moderate

level of prolactin is essential for optimal immune functioning, but high levels have been shown to be immunosuppressive (Benton et al., 1991; Clevenger et al., 1991;Nicolleti et al., 1989). It is therefore possible that stress-induced or illness-related endocrine processes may not only act directly on mammary tumor cells but may also induce immune impairments possibly favoring tumor progression.

References

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561-571.

Benschop RJ, Brosschot JF, Godaert GLR, Smet MBM de, Geenen R, Olff M, Heijnen CJ, Ballieux RE (1994) Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in man. Am J Physiol 266:75-80.

Benton E, Bryant HU, Holaday JW (1991) Prolactin and immune function. In: R. Ader, DL. Felten, N Cohen, eds. Psychoneuroimmunology II. New York:Academic Press.

Berger M, Bossert S, Krieg JC, Dirlich G, Ettmeier W, Schreiber W, Zerssen D von (1987) Interindividual differences in the susceptibility of the cortisol system: an important factor for the degree of hypercortisolism in stress situations. Biol Psychiatry 22:1327-1339.

Biller BMK, Federoff HJ, Koenig JI, Klibanski A (1990) Abnormal cortisol secretion and responses to corticoptropin-releasing hormone in women with hypothalamic amenorrhea. J Clin Endocrinol Metab 70:311-317

Brambilla F, Petraglia F, Facchinetti F, Genazzani A (1986) Abnormal beta-endorphin and beta-lipotropin responses to TRH and LRH administration in primary and secondary affective disorders. Acta Endocrinol 112:481-486.

Brantley PJ, Dietz LS, McKnight GT, Jones GN, Tulley R (1988) Convergence between the daily stress inventory and endocrine measures of stress. J Consult Clin Psychol 56:549-551.

Clevenger C, Altmann S, Prystowsky M (1991) Requirement of nuclear prolactin for interleukin-2-stimulated proliferation of T lymphocytes. Science 253:77-79.

Delahunt JW, Mellsop G (1987) Hormone changes in stress. Stress Med3:123-134.

De Souza EB, Van Loon GR (1982) Stress-induced inhibition of the plasma corticosterone response to a subsequent stressor in rats: a nonadrenocorticotropin-mediated mechanism. Endocrinology 110:23-33.

Dored G, Stefansson S, d'Elia G, Kagedal B, Karlberg E, Ekman R (1990) Corticotropin, cortisol and beta-endorphin responses to the human corticotropin-releasing hormone during melancholia after unilateral electroconvulsive therapy. Acta Psychiatr Scand 82:204-209.

Forsyth IA (1992) The mammary gland. Baillière's Clin Endocrinol Metab 4:809-833.

Gaspard UJ, Romus MA, Gillain D, Duvivier J, Demey-Ponsart E, Franchimont P (1992) Plasma hormone levels in women receiving new oral contraceptives containing ethynil estradiol plus levonogestrel or desogestrel. Contraception 27:577-590.

Gold PW, Calabrese JR, Kling MA, Avgerinos P, Khan I,Gallucci WT, Tomai TP, Chrousos GP (1986) Abnormal ACTH and cortisol responses to ovine corticotropin releasing factor in patients with primary affective disorder. Prog Neuropsychopharmacol Biol Psychiatry 10:57-65.

Gold PW, Kling MA, Khan I, Calabrese JR, Kalogeras K, Post RM, Avgerinos PC, Loriaux DL, Chrousos GP (1987) Corticotropin releasing hormone: relevance to normal physiology and to the pathophysiology and differential diagnosis of hypercortisolism and adrenal insufficiency. Adv Biochem Psychopharmacol 43:183-200.

Harris B, Cook N, Warner N (1988) Anxiety and the dexamethasone suppression test monitored with saliva. Biol Psychiatry 23:698-704.

Hays JH, O'Brian JT (1989) Endocrine and metabolic function in patients with neoplastic disease. In: RB Herberman ed. Influence of the host on tumor development. Dordrecht: Kluwer Academic Publishers.

Hotta M, Shibasaki T, Masuda A (1986) The responses of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. J Clin Endocrinol Metab 62:319-324.

Hubert W, Nieschlag E (1988) Cortisolreaktionen im serum und speichel bei venenpunktionsstress: In: (Haecke R ed) Speicheldiagnostik. GIT-Verlag:Darmstadt

Ingram DM, Nottage EM, Roberts AN (1990) Prolactin and breast cancer risk. Med J Aust 153:469-4732.

Iranmanesh A, Lizarralde G, Johnson ML, Veldhuis JD (1990) Dymamics of 24-hour endogenous cortisol secretion and clearance in primary hypothyroidism assessed before and after partial thyroid hormone replacement. J Clin Endocrinol Metab 70:155-161

Kirschbaum C, Hellhammer DH (1989) Salivary cortisol in psychobiological research: an overview. Neuropsychobiology 22:150-169.

Kitamura T, Kanos S, Shima S, Asai M (1989) Hormonal changes in major depressive disorder. Keio J Med 38:40-52.

Labrie F, Poulin R, Simard J, Zhao HF, Labrie C, Dauvois S, Dumont M, Hatton AC, Poirier D, Mérand Y (1990) Interactions between estrogens, androgens, progestins, and glucocorticoids in ZR-75-1 human breast cancer cells. Ann NY Acad Sci 595:130-148.

Labrie F, Simard J, Launoit Y de, Poulin R, Thériault C, Dumont M, Dauvois S, Martel C, Li S (1992) Androgens and breast cancer. Cancer Detect Prev 16:31-38.

Lopaz-Boado YS, Diez-Itza I, Tolivia J, Lopiz-Otin C (1994) Glucocorticoids and androgens up-regulate the Zn-alpha$_2$ glucoprotein messenger RNA in human breast cancer cells. Breast Cancer Res Treat 3:247-258.

Lesch KP, Muller U, Rupprecht R, Kruse K, Schulte HM (1989) Endocrine responses to growth hormone-releasing hormone, thyrotropin-releasing hormone and corticotropin-releasing hormone in depression. Acta Psychiatr Scand 79:597-602.

Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW, Loriaux DL, Chrousos GP (1987) Acute hypothalamic-pituitary-adrenal responses to the stress of tread-mill exercise. Physiologic adaptations to physical training. N Engl J Med 316:1309-1315.

McLeod RM, Scapagnini U, Thorner MO (1985) Prolactin-Basic and clinical correlates. New York;Springer-Verlag.

McNair DM, Lorr M, Droppleman LF (1971) Profile of Mood States. San Diego:Educational and industrial testing service.

Michelson D, Stone L, Galliven E, Magiakou MA, Chrousos GP, Sternberg EM, Gold PW (1994) Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. J Clin Endocrinol Metab 79:848-853.

Moore JW, Thomas BS, Wang DY (1986) Endocrine status and the epidemiology and clinical course of breast cancer. Cancer Surv 3:537-559.

Mulligan HD, Tisdale MJ (1991) Metabolic substrate utilization by tumour and host tissues in cancer cachexia. Biochem J 277:321-326.

Nicolleti T, Gerli R, Orlandi S, Migliorati G, Rambotti P,Riccardi C (1989) Defective natural killer cell activity in puerperal hyperprolactinemia. J Reprod Immunol 15:113-121.

Oskina IN, Tinnikov AA (1992) Interaction between cortisol and cortisol-binding protein in silver foxes. Comp Biochem Physiol 101:665-668.

Pettingale KW, Watson M, Bhakri HL, Jones H, Tee DEH (1989) Changes in hormonal, immunological and autonomic measures during the performance of laboratory stress task. Stress Med 5:9-15.

Pompe G van der, Antoni MH, Mulder CL, Heijnen C, Goodkin K, De Graeff A, Garssen B, De Vries MJ (1994) Psychoneuroimmunology and the course of breast cancer: an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psychooncology 3:271-288.

Rubin R (1989) Pharmaendocrinology of major depression. Eur Arch Psychiatric Clin Neurosci 238:259-267.

Rupprecht R, Lesch KP, Muller U, Beck G, Beckmann H, Schulte HM (1989) Blunted adrenocorticotropin but normal beta-endorphin release after human corticotropin-releasing hormone administration in depression. J Clin Endocrinol Metab 69:600-603.

Saab PG, Liabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, McCalla J, Cieply LK, Schneiderman N (1992) Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white americans. Psychophysiology 29:384-397.

Spielberger CD (1980) Test manual for the State-Trait Anxiety Inventory -Stai form Y. Palo Alto;Consulting Psychologist Press.

Van den Brink H, Van Wijk M, Bijlsma J (1992) Influence of steroid hormones on proliferation of peripheral blood mononuclear cells in patients with rheumatoid arthritis. Br J Rheumatol 31:663-667.

Von Bardeleben U, Holsboer F (1988) Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. Prog Neuro Psychopharmacol Biol Psychiatry 12:S165-S187.

Chapter 4

The relations of plasma ACTH and cortisol levels with the distribution and function of peripheral blood cells in response to a behavioral challenge in breast cancer; an empirical exploration by means of statistical modeling

Gieta van der Pompe[1], Michael H. Antoni[2], Hugo J. Duivenvoorden[3], and Cobi J. Heijnen[4]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Deparment of Psychology and Psychiatry, Florida

[3] Erasmus University, Faculty of Medicine, Department Medical Psychology and Psychotherapy, Rotterdam

[4] University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', Utrecht

Abstract

The present study explores by means of statistical modeling the relations between ACTH and cortisol levels and distribution and function of peripheral blood cells in response to an acute stressor consisting of a standardized speech task in breast cancer patients with axillary lymphnode metastases and distant metastases. As a control group age-matched women participated in this study. The preliminary findings show that the effect of ACTH on immunoreactivity is related to the health of the donor. In node-positive breast cancer patients and healthy women, ACTH has a modest positive effect on T lymphocyte percentages and on PWM-induced proliferation at baseline and in response to the speech task. In contrast, in breast cancer patients with distant metastases ACTH has a negative effect on T lymphocytes percentages and function at baseline and in response to the stressor. Interestingly, neither ACTH nor cortisol levels were related to natural killer (NK) cell percentages and natural killer cell activity (NKCA). In addition, it appeared that cortisol had a positive effect on CD3 cell percentages when the health of the donor was taken into account. This effect was most distinct on CD3 cells measured at baseline. If replicated on a larger scale, these findings may indicate that the hypothalamic pituitary adrenal axis plays a role in the adaptation of the host defenses in reaction to acute stress, particularly those involving T lymphocytes. Moreover, these findings may suggest that the health of the donor may be an important effect modification factor in the relationships between neuroendocrines and immunoreactivity.

Introduction

An appropriate distribution of immune cells has been suggested to be crucial to the performance of the immune system against tumor cells. Until now, the numbers and percentages of peripheral leukocytes were considered as a reliable representation of the state of distribution of leukocytes in cancer patients. Results from our own group, as those of others, clearly show that progression of breast cancer coincide with significant decreases in peripheral leukocytes (Van der Pompe, Antoni, Heijnen, under review; Contreras Ortiz, Stoliar, 1988). Breast cancer with distant metastases had smaller percentages of T lymphocytes (CD3) and helpher/inducer T (CD4) cells as well as an impaired response of T cells to mitogenic stimulation, whereas the values of node-positive breast cancer patients were reflective of healthy women with the exception of CD4 cells, which were significantly decreased. More recently, several studies have used acute stress paradigms to examine the magnitude and specificity of immune cell changes in healthy volunteers. Specifically, reliable increases in circulating natural killer (NK) cells and cytotoxic/suppressor T (CD8) cells along with decreases in CD4 cells were reported (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, 1991; Bachen, Manuck, Marsland, Cohen, Malkoff, Muldoon, Rabin, 1992; Herbert, Cohen, Marsland, Bachen, Rabin, Muldoon, Manuck, 1994). We also documented changes in distribution and function of peripheral blood cells in response to a speech task in breast cancer patients with either axillary lymphnodes or distant metastases (see Chapter 2). It appeared that the changes in most leukocyte subpopulations in response to the speech task in breast cancer patients were essentially similar to those observed in age-matched healthy women. However, blunted CD8 responses along with significant larger decreases in CD4 cells in response to the task were observed among breast cancer patients with distant metastases. These findings suggest that distribution of some leukocyte subpopulations in response to mild acute stress is intact, although basal values are reduced.

It has been suggested that immune cell distribution as observed during behavioral challenge tests are preferentially sympathetically mediated (Benschop, Nieuwenhuis, Tromp, Godaert, Ballieux, & Van Doornen, 1994). In our study with breast cancer patients, the acute stress-induced changes in immune parameters coincided with significant increases in diastolic and systolic blood pressure and heart rate which can be considered as an indication of autonomic nervous system activation. Hormones related to the hypothalamic pituitary adrenal (HPA) system has also been suggested to be involved in immune cell distribution. Numerous studies using in vitro paradigms have documented the influence of adrenocorticotropin hormone (ACTH) and cortisol on number and function of peripheral blood leukocytes (Ader, Felten, & Nicholas, 1991; Heijnen & Kavelaars, 1991). Evidence for the involvement of ACTH and cortisol on immune cell distribution in response to acute stress has been derived from animal paradigms (reviewed in Ottaway & Husband, 1992). In humans, a few studies have described changes in plasma ACTH and cortisol values in response to mild acute stress (Berger, Bossert, Krieg, Dirlich, Ettmeier, Schreiber, Von Zerssen, 1987; Pettingale, Watson, Bhakri, Jones, Tee, 1989). In addition to the above described immunological changes, the findings of a recent conducted study show that progression of breast cancer is associated with abnormal HPA responses in rest and in response to the speech task (Van der Pompe, Antoni, Heijnen, 1996). It appeared that metastatic breast cancer patients had higher basal cortisol levels than healthy women in addition to a blunted ACTH response and a faster decline in plasma cortisol levels in

response to the task (see Chapter 3). Likewise, conditions of chronic psychological stress such as depressive disorders are also associated with HPA abnormalities including elevated levels of basal cortisol and blunted ACTH response to stimulation with corticotropin releasing hormone (CRH) (Gold, Calabrese, Kling, Khan, Gallucci, Tomai & Chrousos, 1986). Recently it has been emphasized that the relative contribution of endocrines to immunoreactivity may vary as a function of the duration of the depressive illness (Ravindran, Griffiths, Merali & Anisman, 1995). Among patients diagnosed with major depression, ACTH was negatively correlated with total lymphocytes, CD3 and B cells (CD19), whereas among dysthymics, ACTH was unrelated to any of the lymphocyte subsets. A selective inhibitory effect of ACTH and cortisol was also observed in patients with HIV-infection. ACTH and cortisol inhibited the function of NK cells from AIDS patients, whereas these stress hormones did not have an effect on lymphocytes of healthy subjects (Nair, Saravolatz & Schwartz, 1995). In view of these observations it is tempting to speculate that the health of the donor can be a significant source of variation for the association of ACTH and cortisol with immunoreactivity. We, therefore, propose to explore by means of statistical modeling whether there are differences between breast cancer patients diagnosed with axillary lymphnodes or distant metastases and healthy women with respect to the influence of ACTH and cortisol on peripheral blood cells. In order to determine whether ACTH and cortisol sensitivity of immune cells is a dynamic phenomenon, we will also evaluate the possible role of ACTH and cortisol on distribution and function of immune cells to mild acute stress.

Methods

Subjects

Breast cancer patients who were diagnosed with either positive axillary lymph nodes or supraclavicular lymph nodes, skin or distant metastases were included. All patients received first-line endocrine treatment only (tamoxifen). No other type of treatment was administered throughout the course of this study. The group consisted of 23 curatively treated breast cancer patients with positive lymph nodes, but without distant metastases (mean age=60.5, SD=6.1) and 8 patients diagnosed with breast cancer and distant metastases (mean age=54.7, SD=10.8). We also recruited 15 age-matched healthy women (mean age=55.7; SD=4.3). These healthy subjects were recruited through a newspaper advertisement asking for volunteers for a study entitled "Stress, Immunity and Health in Women aged 50 to 65". For details about recruitment see Chapter 2.

Experimental design

After obtaining informed consent, psychological assessment and medical examination, including blood pressure, height and weight, was conducted. In addition, a blood sample was taken for an immunologic baseline profile. Subjects were scheduled for the reactivity session one week later.

Reactivity session. As a model for an acute stressor we used a standardized speech task, which has been shown previously to elicit reliably significant cardiovascular changes consistent with sympathethic nervous system (SNS) activation (Saab, Liabre, Hurwitz,

Frame, Reineke, Fins, McCalla, Cieply, & Schneiderman, 1992). For details about the reactivity model see Chapter 2.

Endocrine measures

Plasma was obtained by centrifugation of EDTA blood (2000 *g*, 10 min at 4°C) and was frozen and stored at -20°C. ACTH was determined with a radioimmunoassay: antiserum was obtained from IgG Corporation USA, and ^{125}I-labeled ACTH from CIS Bioindustries, France. The intra- and interassay coefficients were <10% and 11%, respectively. Prolactin was measured with an immunoenzymetric system on an ES-600 analyzer (Boehringer Mannheim, Germany). The intra- and interassay coefficients were 4% and 6%, respectively. Cortisol levels were determined using a fluorescence polarisation assay on a TDx analyzer (Abbott, USA). The intra- and interassay coefficients of variation were both 6%.

Immune measures

Immune assays included determination of percentages of specific lymphocyte subsets, lymphocyte proliferation to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) and natural killer cell activity (NKCA). These immune measures are described in detail in Chapter 2.

Psychosocial measures

Affective state measures included (1) the Beck Depression Inventory (BDI) consisting of 13 items, (2) the State-Trait Anxiety Inventory (STAI), and (3) the Profile of Mood States (POMS) (Beck, Ward, Mendelson, Mock, Erbaugh, 1961; McNair, Lorr, Droppleman, 1971; Spielberger, 1980). For details about the questionnaire see Chapter 2.

Statistical analysis

Predictive mean matching was used for estimating missing values (< 5%) (Little, 1988). Correlations were calculated between the two baseline endocrine and immune measurements (T1 and T2). The 2nd time-point (T2) was used as the baseline value for data analysis.

Multiple regression was used to explore whether the effects of the endocrine measures on immune measures vary as a function of health-status (i.e., metastatic breast cancer, node-positive breast cancer, healthy). For any variable with a non-normal distribution an appropriate transformation was applied prior to conducting the analyses. With the exception of T lymphocytes (CD3 cells), to all endocrine and immune measures the square root transformation was applied. To index the three different categories of subjects (i.e. metastatic breast cancer, node-positive breast cancer, healthy women) two dummy variables were constructed (Cohen & Cohen, 1983). The two dummy variables were X_1 (coded 2 if the subject has been diagnosed with node-positive breast cancer and coded 1 otherwise) and X_2 (coded 2 if the subject has been diagnosed with metastatic breast cancer and coded 1 otherwise). Two interaction terms were constructed consisting of X_1 multiplied by ACTH or cortisol and X_2 multiplied by ACTH or cortisol, respectively.

Table 1: Prediction of lymphocyte and mononuclear cell phenotype percentages and proliferation to PWM and NKCA by ACTH and by the health of the donor (healthy women, breast cancer patients with axillary lymph node metastases and breast cancer patients with distant metastases).

Dependent variables	Independent variables	Basal levels T2		Speech T3		Recovery 1 T4		Recovery 2 T5	
		ß[1]	p	ß[1]	p	ß[1]	p	ß[1]	p
lymphocytes	ACTH[2]	**.35**	**.03**	**.36**	**.02**	.30	.06	**.35**	**.03**
	ACTH[3]	**.54**	**.03**	**.56**	**.04**	.40	.14	**.63**	**.02**
	X_1	-.11	.57	-.13	.51	-.12	.52	-.25	.19
	ACTH x X_2	-.25	.29	-.22	.35	-.09	.74	-.31	.21
CD3 cells	ACTH[2]	.10	.55	.22	.19	.10	.53	-.00	.99
	ACTH[3]	**.65**	**.01**	**.78**	**.00**	**.67**	**.01**	**.58**	**.02**
	X_1	**-.39**	**.03**	**-.46**	**.02**	**-.41**	**.03**	**-.50**	**.01**
	ACTH x X_2	**-.68**	**.00**	**-.57**	**.02**	**-.65**	**.01**	**-.63**	**.01**
CD4 cells	ACTH[2]	-.04	.82	.00	.98	.06	.70	-.01	.96
	ACTH[3]	.44	.07	**.59**	**.02**	**.64**	**.01**	**.58**	**.02**
	X_1	**-.31**	**.03**	-.31	.10	**-.36**	**.04**	**-.44**	**.02**
	ACTH x X_2	**-.68**	**.00**	**-.71**	**.00**	**-.68**	**.01**	**-.68**	**.01**
NK cells	ACTH[2]	-.14	.40	-.12	.46	-.00	.98	.08	.62
	ACTH[3]	-.40	.08	-.36	.18	-.07	.80	-.42	.09
	X_1	**.54**	**.00**	**.39**	**.05**	.23	.25	**.54**	**.01**
	ACTH x X_2	.14	.53	.13	.59	-.00	.99	.25	.29
Prol. PWM (100 µg/ml)	ACTH[2]	.22	.17	.13	.42	.04	.83	.16	.33
	ACTH[3]	**.74**	**.00**	**.75**	**.00**	**.68**	**.01**	**.72**	**.00**
	X_1	**-.42**	**.02**	**-.38**	**.04**	**-.38**	**.04**	-.35	.07
	ACTH x X_2	**-.79**	**.00**	**-.80**	**.00**	**-.87**	**.00**	**-.73**	**.00**
NKCA	ACTH[2]	-.05	.77	.02	.92	-.09	.57	.19	.25
	ACTH[3]	.06	.82	.16	.55	.13	.59	**.50**	**.04**
	X_1	-.04	.83	-.10	.64	-.25	.20	-.14	.49
	ACTH x X_2	-.16	.49	-.17	.48	-.25	.31	-.44	.06

X_1 is coded 2 for node-positive breast cancer patients and coded 1 for metastastic breast cancer patients and healthy women

X_2 is coded 2 for metastatic breast cancer patients and coded 1 for node-positive breast cancer patients and healthy women

[1] Standardized regression coefficient (statistically significant ß's are bold-face printed)

[2] The main term ACTH is entered only in the regression equation

[3] The main term ACTH and X_1 and the interaction term ACTH by X_2 are entered simultaneously in the regression equation

In accordance with the explorative nature of this study, the all possible regressions strategy was utilized, which means that all possible regressions of *Y* on the *X's* are tested (Jöreskog & Sörbon, 1977; Draper & Smith, 1981; Jöreskog, 1993). Consequently, in any regression equation each independent variable does or does not appear resulting in 2^p equations for p independent variables (i.e. one of the hormones ACTH *or* cortisol, the two dummy-coded variables X_1 and X_2, and the interactions ACTH by X_1 *or* cortisol by X_1, and ACTH by X_2 *or* cortisol by X_2). For the sake of comparability and interpretation, the standardized regression coefficient (ß) was used as a measure of relative importance. The statistical level of

significance was fixed at 0.05. Moreover, the variance inflation factor (VIF)-values (an index of multicollinearity) had to be smaller than 4 for a model to be retained (Glantz & Slinker, 1990). Thus, a model was considered acceptable on the conditions that *all* terms in the regression equation were statistically significant and the VIF-values were within acceptable limits.

Differences between the groups with regard to demographic, biobehavioral and psychosocial variables were tested with univariate analyses of variance (ANOVA) for continuous data and Kruskal-Wallis test for ordinal data.

Results

Demographic and biobehavioral variables

Except for the fact that node-positive breast cancer patients had a lower level of education (Kruskal-Wallis test, $p < .01$), metastatic, node-positive breast cancer patients and healthy controls did not differ significantly on age, number of children, marital status or employment status. In addition, the three groups showed no significant differences on either caffeine intake or cigarette smoking, hours of sleep, frequency of physical exercise, or on their use of pain medication or tranquilizers, although breast cancer patients with distant metastases consumed more alcohol (M=7.38 consumptions a wk) than node-positive breast cancer patients (M=1.18 consumptions a wk), $F(2,41)=3.61$, $p < .05$. Node-positive breast cancer patients did not differ from healthy controls.

Psychosocial variables

There were no group differences in BDI, STAI-DY, or POMS-TMD scores. The psychological responses of breast cancer patients and healthy women are described in detail in Chapter 2.

The effects of ACTH and cortisol on lymphocyte percentages and function at baseline and in response to the task

Regarding the question whether the effect of ACTH on immunological outcome varies as a function of the health of the donor (i.e. metastatic breast cancer, node-positive breast cancer and healthy women) it turned out that of all possible models, one model in which the main term ACTH and the dummy-coded variable X_1 plus the interaction term X_2 multiplied by ACTH were simultaneously entered, was most plausible in the prediction of immunological outcome at baseline and in response to the task.[1] All other tested models

[1] One may argue that in case of the prediction of distribution and function of peripheral blood cells by ACTH and by the health of the donor at baseline and in response to the acute stressor, a hierarchical regression analysis should have been applied. However, one of the limitations of the hierarchical approach is that an interaction (e.g., ACTH by X_1 or ACTH by X_2) can only be entered in a model provided that the main terms are already included (saturated model). However, it has been shown that a variable individually and in interaction with another in one model may give rise to multicollinearity This has to be expected in case of correlations among independent variables of approximately > .80. In consequence of this, the estimation of the standardized regression coefficient(ß's) may be hampered. In the present study, correlations between the independent variable ACTH and the interactions ACTH by X_1 and ACTH by X_2, the main term X_1 and the interaction term ACTH by X_1, and the main term X_2 and the interaction term ACTH by X_2 (at T2, T3, T4 and T5) varied

had to be rejected because the conditions mentioned in the paragraph on statistical analysis were not fulfilled. For the sake of clarity the model comprising only the main effects of ACTH on the immunological outcome will be provided in addition to the aforementioned model. Thus, two models are presented by each dependent variable (see Table 2).

With respect to the prediction of distribution and function of peripheral blood cells (in basal levels and in response to the task) by cortisol and by the health of the donor, the model consisting of the main term cortisol and dummy-coded variables X_1 and X_2 appeared most plausible. Analogous to ACTH, also the model comprising only the main effects of cortisol on immunological outcome are presented (see Table 2).

The effect of ACTH on lymphocyte percentages and function at baseline and in response to the task

Main effects

As shown in Table 1 ACTH levels were significantly positively and uniquely related to total lymphocytes at baseline (T2) unadjusted for the health of the donor: the higher the ACTH levels, the higher the lymphocyte percentages. With the exception of the first post-task time point (T4), ACTH consistently predicted total lymphocyte percentages in response to the task on repeated measurements (T2, T3 and T5). ACTH appeared to be unrelated to the lymphocyte subsets CD3, CD4 and NK cells and to the proliferative response to PWM and NKCA at either baseline (T2) or in response to the task (T3, T4 and T5).

Interaction effects

The model consisting of the main terms ACTH and X_1 and the interaction ACTH by X_2 significantly predicted CD3 cell percentages at baseline (T2) as well in response to the task (T3, T4 and T5) (see Table 2). The non-parallel lines, based on the intercept and unstandardized regression coefficients, in Figure 1 indicate that basal ACTH levels were positively related to basal CD3 percentages in patients with node-positive breast cancer patients and healthy women: the higher ACTH levels, the higher the CD3 percentages (see for details of the calculation of this model Appendix IIa). In contrast, basal ACTH levels were negatively related to basal CD3 percentages in metastatic breast cancer patients: the higher ACTH levels, the lower the CD3 percentages. The same pattern also occurred for CD3 cells measured at T3 and T4. CD4 cell percentages measured at 9 min (T4) and 37 min (T5) after task-exposure also seem to be discernibly predicted by this model. With respect to the prediction of total lymphocyte percentages and NK cell percentages measured in rest and in response to the task this model appeared less plausible.

With respect to PWM-induced T cell proliferation (100 µg/ml), the the main terms ACTH and X_1, and the interaction ACTH by X_2 were shown to be highly significant at

---

between .56 and .85. The VIF-values (i.e., index of multicollinearity) of the parametersin the saturated model were much more higher than 4 and this model had to be rejected. This problem could have been solved by simply removing the interactions from the equations. However, the relation between one of the independent with one of the categories of another variable will not be visible in the regression equation. We, therefore, decided to explore other possible models. In case of percentage of CD3, CD4 and proliferation to PWM at baseline and in response to the speech task, the model including the main terms ACTH, X_1 and the interaction ACTH by X_2 appeared to be most plausible.

Table 2: Prediction of lymphocyte and mononuclear cell phenotype percentages and proliferation to PWM and NKCA by cortisol and by the health of the donor (healthy women, breast cancer patients with axillary lymph node metastases and breast cancer patients with distant metastases).

Dependent variable	Independent variables	Basal levels T2		Speech T3		Recovery 1 T4		Recovery 2 T5	
		ß[1]	p	ß[1]	p	ß[1]	p	ß[1]	p
Lymphocytes	Cortisol[2]	-.00	.99	-.00	.97	-.00	.99	.04	.78
	Cortisol[3]	.03	.90	-.04	.88	.01	.96	.10	.63
	X_1	.16	.43	.19	.37	.04	.87	.01	.94
	X_2	-.10	.69	-.02	.94	-.04	.87	-.14	.53
CD3 cells	Cortisol[2]	-.16	.32	-.11	.49	-.20	.21	-.14	.38
	Cortisol[3]	**.42**	**.04**	.42	.06	.36	.10	.22	.23
	X_1	**-.55**	**.00**	**-.52**	**.01**	**-.56**	**.00**	**-.53**	**.00**
	X_2	**-.82**	**.00**	**-.72**	**.00**	**-.73**	**.00**	**-.54**	**.01**
CD4 cells	Cortisol[2]	-.25	.10	-.25	.11	-.21	.18	-.19	.22
	Cortisol[3]	.22	.29	.28	.18	.40	.06	.21	.22
	X_1	**-.45**	**.02**	**-.46**	**.02**	**-.60**	**.00**	**-.57**	**.00**
	X_2	**-.67**	**.00**	**-.75**	**.00**	**-.81**	**.00**	**-.62**	**.00**
NK cells	Cortisol[2]	.14	.36	.14	.39	.01	.94	.17	.27
	Cortisol[3]	.18	.43	.22	.35	.03	.91	.05	.79
	X_1	.26	.20	.07	.75	.13	.54	**.38**	**.05**
	X_2	-.14	.57	-.18	.47	-.08	.76	.08	.69
Prol. PWM (100 µg/ml)	Cortisol[2]	**-.30**	**.05**	-.29	.06	-.28	.07	-.15	.33
	Cortisol[3]	.10	.60	.11	.56	.26	.15	.12	.47
	X_1	**-.36**	**.05**	-.35	.06	**-.49**	**.01**	-.31	.09
	X_2	**-.65**	**.00**	**-.66**	**.00**	**-.85**	**.00**	**-.58**	**.00**
NKCA	Cortisol[2]	-.22	.15	-.25	.10	-.25	.10	-.11	.47
	Cortisol[3]	-.06	.78	-.07	.74	-.04	.85	.11	.55
	X_1	-.24	.23	-.28	.16	-.36	.07	-.22	.23
	X_2	-.23	.31	-.25	.26	-.26	.25	**-.46**	**.02**

X_1 is coded 2 for node-positive breast cancer patients and coded 1 for metastatic breast cancer patients and healthy women

X_2 is coded 2 for metastatic breast cancer patients and coded 1 for node-positive breast cancer patients and healthy women

[1] Standardized regression coefficient (statistically significant ß's are bold-face printed)

[2] The main term Cortisol is entered only in the regression equation

[3] The main term Cortisol and X_1 and X_2 are entered simultaneously in the regression equation

baseline as well as during the speech task (T3) and at the first post-task time-point (T4). This means that for PWM-induced T cell proliferation measured at these time-points the same pattern occurred as was seen for CD3 cell percentages at baseline (see Figure 1). This model appeared to be less plausible in prediction of natural killer cell activity (NKCA) at any time point.

The effect of cortisol on lymphocyte numbers and functions at baseline and in response to the task: main effects

Main effects

As can be seen in Table 2 basal cortisol values were significantly negatively related to proliferation to PWM (100 μg/ml) at baseline (T2): the higher the cortisol values, the lower PWM-induced lymphocyte proliferation. At T3, T4 and T5, cortisol values were only marginally negatively related to PWM-induced lymphocyte proliferation. Furthermore, cortisol appeared to be unrelated to the total lymphocyte percentages, marginally negatively related to the lymphocyte subsets CD4 and NK cells and NKCA, and marginally positively related to NK cells at either baseline or in response to the task.

Likewise, cortisol values were not related to CD3 percentages at baseline (T2) and in response to the task (T3, T3, T4, T5). Interestingly, the model in which cortisol and the dummy-coded variables X_1 and X_2 are entered simultaneously, significantly predicted CD3 cells at baseline. In Figure 2 the parallel regression lines, based on the intercept and unstandardized regression coefficients, indicate that patients with both node-positive and metastatic breast cancer as well as healthy women, regardless of the differences in their CD3 levels, show increases in basal CD3 percentages with increasing levels of cortisol (see for details of the calculation of this model Appendix IIb). With respect to CD3 levels at T3, T4 and T5, the effect of cortisol and the health of the donor appeared to be less dinstinct. Finally, this model (i.e., cortisol plus X_1 and X_2) appeared less or not plausible with respect to total lymphocyte percentages, CD4 and NK cells and NKCA and PWM-induced lymphocyte proliferation at any time point.

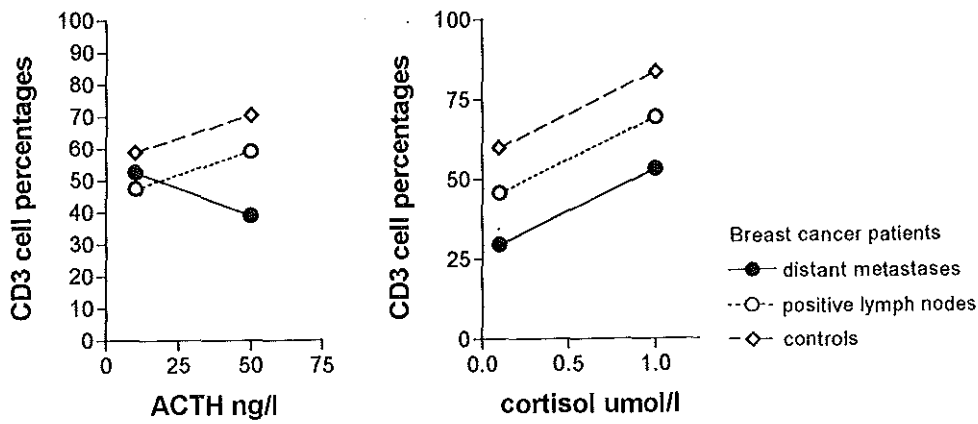

Figure 1-2. The effect of ACTH and cortisol on basal CD3 cell percentages in breast cancer patients with distant metastases and axillary lymph node metastases and in healthy women

Discussion

The scope of the present study was to explore by means of statistical modeling whether the health of the donor can be a source of variation for the association of ACTH and cortisol with distribution and function of peripheral blood cells in breast cancer patients as compared to healthy women. Before discussing the role of ACTH and cortisol in immune cell distribution to the speech task, the influence of these hormones on immune values measured under resting conditions is of interest. The findings of this study show that ACTH levels were positively related to total lymphocyte percentages under resting conditions independent of the stage of the disease. Interestingly, when the model that include the interaction of ACTH with the health of the women (i.e. patient with node-postive and metastatic breast cancer and healthy women) was entered, ACTH had a small positive effect on percentages of CD3 cells and on CD4 cell percentages in node-positive breast cancer patients and healthy women. In contrast, in metastatic breast cancer patients ACTH had a negative effect on CD3 and CD4 cells. Similar effects of ACTH were found for T cell proliferation to PWM. In node-positive breast cancer patients and healthy women, ACTH had a modest positive effect on PWM-induced proliferation, while ACTH was negatively related in patients with metastatic breast cancer. Although these findings are based on a small sample they seem to implicate that, with respect to the extent of breast cancer progression, ACTH acts differently on immune cells expressing membrane markers associated with the CD3 and CD4 phenotype and on the response of T cells to mitogenic stimulation. Data in the literature support the differential effect of ACTH on cellular immune responses (Ader et al., 1991; Fontana, et al., 1987; Heijnen et al., 1987). These data suggest that the *in vitro* effect of neuropeptides on the response of T cells to mitogenic stimulation depends on the metabolic state of the T cell with regard to basal cyclic adenosine monophosphate (cAMP) levels (Kavelaars, Ballieux, & Heijnen, 1990). It has been found that the neuropeptide ß-endorphin increased cAMP levels in cells with low basal levels or decreased cAMP levels in cells with high basal cAMP levels (Kavelaars et al., 1990). Catecholamines and cortisol are known to induce increases in cAMP levels in lymphocytes (Marone & Condorelli, 1982; Durant, 1986). In view of these findings we speculate that the increased cAMP levels, possibly induced by elevated levels of catecholamines or cortisol due to chronic psychological stress or pathophysiological changes that may accompany metastases, may have influenced the sensitivity of the lymphocytes for neuropeptides like ACTH. Thus, it could be that the negative effect of baseline ACTH levels on T cell proliferation in the metastatic breast cancer patients in this study is a result of exposure of lymphocytes to relatively high levels of catecholamines or cortisol. In vitro models have to be developed that investigate the effect of ACTH in interaction with other stress hormones on cAMP levels in lymphocytes of breast cancer patients and normal subjects.

Cortisol appeared to be only modestly negatively associated with percentages of CD3 cells. This relationship reversed after the health of the donor was taken into account. Breast cancer patients and healthy women showed increases in CD3 cells with increasing levels of cortisol regardless of the differences in basal CD3 levels. This finding is consistent with that of others showing that cortisol has a positive effect on the immune response, although the immunosuppressive effect of cortisol have been emphasized more often (Ottaway & Husband, 1992). In line with earlier studies, we observed that cortisol, when not adjusted

for the health of the donor, negatively related to PWM-induced proliferation. In sum, ACTH may selectively inhibit distribution and function of peripheral blood cells in breast cancer patients, whereas cortisol may modulate the immune system independent of the health of the donor. These findings are of interest with respect to the previously observed immune alterations among our group of breast cancer patients. Progressive and clinically stage-related decrements were observed in some lymphocyte subsets and proliferation of T lymphocytes. It is, therefore, likely that ACTH and cortisol may contribute to the inhibition of an effective host response against mammary tumor cells.

Concerning the immunological responses to the speech task, the results of the present study suggest that ACTH is involved in distribution of peripheral leukocytes of breast cancer patients and healthy subjects. Plasma ACTH levels were consistently positively related to total lymphocyte percentages under resting conditions as well as in response to the speech task. Additionally, the selective inhibitory effect of ACTH on cells expressing membrane markers associated with the CD3 and CD4 phenotype as well as on the response to T cells to mitogenic stimulation appeared consistent at all time points. This may imply that ACTH have an influence on distribution and function of T lymphocytes in response to mild acute stress in healthy women and breast cancer patients with positive lymphnodes, whereas increases in ACTH may coincide with significant decreases in CD3 and CD4 cells in breast cancer patients with distant metastases.

In contrast to our expectations, cortisol did not have an effect on distribution and function of peripheral blood cells in response to the task. It has been suggested that the speech task activates preferentially the autonomic nervous system and is not strong enough to activate the HPA-axis. Consistent with this assumption, we observed only modest increases in plasma ACTH levels, whereas cortisol levels of breast cancer patients and healthy women decreased in response to the speech task (Van der Pompe et al., 1996). A possible explanation for the lack of effect of cortisol on immunoreactivity can be that plasma cortisol levels were below the optimum level necessary for influencing lymphocyte migration.

The present study did not find evidence for a parallelism between ACTH and cortisol on the one hand and distribution and function of NK cells on the other. The lack of an effect of ACTH and cortisol can be explained by the suggestion that NK cells were less sensitive than other leukocyte subpopulations. Another explanation of this dicrepancy might also concern the requirement of a strong HPA-axis activation. We, therefore, strongly recommend future studies to use cortisol-associated stressors such as aversive stimuli in stead of effort-related stress models.

Taken the results together, the findings of the present study seem to implicate that the HPA axis plays a role in the adaptation of the host-defenses to acute stress, particularly those mediated by T lymphocytes. The acute-stress induced immune changes seem to be more strongly associated with ACTH, while the basal responses seem to be maintained in part by cortisol. Moreover, the effect of ACTH on distribution and function of T lymphocytes appeared to be bidirectionally dependent on the health of the donor.

The clinical importance of these findings is at present unknown. Research on the relationship between immune system variables and disease progression has thus far been inconclusive (Hacene, Desplace, Brunet, Lidereau, Bourguignat & Oglobine, 1986). However, a diminished response to mitogen-stimulation has been associated with an unfavorable prognosis in several studies (Burford-Mason, Gyte & Watkins, 1989; Seremet, Rudolf, Hrsak & Kastelan, 1992). More research is needed linking the influence of ACTH

Jöreskog KG (1993) Testing statistical equation models. In: (KA Bollen & JS Long, eds). *Testing statistical equation models.* London:Sage

Kavelaars A, Ballieux RE, Heijnen CJ (1990) Differential effects of ß-endorphin on cAMP levels in human peripheral blood mononuclear cells. Brain Behav Immun 4: 171-179

Little RJ (1988) Missing-data adjustments in large surveys. J Bus Econ Stat 6:287-295

Marone G, Condorelli M (1982) Modulation of beta-adrenergic receptor by hydrocortisone in human polymorphonuclear leukocytes. Ric Clin Lab 12:469-477

McNair DM, Lorr M, Droppleman LF (1971) Profile of Mood States. San Diego: Educational and Industrial testing service

Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom E (1991) Immunological changes in young and old adults during brief laboratory stress. Psychosom Med 53:121-132

Ottaway CA, Husband AJ (1992) Central nervous system influences on lymphocyte migration. Brain Behav Immun 6:97-116.

Pettingale KW, Watson M, Bhakri HL, Jones H, Tee DEH (1989) Changes in hormonal, immunological and autonomic measures during the performance of laboratory stress task. Stress Med 5:150-169

Pompe G van der, Antoni MH, Heijnen CJ (1996) Elevated basal cortisol levels and attenuated ACTH and cortisol responses to acute stress in women with metastatic breast cancer. Psychoneuroendocrinology 21:361-374

Pompe G Van der, Antoni MH, Heijnen CJ (1997)The effects of surgical stress and psychological stress on the immune function of operative cancer patients. Psychol Health, in press

Ravindran AV, Griffiths J, Merali Z, Anisman H (1995) Lymphocyte subsets associated with major depression and dysthymia: modification by antidepressant treatment. Psychosom Med 57:555-563

Saab PG, Liabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, McCalla J, Cieply LK, Schneiderman N (1992) Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white americans. Psychophysiology 29:384-397

Seremet M, Rudolf M, Hrsak M, Kastelan M (1992) Prognostic value of some parameters of cellular immunity in breast cancer patients. Acta Med Croat 46:9-13

Spielberger CD (1980). Test manual for the State-Trait Anxiety Inventory - Stai form Y. Palo Alto:Consulting Psychologist Press

Chapter 5

Adjustment to breast cancer

The psychobiological effects of psychosocial interventions

Gieta van der Pompe[1], Michael Antoni[2], Adriaan Visser[1], Bert Garssen[1]

[1]Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Department of Psychology and Psychiatry, Miami

Abstract

This review focuses on the effects of psychosocial interventions on psychological and biological functioning of breast cancer patients. Once in their lifetime one out of eleven women receive a diagnosis of breast cancer. A diagnosis of breast cancer is a severe stressful life event with profound consequences on all aspects of human life. Whether a woman will regain emotional balance and accept the idea of living with a potentially life threatening disease depends on her psychological resiliency. Provision of psychosocial interventions can improve these women's coping abilities and reduce emotional distress and feelings of isolation, and improve psychosexual functioning. Additionally, there exists some evidence that psychotherapy may prolong survival. Prolongation of survival may be related, in part, to an increase in certain aspects of immune function (e.g., natural killer cell activity). This is plausible because the function of the immune system seems to be related to mammary tumor growth. Therefore, future research should examine the degree to which the effects on mammary tumor growth relate to immune system changes.

Introduction

Cancer and its treatment may provoke severe physical and psychological complaints. Breast cancer seems to be more stressful because this disease and its medical treatment can afflict the sense of feminity, perceived sexuality, and fertility of its victims (Irvine, Brown, Crooks, Roberts & Browne, 1991). Several studies have focused on the needs of breast cancer patients in order to provide these women with adequate support by the medical team or by specialized psychosocial services in their adjustment to physical and psychological cancer-related problems (Irvine et al., 1991; Maunsell, Brisson & Deschenes, 1992; Schag, Ganz, Polinsky, Fred, Hirje & Petersen, 1993). Psychotherapy may be an important modality complementing the medical treatment of breast cancer, especially to reduce psychological distress and possibly to affect disease progression (De Vries, 1994; Spiegel, Bloom, Kraemer & Gottheil, 1989).

This article focuses first on the psychological responses of women to breast cancer and its treatment, and on the effects of both individual-based and group psychotherapy programs on emotional and social adjustment of breast cancer patients. Second, the influence of psychosocial factors (adverse life events, psychological responses) and psychotherapy on progression of breast cancer are briefly reviewed. In addition, the biological mechanisms along which these effects may be mediated will be summarized. Figure 1 illustrates this line of reasoning.

Psychosocial problems in breast cancer patients

Many studies have indicated that post-surgical breast cancer patients are facing serious psychologic, physical, sexual and interpersonal problems (Irvine et al., 1991). Consequently, most breast cancer patients experience difficulties in their ability to perform normal daily social activities, and a considerable fraction of these patients (20%-46%) seem to suffer from a moderate to severe emotional morbidity (Irvine et al., 1991; Morris, Greer & White, 1977; Plumb & Holland, 1977).

Patients with cancer seem to improve in their adjustment to their physical and psychological sequelae over time. According to some authors, their level of anxiety and depression appear to be close to general population norms between one and two years postsurgery (Edgar, Rosberger & Nowlis, 1992). On the other hand, it has been shown, in a survey of patients with a variety of cancer types, that cancer survivors assessed their mental well-being 2-3 years after diagnosis less positively than did healthy controls (Sullivan, Cohen & Branehög, 1988). Many breast cancer patients report heightened levels of anxiety and illness-related worries and a reduction of energy and ability to do physical activities (Schag et al., 1993). A significant proportion of breast cancer patients (20-30%) experience a disruption in their quality of life through loss of roles, functional abilities, and problems with social relationships (Irvine et al., 1991; Maunsell et al., 1992) and from severe psychosocial and psychosexual problems (Schag et al., 1993). Most patients rated "fear of cancer recurrence" as a significantly greater concern than the "fear of losing a breast" (Fallowfield, Hall, Maguire & Baun, 1990; Sneeuw, Aaronson, Yarnold, Broderick, Regan, Ross & Goddard, 1992). Body-image and sexual functioning appear to be enhanced

through the use of breast-conserving treatment (Sneeuw et al., 1992; Kiebert, De Haes, & Van de Velde, 1991; Lee, Love, Mitchell, Parker, Rubens, Watson, Fentiman & Hayward, 1992; Pozo, Carver, Noriega, Harris, Robinson, Ketcham, Legaspi, Moffat & Clark, 1993). An important question is which factors may influence adjustment to breast cancer.

Factors associated with adjustment to breast cancer

Several studies have indicated that breast cancer treatment (type of surgery, adjuvant treatment), sociodemographic status (marital and economic status) and the patient's psychological profile (psychiatric pathology, coping styles, social support) are predictors of severe and lasting psychological morbidity following breast cancer diagnosis and treatment (Maunsell et al., 1992).

First, it has been hypothesized that the type of surgery -mastectomy versus lumpectomy- is a risk factor for developing a high level of post-operative psychosocial disturbances. However, several studies provide no solid proof of an overall better psychologic adjustment after breast-conserving treatment; there are no substantial differences between the different treatment modalities in changing cancer-related fears and concerns (Sneeuw et al., 1992; Kiebert et al., 1991; Pozo et al., 1993; Ganz, Coscarelli, Lee, Polinsky & Tan, 1992). In fact, breast-conserved patients, especially younger premenopausal women, reported reduced levels of energy and difficulty requesting help or assistance from their partner, friends or relatives after surgery. This may make them more vulnerable for emotional morbidity (Ganz et al., 1992; Levy, Haynes, Herberman, Lee, McFeeley & Kirkwood, 1992) and suggests that age may interact with treatment type in predicting psychosocial adjustment.

Other studies found that adjuvant chemotherapy led to more severe psychological consequences than adjuvant radiotherapy (Maguire, Tait, Brooke, Thomas & Howat, 1980; Hughson, Cooper, McArdle & Smith, 1986), while another group did not observe differences between the two treatment arms, though they did find evidence for an age interaction effect (Berglund, Bolund, Fornander, Rutqvist & Sjöden, 1991). Younger women who received chemotherapy post-surgery, tended to report a higher frequency of treatment-related symptoms than the younger radiotherapy group, while there no differences for the elder women.

Second, different sociodemographic variables (age, education, marital status and economic status) could not be identified as significant risk factors for psychological morbidity, though the ways these variables (age) interact with different treatment modalities remains to be investigated in a systematic fashion (Schag et al., 1993).

Third, the number of stressful life events and a history of depression before breast cancer diagnosis appear to be strong risk factors for high psychological distress in the 18-month period after initial treatment for breast cancer (Maunsell et al., 1992).

Fourth, several studies provide evidence that the patient's coping styles and the available social recourses are key factors in the adaptation to breast cancer (Irvine et al., 1991). Breast cancer patients who utilized denial as an initial coping strategy appear to experience less psychological distress postoperatively (Watson, Greer, Blake & Shrapnell, 1984). Bloom and Spiegel (1984) found that women with metastatic breast cancer who used

less avoidance or less maladaptive coping behaviors (increased eating, drinking, smoking and sleeping) were better emotionally adjusted. Several studies found that the perceived level of emotional support was also associated with a better adjustment outcome in breast cancer patients (Irvine et al., 1991). More recently published data showed that breast cancer patients who are at low risk for emotional morbidity can be described as resourceful problem solvers, who had coped successfully with other life events and had close family relationships and multiple sources of support (Schag et al., 1993).

To summarize, breast cancer patients have to cope with multiple emotional, psychosexual and social problems, which can last over long periods. The type of surgery per se does not seem to directly contribute to their overall level of psychological distress, though treatment type may interact with age and other demographic variables as it relates to emotional and psychosexual adjustment. Women who already have a considerable burden of stress in their lives, and have ineffective coping styles or limited social resources, may be particularly vulnerable to emotional difficulties after new challenges such as breast cancer diagnosis and treatment.

Effects of psychosocial intervention on adjustment to breast cancer

Accumulating data of studies with different types of cancer patients suggest that psychological interventions may be important for reducing emotional distress, enhancing coping and social support, and improving psychosexual adjustment, especially during key challenges in the cancer patients' lives such as the period shortly before and the year after surgery (Trijsburg, Van Knippenberg & Rijpma, 1992). Several studies have evaluated the effectiveness of several cancer-focused intervention programs with different types cancer patients. These interventions are generally composed of several components and intervention techniques including an emotionally supportive component (to address fear and anxiety about the disease and possibly reduce a sense of social isolation), information provision (about the disease and treatment), behavioral and cognitive techniques to build effective coping strategies (cognitive restructuring) and relaxation training to lower arousal (Trijsburg et al., 1992; Andersen, 1992).

In their review Trijsburg et al. (1992) conclude that tailored counseling, including supportive and informational components with regard to one cancer-related problem (sexuality in gynaecologic cancer and breast cancer patients), has been shown to be effective with respect to preserving self-concept and with respect to reducing distress, fatigue and sexual problems among breast cancer patients. Structured counseling, containing educational and cognitive behavioral instructions and exercises, was shown to ameliorate anxiety and depression. Behavioral interventions and hypnosis were effective at reducing anxiety, pain, nausea, and vomitting; especially as related to chemotherapy side-effects. These effects, however, may have been biased by 1) frequently higher drop-out rates in control groups, and 2) by medical factors (type of cancer, stage of disease, the intensity and duration of medical treatment) contributing to the level of distress during and after medical treatment.

Individual-based psychotherapy with breast cancer patients

Few studies focused primarily on the effectiveness of psychosocial intervention with breast cancer patients. In one study a nurse provided an intervention in the hospital before surgery and every 2 months at home until the patients appeared to be adjusted (Maguire, Tait, Brooke, Thomas & Sellwood, 1980). The counseled women reported fewer physical complaints, responded better psychologically to breast loss and reported fewer difficulties with their social and sexual relationships than patients in the control condition. Although this study employed a randomized design and was based on a reasonable sample of breast cancer patients (N=152), the evaluated intervention lacked a specific theory or content, and the number of sessions varied per patient until "she appeared to be adjusted". This greatly hampers any efforts at replication.

Christensen (1983) invited 20 couples to participate in a 4- week intervention that included discussions of relationships, education, role-play, behavioral practice, and self-image integration. The intervention modestly reduced distress, and significant improvements in sexual satisfaction were found for both women and their partners as compared to a no-treatment control condition. There was, however, no follow-up in this study.

Davis (1986) compared (1) a behavioral therapy such as combined electromyography and temperature control biofeedback along with progressive relaxation training, (2) a cognitive therapy, which entailed positive imagery, self-talk evaluation and relaxation, and (3) a no-treatment control group. The total number in this study were only 25 newly diagnosed breast cancer patients. Both programs were delivered over an 8-week period. Although there were no changes observed over the intervention period in any group, after an 8-month follow-up period there was a significant reduction in state anxiety scores, but no differential improvement between both experimental groups and the no-treatment control group. Therefore, the observed positive changes in psychological outcome measures can not be attributed to the intervention. Since the findings were only based on a limited sample (8-9 patients per condition), it is plausible that limitations in statistical power accounted for the lack of group differences.

Edgar et al. (1992) in order to study the optimal timing of intervention delivery, provided recently diagnosed cancer patients (N=205) including breast cancer patients (N=98) with an intervention either directly after randomization, or 4 months later. For this study, a coping skills intervention was used which included instructions in problem solving, cognitive restructuring, relaxation training, and information about the hospital and health care system. Psychosocial measures were completed for both groups at baseline, and at 4, 8 and 12 months later. Both groups improved with time, but there were greater gains for the group of breast cancer patients who received the intervention 4 months post-randomization. This suggests that patients may require a period of 4 months (to complete their physical recovery from surgery and associated treatment regimens) in order to realize the maximum benefits of psychosocial intervention. The emotional coping of breast cancer patients, however, improved during the year regardless of the intervention timing.

To summarize, both tailored (Maguire et al., 1980) and structured cognitive behavioral counseling (Edgar et al., 1992; Christensen, 1983) revealed positive effects on

several psychosocial variables in early stage breast cancer patients. Some of these studies showed serious flaws with respect to sampling and design (small number of patients and/or no follow-up) as well as the evaluated interventions (lack of theory, unclear content and variable number of sessions). Another issue concerns the format of these interventions, that we discuss below.

Group psychotherapy with the breast cancer patients

As the programs in the above described studies were administered on an individual basis only, they may not have benefitted from the supportive environment created in group psychotherapy (Spiegel & Spira, 1991). As noted previously, social support may have a positive effect on psychosocial adjustment (Irvine et al., 1991). Metastatic breast cancer patients (N=86) assigned to weekly supportive group therapy sessions over a period of one year were significantly more emotionally adjusted, showed fewer maladaptive coping responses, and were less phobic than breast cancer patients receiving a standard medical treatment (Spiegel, Bloom & Yalom, 1981). The psychological benefits of this intervention, however, were only generalizable to metastatic breast cancer patients. It has been hypothesized that patients receiving a group-based psychosocial intervention program benefit from the support provided by their group members.

It is also possible that the social support element of any regular group meeting of breast cancer patients could offer similar benefits to those interventions using specific psychotherapeutic techniques. More generic supportive groups are probably among the most commonly offered, but least studied strategies, in general clinical practice for persons facing chronic or uncertain illnesses, including breast cancer. In contrast with the effects of theory-based psychosocial interventions reviewed above, these generic supportive groups have yielded no or only moderately positive outcomes in patients with several types of cancer (Andersen, 1992). It has been hypothesized that reliance on group support alone may be insufficient to produce any measurable benefit in cancer patients (Jacobs, Ross, Walker & Stockdale, 1983; Telch & Telch, 1986). Regular contacts with fellow cancer patients resulted in decrements of treatment-related negative emotions in one Dutch descriptive study (Van den Borne & Pruyn, 1985).

Besides individually-based interventions, group psychotherapy also results in a decline of psychological distress in breast cancer patients. Because most prior work has not been based upon a theoretical model-driven intervention approach, little has been learned about the psychosocial mechanisms (improved coping strategies, enhanced social support) that underlie the effects of these interventions.

Psychosocial influences on progression of breast cancer

In the past twenty years several studies have been initiated to elucidate the predictive value of psychosocial factors in the course of breast cancer. The burning question of all these studies is: Do psychosocial factors such as mood and personality have an influence on cancer-related mortality? Until now, the results of several studies in the field of psychooncology indicate that various psychosocial factors are associated with disease-free interval

and overall survival of breast cancer patients. Controlling for biological factors such as the size and histopathological characteristics of the tumor and disease stage, psychosocial factors are considered as independent prognostic variables of disease progression.

Based on a review of studies investigating the influence of several psychosocial factors on mammary tumor growth, it has been concluded that the likelihood of a relationship between emotional suppression and tumor progression is reasonably high (Mulder, Van der Pompe, Spiegel, Antoni & De Vries, 1992). Derogatis et al. (1979) showed that long-term survivors revealed higher levels of anxiety, depression and alienation than patients who died within one year. One explanation might be that patients who lived longer may have been better able to communicate their feelings. Several studies have also shown that women with breast cancer who expressed negative affect and anxiety have a better prognosis (Hislop, Waxler, Coldman, Elwood & Kan, 1987; Jensen, 1987; Dean, 1987).

There exists no substantial evidence for a relationship between the level of depression and cancer prognosis. Most studies did not show a relationship between level of depression and progression of breast cancer (Spiegel et al., 1989; Jamison, Burish & Wallston, 1987; Buddeberg, Wolf, Sieber, Riehl-Emde, Bergant, Steiner, Landolt-Ritter & Richter, Barraclough, Pinder, Cruddas, Osmond, Taylor & Perry, 1992). However, it appeared from several studies that women with high levels of hopelessness and helplessness, which is often associated with depression, and low levels of fighting spirit, have shorter survival times (Greer, Morris & Pettingale, 1979).

Besides the initial psychological responses to a breast cancer diagnosis, several studies have also shown that adverse life events are associated with recurrence and overall survival. Breast cancer patients have not only more life stressors prior to the discovery of the primary tumor (Funch & Marshall, 1983; Geyer, 1991; Forsen, 1991) or recurrence of breast cancer (Ramirez, Craig, Watson, Fentiman, North & Rubens, 1989) than controls, but also may be more likely to suffer a relapse or shortened survival time as a function of experiencing elevated levels of life stressors (Funch et al., 1983; Forsen, 1991; Ramirez, 1989). However, a prospective study yielded no evidence that psychosocial adversity is conducive to relapse (Barraclough et al., 1992).

Social support has been found to buffer the negative effects of life stressors on subsequent onset of physical illness in healthy individuals (Sarason, Potter, Sarason & Antoni, 1985). The data about the role of marital status are rather controversial. Being single or being married have both been associated with an increase in survival time (Neale, Tilley & Vernon, 1986; Goodwin, Hunt, Key & Samet, 1987; Cassileth, Walsh & Lusk, 1988; Waxler-Morrison, Hislop, Mears & Kan, 1991). This controversy could be explained by the fact that these studies did not measure directly characteristics of the women's social networks, social support or work experience. In fact, epidemiological studies suggest that adequate social networks are associated with lower cancer incidence (Berkman & Syme, 1979). Waxler-Morrison et al. (1991) found a significant effect of number of supportive friends, number of supportive persons, whether the women worked, the extent of contact with friends, and the size of the social network on survival. From this study it can be concluded that a woman's social network, particularly the context of friendship and work outside the home, may be related to survival.

To summarize, the findings regarding the association between psychosocial factors and progression of breast cancer, are still controversial. Opposite conclusions can arise from differences in the design and analysis of the studies (Goodkin, Antoni, Sevin & Fox, 1993). The importance of controlling for clinicopathological prognostic variables such as tumor stage, histopathologic grading and receptor status has been stressed many times by several authors (Fox, 1980). Intervening behavioral variables, such as diet, smoking and use of alcohol may also be responsible for the inconsistencies in findings across studies (Holland, 1990). In addition, patients may vary in immune system status, which also seems to have value in predicting the course of breast cancer (Herberman, 1989; Mohanty, Nayak & Nanda, 1991). Therefore, to obtain insights into the association between psychosocial factors and survival in breast cancer patients, it seems reasonable to focus on psychobiologic mechanisms that could mediate such relationships. One line of research that provides potentially relevant information in this regard is psychoneuroimmunology (PNI).

The impact of psychosocial factors on immune function and progression of breast cancer

In the past twenty years in the field of PNI an vast number of studies have been published which describe immunological changes in response to different stressors. The majority of these studies described immune responses to acute laboratory stressors and to naturalistic occuring life stressors and more chronic psychiatric disorders in healthy individuals. The number of studies that described these patterns in breast cancer patients is rather small.

The effects of stress on immune function in healthy individuals

Several studies have shown that exposure to stress results in higher levels of endocrines secreted by the hypothalamic pituitary adrenal (HPA) axis and sympathetic adrenomedullary (SAM) system. These hormones can modulate immune function differentially. Both corticotropin releasing hormone (CRH) and adrenocorticotroping hormone (ACTH) can suppress or enhance natural killer cell cytotoxicity (NKCC) and lymphocyte proliferation (Heijnen, Zijlstra, Kavelaars & Ballieux, 1987; Van den Brink, Van Wijk & Bijlsma, 1992). Catecholamines, on the other hand, may enhance NKCC and suppress lymphocyte proliferation (Garbriel, Schwarz, Born & Kindermann, 1992). Recent studies with healthy individuals reveal that exposure to a beta-adrenergic behavioral challenge results in an increase in NKCC and decrease in lymphocyte proliferation (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, Makinodan & Gilmore, 1991). This immunologic reactivity pattern was also found in breast cancer patients. However, the task-induced changes in breast cancer patients were lower compared to age-matched healthy women (Van der Pompe, Antoni & Heijnen, 1994). It may be that sympathetic nervous system-mediated catecholamine outflow and production from the adrenals is important in selectively mobilizing the immunologic response during periods of acute stress.

Chronic stress, on the other hand, results in a decline of immune function. In a separate line of PNI studies researchers have demonstrated that exposure to psychosocial stressors and the experience of psychological distress and affective disorders may result in

alterations in various components of the immune system. Separation or bereavement has been associated with decreased NKCC and proliferative response to various mitogens (Bartrop, Luckhorst, Lazarus, Kiloch & Penny, 1977; Irwin, Daniels, Smith, Bloom & Weiner, 1987; Irwin, Daniels, Bloom, Smith & Weiner, 1987; Kiecolt-Glaser, Fisher, Ogrocki, Stout & Speicher & Glaser, 1987; Kiecolt-Glaser, Kennedy, Malhoff, Fisher, Speicher & Glaser, 1988). Studies relating psychological distress and psychiatric disorders to decrements in functional immune measures are numerous. Based on a recent meta-analysis, it has been concluded that clinical depression was associated with several alterations in cellular immunity including lowered proliferative responsivity of T lymphocytes and NKCC. This analysis also revealed that the depression-immune associations were greater in both older and hospitalized samples (Herbert & Cohen, 1993). Without attempting to cover all the literature, these PNI associations are used as illustrations to point out that behavior may influence immune function.

The effect of stress on immune function in breast cancer patients

Inspired by the above mentioned PNI findings it has been hypothesized that the influence of psychosocial factors on tumor progression is mediated by the immune and neuroendocrine system. This assumption becomes more plausible when we consider that (a) the mammary tumor is sensitive for specific hormones (estrogen and prolactin) (Sutherland, 1987), and (b) the function of the immune system seems to be related to progression of breast cancer (Herberman, 1989). Most cancer patients, including breast cancer patients have an intact immune system at the time of the initial diagnosis, but as disease progresses, T lymphocyte number, proliferative response and NKCC become significantly reduced (Herberman, 1989). Taken into account that in chronically stressed individuals an equal pattern of immunological changes can be observed as in relation to progression of breast cancer, it could be possible that psychosocial factors influence the course of breast cancer. Either by modulating endocrine processes, which are directly related to tumor growth, or indirectly related via decrements in immunologic control over tumor development and metastases. It is beyond the scope of this article to describe into detail the possible mechanisms along which psychosocial stressors may influence progression of breast cancer. In a separate review we focused on possible relations between psychosocial stressors on the one hand and endocrine and immune processes and mammary tumor growth on the other (Van der Pompe, Antoni, Mulder, Heijnen, Goodkin, De Graeff, Garssen & De Vries, 1994). This article is restricted to those PNI studies with breast cancer patient samples.

Until now a very few studies have investigated the influence of psychosocial factors on immunological functioning in breast cancer patients. Levy, Herberman, Whiteside, Sanzo, Lee & Kirkwood (1990) found that the patient's perception of emotional support from spouse or intimate other, perceived support from the patients' physician, and actively seeking social support as a coping strategy were related to greater NKCC. This suggests that emotional support may buffer the influence of stressors on NKCC (Berkman & Syme, 1979). However, breast cancer patients who had a recurrence at the end of the study had a higher NKCC before the start of the adjuvant treatment. One possible explanation might be that the higher NKCC was an attempt of the immune system to compensate for the

greater threat posed by an aggressive tumor in order to control the spread of the disease. Higher NKCC in the follow-up period after the adjuvant treatment predicted a greater disease-free interval. With respect to mood and disease outcome elevated distress levels at baseline and in the follow-up predicted shorter time to recurrent disease (Levy, Herberman, Lippman, D'Angelo & Lee, 1991). Up to this point, no studies are available that provide insight into the relationship between psychosocial factors on the one hand and immune function and disease progression on the other.

On the basis of these sparse data we cannot draw any firm conclusions that PNI mechanisms are linked to breast cancer progression. The state of the art does allow us to draw the following conclusions: (1) The results of several studies lend support to the assumption that psychosocial factors are related to progression of breast cancer, (2) mammary tumors are hormonal sensitive, (3) activity of the immune system can influence the outcome of this breast disease, and (4) in chronically stressed individuals the same pattern of immunological changes can be observed as in relation to progression of breast cancer.

Intervention-related immunological changes in breast cancer patients

Over the past ten years different research groups initiated intervention studies to obtain information about the effect of psychosocial intervention on biological functioning (survival, immune and endocrine parameters). In addition to previously described psychological improvements, Spiegel et al. (1989) showed that patients with metastatic breast cancer randomized to a one year group psychotherapy program lived significantly longer than patients randomized to standard oncologic care only. The main goals of this psychotherapy program were to increase emotional expression of negative affect and to help patients to receive and give support to fellow patients in dealing with their breast cancer-related concerns and problems (Spiegel et al., 1989). The increase in survival time may be directly related to these therapeutic components, or indirectly due to better diet or more exercise as a result of lower levels of depressed mood. Survival plots indicated that divergence in survival began at 20 months after entry. This might suggest that only a select group of patients benefitted from the intervention. Both psychological and immunological characteristecs of these patients need to be identified. One possibility might be that this group of patients had better coping styles at baseline as compared to patients with shorter survival times and "learned" to deal more effectively with their disease. It could also be possible that these patients had a better immune function at study entry. Lowering distress may have resulted in a less attenuated immune response, which may have resulted in reduction of metastatic spread. These hypotheses can be tested in studies concurrently measuring endocrine and immune parameters hypothesized to be correlated with survival.

Some research groups (Gruber, Hall, Hersch & Dubois, 1988; Levy, 1990; Fawzy, Kemmeny, Fawzy & Elashoff, 1990) evaluated the effect of psychosocial interventions on immune function in cancer patients. Levy et al. (1988) evaluated psychosocial and immunological changes in a randomized trial with colon cancer and malignant melanoma patients. Patients receiving an individually administered cognitive-behavioral therapy showed an increase in NK cells and a trend towards higher NKCC compared to patients in the control condition. The same pattern emerged in a randomized prospective study with

post-surgical patients with malignant melanoma, who were assigned to either a 6-week structured psychiatric group intervention or to a standard medical care control condition (Fawzy et al., 1990). Gruber et al. (1988) tested the efficacy of a guided imagery and a relaxation program of one year in a mixed group of cancer patients with metastatic disease. This research team noted increases in several immune measures compared to baseline, though total white blood cell counts did not change. In all three studies the pattern of psychological changes paralleled the immune measures. That is, decrements in distress were accompanied by increments in NK cell counts and activity (Gruber et al., 1988; Levy, 1990; Fawzy, Cousins, Fawzy & Kemeny, 1990). These observations demonstrate the possibility of positively affecting immunologic processes by psychosocial intervention in cancer patients. However, whether these immunological effects have any impact on the course of the disease remains unknown.

Conclusions

A diagnosis of breast cancer and its treatment is a severe stressful life event with profound consequences on all aspects of human life. Breast cancer patients have specific needs and concerns which in a significant proportion of patients can last longer periods. Whether a patient will regain emotional balance and accept the idea of living with a potentially life threatening disease is dependent on her psychological resiliency: women with a depressive predisposition, ineffective coping styles and a high level of stressful life events in their current lives may have an increased risk to develop psychiatric symptoms. The type of surgery they receive (i.e., lumpectomy or mastectomy) may also have a predictive value, but the impact of this variable is outweighed by the fear for a recurrence among early stage breast cancer patients. In metastatic breast cancer patients the fear for pain, death and dying are most predominant.

In the coming years no breaktrough in the medical treatment of cancer is expected. So, psychosocial care of patients and their families remains important. Several psychotherapy programs have been shown to increase quality of life of breast cancer patients. Although the programs varied in their content and procedural variables (e.g., number of sessions), overall they seemed to be effective in decreasing psychological distress in breast cancer patients. However, some programs lacked specific theory or content, which may hamper any effort at replication and (or) transference to health care workers. Although the concerns and of breast cancer patients are common across different stages (confrontation with a life-threatening disease), there are also differences. Our work suggests that major concerns of metastatic breast cancer patients focus on existential issues: fear for pain, death and dying, concerns with respect to family and the awareness of a shortened life-perspective. The major concerns of breast cancer patients with primary disease, on the other hand, focus on the fear for recurrence, and problems associated with surgery and/or adjuvant treatment (chemotherapy), which can afflict feminity, body-image, sexuality, and, in young women, fertility (Van der Pompe, Antoni & Heijnen, 1994). Therefore, future research should tailor psychotherapy programs to the specific needs and concerns of non-metastatic vs. metastatic breast cancer patients.

There exists some evidence that psychotherapy may prolong survival of (metastatic) breast cancer patients. Prolongation of survival time may be related to an increase in immune function. Future research should pay attention to the effects of psychotherapy correlated with immune function on the one hand and mammary tumor growth on the other. Most early stage breast cancer patients have an intact immune system at the time of their initial diagnosis. As disease progresses immune function becomes significantly reduced. Therefore, it can be assumed that the likelihood of a relationship between psychotherapy and immune function on the hand and survival on the other decreases as disease progresses. The core question is: What can be done to prevent immunosuppression in early stage breast cancer? Surgical stress impairs NKCC in patients with sarcomas and other solid tumors (Pollock, Lotzova & Stanford, 1992). In addition, adjuvant treatment (radio- and chemotherapy) results in a decreased immune function (Brenner & Margolese, 1991). Thus, in the period surrounding the diagnosis/treatment cancer patients may have a high risk to develop (micro)metastases. Provision of individual or group psychotherapy can improve the quality of life of these patients and possibly by prevention of immunosuppression decrease the risk of metastases and prolong survival.

References

Andersen B (1992) Psychological interventions for cancer patients to enhance the quality of life. JCCP 66:552-568

Barraclough J, Pinder P, Cruddas M, Osmond C, Taylor I, Perry M (1992) Life events and breast cancer prognosis. Br Med J 304:1078-1081

Bartrop R, Luckhorst E, Lazarus L, Kiloch L, Penny R (1977) Depressed lymphocyte function after bereavement. Lancet 83:834-836

Berglund G, Bolund C, Fornander T, Rutqvist L, Sjöden P (1991) Late effects of adjuvant chemotherapy on quality of life among breast cancer patients. Eur J Cancer 27:1075-1081

Berkman L, Syme S (1979) Social networks, host resistance, and mortality: a nine year follow-up study of Alameda County residents. Am J Epidemiology 2:186-204

Bloom J, Spiegel D (1984). The relationship of two dimensions of social support to the psychological well-being and social functioning of women with advanced breast cancer. Soc Sci Med 19:831-837

Borne HW van den, Pruyn J (1985) Lotgenoten contacten. Assen:Van Gorcum

Brenner B, Margolese R (1991) The relationship of chemotherapeutic and endocrine intervention on natural killer cell activity in human breast cancer. Cancer 68:482-488

Brink H van den, Wijk M van, Bijlsma J (1992) Influence of steroid hormones on proliferation of peripheral blood mononuclear cells in patients with rheumatoid arthritis. Br J Rheumatol 31:663-667

Buddeberg C, Wolf C, Sieber M, Riehl-Emde A, Bergant A, Steiner R, Landolt-Ritter C, Richter D (1991) Coping strategies and course of disease of breast cancer patients. Psychother Psychosom 55:151-157

Cassileth B, Walsh W, Lusk E (1988) Psychosocial correlates of cancer survival: a subsequent report 3 to 8 years after cancer diagnosis. J Clin Oncol 6:1753-1759

Christensen D (1983) Postmastectomy couple counseling: An outcome study of a structured treatment protocol. J Sex & Mar Ther 9:266-275

Davis H (1986) Effects of biofeedback and cognitive therapy on stress in patients with breast cancer. Psychol Reports 59:967-974

Dean Ch (1987) Psychiatric morbidity following mastectomy: preoperative predictors and type of illness. J. Psychosom Res 33:385-392

Derogatis L, Abeloff N, Melisaratos N (1979) Psychological coping mechanisms and survival time in metastatic breast cancer. JAMA 242:1504-1508

Edgar L, Rosberger Z, Nowlis D (1992) Coping with cancer during the first year after diagnosis. Assessment and intervention. Cancer 69:817-828

Fallowfield L, Hall A, Maguire G, Baun M (1990) Psychological outcomes of different treatment policies in women with early breast cancer outside a clinical trial. BMJ 301:575-580

Fawzy F, Kemeny M, Fawzy N, Elashoff R (1990) A structured psychiatric intervention for cancer patients. II. Changes over time in immunological measures. Ach Gen Psychiatry 47:729-735

Fawzy F, Cousins N, Fawzy N, Kemeny M (1990) A structured psychiatric intervention for cancer patients. I. Changes over time in methods of coping and affective disturbance. Arch Gen Psychiatry 47:720-725

Forsen A (1991) Psychosocial stress as a risk for breast cancer. Psychother Psychosom 55:176-185

Fox B (1980) Psychosocial factors and the immune system in human cancer. In: Psychoneuroimmunology (R. Ader ed). New York:Academic Press

Funch D, Marshall J (1983) The role of stress, social support and age in survival from breast cancer. J Psych Res 27:77-83

Gabriel H, Schwarz L, Born P, Kindermann W (1992) Differential mobilization of leucocyte and lymphocyte subpopulations into the circulation during endurance exercise. Eur J Appl Physiol 65:529-534

Ganz P, Coscarelli C, Lee J, Polinsky M, Tan S (1992) Breast conservation versus mastectomy. Is there a difference in psychological adjustment or quality of life in the year after surgery? Cancer 69:1729-1738

Geyer S (1991) Life events prior to manifestation of breast cancer: A limited prospective study covering eight years before diagnosis. J Psychosom Res 35:355-363

Goodkin K, Antoni M, Sevin B, Fox B (1993) A partially testable predictive model of psychosocial factors in the etiology of cervical cancer. II. Bioimmunological, psychoneuroimmunological, and socioimmunological aspects, critique and prospective integration. Psychooncology 2:99-121

Goodwin J, Hunt W, Key C, Samet J (1987) The effect of marital status on stage, treatment and survival of cancer patients. J Am Med Assoc 258:3125-3130

Greer S, Morris T, Pettingale K (1979) Psychological response to breast cancer: effect on outcome. Lancet ii: 785-787

Gruber B, Hall N, Hersch S, Dubois P (1988) Immune system and psychological changes in metastatic cancer patients using relaxation and guided imagery: a pilot study. Scan J Beh Ther 17:25-46

Heijnen C, Zijlstra J, Kavelaars A., Ballieux R (1987) Modulation of the immune response by POMC-derived peptides. I. Influence on proliferation of human lymphocytes. Brain Behav Immun 1:284-291

Herberman R (1989) Influence of the host on tumor development. Dordrecht:Kluwer Academic Publishers

Herbert T, Cohen S (1993) Depression and immunity: a metaanalytic review. Psychol Bull 113:472-486

Hislop T, Waxler N, Coldman A, Elwood J, Kan L (1987) The prognostic significance of psychosocial factors in women with breast cancer. J Chron Dis 40:729-735

Holland J (1990) Behavioral and psychosocial risk factors in cancer: Human studies. In Handbook of Psychooncology (J. Holland & J. Rowland eds). New York:Oxford University Press

Hughson A, Cooper A, McArdle, Smith D (1986). Psychological impact of adjuvant chemotherapy in the first two years after mastectomy. Br Med J Clin Res 293:1268-1271

Irvine D, Brown B, Crooks D, Roberts J, Browne G (1991) Psychosocial adjustment in women with breast cancer. Cancer 67:1097-1117

Irwin M, Daniels M, Smith T, Bloom E, Weiner H (1987) Impaired natural killer cell activity during bereavement. Brain Behav Immun 1:98-104

Irwin M, Daniels M, Bloom E, Smith T, Weiner H (1987) Life events, depressive symptoms, and immune function. Am J Psych 144:437-441

Jacobs C, Ross R, Walker I, Stockdale F (1983). Behavior of cancer patients: a randomized study of the effects of education and peer support groups. Am J Clin Oncol 6:347-353

Jamison R, Burish T, Wallston K (1987) Psychogenic factors in predicting survival of breast cancer patients. J Clin Oncol 5:768-772

Jensen M (1987) Psychobiological factors predicting the course of breast cancer. J Personal 55:317-342

Kiebert G, Haes J de, Velde C van de (1991) The impact of breast-conserving treatment and mastectomy on the quality of early-stage breast cancer patients: A review. J Clin Oncol 9:1059-1070

Kiecolt-Glaser J, Fisher L, Ogrocki P, Stout J, Speicher C, Glaser R (1987) Marital quality, marital disruption and immune function. Psychosom Med 49:13-34

Kiecolt-Glaser J, Kennedy S, Malhoff S, Fisher L, Speicher C, Glaser R (1988) Marital discord and immunity in males. Psychosom Med 50:213-229

Lee M, Love S, Mitchell J, Parker E, Rubens R, Watson J, Fentiman I, Hayward (1992) Mastectomy or conservation for early breast cancer: Psychological morbidity. Eur J Cancer 28:1340-1344

Levy S, Herberman R, Whiteside T, Sanzo K, Lee J, Kirkwood J (1990) Perceived social support and tumor estrogen/progesterone receptor status as predictors of natural killer cell activity in breast cancer patients. Psychosom Med 52:73-85

Levy S (1990) Cognitive behavioral intervention with cancer patients and normal populations: Effects on natural immunity. Paper presented at conference on Coping with cancer and beyond. Cancer treatment and mental health, Rotterdam

Levy S, Herberman R, Lippman M, D'Angelo T, Lee J (1991) Immunological and psychosocial predictors of disease recurrence in patients with early-stage breast cancer. Behav Med:67-75

Levy S, Haynes L, Herberman R, Lee J, McFeeley S, Kirkwood J (1992) Mastectomy versus breast conservation surgery: Mental health effects at long-term follow-up. Health Psych 11:349-354

Maguire P, Tait A, Brooke M, Thomas C, Sellwood R (1980) Effect of counseling on the psychiatric morbidity associated with mastectomy. BMJ 281:1454-1456

Maguire P, Tait A, Brooke M, Thomas C, Howat J, Sellwood R, Bush H. (1980) Psychiatric morbidity and physical toxicity associated with adjuvant chemotherapy after mastectomy. BMJ 281:1179-1180

Maunsell E, Brisson J, Deschênes L (1992) Psychological distress after initial treatment of breast cancer. Cancer 70:120-125

Mohanty I, Nayak M, Nanda B (1991) Cell mediated immune status in breast carcinoma. Indian J Pathol Microbiol 34:1-6

Morris T, Greer H, White P (1977) Psychological and social adjustment to mastectomy: A 2-year follow-up study. Cancer 40:2381-2387

Mulder C, Pompe G van der, Spiegel D., Antoni M, Vries M de (1992) Do psychosocial factors influence the course of breast cancer? Psychooncology 1:155-167

Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom E, Makinodan T, Gilmore S (1991) Immunological change in young and old adults during brief laboratory stress. Psychosom Med 53:121-132

Neale A, Tilley B, Vernon S (1986) Marital status delay in seeking treatment and survival from breast cancer. Soc Sci Med 23:305-313

Plumb M, Holland J (1977) Comparative studies of psychological function in patients with advanced cancer. I. Self-reported depressive symptoms. Psychosom Med 39:264-276

Pollock R, Lotzova E, Stanford S (1992) Surgical stress impairs natural killer cell programming of tumor for lysisin patients with sarcomas and other solid tumors. Cancer 8:2192-2202

Pompe G van der, Antoni M, Heijnen C (1994) Cellular immune changes following standardized laboratory stress in breast cancer patients compared to age-matched healthy controls. Paper presented at 52nd annual meeting of the American Psychosomatic Society, Boston

Pompe G van der, Antoni M, Heijnen C (1994) Effectiveness of a group psychotherapy program on immunologic and neuroendocrine parameters in non-metastatic and metastatic breast cancer patients. Paper presented at 52nd annual meeting of the American Psychosomatic Society, Boston

Pompe G van der, Antoni M, Mulder C, Heijnen C, Goodkin K, Graeff A de, Garssen B, Vries M de (1994) Psychoneuroimmunology and the course of breast cancer; an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psychooncology 3:271-288

Pozo C, Carver C, Noriega V, Harris S, Robinson D, Ketcham A, Legaspi A, Moffat F, Clark K (1993). Effects of mastectomy versus lumpectomy on emotional adjustment to breast cancer: A prospective study of the first year postsurgery. J Clin Oncol 8:1292-1298

Ramirez A, Craig T, Watson J, Fentiman I, North W, Rubens R (1989) Stress and relapse of breast cancer. Br Med J 298:291-293

Sarason I, Potter, E, Sarason B, Antoni M (1985) Life events, social support and physical illness. Psychosom Med 47:156-163

Schag C, Ganz P, Polinsky M, Fred C, Hirje K, Petersen L (1993) Characteristics of women at risk for psychosocial distress in the year after breast cancer. J Clin Oncol 11:783-793

Sneeuw K, Aaronson N, Yarnold J, Broderick M, Regan J, Ross G, Goddard A (1992) Cosmetic and fuctional outcomes of breast conserving treatment for early stage breast cancer. 2. Relationship with psychosocial functioning. Radother Oncol 25:160- 166

Spiegel D, Bloom J, Yalom I (1981) Group support for patients with metastatic breast cancer. Arch Gen Psychiatry 38:527-533

Spiegel D, Bloom J, Kraemer H, Gottheil E (1989) Effect of psychosocial treatment on survival of patients with metastatic breast cancer. Lancet ii:888-891

Spiegel D, Spira J (1991) Supportive-expressive group therapy: A treatment manual of psychosocial intervention for women with recurrent breast cancer. Psychosocial treatment laboratory, Stanford University School of Medicine

Sullivan M, Cohen J, Branehög I (1988) A psychological study of cancer survivors in California, USA and the western region of Sweden, Gothenburg, Gothenburg University and Los Angeles, UCLA

Sutherland D (1987) Hormones and cancer. In The basic science of oncology (I. Tannock, R. Hill eds). Toronto:Pergamon Press

Telch C, Telch M (1986) Group coping skills instruction and supportive group therapy for cancer patients: A comparison of strategies. JCCP 54:802-808

Trijsburg R, Knippenberg F van, Rijpma S (1992) Effects of psychological treatment on cancer patients: a critical review. Psychosom Med 54:489-517

Vries M de (1994) Mensen met kanker staan nog vaak in de kou. Het tekort van de geestelijke zorg in Nederland. Medisch Contact 49

Watson M, Greer S, Blake S, Shrapnell K (1984) Reaction to a diagnosis of breast cancer: Relationship between denial, delay, and rates of psychological morbidity. Cancer 53:2008-2012

Waxler-Morrison N, Hislop T, Mears B, Kan L (1991) Effects of social relationships on survival for women with breast cancer. Soc Sci Med 33:177-183

Chapter 6

Prediction of psychological adjustment to breast cancer after participation in a group psychotherapy program: an empircal exploration

Gieta van der Pompe[1], Michael Antoni[2], Hugo J. Duivenvoorden[3]

Klazien de Vries-Kragt[1], Floor Pelgrim[1], Adriaan Visser[1] & Marco J. de Vries[1]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] University of Miami, Department of Psychology and Psychiatry, Miami

[3] Erasmus University, Faculty of Medicine, Department of Medical Psychology and Psychotherapy, Rotterdam

Abstract

There is growing evidence that the efficacy of group-based psychological interventions with somatically ill patients may vary as a function of patients' individual differences in distress levels and other stress moderating variables. The present study explored the degree to which the effectiveness of a time-limited group psychosocial intervention program for women adjusting to a breast cancer diagnosis depends on patients' baseline psychological profile. Thirty-two breast cancer patients (23 post-surgical breast cancer patients and 9 recently diagnosed with metastatic disease) were randomly assigned to either a 13-weeks experiential-existential group psychotherapy program or to a waiting-list control condition. Patients in the intervention group were encouraged to express their emotions by self-disclosure, sharing fears and other feelings about the illness and change their life-perspective in a more meaningful way. The major finding of this study is that the group psychotherapy program is most effective in those breast cancer patients presenting with elevated levels of depression or maladaptive coping strategies such as disengagement and alcohol abuse; patients who entered the study with lower distress or less use of maladaptive coping strategies showed no benefits of the intervention. The results show that determination of psychological profile at baseline may determine to which degree psychotherapy is beneficial for breast cancer patients. Possible explanations for findings along with strong recommendations for research on larger scale are provided.

Introduction

Between 20% and 40% of all breast cancer patients suffer from a serious emotional disturbances in the periods following diagnosis and treatment (Plumb & Holland, 1977; Morris, Greer & White, 1977; Irvine, Brown, Crooks, Roberts & Browne, 1991). These figures suggest that a considerable number of breast cancer patients are in need of, and possibly benefit from professional help. At present several studies have provided evidence that psychosocial intervention programs reduce emotional distress and improve coping abilities (Andersen, 1992; Trijsburg, Van Knippenberg & Rijpma, 1992). Much of this research had operated from the assumption that most breast cancer patients are likely to benefit from psychosocial interventions. Despite the intuitive 'preventive' measure that psychosocial intervention should be offered to all breast cancer patients, there is little evidence that these types of clinical services are equally useful to all patients. It has been suggested that it may be more effective to offer psychosocial services only to those at risk for developing emotional problems (Maguire, 1995). These risk factors may include current affect state or other intermediary factors such as maladaptive coping that may increase the likelihood of poor psychological adjustment (Mulder, Van der Pompe, Spiegel, Antoni & De Vries, 1992). However, until now it is unknown whether the degree of effectiveness of psychological treatment in breast cancer patients depends on the baseline psychological profile. The primary aim of present study was to explore whether breast cancer patients' psychological profile modulate the efficacy of 13-weeks experiential-existential group psychotherapy program.

Methods

Patients

Breast cancer patients who have been treated for primary breast cancer and were diagnosed with either positive axillary lymph nodes ($T_{1-3}N_{1-3}M_0$) or supraclavicular lymph nodes, skin or distant metastases ($T_{1-4}N_{1-3}M_1$) were included in the study. The patients diagnosed with positive axillary lymph nodes joined the study 4 months after surgery. The patients with distant metastases entered the study after notification of the recurrence. All patients received first-line endocrine treatment only (tamoxifen). No other type of treatment was administered throughout the course of this study. Excluded were breast cancer patients with brain metastases or other malignancies.

For the recruitment of these patients several hospitals in the western region of the Netherlands participated in this study. Eligible patients were invited by their clinicians to participate in a study investigating the effect of psychosocial intervention program on adjustment to cancer. Interested patients completed a screening session in which a more elaborate description of the study was provided, including issues of confidentiality right to refuse participation at any time without loss of optimal treatment, time commitments and study benefits. After this procedure patients were asked to sign an informed consent form. Only patients having signed an informed consent form were included in the study. This

screening procedure resulted in a cohort of 32 breast cancer patients aged 50 to 70 years (mean=58.8; sd=8.0). The group consisted of 23 curatively treated patients with positive lymph nodes, but without distant metastases (mean age=60.5; sd=6.1) and 9 patients diagnosed with breast cancer and distant metastases (mean age=54.7; sd=10.8). Of the 15 originally assigned to the intervention-group, 1 patient was excluded from the analysis because she was to ill to attend the sessions. Out of the 17 assigned to the waiting-list control condition, 3 patients were excluded because they were too ill to paticipate in post-treatment assessment or had died. The final sample consisted of 14 patients in the intervention-group and 14 patients in the waiting-list control condition.

Study procedures

After having completed psychosocial questionnaires, patients were randomly assigned to either the experiential-existential group psychotherapy (EEGP) or the waiting-list control condition. The patients in the EEGP condition were scheduled for therapy sessions. Patients assigned to the waiting-list control condition were informed that they would be offered the opportunity to complete the psychosocial group support at the end the study period. At the end of intervention period patients were scheduled to complete post-treatment psychosocial questionnaires.

Group psychotherapy condition

Theoretical background

The intervention program is based on the principles of the experiential-existential therapy. This approach aims at changing patients' experience of life and to facilitate existential reorganization. This is a dynamic approach and its focus is on fundamental concerns of patients' life: fear of death; experienced limitations of freedom; existential isolation; relationships with family, relatives and with the medical profession; autonomy vs. helplessness and dependency and meaning of life (Yalom, 1980). According to the existential approach those individuals who confront their existential anxiety related to the illness are hypothesized to adjust more adequately than those denying it (Yalom, 1980).

Intervention goals

During the EEGP program the existential crisis provoked by the confrontation with a life-threatening illness was worked through: (a) patients were encouraged to express their emotions, sharing fears and concerns and addressing feelings about illness and death and previous life experiences. (b) Body awareness exercises and relaxation were used to reduce psychological distress. (c)Social isolation was reduced by support from the members of the group and by stimulating involvement in or improving meaningful relationships. Interpersonal conflict resolution skills (i.e., assertiveness training) were communicated and practiced in the sessions.

Cancer-specific focus

Members were encouraged to address the issues they considered important and which emerged during the intervention. Although breast cancer patients across stages of the disease have similar concerns and needs such as confrontation with a life-threatening disease, there are also differences. Prior work at our institute and by others indicated that major concerns of metastatic breast cancer patients are existential issues such as fear of pain, death and dying, concerns with respect to family and the consequences of a shortened life-perspective. The major concerns of breast cancer patients without distant metastases are the fear of recurrence, and problems associated with surgery and adjuvant treatment (predominantly chemotherapy) which can affect their self-perceptions of feminity, body-image, sexuality and, in younger women, fertility (Van der Pompe, Antoni, Visser, Garssen, 1996). Although all patients in the EEGP condition were offered the same group psychotherapy program, node-positive breast cancer patients and metastatic breast cancer patients were, because of the above decribed differences in issues and concerns, treated in in separate groups.

Structure and format

The therapy was semi-structured; the topics and the goals of each session were outlined in a manual developed before the commencement of the study. The order in which these topics and goals were addressed was decided by the therapists.The intervention period consisted of 13 sessions held weekly for 2.5 hours.

Therapists qualifications

The groups were led by two psychotherapists who were experienced in conducting group psychotherapy with cancer patients, particularly with breast cancer-related issues. The main function of the therapists was to help the women to become more aware of incongruences between emotional, cognitive and behavioral patterns and to aim at meaningful life goals in the context of a shortened life expectancy. The therapists refrained from giving advice, and worked actively with the participants to help develop insights and solutions to their problems.

Waiting-list control condition

Patients in the waiting-list control (WLC) condition received EEGP after a waiting period of four months. During this waiting period they did not receive any psychosocial or psychiatric treatment from sources outside the context of this study.

Demographic characteristics

At the start of the study several demographic characteristics of the patients were assessed including age, partnership status, number of children, education, and employment status.

Psychosocial Measures

Emotional adjustment

Affective state measures included Beck Depression Inventory comprising 13 items (α = 0.82) (Beck, Mendelson, Mock & Erbaugh, 1961; Bouman, Luteijn, Albersnagel & Van der Ploeg, 1985), the State-Trait Anxiety Inventory (STAI) (α's = 0.66 and 0.94 for state- and trait-scale of the STAI respectively) (Spielberger, 1980, Van der Ploeg, Defares & Spielberger, 1980), and the Profile-of-Mood States (32-item POMS scale α = 0.91) (McNair, Lorr & Droppleman, 1971; Wald & Mellenberg, 1990). For all items of the STAI, subjects were asked to indicate how they felt at present and in general on a 4-point scale (1="not at all" to 4="extremely"). The Profile of Mood States (POMS) consisting of five dimensions: depression, anger, fatigue, vigor and tension. For each item, subjects were asked to indicate how they felt, using a 4-point intensity scale ranging from 1 ("not at all") to 4 ("extremely"). The total mood disturbance (TMD) score is computed by adding the negative scales and substracting 'vigor'. All these scales have been translated and validated for a Dutch population (Bouman, Luteijn, Albersnagel & Van der Ploeg, 1985; Van der Ploeg, Defares & Spielberger, 1980; Wald & Mellenberg, 1990).

Coping

To measure coping a translated version of the COPE designed by Carver et al. (1989) was used. The original index incorporates 13 distinct scales. The conceptualization of the scales is based on the Lazarus model of coping with stress (Lazarus & Folkman, 1984; Carver, Scheier & Weintraub, 1989). On the basis of Lazarus' model of coping with stress the subscales are grouped into three categories: (1) problem-focused coping, which is composed of active coping, planning, suppression of competing activities, restraint coping, and seeking social support for instrumental reasons (20 items, α = 0.87); (2) emotion-focused coping, which is composed of seeking social support for emotional reasons, positive reinterpretation and growth, acceptance, turning to religion (17 items, α = 0.80); and (3) maladaptive coping, composed of focusing on and venting of emotions, denial, behavioral disengangement, mental disengagement, alcohol-drug disengangement (20 items, α = 0.61). In the analyses of the present study the three total scores (i.e., problem-focused, emotion-focused, and maladaptive coping) have been used. Subjects checked the degree of coping for each scale on a 4-point intensity scale, ranging from 1 ("not at all") and 4 ("extremely"). Before the commencement of the present study, the translated version of the COPE has been tested on a large scaled basis (Van der Pompe, manuscript in preparation).

Emotional Expression

Emotional Expression was measured with a Dutch adaptation (Van der Ploeg, Kleijn, Mook, Van Donge, Pieters & Leer, 1989) of the Emotional Expression Scale of Watson & Greer (1983). The extent to which patients control, suppress or express anger, anxiety, and depression are measured on a 4-point scale in each of the 18 items comprising this measure. In the present study the subscales emotional expression-in (6 items, α = 0.80) and emotional expression-out (6 items, α = 0.90) have been used. The validity of this scale has

been established (Van der Ploeg et al., 1989).

Social support

Characteristics of the social support system of the patient were measured with the social support scale of Van Sonderen (1991). This 49-items list measures the availability of social support and the discrepancy between the social support desired and that actually offered to the patient. Subjects were asked to indicate how often they felt supported by their network on a 4-point scale ranging from 1 ("never") to 4 ("often"). In addition they indicated the degree of satisfaction with the support provided by their network on a 4-point scale, ranging from 1 ("too less") to 4 ("too often"). The questionnaire has five subscales: 1. every day emotional support, 2. emotional support in problem situations, 3. appreciation, 4. instrumental support, 5. informational support. In the analyses we will use two total scores: the availability ($\alpha = .90$) and the satisfaction score ($\alpha = .90$). The validity of this scale has been established (Sonderen, 1991).

Statistical Analysis

Predictive mean matching (Little, 1988) was used in estimating missing values for psychological outcome variables provided that the variable had < 15% of cases missing.

Multiple regression was used to explore whether the effect of EEGP on post-intervention psychological measures can be predicted by the respective psychological baseline characteristics (Cohen & Cohen, 1983). For any variable with a non-normal distribution an appropriate transformation was applied prior to conducting the regression analyses. To index the EEGP condition and the WLC condition, the variable 'Condition' was coded as 1 for EEGP and coded 2 for WLC. Interaction terms were constructed, consisting of the variable Condition multiplied by baseline psychological characteristics. The standardized regression coefficient (ß) was used as a measure of relative importance of each predictor. The statistical level of significance was fixed at 0.05. Moreover, the variance inflaction factor (VIF)-values (an index of multicollinearity) had to be smaller than 4 (Glantz & Slinker, 1990). Thus, a regression model was considered plausible if the predictor variables and the interaction term were statistically significant provided that the VIF-values within acceptable limits.

The sequence of regression equations were run as follows: First, to examine the efficacy of the intervention on psychological outcome only the variable 'Condition' was entered in the regression equation. Second, to explore whether the effect of EEGP on psychological outcome variables could be predicted by relevant baseline values on outcome measures, three different regression models were tested: in the first series of regression analyses the model consisting of the main term Baseline Value and the variable 'Condition' plus the interaction term Condition x Baseline Value corresponding to the outcome variable was entered, in the second series of regression analyses the model consisting of the variable 'Condition' and the respective interaction term was entered, and in the third step the interaction Condition by Baseline Value was entered.

Table 1: Means (SD) of baseline psychological distress levels in breast cancer patients and in norm-groups (healthy subjects and patients diagnosed with a psychiatric disease).

Psychological characteristic	Research-subjects		Norms		Norm-population
	Mean	SD	Mean	SD	
Beck Depression Inventory					
Total Score	8.0	6.2	2.8	3.5	Healthy subjects
			11.5	7.2	Psychiatric in-patients (Bouman et al., 1985)
State-Anxiety Inventory					
State Anxiety	42.9	7.3	37.0	11.8	Healthy women aged 51-60 yrs.
			40.8	12.2	Neurotic women
			51.8	10.8	Women with anxiety-neurosis
Trait Anxiety	40.6	10.8	38.7	8.9	Healthy women aged 51-60 yrs.
			45.8	11.4	Neurotic women
			54.5	11.2	Women with anxiety-neurosis (Van der Ploeg et al., 1980)
Profile-of-Mood States					
Total Mood disturbance (TMD)	47.1	16.4			Healthy women (Wald & Mellenbergh, 1990)
Depression	12.4	5.5	2.6	4.5	
Anger	10.2	4.4	3.6	4.1	
Fatigue	12.0	6.2	4.6	5.3	
Vigor	14.5	4.8	11.4	4.9	
Tension	12.5	4.5	5.1	4.9	

Differences between the groups with regard to the demographic, biobehavioral and baseline psychosocial variables were tested with univariate analyses of variance (ANOVA) for continuous data and Kruskal-Wallis test for ordinal data.

Results

Participant Characteristics

Most of the patients (81%) were married or lived with a partner; 77% had at least one or more children. The majority of our subjects (67%) were unemployed, and 48% were high school educated. There were no significant differences on these demographic variables between the EEGP group and the WLC group.

Baseline distress levels

In our sample, the scores on the Beck Depression Inventory (BDI) were within symptomatic ranges for depression for 27.3% of our population (Table 1) (Bouman et al., 1985). The scores on State and Trait Anxiety Inventory (STAI-DY) were within the range of patients presenting with neurosis for 67.7% and 27.3% respectively of the breast cancer patients (Van der Ploeg, 1980). The scores on the POMS-TMD were for 72.7% of our population higher than those observed in women with severe sleep disturbances (Wald & Mellenberg, 1989). To summarize, this sample of breast cancer patients showed a broad distribution of women experiencing mild to moderate levels of mood disturbance. There were no statistically significant differences in the BDI-, POMS-TMD- and state and trait anxiety score between patients assigned to the EEGP condition and the WLC condition. Neither could we obtain differences in any of these psychosocial measures between node-positive breast cancer patients and those with metastatic disease, which justified to a certain extent not taking the stage of the disease into account.

The effect of EEGP on psychological distress levels

The regression analyses showed that except for the POMS-subscale Tension there was no effect of the main term Condition on psychological outcome (Table 2, Column 2). The unadjusted negative regression weight (ß = -.41) of the Condition in prediction of the level of Tension indicates that the WLC group had lower post-treatment levels of tension than patients in the EEGP group.

The effect of EEGP on psychological distress levels adjusted for respective baseline values

In the first series of regression analyses the model consisting of both main terms Baseline Value and Condition plus the Condition by Baseline Value interaction was tested. In case of all dependent variables (i.e., psychological distress measures, coping, emotional expression, and social support) this model had to be rejected because the aforementioned conditions (see chapter on statistical analyses) were violated (data not presented).[1]

---

[1]Because the purpose of the pr escnt study is toexplore whether the effect of EEGP on psychological outcome is related to patients' respective baseline psychological values, we decided to explore different models.The models described in this chapter appeared to be most plausible and relevant in predicting post-intervention psychological values. The saturated model (i.e., main term Baseline Value and Condition, and the interactionterm Baseline Value by Condition) had to be rejected due to multicollinearity. The correlations between the baseline POMS-TMD, BDI-depression, and the Anxiety (trait) score individually and in interactionwith the variable Condition were .85, .53 and .58, all p's > .001. These high significant correlations have hampered the estimation of the standardized regression coefficients (ß's).

Table 2: Multiple regression analyses for psychological outcome measures

OUTCOME MEASURES	Condition[1]		Condition x Baseline Value[1]		Condition[2]		Condition x Baseline Value[1]	
	ß[3]	p	ß[3]	p	ß[3]	p	ß[3]	p
POMS								
Total Mood Disturbance (TMD)	-.23	.25	.23	.25	-.72	.00[c]	.71	.00[c]
Depression	-.06	.77	.55	.00[c]	-.43	.02[a]	.76	.00[c]
Anger	.20	.32	.57	.00[c]	-.20	.33	.69	.00[c]
Fatigue	-.23	.25	.52	.01[a]	-.49	.00[c]	.71	.00[c]
Vigor	-.13	.51	.35	.08	-.58	.01[a]	.71	.00[c]
Tension	-.41	.04[a]	.25	.21	-.72	.00[c]	.62	.00[c]
BDI								
Depression	-.11	.61	.62	.00[c]	-.39	.01[b]	.75	.00[c]
STAI								
State Anxiety	-.24	.23	-.01	.96	-[4]	-	-	-
Trait Anxiety	-.35	.08	.15	.46	-.94	.00[c]	.82	.00[c]

[1] The main term Condition and the interaction term Condition x BaselineValue (corresponding to outcome measure) are entered separately in the regression equation; Condition is coded 1 for EEGP condition and coded 2 for waiting-list controls

[2] Both the main term and the interaction term are entered simultaneously in the regression equation; Condition is coded 1 for EEGP condition and coded 2 for waiting-list controls

[3] Standardized regression coefficient

[4] - is printed when the assumption VIF < 4 is violated.

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

In the second series of analyses (Table 2, Column 3), the main term Condition and the interaction term Condition x Baseline Value were entered into the regression model. It turned out that the Total Mood Disturbance (TMD) score (ß = -.72; ß = .71, respectively), BDI-depression score (ß = -.39, ß = .75, respectively), and Trait Anxiety score (ß = -.94; ß = .82, respectively) were significantly predicted by both terms. The counterintuitive negative regression weights of the main term Condition post-test TMD, BDI-depression and Trait Anxiety seem to indicate that patients in the WLC condition have as a group in this type of model, significantly lower levels of mood disturbances, depression and trait anxiety than patients in the EEGP condition. The significant interaction term Condition x baseline Mood Disturbances, Condition x baseline BDI-depression, and Condition x baseline Trait Anxiety respectively modified this relationship in opposite direction. The regression lines

in Figure 1 based on the intercept and unstandardized regression coefficients, show that patients in the EEGP group who had a high BDI-depression score at study entry had a lower BDI-depression score than patients in the WLC condition (see for details of the calculation of the model Appendix III).

With respect to post-test State Anxiety levels, the model Condition plus Condition x baseline State Anxiety interaction had to be rejected because corresponding VIF-values exceeded the acceptable VIF limit of 4.

The third series of regression analyses testing the interaction term Condition x Baseline Values (Table 2, Column 4) did not explain a significant proportion of the variance of the Total Mood Disturbance (TMD) scores, but significantly predicted the POMS Depression subscale scores (ß = .55), Anger (ß = .57) and Fatigue (ß = .52). Likewise, the level of BDI-Depression post-test could also be significantly predicted by this model (ß = .62). These findings are considered less valid because the interaction Condition x Baseline Value forms part of the previously described model consisting of the main term Condition and the Condition x Baseline Value interaction which significantly predicted post-treatment TMD-score and BDI-depression.

Finally, the unadjusted regression weight of the interaction term predicting both State Anxiety and Trait Anxiety was not significant.

The effect of EEGP on coping, emotional expression and social support

As shown in Table 3 (Column 2) the variable Condition was unrelated to the post-intervention levels of coping, emotional expression and social support (Table 3, Column 2). The negative regression weight of the condition predicting Emotions-In became marginally significant (ß = -.37). This may indicate that patients in the intervention-group were more expressive post-test than controls.

The effect of EEGP on coping, emotional expression social support adjusted for respective baseline values

In the first series of regression analyses entering the Condition plus Condition x Baseline Value (Table 3, Column 3) did significantly increase the variance for Maladaptive Coping (ß = -.82; ß = .76, respectively) and Emotions-Out (ß = -.82; ß = .86, respectively). This may indicate that patients who participated in the EEGP used less maladaptive coping strategies or were less expressive post-test than patients in the WLC condition but this differential effect appeared to occur relatively more in patients who were inclined to use these maladaptive strategies and were more emotionally expressive at study entry. This model had to be rejected with respect to post-test Problem- and Emotion-Focused Coping and both Social Support outcome measures (Support Availability and Satisfaction) because of the aforementioned conditions (see paragraph on statistical analysis) were violated.

Table 3: Multiple regression analyses for coping styles, emotional expression and social support

OUTCOME MEASURES	Condition[1]		Condition x Baseline Value[1]		Condition[2]		Condition xBaseline Value[2]	
	ß[3]	p	ß[3]	p	ß[3]	p	ß[3]	p
COPE								
Problem-focused coping	.06	.79	.54	.00[c]	-[4]	-	-	-
Emotion-focused coping	.19	.33	.54	.00[c]	-	-	-	-
Maladaptive coping	-.17	.40	.07	.73	-.82	.03[a]	.76	.04[a]
Rationality & Emotionality								
Emotions-In	-.37	.06	-.23	.25	-.43	.12	.09	.76
Emotions-Out	-.28	.15	.53	.00[c]	-.82	.00[c]	.86	.00[c]
Social Support								
Support Availability	-.26	.23	-.04	.87	-	-	-	-
Support Satisfaction	-.32	.14	-.05	.82	-	-	-	-

[1] The main term Condition and the interaction term Condition x Baseline Value (corresponding to outcome measure) are entered seperately in the regression equation; Condition is coded 1for the EEGP condition and coded 2 for the waiting-list controls

[2] Both the main term and the interaction term are entered simultaneously in the regression equation; Condition is coded 1 for the EEGP condition and coded 2 for waiting-list controls

[3] Standardized regression coefficient

[4] - is printed when assumption VIF < 4 is violated.

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

In the second series of regression analyses, the interaction terms Condition x Problem-focused Coping and Condition x Emotion-focused Coping were significant. This means that patients in the WLC condition, who were inclined to use problem-focused coping at entry, tended to use relatively more problem-focused coping strategies post-test than did patients in the EEGP condition with similar high coping levels at baseline (Table 3, Column 4) (for both outcome measures: ß = . 54).

This model also appeared to be the best fit for predicting post-treatment Emotions-Out parameter (ß = .53), while Emotions-In could not be predicted by this model. Thus, controls classified as high emotions-out patients showed higher post-treatment emotions-out scores than EEGP participants who were similarly classified at baseline. We consider this finding less valid because the interaction Condition x Baseline Value model forms part of the previously described model consisting of the main term Condition and the Condition x Baseline Value interaction, which significantly predicted Emotions-Out. Finally, this model did not improve prediction of post-treatment levels of social support or support satisfaction.

To summarize, these preliminary results suggest that the efficacy of EEGP on psychological distress, coping, and emotional expression depends on the patients' psychological profile at study entry.

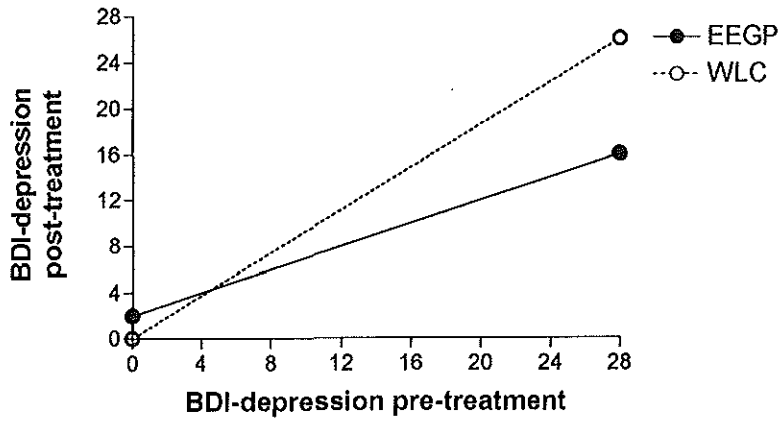

Figure 1. The effect of EEGP on BDI-depression compared to the WLC condition in breast cancer patients

Discussion

Before discussing the findings, we want to emphasize that due to the small sample size the present study is exploratory. Nevertheless, the results seem to implicate that the psychological benefit of a 13-weeks group psychotherapy program for breast cancer patients depended on patients' psychological profile at study entry. Breast cancer patients with higher levels of mood disturbances, depression and (trait) anxiety at baseline who were assigned to the intervention had lower scores after conclusion of the intervention-period compared to those in the WLC condition who had similar levels of psychological distress at entry. This effect appeared to be most significant in the post-treatment depression levels, while only small decreases in mood disturbances and trait anxiety levels could be obtained.

We did not find changes in the state anxiety levels. One explanation for this finding could be that the thirteen sessions were too few to deal effectively with high levels of (existential) anxiety. Because patients are uncertain about outcome of the disease, anxiety levels are realistic and therefore likely to persist. Another viable explanation for not finding the expected results is that the STAI may not capture the existential anxiety related to cancer diagnosis. We, therefore, encourage future studies to make use of the interview method tailored to the specific breast cancer related concerns and fears and to ask patients to comment on changes in these concerns and fears and to indicate to which condities or strategies of the intervention program the changes are attributed.

With regard to changes in coping and emotional expression a similar pattern of findings emerged. We found that patients after EEGP were less inclined to use maladaptive coping (e.g., behavioral and mental disengagment and alcohol-drug abuse) as compared to the WLC group but again only when we took into account their baseline use of these coping strategies. Others have observed changes in coping strategies in cancer patients participating in group-based interventions ranging from 6 weeks (Fawzy, Cousins, Fawzy, Kemeny, Elashoff & Morton, 1990) to 12 months (Spiegel et al., 1981). In addition to lower mood disturbance scores after a 1-year supportive group therapy, Spiegel and colleagues (1981) found a decline in the use of maladaptive strategies of managing stress (e.g., such as overeating, smoking or drinking) than did the members of the control condition. Their findings differ from ours in the sense that they found main effects, while we found effects of EEGP after taking into account the condition in interaction with maladaptive coping at baseline. Furthermore, we found that patients in the intervention group were on the average more emotionally expressive at post-treatment than patients in the WLC group, but when we took into account baseline levels highly emotional expressive patients in the intervention group became less expressive than controls.

It appeared that in patients with relatively high levels of adaptive (problem- and emotion-focused) coping at baseline, those assigned to the WLC condition tended to use more adaptive coping, either emotion- or problem-focused, than patients following the intervention program. Several explanations for this finding are possible. In order to overcome their existential crisis patients in our program are encouraged to express their concerns and fears they have about their illness and death and dying. In patients more able to communicate their feelings, a shift in coping styles may have occurred from adaptive coping styles such as acceptance of the disease and positive reinterpretation and growth, to coping styles more facilitative of external, conscious expression of negative emotions such as anger and frustation (Derogatis, Abeloff & Melisaratos, 1979). These patients as shown by the results of Derogatis et al. may appear less well adjusted to their illness than patients whose coping styles involve suppression of negative affect. Importantly, Derogatis et al. reported that a less well adjusted psychological profile was associated with a long-term survival. On the other hand, it might also be possible that patients who are normally able to deal effectively with their problems become dependent on the group (Kibel, 1992). These results underline that it is of paramount importance to screen out patients who will *not* benefit from a given treatment.

Another possible reason for not finding the expected effects of EEGP on coping could be that the analyses were restricted to pre- and post-treatment measurements that were too close in time. Other studies, following patients for at least six months after the end of treatment, found convincing effects (Fawzy, Cousins, Fawzy, Kemeny, Elashoff & Morton, 1991). This may also explain why we found only slight changes in mood disturbances and no significant changes in the intervention group as compared with the WLC group in social support.

Many studies have found in different populations that adaptive coping methods (either emotion- or problem-focused coping) and social support reduce distress levels (Lazarus & Folkman, 1984; Cohen & Wills, 1985). As our intervention program did not

result in significant increases in adaptive coping and social support, it is possible that the intervention-related reductions in depression found in those with relative high BDI-scores are mediated by other coping methods (as suggested earlier coping strategies more facilitative of externalization of negative affect) than those measured by the COPE. It would be interesting to determine whether changes in the coping methods or social support are related to the changes in distress levels and whether these relationships are different for patients in the intervention or control group.

To conclude, the results of present preliminary study seem to indicate that an EEGP intervention program is only effective in those breast cancer patients presenting distress elevations, especially high levels of depression, and frequent use of maladaptive coping strategies for managing their disease. Before drawing firm conclusions research on a larger scale is needed to replicate and expand these findings. If in case of a replication the patients' baseline psychological profile remains critical in facilitating psychological adjustment to breast cancer by EEGP, we are in principle able to select only those patients who will benefit from this type of treatment. However, one has to be cautious to extrapolate the results of studies as these to a clinical setting. The psychological profile of patients volunteering for a research project may be different from that of patients who apply for psychosocial treatment in a clinical setting. In addition, it is possible that those patients who will *not* be eligible for EEGP may benefit from another type of treatment. Therefore, an interesting route to investigate is which type of treatment (e.g., relaxation training alone or in combination with EEGP *or* group support compared with EEGP) may modify the effect of the determinants of psychological outcome. Both clinical data (e.g., age, presence of chronic disease, tumor size, number of positive axillary nodes, type of primary surgery and adjuvant treatment) and psychosoial information derived from interviews and psychological questionnaires need to be included in the regression analysis. This research strategy will enable us to develop intervention programs tailored to the needs of the individual patient.

To summarize, the present results show that EEGP may be of value in the care of cancer patients. The effects of EEGP have been tested in prediction analysis, which revealed that the effectiveness of the program for women adjusting to breast cancer diagnosis and treatment depends on their baseline psychological profile.

References

Andersen, B (1992). Psychological interventions for cancer patients to enhance the quality of life. Journal of Consulting and Clinical Psychology 66:552-568

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561-571

Bouman TK, Luteijn F, Albersnagel FA, Ploeg FAE van der. (1985) Enige ervaringen met de Beck Depression Inventory (BDI). Gedrag 13:1324.

Carver C, Scheier M, Weintraub J (1989) Assessing coping strategies: A theoretically based approach. Journal of Personality and Social Psychology 56:267-283

Cohen J & Cohen P (1983) *Applied multiple regression/correlation analysis for the behavioral sciences (2nd edition).* Hillsdale:NJ:Erlbaum

Cohen S & Wills TA (1985) Stress, social support, and the buffering hypothesis. Psychological Bulletin 98:310-357

Edgar L, Rosberger Z, Nowlis D (1992) Coping with cancer during the first year after diagnosis. Cancer 69:817-828

Glantz SA & Slinker BK (1990) *Primer of applied regression and analysis of variance.* New York:McGraw-Hill

Fawzy F, Cousins N, Fawzy N, Kemeny M, Elashoff R, Morton D (1990) A structured psychiatric intervention for cancer patients: I. Changes over time in methods of coping and affective disturbance. Arch Gen Psychiatry 47:720-725

Derogatis LR, Abeloff MD, Melisaratos N (1979) Psychological coping mechanisms and survival time in metastatic breast cancer. JAMA 242:1504-1508

Irvine D, Brown B, Crooks D, Roberts J, Browne G (1991) Psychological adjustment in women with breast cancer. Cancer 67:1097-1117

Kibel HD (1992) The clinical application of object relations theory. In: *Handbook of contemporary group psychotherapy. Contributions from object relations, self psychology, and social systems theories* (Klein, R.H., Bernard, H.S., Singer, D.L., eds). Madison: Connecticut

Lazarus RS & Folkman S (1984) *Stress, appraisal, and coping.* New York: Springer

Little RJ (1988) Missing-data adjustments in large surveys. Journal of Business & Economic Statistics 6:287-295

Maguire P (1995) Psychosocial interventions to reduce affective disorders in cancer patients: research priorities. Psycho-oncology 4:113-119

Maguire P, Tait A, Brooke M, Thomas C (1980) Effect of counselling on the psychiatric morbidity associated with mastectomy. British Medical Journal 281:1454-1456

Morris T, Greer H, White P (1977) Psychological and social adjustment to mastectomy: A 2-year follow-up study. Cancer 40:2381-2387

Mulder CL, Pompe G van der, Spiegel D, Antoni MH, Vries MJ de (1992) Do psychosocial factors influence the course of breast cancer? A review of recent literature, methodological problems and future directions. Psychooncology 1:155-167

McNair DM, Lorr M, Droppleman LF (1971) *Profile of Mood States.* San Diego: Educational and industrial testing service

Ploeg HM van der, Defares PD, Spielberger CD (1980). *Handleiding bij Zelfbeoordelingsvragenlijst (ZBV).* Lisse: Swets & Zeitlinger, The Netherlands

Ploeg HM van der, Kleijn W, Mook J, Donge M van, Pieters A, Leer JW (1989) Rationality and antiemotionality as a risk factor for cancer: concept differentiation. Journal of Psychosomatic Research 33:217-225

Plumb M & Holland J (1977) Comparative studies of psychological function in patients with advanced cancer. I Self-reported depressive symptoms. Psychosom Med 39: 264-276

Pompe G van der, Antoni MH, Visser A, Garssen B (1996) Adjustment to breast cancer: The psychobiological effects of psychosocial interventions. Patient Education and Counselling

Sonderen E van (1991) Het meten van sociale steun *(The measurement of social support).* Thesis:University Press Groningen

Spielberger CD (1980) *Test manual for the State-Trait Anxiety Inventory -Stai form Y,* Palo Alto: Consulting Psychologist Press

Spiegel D, Blood JE, Yalom I (1981) Group support for patients with metastatic cancer. Arch Gen Psychiatry 38:527-533

Taylor SE (1983) Adjustment to threatening events. A theory of cognitive adaptation. American Psychologist 38:1161-1173

Taylor SE, Kemeny ME, Aspinwall L, Schneider S, Rodriguez R, Herbert M (1992) Optimism, coping psychological distress, and high-risk sexual behavior among men at risk for acquired immunodeficiency syndrome (AIDS). Journal of Personality and Social Psychology 63:460-473

Trijsburg R, Knippenberg F van, Rijpma SE (1992) Effects of psychological treatment on cancer patients: a critical review. Psychosom Med 54:489-517

Wald FDM & Mellenberg GJ (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). Nederlands Tijdschrift voor de Psychologie 45:86-99

Yalom ID (1980) *Existential Psychotherapy.* New York: Basic Books.

Chapter 7

The effect of a group psychotherapy program on endocrine and immune function in breast cancer patients

Gieta van der Pompe,[1] Hugo J. Duivenvoorden[2], Michael H. Antoni[3], Adriaan Visser[1] & Cobi J. Heijnen[4]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam

[2] Erasmus University, Faculty of Medicine, Department Medical Psychology and Psychotherapy, Rotterdam

[3] University of Miami, Department of Psychology and Psychiatry, Miami

[4] University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', Utrecht

Abstract

Cancer patients who had been treated for breast cancer and were diagnosed with either positive axillary lymph nodes or distant metastases were randomly assigned to either a 13-week experiential-existential group psychotherapy (EEGP) program or a waiting list control (WLC) condition. Endocrine and immune measures were obtained before and after the intervention-period. The major findings of this study are that after the 13 weeks of the experiment patients in the EEGP group showed lower levels of plasma cortisol and lower levels of prolactin as well as lower percentages of natural killer cells, CD8 cells and CD4 cells in addition to a lower proliferative response to pokeweed mitogen than patients in WLC group. Importantly, this was only found in those breast cancer patients presenting relatively high endocrine and immune baseline levels suggesting that the patients' profile with regard to endocrine and immune function at the start of a program can be an important effect modification factor. If replicated on a larger scale, the current results may be relevant for the treatment of breast cancer.

Introduction

Several studies have shown that psychosocial intervention can reduce emotional distress and improve coping abilities in patients dealing with different types of cancer (Andersen, 1992; Trijsburg, Van Knippenberg, Rijpma, 1992). There is also some indication that such interventions induce changes in cellular immune function in cancer patients and those with other life-treatening diseases (Mulder, 1994; Van der Pompe, Antoni, Visser, Garssen, 1996). Fawzy, Kemeny, Fawzy and Elashoff (1996) evaluated psychosocial and immune effects in a randomized trial with post-surgical malignant melanoma patients. Patients receiving a short-term structured cognitive-behavioral group intervention showed increases in percentages of large granular lymphocytes and natural killer (NK) cells as compared to patients in the control condition. Recently Schedlowski, Jung, Schimanski, Tewes and Schmoll (1994) tested the efficacy of 10-week cognitive-behavioral intervention program in which they used relaxation and guided imagery as well as teaching stress- and illness-related coping skills in post-surgical early stage breast cancer patients. These authors observed increases in numbers of circulating lymphocytes after therapy. These observations underline the possibility that immunologic processes can be affected by psychosocial interventions in patients with different types of cancer, at least in the early stages of the disease.

Breast cancer patients across different stages of their disease show variability in levels of immune parameters (Contreras Ortiz, Stoliar, 1986; Burford-Mason, Gyte, Watkins, 1989). In general, early stage breast cancer patients have a normal immune function, but as the disease progresses immune function becomes reduced (Contreras Ortiz et al., 1986; Burford-Mason et al., 1989). In addition, it has been found that progression of breast cancer is associated with physiologic adaptations that result in increased cortisol production (Drafta, Stroe, Schindler, Toedosiu, Gozariu, Drafta, 1981; Hays, O'Brian, 1989; Van der Pompe, Antoni, Heijnen, 1996). In view of these findings we hypothesize that the efficacy of an intervention program on endocrine and immune measures in breast cancer patients may depend on patients' endocrine and immune levels at the start of a program (Van der Pompe et al., 1996). The present study has been undertaken to explore whether the effect of a 13-week Experiential-Existential Group Psychotherapy (EEGP) program on endocrine and immune outcome is related to patients'respective baseline endocrine and immunological values.

Methods

Subjects

Breast cancer patients who had been treated for primary breast cancer and were diagnosed with either positive axilliary lymph nodes ($T_{1-3}\,N_{1-3}\,M_0$) or supraclavicular lymph nodes, skin or distant metastases ($T_{1-4}\,N_{1-3}\,M_1$) were included in the study. All patients received first-line endocrine treatment only (tamoxifen). No other type of treatment was administered throughout the course of this study. The group consisted of 23 curatively treated

breast cancer patients with positive lymph nodes, but without distant metastases (mean age=60.5; SD=6.1) and 8 patients diagnosed with breast cancer and distant metastases (mean=54.7; SD=10.8). Of the 15 originally assigned to the intervention group, 4 patients were excluded from the analysis because they were too ill to attend all sessions. Of the 16 assigned to the control condition, 3 patients were excluded because they were to ill to participate in post-treatment assessment or had died, 1 patient refused post-treatment assessment. The final sample consisted of 11 patients in the intervention group and 12 patients in the control condition.

A group of 15 age-matched healthy women (mean age=55.69; SD=4.27) were recruited through a newspaper advertisement asking for volunteers for the study "Stress, Immunity and Health in Women aged 50 to 65". For details about recruitment see Chapter 2.

Study procedure

Patients filled out a series of psychosocial questionnaires and two peripheral blood samples were collected for endocrine and immunological analyses after a rest period, one at 27 and one at 30 min following venipuncture. Patients were randomly assigned to either the experiential-existential group psychotherapy (EEGP) or waiting list control condition. The patients assigned to the control condition were informed that they would be offered the opportunity to complete the group psychotherapy program at the end of the study period. After the 13-weeks intervention period all patients were scheduled to complete psychosocial questionnaires and to give two blood samples for endocrine and immunological analyses.

Group psychotherapy condition

This intervention program is based on the principles of the experiential-existential therapy (Yalom, 1983). The intervention period consisted of 13 sessions held weekly for 2.5 hours. The groups were led by two psychotherapists who were experienced in conducting group psychotherapy with cancer patients. The theoretical background, the intervention goals, structure and format of the program as well as therapist qualifications are described in detail in Chapter 6.

Waiting-list control condition

Patients in the waiting-list control (WLC) condition received EEGP after a waiting period of four months. During this waiting period they did not receive any psychosocial or psychiatric treatment from sources outside the context of this study.

Demographic and biobehavioral measures

At the start of the study several demographic characteristics of the patients were assessed

including partnership status, number of children, education, and employment status. In addition, a set of biobehavioral factors such as cigarette smoking and caffeine or alcohol consumption were assessed wich are known to modulate immune function.

Psychosocial measures

Affective state measures included the Beck Depression Inventory (BDI), the State-Trait Anxiety Inventory (STAI), and the Profile of Mood States (POMS) (Beck, Ward, Mendelson, Mock, Erbaugh, 1961; Spielberger, 1980; McNair, Lorr, Droppleman, 1971). For details about the questionnaires see Chapter 6.

Endocrine measures

EDTA plasma was obtained by centrifugation (2000 *g*, 10 min at 4°C) and was frozen and stored at -20°C. Cortisol levels were determined using a fluorescence polarisation assay on a TDX analyzer (Abbott, USA). The intra- and interassay coefficients of variation were both 6%. Prolactin was measured with an immunoenzymetric system on an ES-600 analyzer (Boehringer Mannheim, Germany). The intra- and interassay coefficients were 4% and 6%, respectively. ACTH was determined with a radioimmunoassay: antiserum obtained from IgG Corporation USA, and ^{125}I-labeled ACTH from CIS Bioindustries, France. The intra- and interassay coefficients were <10% and 11%, respectively.

Immune measures

Subsets of peripheral blood cells

Lymphocytes and granulocytes/ml were determined in whole blood using an automated closed tube sampler (Technikon H1 system). Lymphocyte subset analysis was performed by incubating 100 µl of whole heparinized blood with one of the following combinations of conjugated monoclonal antibodies (Becton and Dickinson, USA): CD4, CD8, CD3 and CD16/56. Subsequently, red blood cells were lysed (lysing solution Becton and Dickinson) and washed with PBS containing 0.1% sodium-azide. Cells were analyzed using a Flow cytometer (FACSscan, Becton and Dickinson).

Natural killer cell activity (NKCA)

NKCA was determined in heparinized whole blood with some modifications of the original protocols (Ottenhof, Morales, Baines, 1981; Baron, Klimas, Fischl, Fletcher, 1985). K562 was used as a target cell line: cells were labelled with $Na^{51}CrO_4$ (3.7 MBq; Amaersham UK) for one hour at 37°C, 5% CO_2. After labelling cells were washed twice and resuspended in RPMI-1640 (Gibco USA). Undiluted blood (100ul) and serial dilutions in medium were dispensed into a 96 well round-bottom tissue culture plate. A fixed number of labelled K562 cells was added to each well (100ul; 10,000 cells per well). The plates

were centrifuged for 5 min (100 x g), and incubated at 37°C. After 4 hours, plates were centrifuged again and supernates (100 ul) of triplicate samples were counted in a gamma-counter for 4 min. Maximum ^{51}Cr release and spontaneous release were determined in wells containing 1% Triton-X100 or medium respectively. Specific ^{51}Cr release was calculated as follows: % release = (ER - SR)/(TR-SR) x 100, where ER = mean cpm experimental release, SR = mean cpm spontaneous release, TR = total release.

Proliferative responses

Lymphocyte proliferative responses were tested according to the method as described by Bloemena, Roos, Van Heijst, Vossen, and Schellekens (1989). Heparinized blood was diluted 10 x with RPMI-1640 supplemented with penicillin (100 IU per ml) and streptomycin (100 ug/ml). L-glutamine (2mM). 100 ul of the diluted blood was incubated with 50 ul of Phytohaemagglutinin (PHA, Wellcome Diagnostics, UK) in various dilutions or with 50 µl of Pokeweed Mitogen (PWM, Gibco USA) in 96 well round-bottom plates. Proliferative responses were determined after 4 days of culture (5% CO_2, 37°C) by measuring incorporation of ^{3}H-thymidine (37 KBq, Amersham UK), added 16-18 hours before harvesting the cultures.

Data analyses

Correlations were calculated between the two baseline endocrine and immunological measurements (at 27 and 30 min post-venipuncture). Since all immune and endocrine measures showed high correlations between baseline sampling points ($r > .70$), the 2nd time-point measurement (30 min) was used as the baseline value for data analysis, because at this time patients had a greater chance to recover from homeostatic dysregulation caused by insertion of the catheter (Harris, Cook, Warner, 1988).

Multiple regression analyses were used to explore whether the effect of EEGP on endocrine and immune measures can be predicted by the pre-intervention values corresponding to the outcome variable (Jöreskog, Sörben, 1977; Jöreskog, 1993). For any variable with a non-normal distribution an appropriate transformation was applied prior to conducting the analyses. To index the EEGP condition and the WLC condition, the variable 'Condition' was coded 1 for EEGP and coded 2 for WLC. Interaction terms were constructed consisting of the variable Condition multiplied by baseline endocrine or immune values. The standardized regression coefficient (ß) was used as a measure of relative importance of each predictor. The statistical level of significance was fixed at 0.05. Moreover, the variance inflation factor (VIF)-values (an index of multicollinearity) had to be smaller than 4 for a model to be retained (Glantz, Slinker, 1990). Thus, a regression model was considered plausible if the predictor variables and interaction term were statistically significant provided that the VIF-values were within acceptable limits.

The sequence of regression equations were run as follows: First, to examine the efficacy of EEGP on endocrine and immune measures the variable 'Condition' was entered. Second, to explore whether the effect of EEGP on endocrine and immune measures could

be predicted by the baseline values corresponding to the outcome variables, three different regression models were tested: in the first series of regression analyses the model consisting of the main term Baseline Value (either immune or endocrine) and the variable 'Condition' plus the interaction term Condition by Baseline Value was entered, in the second series of regression analyses the model consisting of the variable 'Condition' and the respective interaction term was entered, and in the third step only the interaction Condition by Baseline Value was entered.[1] In addition, correlations between changes (i.e., the arithmetic difference between a immune *or* endocrine measure taken post-treatment and a immune or endocrine measure taken at study entry) in outcome measures and changes in psychological distress measures were calculated.

In order to test for differences in baseline endocrine and immune values between metastatic breast cancer patients, early stage breast cancer patients in comparison with a norm-group of age-matched healthy women, we performed univariate analysis using Group (i.e., metastatic breast cancer patients, node-positive breast cancer patients, and age-matched healthy women) as the between groups factors. Where the univariate F-test was significant, Tukey's HSD procedure for post-hoc comparisons was performed to determine which pairs of groups were significantly different.

Differences between the groups with regard to demographic, biobehavioral, and psychosocial variables were tested with univariate analyses of variance (ANOVA) for continuous data and Kruskal-Wallis test for ordinal data.

Results

Demographic and biobehavioral variables

Most of the patients (81%) were married or lived with a partner; 77% had at least one or more children. The majority of our subjects (67%) were unemployed, and 48% had a high school education. There were no significant differences with respect to these demographic variables between the women assigned to the EEGP condition and those in the WLC condition. There were no significant group differences in either caffeine intake, cigarette smoking or alcohol consumption, hours of sleep, frequency of physical exercise, nor in their use of pain medication or tranquilizers between patients assigned to the EEGP condition and the WLC condition.

---

[1] Because the purpose of the present study is to *explore* whether the effect of EEGP on endocrine immune outcome is related to patients' respective baseline endocrine and immune values, we decided to explore different models. The models described in the manuscript appeared to be most plausible and relevant in predicting post-intervention endocrine and immune values. The satura ted model (i.e., the main term Baseline Value and Condition, and the interaction term Baseline Value by Condition) had to be rejected due to multicollinearity. The correlations between the baseline endocrine and immune values individually and in interaction with Condition were between .41 and .98, all p's < .05. These high significant correlations have hampered the estimation of the standardized regression coefficients (ß's).

Table 1: Descriptive statistics (means and standard dev iations) and testing (univariate analyses of variance) for dependent variables.

Variables	Metastastic	Node-positive	Controls	*F*
ACTH	33.00 (5.26)	37.23 (10.64)	30.20 (7.65)	2.10, p=.137 df=2,39
Cortisol	.70 (.20)	.49 (.17)	.29 (.08)	13.84, p=.000 [c] df=2,39
Prolactin	.24 (.32)	.11 (.03)	.15 (.10)	.24, p=.791 df=2,42
CD3%	46.14 (19.15)	55.39 (11.13)	64.93 (6.94)	6.00, p=.005 [b] df=2,42
CD4%	27.44 (10.23)	36.97 (9.69)	46.07 (10.99)	6.97, p=.003 [b] df=2,42
CD8%	19.29 (13.56)	25.83 (9.72)	24.11 (9.41)	1.18, p=.316 df=2,45
NK%	7.32 (7.42)	11.15 (4.79)	7.71 (3.79)	3.49, p=.040 [a] df=2,42
NKCA	44.40 (18.53)	46.42 (15.53)	57.25 (19.01)	2.25, p=.118 df=2,44
Proliferation PWM (100 µg/ml)	1268 (1319)	3552 (2867)	5354 (2801)	6.17, p=.004 [b] df=2,44

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

Psychosocial distress: baseline levels

In our sample, the scores on the BDI were within symptomatic ranges for depression for 27.3% of our population (Bouman, Luteijn, Albersnagel, Ploeg, 1985). The scores on State and Trait Anxiety Inventory (STAI-DY) were within the range of neurosis for 67.7% and 27.3% respectively of the breast cancer patients (Van der Ploeg, Defares, Spielberger, 1980). The scores on the POMS-TMD were for 72.7% of our population higher than those observed in women with severe sleep disturbances (Wald, Mellenberg, 1990). In summary, this group of breast cancer patients showed a broad distribution of women experiencing mild to moderate levels of psychological distress. There were no statistically significant differences in the BDI-, STAI- and POMS-TMD score between patients assigned to the EEGP condition and the WLC condition. Neither could we obtain differences in any of these psychosocial measures between node-positive breast cancer patients and those with distant metastases, which justified to a certain extent not taking the stage of the disease into account. Moreover, there were no statistically significant differences between breast cancer patients and healthy women in psychological distress levels.

Endocrine and immune measures: baseline values

In order to get insight in the patients' endocrine and immune status, their baseline endocrine

and immune values were compared with those of a group of age-matched healthy women. As summarized in Table 1, it appeared that breast cancer patients had significantly higher baseline cortisol values than the norm group consisting of age-matched healthy women. Metastatic breast cancer patients had significantly higher baseline cortisol levels than node-positive breast cancer patients, $p < 05$. There were no significant group differences in either baseline ACTH or baseline prolactin (PRL) levels.

With respect to the immune measures, metastatic breast cancer patients showed significantly lower percentages of CD3 and CD4 cells, and proliferative responses to PWM (100 μg/ml) than age-matched healthy women. Node-positve breast cancer patients had significantly lower percentage of CD4 cells than age-matched healthy women and higher percentages of NK cells than either metastatic breast cancer patients or healthy women (both $p's < .05$), but did not differ from either of these groups on other immune and endocrine measures. There were no group differences in total lymphocyte and CD8 cell percentages, and natural killer cell activity (NKCA).

These findings show that breast cancer patients, especially those diagnosed with distant metastases, differ significantly from healthy women. However, with the exception of baseline plasma cortisol levels and percentages of NK cells, there were no significant differences in the baseline levels of ACTH and PRL and no significant differences in the percentages of lymphocytes expressing CD3-, CD4-, CD8-associated markers, PWM-induced lymphocyte proliferation and NKCA between node-positive breast cancer patients and metastatic breast cancer patients. On the basis of these findings we decided to combine both groups of breast cancer patients and not to adjust for disease-stage in the regression analyses.

The effect of EEGP on endocrine and immune levels

As shown in Table 2 (column 2) the variable Condition was unrelated to most endocrine and immune measures. The positive regression weight of the variable 'Condition' in prediction of NK (CD16/56) percentages was marginally significant (ß = .38) indicating that the EEGP group had post-treatment a lower percentage of NK cells in the peripheral blood than the WLC group.

The effect of EEGP on endocrine and immune values adjusted for baseline values corresponding to the outcome variables

In the first series of regression analyses the model consisting of both main terms Baseline Value and Condition plus the Condition by Baseline Value interaction was tested. In the case of all dependent variables this model had to be rejected because the aforementioned conditions (see the paragraph on statistical analysis) were violated (data not presented).

Table 2: Prediction of ACTH, prolactin, cortisol and of lymphocyte and mononuclear cell phenotype percentages and NKCA and proliferation to PWM by the Treatment and by the baseline values corresponding to outcome variable

Dependent variables	Condition[1]		Condition[2]		ConditionxBaseline Value[2]		ConditionxBaseline Value[1]	
	ß[3]	p	ß[3]	p	ß[3]	p	ß[3]	p
ENDOCRINE								
ACTH	-.16	.58	-[4]	-	-	-	.06	.77
Cortisol	-.21	.36	.41	.09	.90	.00	.62	.00
Prolactin	.21	.34	-	-	-	-	.53	.01
IMMUNE								
CD3	-.19	.40	-	-	-	-	.32	.15
CD4	-.13	.58	-.75	.01	.88	.00	.35	.11
CD8	.06	.79	-.52	.01	.96	.00	.65	.00
CD16/56	.38	.08	-	-	-	-	.62	.00
NKCA	.29	.20	.27	.42	.03	.93	.22	.23
Proliferation to PWM (100 mg/ml)	-.22	.32	-.72	.00	.90	.00	.51	.02

[1] Both the main term and the interaction term are entered simultaneously in the regression equation; Condition is coded 1 for E EGP condition and coded 2 for waiting-list controls

[2] The main term Condition and the interaction term Condition x Baseline Value (corresponding to outcome measure) are entered separately in the regression equation; Condition is coded 1 for EEGP and coded 2 for waiting-list controls

[3] Standardized regression coefficient

[4] - is printed when the assumption VIF < 4 is violated.

In the second step of our regression strategy in which the main term Condition and the interaction term Condition x Baseline Value were entered in the regression model model, it turned out that post-treatment CD4 cell and CD8 cell percentages, and mitogen-induced proliferative responses to PWM were significantly predicted by both terms (Table 2, column 3). The non-parallel lines in Figure 1, based on the intercept and unstandardized regression coefficients (see for details of the calculation of this model Appendix A), show that patients in the EEGP group who had a high percentage of CD8 cells at study entry showed a lower percentage post-treatment than patients with high baseline levels in the WLC condition. Specifically, patients in the EEGP group who had, for example, 50% CD8 cells in the peripheral blood showed a reduction of approx. 28%, while the levels of patients in the WLC condition with similar baseline levels remained unchanged. The same pattern as was seen for percentages of CD8 cells did also occur for percentages of CD4 cells and mitogen-induced proliferative responses to PWM. This model appeared to be

less plausible with respect to the prediction of cortisol and implausible with respect to the prediction of ACTH, PRL and the cells expressing the membrane markers CD3, CD16/56 (NK) and NKCA.

In the third step the interaction term Condition x Baseline Values model (Table 2, Column 4) predicted significantly post-treatment PRL (ß = .53) and cortisol values (ß = .62). As evident in Figure 2 the non-parallel lines, which are based on the intercept and the unstandardized regression coefficients (see for details of the calculation of this model Appendix B), illustrate that patients in the EEGP group with relatively high baseline PRL values had lower post-intervention PRL levels than WLC's with high baseline values. Specifically, patients in the EEGP condition who showed, for example, a prolactin value of 0.70 IU/l displayed a reduction of approximately 63%, while those in the WLC condition with similar PRL levels at study entry displayed a reduction of 36% at the end of the study. Likewise, with regard to NK cell percentages, the significant interaction Condition x Baseline Value (ß = .62) shows that patients in the EEGP group with relatively higher baseline NK cell percentages had lower post-test values than their high baseline counterparts in the intervention group. Thus, the same pattern as was seen for PRL did occur for NK cell percentages

As can be seen in Table 2 this model also significantly predicted post-treatment CD8 cell percentages (ß = .65), and mitogen induced proliferation (PWM 100 μg/ml) (ß = .51). However, these findings are less valid because the interaction Condition x Baseline Value model forms part of the previously described model consisting of the main term Condition and the Condition x Baseline Value interaction which significantly predicted percentages of CD8 cells as well as mitogen induced proliferation to PWM.

To summarize, these preliminary results indicate that the efficacy of EEGP on several endocrine and immune measures depend largely on patients' immune and endocrine levels at study entry.

Correlations between psychological, endocrine and immune changes

To investigate the degree to which psychological distress changes paralleled immune and endocrine changes after the 3 month intervention period, we correlated changes in psychological distress levels with changes in endocrine and immune parameters in the combined groups. No significant correlations were found between changes in endocrine levels and changes in psychological distress measures.

Strikingly, alterations in Trait Anxiety were consistently and significantly positively related to changes in CD4 cell percentages and proliferation of peripheral blood lymphocytes to PWM (100 μg/ml), all p's < .05. Likewise, the BDI-depression scores changes were significantly and positively correlated with percentages of NK cells ($p < .05$) and POMS TMD-score changes were positively correlated with pre-post changes in proliferative responses to PWM (100 μg/ml) ($p < .01$). No significant correlations between psychological distress and percentages of CD8 cells were found.

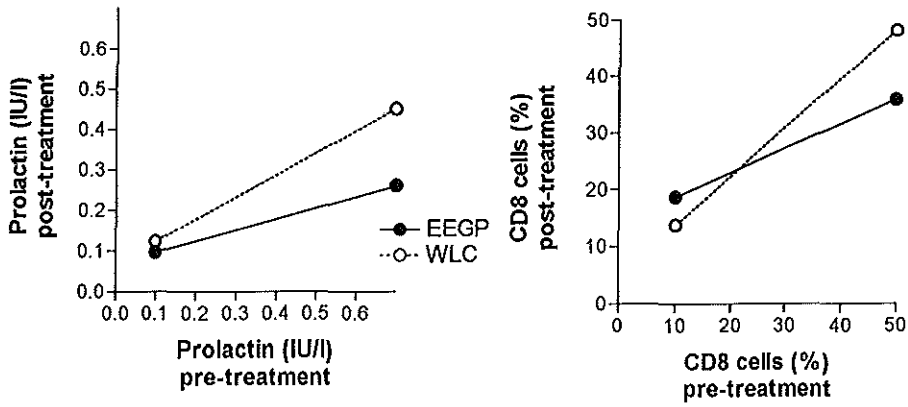

Figure 1-2. The effect of EEGP on plasma PRL levels and on percentages of CD8cells compared to the WLC condition.

Discussion

The present study has been undertaken to explore the effect of a short-term experiential-existential group psychotherapy program on endocrine and immunological parameters in breast cancer patients. The preliminary results show that this program had only a marginal effect on ACTH, PRL and cortisol plasma levels. But, interestingly, when we took the interaction between the condition and baseline levels into account, it appeared that EEGP patients with relatively high plasma PRL and cortisol values at study entry had lower post-treatment levels than their high baseline counterparts in the WLC. These findings seem to indicate that EEGP decreases the activity of the hypothalamic-pituitary-adrenal (HPA) system.

Equivalent to the endocrines, EEGP, when not adjusted for baseline values, did not have a significant effect on any of the immune measures. However, EEGP patients with a higher percentage of lymphocytes expressing membrane markers CD4, CD8 and the CD16/56 (NK) phenotype at baseline had lower percentages post-treatment than their counterparts in the WLC with similar levels. At first glance, this finding might be regarded to be unfavorable for EEGP if it is accepted tht lymphocytes, especially those expressing CD16/56 and CD8 markers, play a meaningful role in inhibiting the development and growth of (micro)metastases. However, we do not know whether NK cells in the peripheral blood compartment actually play a role in killing solid tumors. Increase of NK cell percentage may be just a sign of immune activation by the tumor or the chronic stress of the illness. It has been suggested that a lower percentage of NK cells and CD8 cells in the peripheral blood may be associated with a better prognosis

(Herberman, Wiltrout, Gorelik, 1987; Levy, Herberman, Whiteside, Sanzo, Lee, Kirkwood, 1990; Levy, Herberman, Lippman, D'Angelo, Lee, 1991). In addition, it has been proposed that the percentage of NK cells in the peripheral blood is a reflection of the host response to the degree of aggressiveness of the tumor process (Herberman et al., 1987). Consistent with this suggestion is the finding that node-positive breast cancer patients in this study had at baseline a significantly higher NK cell percentage in the peripheral blood than their healthy counterparts.

Changes in lymphocyte migration patterns resulting in changes in percentages of NK cells may also occur in response to neuroendocrine signals (Ottaway, Husband, 1992). In animals exposed to short-lasting stressors, the production of ACTH and concomittant release of circulating corticosteroids by the adrenal cortex have been correlated with changes in lymphocyte migration patterns (Ottaway et al., 1992). Thus, it could be that the lower percentage of lymphocytes after EEGP is the result of lower levels of glucocorticoids, possibly associated with a reduction of chronic stress levels. Consistent with this assumption is the finding that the extent of change in the percentage of cells expressing CD4 and NK markers was significantly and positively correlated with the extent of change in several mood states. Specifically, subjects who showed the largest increases in percentage of CD4 cells and in percentage of NK cells were those who reported the largest increases in trait anxiety levels and depression scores, respectively.

No significant changes emerged with respect to NKCA. We did find, however, that patients in the EEGP condition had post-treatment a smaller proliferative response to PWM (100 μg/ml) than patients in the WLC condition. This effect occurred only in patients with the highest basal PWM values. It is unclear yet whether the changes in PWM-induced proliferation are due to changes in selective redistribution of lymphocytes or due to altered endocrine mediators interfering with lymphocyte function (Kavelaars, Ballieux, Heijnen, 1990).

Tamoxifen has been shown to be associated with changes in number and function of human peripheral blood lymhocytes (Paterson, Grimshaw, Webster, 1989). The increases induced by tamoxifen in T lymphocyte counts and function have been claimed to be the cause of treatment success in breast cancer patients through immune mechanisms (Yonemoto, Schick, Albano, Fujisawa, Waldman, 1977). Since all patients received tamoxifen throughout the course of the study, it can not explain the present findings. There were also no differences in life-style factors such as caffeine intake, cigarette smoking, or alcohol consumption between the EEGP and the WLC group.

In contrast to our findings, Fawzy et al. (1990) found a higher percentage of NK cells after participation in an intervention program among post-surgical melanoma patients. A possible explanation might be that this study focussed on early stage cancer patients who were curatively treated and may have been free of tumor at the time of the intervention, whereas a large number of the patients in the current study were likely to suffer from a progressive disease at time of testing.

Our findings do support the notion that the efficacy of EEGP on endocrine and immune measures depends largely on patients' endocrine and immune levels at study entry. A disadvantage of the present study is that our sample was too small to distinguish between the effects in metastatic and node-positive breast patients, respectively. The

inclusion of more predictor variables in the regression models resulted in an unstable solution (VIF-values > 4) for all endocrine and immune outcome measures. However, except for plasma cortisol levels and percentages of NK cells, there were no statistically significant differences between node-positive breast cancer patients and metastatic breast cancer patients in any of the endocrine and immune values measured at the start of the study, which justified combining both groups of patients (see Table 1). Note the analyses are performed on transformed data (logarithmic transformation). The results of the present study can only be compared with those of other studies where the results are transformed in an equal manner.

Furthermore, it should be noted that the correlations between immune cell changes and psychological changes are based on the combined groups. Because women with metastatic disease may differ from those with more early disease in terms of the psychosocial stress they are dealing with, large scaled intervention studies need to be initiated to determine relations between psychological changes and immune cell changes at different stages of disease.

The results of the current study seem to indicate that EEGP may normalize the function of the endocrine and immune system. If this is confirmed by a replication on a larger scale, the results may be relevant for the management of breast cancer. EEGP seemed to reduce PRL and cortisol levels in those breast cancer patients displaying high plasma levels at study entry. It has been suggested that PRL as well as cortisol stimulate mammary tumor growth (Labrie, Poulin, Simard, Zhao, Labrie, Dauvois, Dumont, Hatton, Poirier, Merand, 1990; Moore, Thomas, Wang, 1986). How these hormonal effects as well as the stabilizing effects of EEGP on the redistribution patterns of some peripheral blood lymphocyte subsets in breast cancer patients relate to clinical outcome remains a challenge for future research. One of the interesting routes to investigate is whether EEGP will influence the formation of (micro)metastases possibly occurring directly after surgery (Baum, 1996). This may be 'the' critical period during which the immune system plays a pivotal role in eliminating tumor cells.

References

Andersen B (1992) Psychological interventions for cancer patients to enhance the quality of life J Consult Clin Psychol 66: 552-568

Baron GC, Klimas NG, Fischl MA, Fletcher MA (1985) Decreased natural cell-mediated cytotoxicity per effector cell in acquired immunodeficiency syndrome. Diagn Immunol 3:197-204.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561-571

Bloemena E, Roos MTL, Van Heijst JLAM, Vossen JMJJ, Schellekens PTA (1989) Whole-blood lymphocyte cultures. J Immunol Methods 122:161-167.

Bouman TK, Luteijn F, Albersnagel FA, Ploeg FA (1985) Enige ervaringen met de Beck Depression Inventory (BDI). Gedrag 13:13-24

Burford-Mason A, Gyte G, Watkins S (1989) Phytohaemagglutinin responsiveness of peripheral lymphocytes and survival in patients with primary breast cancer. Breast Cancer Res Treat 13:243-250.

Contreras Ortiz O, Stoliar A (1986). Immunological changes in human breast cancer. Eur J Gynaecol Oncol 6:502-514

Drafta DS, Stroe E, Schindler EE, Toedosiu T, Gozariu L, Drafta D (1981) Adrenal function in early and metastatic breast cancer patients. Endocrinology 19:115-121.

Fawzy F, Kemeny M, Fawzy N, Elashoff R (1990) A structured psychiatric intervention for cancer patients. II. Changes over time in immunological measures. Arch Gen Psychiatry 47: 729-735

Glantz SA, Slinker BK (1990) *Primer of applied regression and analysis of variance.* New York:McGraw-Hill

Harris B, Cook N, Warner N (1988) Anxiety and the demamethasone suppression test monitored with saliva. Biol Psychiatry 23:698-704.

Hays JH, O'Brian JT (1989) Edocrine and metabolic function in patients with neoplastic disease. In: RB Herberman ed. *Influence of the host on tumor development.* Dordrecht: Kluwer Academic Publishers

Herberman R, Wiltrout R, Gorelik E (1987) *Immune responses to metastases.* Boca Rotan:CRC Press

Jöreskog KG, Sörbon D (1977) Statistical models and methods for analysis of longitudinal data. In: DJ Aigner, AS Goldberger (eds). *Latent variables in socioeconomic models.* Amsterdam:North Holland

Jöreskog KG (1993) Testing statistical equation models. In: KA Bollen & JS Lang (eds). *Testing statistical equation models.* London:Sage

Kavelaars A, Ballieux RE, Heijnen CJ (1990) Differential effects of ß-endorphin on cAMP levels in human peripheral blood mononuclear cells. Brain Behav Immun 4:171- 179.

Labrie F, Poulin R, Simard J, Zhao HF, Labrie C, Dauvois S, Dumont M, Hatton AC, Poirier D, Merand Y (1990) Interactions between estrogens, androgens, progestins, and glucocorticoids in ZR-75-1 human breast cancer cells. Ann NY Acad Sci 575:130-148.

Levy SM, Herberman RB, Whiteside T, Sanzo K, Lee J, Kirkwood J (1990) Perceived social support and tumor estrogen/progresterone receptor status as predictors of natural killer cell activity in breast cancer patients. Psychosom Med 52:73-85

Levy SM, Herberman RB, Lippman M, D'Angelo T, Lee J (1991) Immunological and psychosocial predictors of disease recurrence in patients with early-stage breast cancer. Beh Med 67-75.

McNair DM, Lorr M, Droppleman LF (1971) *Profile of Mood States.* San Diego, Educational and Industrial testing service

Mohanty I, Nayak M, Nanda B (1991) Cell mediated immune status in breast carcinoma. Indian J Pathol Microbiol 34:1-6.

Moore JW, Thomas BS, Wang DY (1986) Endocrine status and the epidemiology and clinical course of breast cancer. Cancer Surv 3:537-559.

Mulder CL (1994) *Psycho-immunology and HIV infection. Biopsychosocial determinants of distress, immunological parameters, and disease progression in homosexual men infected with human immunodeficiency virus-1.* Rotterdam:Dissertation

Ottaway CA, Husband AJ. Central nervous system influences on lymphocyte migration. Brain Behav Immun 6:97-116.

Ottenhof PC, Morales A, Baines MG (1981) Quantitation of a whole blood assay for human natural killer cell activity. J Immunol Methods 425:305-318.

Paterson AG, Grimshaw AD, Webster DJT (1989)The predictive values of T-lymphocyte counts in breast cancer patients treated by endocrine manipulation. Eur J Surg Oncol 131-137.

Ploeg HM van der, Defares PD, Spielberger CD (1980) *Handleiding bij Zelfbeoordelings-vragenlijst (ZBV).* Lisse:Swets & Zeitlinger

Pompe G van der, Antoni MH, Visser A, Garssen B (1996) Adjustment to breast cancer: The psychobiological effects of psychosocial interventions. Pat Edu Coun 28:209-219

Pompe G van der, Antoni MH, Heijnen CJ (1996) Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. Psychoneuroendocrinology 21:361-374.

Schedlowski M, Jung C, Schimanski G, Tewes U, Schmoll HJ (1994) Effects of behavioral intervention on plasma cortisol and lymphocytes in breast cancer patients: an exploratory study. Psychooncology 3:181-187

Spielberger CD (1980) *Test manual for the State-Trait Anxiety Inventory -Stai form Y.* Palo Alto:Consulting Psychologist Press

Trijsburg R, Knippenberg F van, Rijpma S (1992) Effects of psychological treatment on cancer patients: A critical review. Psychosom Med 54:489-517.

Wald FDM, Mellenberg GJ (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). *Ned Tijdschr Psychol* 45:86-99.

Yalom I (1983) *Existential Psychotherapy.* New York: Basic Books.

Yonemoto RH, Schick P, Albano W, Fujisawa T, Waldman SR (1977) Immune responses in the treatment of advanced carcinoma of the breast. Effects of adrenalectomy. Arch Surg 112:991-996

Chapter 8

The effect of a group psychotherapy program on the reactivity to acute stress on the cardiovascular, endocrine and immune system in breast cancer patients

Gieta van der Pompe[1], Michael H. Antoni[2], Hugo J. Duivenvoorden[3], Alexander de Graeff[4], R.F.A. Simonis[5], Sytze G.L. van der Vegt [6] & Cobi J. Heijnen[7]

[1] Helen Dowling Institute for Biopsychosocial Medicine, Rotterdam, The Netherlands

[2] Erasmus University, Faculty of Medicine, Department of Medical Psychology and Psychotherapy

[3] University of Miami, Department of Psychology and Psychiatry, Florida, USA

[4] University Hospital Utrecht, Utrecht, The Netherlands

[5] Hospital 'Groene Hart', Gouda, The Netherlands

[6] Hospital Oudenrijn, Utrecht, The Netherlands

[7] University Hospital for Children and Youth "Het Wilhelmina Kinderziekenhuis', Utrecht

Abstract

In this study we explored the effect of a 13-week experiential-existential group psychotherapy program (EEGP) on the reactivity to a psychological stressor (speech task) in a cohort of breast cancer patients who were diagnosed with either axillary lymph node metastases or distant metastases. The task-induced stress was measured by its effect on cardiovascular, endocrine and immune functions. It appeared that the mean of the plasma prolactin values measured during the speech task of patients after participation in the EEGP were similar to those at study entry. However, the plasma prolactin values of the waiting list control (WLC) patients obtained during the speech task performed after the 3 month intervention period exceeded their PRL values measured at study entry. With respect to the immune system, the EEGP group showed a smaller increase in natural killer (NK) cell percentage induced by the speech task post-intervention than before EEGP participation. In contrast, the NK cell percentages of the WLC patients measured post-treatment were higher than those measured at study entry. Speech task-induced changes in proliferative responses to phytohaemagglutinin (PHA) increased post-intervention in patients in the EEGP condition. In contrast, the mean of the task-induced proliferative responses to PHA show pre-post intervention decreases in WLC patients. The results of this preliminary study suggest that the EEGP may influence the immune and neuroendocrine system of patients with breast cancer.

Introduction

Various behavioral intervention programs such as self-relaxation by progressive muscle relaxation training and autogenic training have been associated with alterations in cardiovascular and plasma hormone levels consistent with decreases in the activity of the sympathetic nervous system (SNS) and the hypothalamic pituitary adrenal (HPA) axis (Hoffman, Benson, Arns, Stainbrook, Landsberg, Young, Gill, 1981; Morrel, Hollandsworth, 1986; Mills, Schneider, Hill, Walton, Wallace, 1990). Recently, Schedlowski et al. (1994) found decreases in plasma cortisol levels and increases in numbers of circulating lymphocytes in early-stage breast cancer patients after participation in a 10-week behavioral intervention program consisting of relaxation and guided imagery. In a randomized trial with post-surgical patients with melanoma, the patients who were assinged to a 6 week structured psychiatric group intervention showed increases in NK cells compared to patients in the control condition (Fawzy, Kemeny, Fawzy, Elashoff, 1990). The authors showed that interventions aiming at reducing psychological stress may interfere with baseline levels of endocrine and immune reactivity.

The above described studies evaluated the effect of therapy programs based on the (cognitive-) behavioral therapy on endocrine and immune function in healthy subjects and cancer patients. Spiegel et al. (1989) developed an intervention program, predominantly based on the existential therapy and group support and tailored to the specific needs of metastatic breast cancer patients. Patients with metastatic breast cancer who participated in this group psychotherapy program lived significantly longer than patients randomized to standard oncologic care only. A plausible explanation for the increase in survival can be that this grouppsychotherapy program reduced the activity of the SNS and the HPA-axis and altered the immune function, which may have influenced tumor growth. We consider it, therefore, fruitful to investigate whether this type of therapy may influence the activity of the SNS and the HPA axis.

The present study has been undertaken to explore the effect of an experiential-existential group psychotherapy (EEGP) program on cardiovascular, endocrine and immune responses in breast cancer patients. This EEGP program is tailored to the specific needs of breast cancer patients, and is in line with the program evaluated by Spiegel et al. (1989) in their study with metastatic breast cancer patients. The patients had been treated with a curative intention for breast cancer and were diagnosed with either axillary lymph node metastases or distant metastases. In order to explore possible effects of EEGP on the cardiovascular, endocrine and immune system, we investigated the cardiovascular, endocrine and immune responses of breast cancer patients to an acute stressor, a speech task, immediately before and after EEGP. The rationale for using an acute laboratory stressor is that there is a growing literature supporting the notion that by using a reactivity model more reliable information can be obtained about the organizational level of the neuroendocrine system as well as the sensitivity of the immune system to the endocrine signals than by determining baseline values. Several studies have focused on cardiovascular and cellular immune changes in response to acute stressors such as mental arithmetic or speech task and reported reliable increases in blood pressure levels and circulating natural killer (NK) cells

and T-suppressor/cytotoxic (CD8) cells and natural killer cell cytotoxicity (NKCC) (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, 1991; Bachen, Manuck, Marsland, Cohen, Malkoff, Muldoon, Rabin, 1992; Herbert, Cohen, Marsland, Bachen, Rabin, Muldoon, Manuck, 1994). Additionally, a few studies have decribed changes in plasma cortisol values of healthy donors in response to brief stress challenges (Pettingale, Watson, Bhakri, Jones Tee, 1989; Berger, Bossert, Krieg, Dirlich, Ettmeier, Schreiber, Vahn Zerssen, 1987). Our findings in a recent study show that progression of breast cancer is associated with abnormal SNS and HPA responses in rest and in response to a speech task (see Chapters 2 and 3). Breast cancer patients, particularly those with distant metastases, had significantly higher basal heart rate values and basal cortisol levels than healthy age-matched women in addition to a blunted ACTH response to the speech task.

Methods

Patients

Breast cancer patients who had been treated for primary breast cancer and were diagnosed with either positive axillary lymph nodes (T_{1-3} N_{1-3} M_0) or supraclavicular lymph nodes, skin or distant metastases (T_{1-4} N_{1-3} M_1) were included in the study. All patients received tamoxifen, either as adjuvant treatment or as first-line treatment for distant metastatic disease. No other type of treatment was administered throughout the course of this study. The group consisted of 23 curatively treated breast cancer patients with axillary lymph node metastases, but without distant metastases (mean age=60.5; SD=6.1) and 8 patients diagnosed with breast cancer and distant metastases (mean 54.7; SD=10.8). Of the 15 originally assigned to the intervention-group, 4 patients were excluded from the analysis because they were too ill to attend all sessions. Of the 16 assigned to the control condition, 3 patients were excluded because they were too ill to participate in post-treatment assessment or had died, and 1 additional patient refused the post-treatment assessment. The final sample available for analysis consisted of 11 patients in the intervention-group and 12 patients in the control group.

Experimental manipulation

At their initial visit to our research facility all patients' blood pressure, height and weight and use of medication were recorded and they were scheduled for the reactivity session one week later. Patients were asked to follow a low monoamine and caffeine-free diet on the day prior to the reactivity session.

Reactivity session. Subjects arrived at the laboratory at 9 AM and were seated in a comfortable chair. A thromboresistent butterfly needle (model no NT5-19) was inserted into the antecubital vein of the arm opposite to the surgical site to prevent the possible effects of oedema and connected to a Dakmed Ambulatory Withdrawal Pump (model ML 6-5S3R) via a sterile heparinized tubing set. The experiment started with a 30 min pre-task rest period following venipuncture during which subjects watched a non-stressful movie.

Subsequently, they were asked to prepare a speech (for 4 min) about a potentially threatening personal situation ("How would you react if you were wrongly accused of stealing a coat in a shop?") and then recite it aloud (for 4 min) while being videotaped. The reactivity session ended with a 32 min post task period during which subjects again watched a non-stressfull movie. A total of 5 blood samples for endocrine and immune measures were collected: during the pre-task baseline period (27 and 30 min post-venipuncture), task performance (5 min post-task onset) and the post-task period (9 and 37 min post-task onset). The schedule of this session is presented in Chapter 2.

After the reactivity session, patients were randomly assigned to either the experiential-existential group psychotherapy (EEGP) program or to the control condition. The intervention group patients were scheduled for therapy sessions. Subjects assigned to the control condition were offered the opportunity for EEGP after a 13-week waiting period. At the end of the 13-week intervention or waiting period, both groups of patients were scheduled for a second (post-treatment) reactivity session. To minimize the effect of habituation to the reactivity task, we changed the content of the task from the stolen coat scenario to one in which the patient was asked "How would you react if you were wrongly accused of damaging a car". All measurements in each of the reactivity sessions were identical. The tasks used in the pre- and post-treatment reactivity sessions were well-standardized challenges previously shown to reliably elicit cardiovascular changes of similar magnitude consistent with sympathetic activation (Saab et al., 1992).

Group psychotherapy condition

This EEGP intervention program is based on the principles of the experiential-existential therapy tradition (Yalom, 1983). The intervention period consisted of 13 sessions held weekly for 2.5 hours. The groups were led by two psychotherapists who were experienced in conducting group psychotherapy with cancer patients. The theoretical background, the intervention goals, the structure and the format of the program as well as the therapist qualifications are described in detail in Chapter 6.

Waiting-list control condition

Patients in the waiting-list control (WLC) condition received EEGP after a waiting period of four months. During this waiting period they did not receive any psychosocial treatment from sources outside the context of this study.

Demographic variables and biobehavioral variables

At the start of the study several demographic characteristics of the patients were assessed including age, partnership status, number of children, education, and employment status. In addition, a set of biobehavioral factors such as caffeine and alcohol intake, cigarette smoking, hours of sleep, frequency of physical exercise, and the use of pain medication or tranquillizers were assessed that are known to modulate immune function.

Psychosocial measures

Affective state measures included the Beck Depression Inventory and the Profile-of-Mood-States (Beck, Ward, Mendelson, Mock, Erbaugh, 1961; McNair, Lorr, Droppleman, 1971). We measured emotional expression with a Dutch adaptation of the Emotional Expression Scale of Watson & Greer (1983) and the characteristics of the social support system of the patient with the social support scale of Van Sonderen (1991). For details about the questionnaires see Chapter 6.

Cardiovascular measures

Changes in heart rate, diastolic blood pressure and systolic blood pressure were measured during the reactivity sessions by Finapress (no. 4, Toegepast Natuurwetenschappelijk Onderzoek [TNO]). Heart rate ("interbeat interval") and systolic and diastolic blood pressure were sampled at each heart beat during the pre-task neutral period, speech task period, and post-task period. Data were recorded on magnetic tape for later analysis. Mean values per 30 seconds were determined for heart rate, and systolic and diastolic blood pressure during the last fourteen 30 seconds blocks of the pre-task period, all blocks recorded during the speech task, the first 2 blocks directly after the task (first post-task time point), and the last 8 blocks of sampling (second post-task time point).

Endocrine measures

Plasma was obtained by centrifugation of EDTA blood (2000 *g*, 10 min at 4°C) and was frozen and stored at -20°C. Cortisol levels were determined using a fluorescence polarisation assay on a TDX analyzer (Abbott, USA). The intra- and interassay coefficients of variation were both 6%. Prolactin was measured with an immunoenzymetric system on an ES-600 analyzer (Boehringer Mannheim, Germany). The intra- and interassay coefficients were 4% and 6%, respectively. ACTH was determined by a radioimmunoassay: antiserum was obtained from IgG Corporation USA, and ^{125}I-labeled ACTH from CIS Bioindustries, France. The intra- and interassay coefficients were <10% and 11%, respectively.

Immune measures

Immune assays included determination of percentages of specific lymphocyte subsets, lymphocyte proliferation to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) and natural killer cell activity (NKCA). These immune assays are described in detail in Chapter 7.

Statistical analysis

Predictive mean matching was used for estimating missing values of outcome measures (in case <10%) (Little, 1988).

Differences between patients in cardiovascular, endocrine and immune responsivity to the task measured before and after the intervention period assigned to the group psychotherapy program (EEGP) and those in the control condition were analyzed. The method used was a multivariate analysis of variance (MANOVA) for repeated measurements. This was a 2 (Condition: EEGP vs. WLC) x 2 (Time point: pre-EEGP [PRE] vs. post-EEGP [POST]) x 5 (Task: time points within a reactivity session [T1,T2,T3,T4, and T5]) design. If a variable had a nonnormal distribution an appropriate transformation was applied prior to conducting the analyses.

The strategy for summarizing the findings will be as follows: All cardiovascular, hormonal and immunological outcome measures that yield a significant second order interaction Condition x Time Point x Task will be described first. If a second order interaction is significant for a given outcome variable, then the first order interaction Condition x Time Point is no longer considered valid. Only if the second order interaction is not statistically significant, the significant first-order interaction will be described.

In addition, correlations between change scores of outcome measures and change scores of psychological parameters measured before and after the intervention. In case of a significant first order interaction change scores are calculated by subtracting the mean values measured during the reactivity session before the intervention from the mean values measured during the reactivity session after the intervention period ($m\text{POST}_{(T1,T2,T3,T4,T5)}$ - $m\text{PRE}_{(T1,T2,T3,T4,T5)}$). In case of a second order interaction change scores are calculated by subtracting the acute stress-induced change at the second time point from the change at the first time point (i.e., $\Delta\text{POST}_{(T2-T1)} - \Delta\text{PRE}_{(T2-T1)}$).[1] Change scores of psychological distress, expression of emotion and social support are calculated by subtracting the post-treatment levels from the pre-treatment levels.

Results

Demographic and biobehavioral variables

Most of the patients (81%) were married or living with a partner, and 77% had one or more children. The majority of our patients (67%) was unemployed, and 48% were high school educated. There were no significant differences in these demographic variables between the EEGP condition and the WLC condition. There were also no significant group differences in either caffeine intake, cigarette smoking or alcohol consumption, hours of sleep, frequency of physical exercise, nor on their use of pain medication or tranquilizers.

[1] Since all endocrine and immune measures showed high significant correlations between baseline sampling points (T1 and T2), the second time point (T2) was used as the baseline value for calculating change scores

Table 1: Means (and sd) of diastolic, systolic blood pressure and heart rate during speech task in EEGP patients and WLC patients pre-post treatment

	EEGP		WLC			CxTxT[1]	CxT[2]
Time points	PRE	POST	PRE	POST	NORM - GROUP	F p	F p
	DBP						
T1	78.6 (10.8)	77.5 (15.2)	79.5 (15.1)	75.3 (12.3)	70.4 (6.7)	1.99 .11	.09 .76
T2	78.7 (10.9)	77.3 (15.3)	79.3 (10.9)	75.1 (12.3)	71.4 (7.5)		
T3	93.1 (10.9)	85.5 (14.3)	95.2 (17.9)	94.2 (12.6)	84.7 (12.3)		
T4	84.1 (10.9)	79.8 (14.3)	89.7 (18.5)	84.8 (12.8)	77.2 (10.7)		
T5	77.2 (10.3)	75.3 (15.3)	78.8 (17.6)	71.5 (11.8)	68.1 (14.0)		
	SBP						
T1	133.3 (21.4)	125.6 (26.9)	124.4 (20.0)	128.2 (19.8)	125.3 (19.1)	1.05 .39	.63 .44
T2	134.3 (23.9)	125.9 (27.8)	126.7 (22.9)	145.5 (72.2)	129.3 (18.4)		
T3	167.0 (36.5)	157.4 (32.9)	173.9 (36.7)	160.8 (33.2)	156.4 (30.7)		
T4	152.6 (29.7)	141.5 (34.8)	156.1 (40.3)	152.1 (34.6)	144.7 (26.7)		
T5	138.4 (24.5)	138.2 (24.6)	134.0 (24.4)	133.2 (22.0)	131.4 (16.3)		
	HR						
T1	69.2 (12.8)	73.2 (25.4)	66.5 (10.9)	69.0 (13.6)	61.8 (7.8)	1.30 .28	.31 .59
T2	69.5 (13.1)	72.9 (25.7)	66.4 (11.6)	68.1 (13.2)	63.7 (8.2)		
T3	88.1 (14.5)	98.1 (39.4)	88.3 (18.8)	83.0 (15.5)	77.2 (12.6)		
T4	81.2 (14.1)	84.2 (31.9)	78.6 (16.6)	80.3 (18.4)	72.7 (10.8)		
T5	72.7 (12.9)	79.5 (26.4)	71.6 (12.2)	71.8 (20.7)	65.2 (8.0)		

[1] Condition x Time Point x Task interaction
[2] Condition x Time Point interaction

The effect of EEGP on cardiovascular, endocrine and immunologic responses to the speech task

First, we investigated whether there were differences in changes in diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate (HR) in response to the speech task measured before and directly after the 3 month intervention-period between patients assigned to the EEGP and those in the WLC condition. The current results show (see Table 1) that the repeated measures MANOVA's for DBP, SBP and HR yielded no significant Condition x Time Point x Task interactions *or* Condition x Time Point interactions. Therefore the EEGP intervention did not appear to influence participants' cardiovascular responsivity to the behavioral challenge.

As can be seen in Table 2, the Condition x Time Point x Task interactions were not statistically significant for ACTH, cortisol or prolactin (PRL). However, there was a significant Condition x Time point interaction for PRL. This finding means that there were differences in the mean PRL values measured during the speech task after the 3 month intervention period as compared to those measured during the speech task at the start of the study between the EEGP group patients and the WLC group patients, $F(1,20)= 5.57$, $p < .05$. As evident in Figure 1, in WLC patients the *mean* PRL values measured during the

Table 2: Means (and sd) of ACTH, cortisol and prolactin during speech task in EEGP patients and WLC patients pre-post treatment

	EEGP		WLC			CxTxT[1]	CxT[2]
Time points	PRE	POST	PRE	POST	NORM-GROUP	F p	F p
	ACTH						
T1	38.6 (8.4)	39.2 (9.1)	37.5 (11.0)	35.3 (10.6)	31.2 (8.8)	1.35 .26	.11 .74
T2	37.9 (7.9)	37.9 (9.6)	36.7 (11.6)	35.7 (10.9)	30.2 (7.1)		
T3	39.8 (8.7)	38.1 (9.4)	39.6 (13.9)	37.5 (11.7)	31.8 (10.0)		
T4	38.7 (8.4)	37.4 (9.3)	39.9 (14.1)	37.4 (11.2)	35.3 (14.0)		
T5	36.6 (6.3)	34.3 (7.2)	36.3 (12.9)	33.9 (11.1)	29.3 (6.6)		
	Cortisol						
T1	.57 (.24)	.53 (.24)	.48 (.12)	.42 (.10)	.29 (.08)	.69 .60	.05 .83
T2	.57 (.24)	.53 (.26)	.47 (.12)	.41 (.09)	.29 (.08)		
T3	.54 (.21)	.49 (.26)	.45 (.11)	.40 (.09)	.27 (.06)		
T4	.53 (.21)	.52 (.23)	.47 (.13)	.41 (.09)	.27 (.06)		
T5	.45 (.17)	.43 (.23)	.40 (.13)	.32 (.08)	.25 (.07)		
	Prolactin						
T1	.11 (.03)	.11 (.02)	.10 (.02)	.12 (.03)	.15 (.11)	1.25 .30	5.57 .03[b]
T2	.11 (.02)	.11 (.02)	.10 (.02)	.12 (.04)	.15 (.10)		
T3	.11 (.03)	.10 (.03)	.10 (.02)	.12 (.04)	.15 (.10)		
T4	.11 (.03)	.11 (.02)	.10 (.03)	.13 (.05)	.16 (.11)		
T5	.10 (.03)	.10 (.03)	.09 (.02)	.11 (.03)	.13 (.08)		

[1] Condition x Time Point x Task interaction
[2] Condition x Time Point interaction
[a] $p < .10$; [b] $p < .05$; [c] $p < .01$

speech task (T1, T2, T3, T4, and T5) increased by 22% over baseline (PRE) [($m\text{POST}_{(T1,T2,T3,T4,T5)}$ - $m\text{PRE}_{(T1,T2,T3,T4,T5)}$)/$m\text{PRE}_{(T1,T2,T3,T4,T5)}$ x 100].[2] In contrast, the mean PRL values measured during the reactivity session in EEGP patients after the intervention period were essentially similar (i.e., decrease of 2% over baseline (PRE)) to the mean of PRL values measured during the reactivity session at study entry. The Condition x Time Point interactions were not statistically significant for either ACTH or cortisol.

With respect to the effect of EEGP on distribution of peripheral blood cells (see Table 3), the Condition x Time-points x Task interaction did reach significance for percentages of NK cells, $F(4,80)=3.05$, $p < .05$. This significant interaction indicates that there were differences in the NK cell percentages measured during the speech task (T1 thru T5) after the intervention period (POST) as compared to those measured during the speech task (T1 thru T5) at the study entry (PRE) between the EEGP patients and the WLC patients. As can be seen in Figure 2[2], at study entry EEGP patients showed increases of 80% and

[2] A significant first order interaction is displayed as the mean of the repeated endocrine or immune measures (T1, T2, T3, T4 and T5) in response to the task. A significant 2nd order interaction is in the present study displayed on a point-by-point basis only.

97% of the baseline (T2) in NK cell percentages in response to the speech task (T3) and at the first post-task time-point (T4). After the 3 month intervention period, there was only an increase in NK cell percentages of 41% and 67%. In contrast, patients in the WLC group showed at study entry increases in NK cell percentages of 39% and 46% of the baseline (T2) in response to the task on T3 and T4. After 3 months the NK cell percentages increased with the same magnitude (38% and 53%, respectively).

The results in Table 3 show also that the repeated measures MANOVA's for percentages of total lymphocytes and lymphocytes expressing CD3, CD4, CD8 markers yielded no significant Condition x Time Point x Task interactions nor Condition x Time point interactions. With respect to the functional measures, the repeated measures MANOVA yielded a significant Condition x Time Point x Task interaction for NKCA, $F(4,80)=3.48$, $p < .05$ (see Table 3) suggesting that the NKCA of patients in the EEGP and the WLC decreased as compared to the NKCA at study entry. However, the decrease in NKCA appeared to be more profound in patients assigned to EEGP. We hypothesized that this effect of the intervention on NKCA is related to changes in percentages of NK cells. To test this hypothesis, a multivariate analysis of covariance (MANCOVA) model for repeated measures was used for NKCA with NK cell percentage as covariate. It was found that when adjusted for NK cell percentages, the Condition x Time Point x Task interaction for NK cell percentage was no longer significant, indicating that the differences in NKCA can be attributed to changes in NK cell numbers.

Additionally, the Condition x Time Point x Task interaction did not reach significance for the phytohaemagglutinin (200 µg/ml) proliferative response of peripheral blood leukocytes. However, the Condition x Time Point interaction was significant for the PHA proliferative response (200 µg/ml). This finding suggests that there were differences in the *mean* PHA proliferative responses measured during the reactivity session (T1 thru T5) after the intervention period (POST) as compared to those measured during the reactivity session (T1 thru T5) at study entry (PRE) between the EEGP group and the WLC group, $F(1,20)=3.12$, $p < .10$. As illustrated in Figure 3, the mean PHA proliferative responses of patients in the EEGP group increased by 14% over baseline (PRE) at the end of the study, while the mean PHA proliferative responses of patients in the WLC group decreased by 33% from baseline (PRE) $[(m\text{POST}_{(T1,T2,T3,T4,T5)} - m\text{PRE}_{(T1,T2,T3,T4,T5)} / \text{mPRE}_{(T1,T2,T3,T4,T5)} \times 100]$ at the end of the study.

These results suggest that participation of breast cancer patients in this EEGP program alter the endocrine and immune reactivity to the speech task.

Correlations between changes in acute stress-induced responses and changes in psychological function

To investigate the degree to which (significant) changes in acute stress-induced plasma PRL levels and percentage of NK cells and PHA-induced proliferation paralleled changes in psychological distress, emotional expression and social support after the 3 month intervention period, correlations were conducted between changes in acute stress-induced responses and changes in the psychological parameters.

Table 3: Means (and sd) of lymphocyte and mononuclear cel phenotype percentages during speech task in EEGP patients and WLC patients pre- and post-treatment

	EEGP		WLC			CxTxT[1]	CxT[2]
Time point	PRE	POST	PRE	POST	NORM-GROUP	F p	F p
	Total count						
T1	31.8 (10.6)	33.0 (10.5)	37.5 (11.0)	34.7 (10.8)	30.4 (8.7)	.67 .61	.23 .92
T2	30.8 (10.6)	32.6 (9.5)	38.5 (12.0)	32.9 (7.9)	29.7 (8.7)		
T3	34.5 (11.1)	32.3 (10.8)	39.9 (12.6)	33.8 (8.6)	32.3 (8.7)		
T4	34.1 (9.4)	33.7 (10.6)	40.5 (15.3)	34.3 (8.5)	34.5 (8.2)		
T5	30.4 (9.1)	33.4 (12.3)	36.2 (11.8)	32.9 (9.4)	30.8 (8.4)		
	CD3 cells						
T1	52.2 (13.2)	57.6 (12.0)	54.3 (16.1)	56.9 (12.1)	65.2 (7.7)	.53 .72	1.34 .26
T2	53.8 (12.9)	59.0 (9.6)	53.6 (14.9)	54.9 (12.4)	64.9 (6.8)		
T3	49.1 (10.4)	56.1 (11.6)	48.4 (18.7)	53.0 (12.1)	59.8 (8.1)		
T4	47.5 (11.9)	54.6 (12.0)	49.8 (15.6)	51.9 (13.3)	60.6 (8.1)		
T5	52.7 (13.6)	57.9 (13.5)	54.3 (13.9)	54.0 (14.1)	65.9 (5.6)		
	CD4 cells						
T1	36.7 (11.6)	40.3 (12.3)	35.0 (10.5)	38.8 (10.9)	44.8 (11.0)	.75 .56	.10 .76
T2	36.2 (11.6)	41.5 (12.5)	35.0 (11.1)	38.7 (11.1)	46.1 (11.2)		
T3	32.1 (9.5)	38.9 (11.7)	31.5 (10.9)	36.1 (10.1)	40.9 (10.5)		
T4	29.7 (9.3)	36.4 (10.4)	29.5 (9.3)	33.9 (10.1)	39.7 (11.0)		
T5	36.0 (11.5)	41.0 (10.9)	34.9 (11.7)	39.7 (10.9)	47.9 (8.5)		
	CD8 cells						
T1	25.5 (9.6)	25.7 (10.3)	24.6 (11.6)	26.3 (7.4)	25.0 (10.1)	.53 .71	1.16 .29
T2	24.9 (10.4)	25.1 (10.5)	24.5 (10.6)	26.1 (7.9)	24.1 (9.4)		
T3	28.9 (11.2)	28.0 (11.4)	26.8 (13.0)	28.8 (8.4)	27.6 (9.8)		
T4	29.6 (11.7)	29.1 (11.5)	27.7 (12.3)	30.2 (9.0)	28.6 (10.4)		
T5	25.7 (10.5)	25.8 (9.9)	23.4 (10.3)	25.8 (7.8)	22.7 (7.5)		
	NK cells						
T1	9.7 (5.0)	8.9 (3.9)	12.4 (6.0)	12.3 (5.2)	8.0 (3.9)	3.05 .02[b]	.66 .43
T2	9.2 (4.9)	8.5 (3.5)	12.4 (5.4)	12.1 (5.6)	7.7 (3.7)		
T3	16.6 (8.5)	12.0 (5.5)	17.2 (9.2)	16.5 (7.8)	13.6 (6.5)		
T4	18.1 (8.9)	14.2 (6.8)	18.1 (8.9)	18.1 (8.8)	15.2 (7.2)		
T5	9.8 (4.6)	9.7 (4.5)	9.8 (4.6)	12.5 (8.3)	7.5 (3.2)		
	NKCA						
T1	30.8 (10.7)	19.7 (5.7)	37.7 (14.6)	23.9 (7.9)	40.8 (17.3)	3.48 .01[b]	.01 .94
T2	29.7 (9.1)	21.1 (5.6)	37.8 (15.2)	26.1 (8.1)	40.3 (17.1)		
T3	42.1 (12.1)	24.8 (9.3)	49.7 (16.8)	33.5 (12.6)	56.0 (16.7)		
T4	45.4 (11.9)	33.0 (11.2)	52.3 (16.2)	36.9 (12.8)	54.7 (14.6)		
T5	33.1 (10.8)	24.2 (7.7)	44.6 (14.7)	24.8 (11.8)	37.9 (12.9)		
	PHA						
T1	22708(11401)	24843(22307)	21075(14240)	13910(11565)	34094(15237)	.58 .68	3.12 .09[a]
T2	21475(11422)	26439(21942)	20649(14478)	14789(10075)	30707(14413)		
T3	22273(10356)	22907(22178)	21012(11796)	14203(9554)	29052(14275)		
T4	21274(11036)	24655(14603)	21256(14505)	13956(8129)	29224(16033)		
T5	23131(13840)	27288(23937)	24011(16487)	15374(10567)	30969(12697)		

[1] Condition x Time Point x Task interaction

[2] Condition x Time Point interaction

[a] $p < .01$; [b] $p < .05$; [c] $p < .01$

No significant correlations were found between changes in above mentioned biological parameters and changes in psychological distress measures and availability of social support. However, changes in emotional expression appeared to be significantly related to changes in the acute stress-induced responses. Changes in mean plasma PRL levels were significantly negatively related to changes in Emotions-Out ($r = -.37$, $p < .05$). Increases in mean plasma PRL levels as observed in patients who did not receive EEGP coincide with a decrease in emotional expression. A similar pattern emerged for changes in acute stress-induced NK cell responses. Smaller increases of NK cells in the peripheral blood coincided with increases in Emotions-Out ($r = -.58$, $p < .01$). Additionally, change in PHA-induced proliferation were significantly negatively related to changes in control of emotion ($r = -.47$, $p < .05$).

Discussion

This study describes the effect of a short-term EEGP program on changes in cardiovascular, endocrine and immunological variables in response to a behavioral challenge test in breast cancer patients. We descibed in the Chapters 2 and 3 that this test elicited significant changes in cardiovascular, endocrine and immune responses. Specifically, marked increases were found in cardiovascular parameters (DBP, SBP and HR), but rather small increases in ACTH and decreases in plasma cortisol and plasma PRL in breast cancer patients as a group. On the level of the immune system, task-induced increases were observed in the total lymphocyte count, and in the percentage of lymphocyte cells expressing the CD8 and CD16/56 markers, as well as of NKCA. In addition, decreases in the percentage of CD4 cells and the proliferative response to PHA and PWM were found. These results are in line with earlier established findings on acute-stress induced changes obtained in healthy men (Naliboff et al., 1991; Bachen et al., 1992; Herbert et al., 1994; Pettingale et al., 1989; Berger et al., 1987).

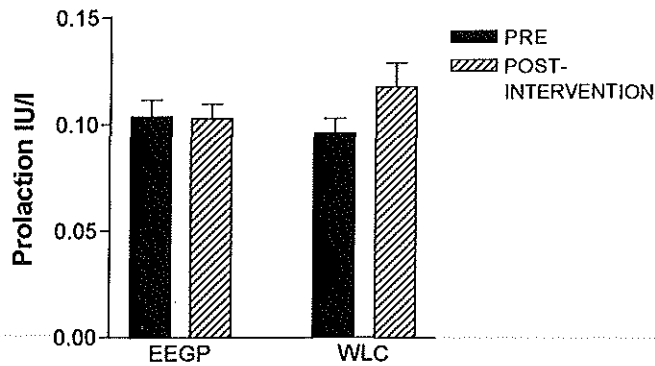

Figure 1. The effect of EEGP on the mean plasma prolactin levels measured during the reactivity session as compared to the WLC condition

Our present results show that there are no differences in the pre-post task-induced DBP, SBP and HR responses of patients in the EEGP and those in the WLC condition. The DBP, SBP and HR changes measured before and after the intervention period in patients assigned to EEGP as compared to that in the WLC condition, suggest a similar level of activation of the sympathoadrenal system in both groups. Neither did we found significant differences between the task-induced ACTH and cortisol changes of patients after participation in EEGP and those in the WLC condition. Interestingly, we could observe differences in the level of PRL. The plasma PRL levels obtained during the reactivity session of patients after participation in the EEGP were similar to their PRL levels during the reactivity session at the start of the study. In contrast, the PRL values of WLC patients at the time points mentioned above actually exceeded their PRL levels observed at study entry. Diagnostically, high PRL values have been associated with a poorer prognosis (McLeod, Scapagnini, Thorner, 1985; Barni, Lissoni, Tancini, Crisino, Paolorossi, Rovelli, Fumagalli, Ferri, 1986; Ayala, Lissoni, Archili, Barni, Roveda, Giacomelli, Bissi, 1990). Therefore the fact that no further increases in PRL were observed in time in the group of patients that were treated with EEGP, suggest that this intervention may have a favorable effect on the progression of the disease.

As presented in Table 2, the PRL values measured before and after the intervention of patients in the EEGP and the WLC group were lower than those obtained in healthy women. These lower task-induced PRL values of our patients may be explained by the use of tamoxifen. Tamoxifen has been shown to reduce baseline PRL levels and the PRL increase in response to thyrotropin-releasing hormone (TRH) in postmenopausal women with advanced breast cancer (Van der Geest, Sluiter, Doorenbos, Reitsma, 1983). Notwithstanding the fact that Tamoxifen administration reduced PRL levels, the PRL levels of patients who did not join the intervention increased after the intervention period.

On the level of the immune system, we came across some interesting findings. Breast cancer patients had a higher (not significant) response to the speech task with respect to NK cell percentages than healthy women at the time before the intervention (see Table 3). After the intervention, patients in the EEGP group had a lower reactivity. In contrast, the task-induced NK cell response of patients in the WLC group was of a similar magnitude as the NK cell response measured at baseline. Although we found similar increases in all cardiovascular variables for both the patients in the EEGP group and those in the WLC group at study entry and after the intervention period, the smaller increases in NK cell percentages of our patients after participation in the EEGP program may be due to changes in the sensitivity of the NK cells for catecholamines.

Catecholamines can bring about changes in NK cells redistribution by binding to high affinity $ß_2$-adrenergic receptors (Mills, Dimsdale, 1993). Elevated levels of catecholamines have been shown to result in a decreased sensitivity of $ß_2$-adrenergic receptors (Motulsky, Cunningham, De Blasi, Insel, 1986), possibly resulting in a low or no increase (i.e., hyporeaction) in NK cells in response to acute stress. Alternatively, normal or low resting catecholamines have been associated with a high density or sensitivity of $ß_2$-adrenergic receptors (Pacak, Nedvidkova, Horvath, Frantik, Husek, Pacovsky, 1989),

possibly resulting in a high increases (i.e., hyperreaction) in NK cells in response to acute stress. The fact that breast cancer patients had a higher NK cell response to the speech task than healthy women can be explained by the suggestion that the NK cells of breast cancer patients were highly sensitive for acute stress. Thus, it may be plausible that the smaller increases in NK cells in response to the speech task observed in our patients after EEGP may be due to a decreased sensitivity of NK cells for acute stress.

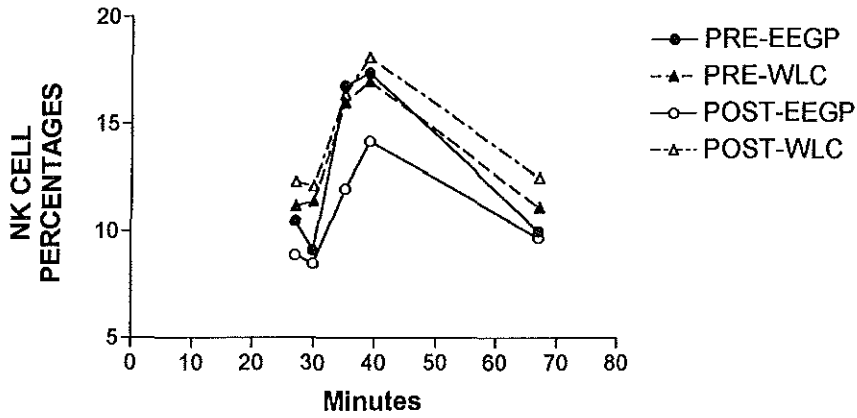

Figure 2. The effect of EEGP on NK cell percentages in response to the speech task as compared to the WIC condition

Chronic stress have been shown to affect redistribution of NK cells in response to acute stress (Benschop et al., 1994). Thus, the smaller increases in NK cells to acute stress may be associated with a reduction in chronic stress, which we have observed in patients in the EEGP group, particularly in those who presented high stress levels at the entry of the study (data not shown). In the current study a lower NK cell reactivity was significantly and negatively related with expression of emotion, which, in turn, was negatively correlated with fatigue (POMS subscale; $r = -.35, p < .05$). The fact that cardiovascular responses were not affected by EEGP can be explained by the suggestion that blood pressure levels are less likely to be affected by psychological stress and intermediate variables such as expression of anxiety and fear.

We also found a significant condition by time point by task interaction for NKCA which suggests that the pre-treatment task-induced changes in NKCA were significantly different from those after 3 months both for the EEGP and WLC group. However, after adjusting for changes in NK cell percentages this interaction was no longer valid. This may indicate that the differences in NKCA can be attributed to changes in distribution in NK cells.

Additionally, we found that at study entry patients assigned to EEGP and those in the WLC condition displayed similar mean PHA-proliferation responses to the speech task.

After the intervention period, EEGP patients increased in their responses, while WLC patients had decreased mean PHA-proliferation responses to the task. Research on the clinical importance of immune system variables in predicting disease progression has thus far been inconclusive (Hacene et al., 1986). However, diminished response to mitogen-stimulation has been associated with an unfavorable prognosis in several studies (Burford-Mason et al., 1989; Seremet et al., 1992). However, more research is needed to show that increases in lymphocyte proliferative response can be regarded as a favorable effect of the EEGP program. Our results may suggest that EEGP may interfere with the capacity of T lymphocytes to react to neuroendocrine mediators induced by the speech task. Since it is plausible that this effect will also occur after exposure to stressors in daily life, the EEGP may influence the net T cell activity.

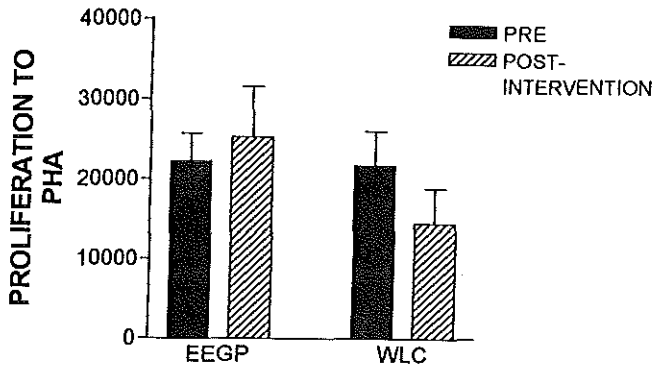

Figure 3. The effect of EEGP on mean PHA-proliferation measured during the reactivity sessions as compared to the WLC condition

EEGP aims at facilitating grief by encouraging patients to express their emotions in a non-judging supportive environment. As described above, smaller increases in NK cells in response to acute stressor observed in the EEGP group coincided with a decrease in emotional expression. NK cell reactivity of WLC's did not change and showed in addition to that increases in PRL values, which appeared to be related to decreases in expression of emotion. Moreover, subjects who showed the largest increases in PHA-induced proliferation were those who reported the largest decreases in control of emotion ($r = -.47$, $p < .05$). These data seem to suggest that EEGP may influence immune function of breast cancer patients by altering the tendency to suppress emotions.

A limitation of the present study is that our sample is too small to establish significant differences in reactivity between breast cancer patients with axillary lymph node metastases and breast cancer patients with distant metastases. We, therefore, would like to stress that this study is exploratory. However, except for plasma ACTH ($F[1,29]=3.43, p$

= .02) and percentages of CD8 cells (F[1,29]=2.93, p = .04, there were *no* statistically significant differences between breast cancer patients with lymph node metastases and those with distant metastases in any of the cardiovascular, endocrine and immune changes in response to the task measured at the start of the study. These findings may justify that we did not control for disease stage in the MANOVA model. Anyhow, the division of patients with axillary lymph node metastases from those with distant metastases can be to a certain extent arteficial because the absence of a clinical observable recidive will not guarantee that the first group of patients has no distant metastases at the time of testing. Of our cohort, five patients originally diagnosed with axillary lymph node metastases, who were assigned to either the EEGP group or the WLC group, appeared to have distant metastases after the conclusion of this study.

In addition, in this study the use of multiple statistical tests may cause a high risk of inflation of type 1 error. In general the rule of thumb is that for one out of every twenty tests, one is a product of chance. However, we found three significant tests of thirteen, which is more than can be expected by chance. Another caveat is in order. Habituation to the speech task may have occurred, since the same task has been offered more than once in the present study. This subsequently might have influenced the response to the task measured after the intervention. However, habituation has probably not influenced the results of this study because the cardiovascular changes to the task were similar before and after the intervention.

In conclusion, the preliminary findings of this study seem to indicate that EEGP may alter the reactivity to stress on the level of the endocrine (i.e., PRL) and immune system (i.e., proliferative response to PHA and NK cell percentages). The clinical relevance of these hormonal and immune system changes remains to be established. The results of the present study do justify studies investigating the effect of EEGP on the reactivity to acute stress on the endocrine and immune system correlated with breast cancer recurrence and survival on a large scaled basis.

References

Ayala D, Lissoni P, Archili C, Barni S, Roveda G, Giacomelli M, Bissi A (1990) Effect of mastectomy versus mastectomy plus adjuvant ovariectomy on prolactin response to TRH in breast cancer. J Biol Regul Homeost Agents 4:150-152

Bachen EA, Manuck SB, Marsland AL, Cohen S, Malkoff SB, Muldoon MF, Rabin BS (1992) Lymphocyte subset and cellular immune responses to a brief experimental stressor. Psychosom Med 54:673-679

Barni S, Lissoni P, Tancini G, Crispino S, Paolorossi F, Rovelli F, Fumagalli G, Ferri L (1986) Prolactin response to thyrotropin-releasing hormone in early and advanced human breast cancer. Tumori 72:399-403

Benschop RJ, Brosschot JF, Godaert GLR, Smet M de, Geenen R, Olff M, Heijen CJ, Ballieux RE (1994) Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in man. Am J Physiol 266:75-80

Berger M, Bossert S, Krieg JC, Dirlich G, Ettmeier W, Schreiber W, Zerssen D von (1987) Interindividual differences in the susceptibility of the cortisol system: an important factor for the degree of hypercortisolism in stress situations. Biol Psychiatry 22:1327-1339

Burford-Mason A, Gyte GML, Watkins (1989) Phytohaemagglutinin responsiveness of peripheral lymphocytes and survival in patients with primary breast cancer. Br Canc Res Treat 13:243-250

Fawzy F, Kemeny M, Fawzy N, Elashoff R (1990) A structured psychiatric intervention for cancer patients. II. Changes over time in immunologic measures. Arch Gen Psychiatry 47:729-735

Geest S van der, Sluiter WJ, Doorenbos H, Reitsma WD (1983) Tamoxifen and prolactin secretion in postmenopausal women with advanced breast cancer. Neth J Med 26:99-103

Hacene K, Desplaces A, Brunet M, Lidereau R, Bourguignat A, Oglobine J (1986) Competitive prognostic value of clinicopathologic and bioimmunologic factors in primary breast cancer. Cancer 57:245-250

Herbert TB, Cohen S, Marsland AL, Bachen EA, Rabin BS, Muldoon MF, Manuck SB (1994) Cardiovascular reactivity and the course of immune response to an acute psychological stressor. Psychosom Med 56:337-344

Hoffman JW, Benson H, Arns PA, Stainbrook GL, Landsberg L, Young JB, Gill A (1981) Reduced sympathetic nervous system response associated with the relaxation response. Science 215:190-192

Little RJ (1988) Missing-data adjustments in large surveys. J Bus Eco Stat 6:287-295

McLeod RM, Scapagnini U, Thorner MO (1985) Prolactin: Basic and clinical correlates. New York:Springer-Verlag

Mills PJ, Schneider RH, Hill D, Walton KG, Wallace RK (1990) Beta-adrenergic receptor sensitivity in subjects practicing transcedental meditation. J Psychosom Res 34:29-33

Mills P, Dimsdale JE (1993) The promise of adrenergic receptor studies in psychophysiologic research II: Applications, limitations, and progress. Psychosom Med 55:448-457

Morrell EM, Hollandsworth JG (1986) Norepinephrine alterations under stress conditions following the regular practice of meditation. Psychosom Med 48:270-277

Motulsky HJ, Cunningham EMS, Blasi A de, Insel PA (1986) Desensitization and redistribution of ß-adrenergic receptors on human mononuclear leukocytes: Am J Physiol 250:E583-E590

Naliboff B, Benton, D, Solomon G, Morley J, Fahey J, Bloom E (1991) Immunological changes in young and old adults during brief laboratory stress. Psychosom Med 53:121-132

Pacak K, Nedvidkova J, Horvath M, Frantik E, Husek P, Pacovsky V (1989) High cardiovascular reactivity to psychological challenge associated alternatively with high density of beta-adrenergic receptors or with high resting level of catecholamines: Activ Nerv Super 31:96-99

Pettingale KW, Watson M, Bhakri HL, Jones H, Tee DEH (1989) Changes in hormonal, immunological and autonomic measures during the performance of laboratory stress task. Stress Med 5:150-169

Saab PG, Liabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, McCalla J, Cieply LK, Schneiderman N (1992) Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white americans. Psychophysiology 29:384-397

Schedlowski M, Jung C, Schimanski G, Tewes U, Schmoll HJ (1995) Effects of behavioral intervention on plasma cortisol and lymphocytes in breast cancer patients: an exploratory study. Psychooncology 3:181-187

Seremet M, Rudolf M, Hrsak M, Kastelan M (1992) Prognostic value of some parameters of cellular immunity in breast cancer patients. Acta Med Croat 46:9-13

Spiegel D, Bloom J, Kraemer H, Gottheil (1989) Effect of psychosocial treatment on survival of patients with metastatic breast cancer. Lancet ii:888-891

Yalom I (1983) Existential Psychotherapy. New York: Basic Books

Chapter 9

General discussion and conclusions

Cardiovascular, endocrine and immune responses to acute stress inwomen with breast cancer and healthy women

The studies described in the first part of the present thesis were designed to investigate how an acute stressor influences the cardiovascular, the endocrine and the immune system of breast cancer patients and of healthy women. In this respect we determined on the one hand the response to the stressor of healthy age-matched control women and breast cancer patients as a group. On the other hand, we investigated whether breast cancer patients respond differently to an acute stressor compared to healthy age- and sex-matched controls. Eligible patients were those who had been treated with a curative intention for breast cancer and were diagnosed with either positive axillary lymph nodes, or supraclavicular lymph nodes, skin or distant metastatases.

As a model for an acute stressor we used a standardized speech task, which has been shown previously to elicit reliably significant cardiovascular changes consistent with SNS and HPA system activation by Saab et al. (1992). The reason for using a reactivity model is that the reproducibility of individual variability of subjects' basal immune responses has been shown to be low. Initial data suggest that information on the organizational level of the neuroendocrine system and the sensitivity of the immune system to neuroendocrine mediators can better be obtained in a reactivity model than by only measuring baseline responses (Mills, Haeri, Dimsdale, 1995; Marsland, Manuck, Fazzari, Stewart, Rabin, 1995).

First, we will address the effect of the speech task on immune and endocrine responses of the total group of subjects, unadjusted for the health of the donor. The results of this study have been discussed in a broad context of previously established findings regarding the effect of an acute psychological stressor on immune and endocrine responsivity in healthy donors. Second, we have focussed on research on possible differences in response patterns between breast cancer patients and healthy subjects. We have examined changes in the hypothalamic pituitary adrenal (HPA) axis in response to the speech task in breast cancer patients. In addition we have described the alterations in the speech task-induced distribution and function of peripheral blood cell in relation to progression of breast cancer.

Cardiovascular, endocrine and immune responses to acute stress in women with breast cancer and healthy women unadjusted for the health status

The results described in Chapter 2 show that the speech task induced changes in the distribution and function of lymphocytes of the subjects from the three groups combined. Specifically, notable increases have been found in percentages of CD8 and CD16/56 (NK) cells, and decreases in percentages of CD3, CD4, and CD19 cells. This pattern of changes in peripheral blood cells is consistent with earlier findings obtained in healthy subjects (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, 1991; Bachen, Manuck, Marsland, Cohen, Malkoff, Muldoon, Rabin, 1992; Zakowski, McAllister, Deal, Baum, 1992; Herbert, Cohen, Marsland, Bachen, Rabin, Muldoon, Manuck, 1994). In addition, marked elevations in blood pressure and heart rate were observed. It has been well-documented that elevations

in cardiovascular functions are accompanied by increases in plasma catecholamines (Dimsdale, Moss, 1983; Eisenhofer, Lambie, Johnson, 1985; Tischenkel, Saab, Schneiderman, 1989). Accumulating evidence indicates that redistribution of lymphocytes, especially NK cells, can, in part, be attributed to changes in catecholamines (Kappel, Tvede, Galbo, Haahr, Kjaer, Linstow, Klarlund, Pederson, 1991).

Catecholamines can bring about changes in lymphocyte redistribution by binding to high affinity $ß_2$-adrenergic receptors expressed by all lymphoid cells, which results in the activation of adenylate cyclase and subsequently increased intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP) (Ottoway, Husband, 1992; Scarpace, Tumer, Mader, 1991; Chambers, Cohen, Perlman, 1993). It has been shown that the density and affinity of the $ß_2$-adrenergic receptors on CD8 cells and NK cells are higher than on CD4 cells (Larsson, 1985; Landman, Burgisser, Wesp, Buhler, 1984; Kahn, Sonsoni, Silverman, Englemann, Melmon, 1986; Fuchs, Albright, Albright, 1988; Maisel, Folwer, Rearden, Motulsky, Michel, 1989), which possibly explains why CD8 cells and NK cells outnumber other subsets such as CD4 cells in the circulation after the subject has been exposed to an acute stressor. It is still unclear whether the changes in the distribution of lymphocyte subsets after acute stress are solely due to the flux of subpopulations of mononuclear cells that may have relatively high numbers of $ß_2$-adrenergic receptors or due to a transient increase in number (up-regulation) of $ß_2$-adrenergic receptors. Until now it was generally assumed that the cells were recruited from the bone marrow or lymphnodes which are known to have a large reserve of mature mononuclear cells estimated to be 10-fold greater than the number of cells in the circulation at any given time (Dale, 1980). Alternatively, it has been suggested that a large number of NK cells are already present in the circulation, and that most of these cells are adhering to the vessel wall, being thus less accessible to collection of blood by venipuncture (Benschop, Nieuwenhuis, Tromp, Godaert, Ballieux, Van Doornen, 1994). Benschop et al. (1994) have shown in an in vitro model of NK cell adherence to the endothelium, that catecholamines decrease selectively the adherence of NK cells to endothelial cells. This may serve as the mechanism responsible for the apparent increase of NK cells in the peripheral circulation after acute stress. Therefore the marginal zone in the vessels may a source for the acute increase in $CD8^+$ and $CD16/56^+$ cells in the circulation.

In addition to the regulatory role of catecholamines, there are a number of other regulatory mechanisms. Cortisol and adrenocortiocotropin hormone (ACTH) have been suggested to have prominent actions on migration of lymphocytes (Ottoway et al., 1992). In our experiment we measured the hormones: ACTH, cortisol and prolactin (PRL). The results in Chapter 3 show that plasma ACTH and PRL levels increased in response to the task in breast cancer patients and healthy women as a group. These observations are essentially similar to the ACTH and PRL responses to acute stress obtained in healthy men (Schulte, Bamberger, Elsen, Herrmann, Bamberger, Barth, 1994; Schedlowski, Wiechert, Wagner, Tewes, 1992). . However, plasma cortisol levels decreased in response to the speech task, unadjusted for the health of the donor. Until now, it has often been observed that acute psychological stressors in humans do not give rise to an increase in cortisol levels (Berger, Bossert, Krieg, Dirlich, Ettmeier, Schreiber, Von Zeissen, 1987; Benschop, Brosschot, Godaert, De Smet, Geenen, Olff, Heijnen, Ballieux, 1994). Our control group

as well as the breast cancer patients displayed higher levels of psychological distress than the norm. Therefore the HPA axis activity and consequently the metabolic activity of the body may be higher in our control group than in a norm group. This may lead to a decrease in cortisol. Wild silver foxes also have decreased cortisol levels in reaction to acute stress in winter, while they showed increased levels in summer (Oskina, Tinnikov, 1992). Since cortisol is known to regulate metabolic activity, the more intensive metabolic proceses in these animals in winter than in summer may result in a maximum activity of the HPA system which, in turn, may result in a reversed reaction to acute stress.

In Chapter 4, we show that plasma ACTH and cortisol values were positively related to redistribution of CD3 cells in rest as well as in response to the speech task. These preliminary findings suggest that the hormones belonging to the HPA axis interfere with the immune response to psychological stressors. More research is needed to clarify the mechanism how cortisol and other neurohormonal factors such as ACTH, prolactin and ß-endorphin can affect T cell migration in response to acute stress. However, peripheral blood lymphocytes express receptors for all the hormones mentioned above (Johnson, Torres, 1994; Leite-de-Moraes, Touraine, Kelly, Kuttenn, Dardenne, 1995; Bhargava, 1990).

Stress also interferes with the function of peripheral blood cells via the secretion of catecholamines and hormones of the HPA-axis like cortisol, ACTH, ß-endorphin and prolactin (Kappel et al., 1991; Weicker, Werle, 1991; Schedlowski, Falk, Rohne, Wagner, Jacobs, Tewes, Schmidt, 1993; Sauer, Polack, Wikinski, Holsboer, Stalla, Arzt, 1995). The results of the present study show that the speech stressor induces marked increases in NKCA. We observed a significant decrease in proliferation of T lymphocytes to PWM (100 µg/ml). No significant changes in the proliferative response to PHA could be found. Which hormone(s) or neurotransmitters are responsible for the modulation of the function of the cells has not been determined. Benschop et al. (1994) have shown that the catecholamines are responsible for the increased function of NK cells. It is not known yet whether cortisol are responsible for the modulation of T cell function. However, preliminary data of Heijnen et al. show that acute stress has a direct effect on NK cell function (within 3 minutes) whereas only 10-15 minutes after the acute stressor a modulation of T cell function could be observed. The latter is in favor with the hypothesis that T cell function is influenced by hormones of the HPA-axis such as cortisol.

In conclusion, the results of present have shown that the task elicited significant changes in heart rate and blood pressure values, hormones belonging to the HPA axis, and changes in the distribution and function of peripheral blood cells.

Cardiovascular, endocrine and immune responses to acute stress in women with breast cancer and healthy women adjusted for the health of the donor

Evidence of a hyperactive adrenal gland in patients with metastatic breast cancer

In this study (Chapter 3) we were particularly interested whether progression of breast cancer is associated with an altered HPA axis activity. The results of the present seem to indicate that breast cancer is associated with a hyperactive adrenal gland resulting in chronically increased baseline cortisol levels.

These increased levels may be due to the stress related to this chronic-progressive illness in combination with the administration of tamoxifen.

As already mentioned above, the plasma cortisol values of both breast cancer patients and healthy women declined in response to the task, but those of metastatic breast cancer patients declined faster. It has been explained that an aberrant cortisol response to acute stress may be associated with a higher activity of the HPA-axis and consequently with a higher metabolic activity of the body. The higher activity of the HPA-axis may be associated with the relatively higher levels of psychological distress observed in our control women and breast cancer patients. Consistent with this assumption, we found that depressive symptomatology correlated negatively with the cortisol response to speech task as well as with the change in plasma cortisol measured during exercise on the bicycle ergometer. The chronic stress levels may shed some light on the diminished cortisol response in both breast cancer patients and healthy women, but it remains a point to be established why metastatic breast cancer patients demonstrated a faster cortisol decline to the speech task. We suggest that the faster decline of the cortisol values in metastatic breast cancer patients is possibly caused by increased metabolic clearance of cortisol due to increased utilization of metabolic substrates often observed in the presence of tumor (Mulligan, Tisdale, 1991).

Additionally, metastatic breast cancer patients showed a smaller increase in plasma ACTH values in response to the task as compared to node-positive breast cancer patients and healthy women. In view of these findings we speculate that this may associated with higher basal plasma cortisol values in this group of patients and suggest that the pituitary corticotroph cell in metastatic breast cancer patients is appropriately restrained by the negative feed back relatively high cortisol basal levels.

Alterations in the immune responses to acute stress in patients with metastatic breast cancer

It is an interesting phenomenon that we observed differences in redistribution of total lymphocytes and the cell subtype CD8 in response to the speech task between metastatic breast cancer patients and healthy controls after their health status was taken into account (Chapter 2). Metastatic breast cancer patients had higher increases in lymphocyte percentages in response to the speech task than healthy women. Although a fully satisfactory explanation is lacking at present, lymphocyte hyperresponsiveness in metastatic cancer may be associated with alterations in the sympathetic nervous system (SNS) and the HPA axis. At first glance the hypothesis that the greater increase in lymphocytes in response to the speech task may be associated with changes in the SNS system is rather tentative. Except for a higher heart rate in rest, there were no differences in blood pressure values in rest nor in response to the task between the breast cancer group and controls. However, it has been shown in humans that chronic stress may affect immunologic, but not cardiovascular responsiveness to acute psychological stress (Benschop et al., 1994). This finding seems to indicate that there may occur changes in the sensitivity of β_2-adrenergic receptors of peripheral blood cells for catecholamines, while these are not detectable in the blood pressure values. Thus, it could be that the lymphocytes of metastatic breast cancer patients were more sensitive for catecholamines than those of healthy women despite similar increases in cardiovascular parameters. In Chapter 4 we described that the increases in

plasma ACTH levels were associated with increases in total lymphocytes in the peripheral blood compartment, unadjusted for the health of the donor. Since plasma ACTH levels of metastatic breast cancer patients and healthy women were essentially similar, the greater increase in lymphocytes obtained in metastatic breast cancer patients can not be attributed to higher levels of ACTH.

In contrast to the hyperresponsiveness of total lymphocytes to the task, lymphocytes expressing CD8 markers of metastatic breast cancer patients did not change in response to the task. The nonresponsiveness of CD8 cells is in sharp contrast with the significant increases in CD8 cells found in women without breast cancer or breast cancer patients with axillary lymph node metastases. The nonresponsiveness of CD8 cells for acute stress may be associated with a decreased sensitivity for hormones such as catecholamines or cortisol. As already mentioned in this discussion, it is not yet known which hormone(s) or neuroendocrine mediators may account for the changes in peripheral blood cells in reponse to acute stress. It has been shown that elevated levels of catecholamines may result in a decreased sensitivity of $ß_2$-adrenergic receptors, which, in turn, resulted in a low or no increase in NK cells in response to acute stress (Benschop et al., 1994; Motulsky, Cunningham, De Blasi, Insel, 1986). However, since NK cells of metastatic breast cancer patients are still reactive to acute stress may suggest that other hormones and their receptors are involved in the non-responsiveness of CD8 cells in metastatic cancer.

The pathophysiological significance of the low response of CD8 cells to the speech task in metastatic cancer patients is at present unknown. The increases in lymphocytes in response to acute stress can be regarded as a 'preventive measure' of the host against infections, which can be the result of tissue damage caused by stress. As a first line of defense NK cells and macrophages will be attracted to damaged tissue sites. CD8 cytotoxic T lymphocytes (CTL) become active in the defense against foreign cells after antigen recognition. Both NK and CD8 cells have been suggested to play a role in the inhibition of tumor growth. Until recently CTL's were assumed to be only involved in the inhibition of the growth of virally- and UV-light induced tumors such as lymphomas and human melanomas. Yet, mammary tumors as well as other spontaneous tumors have been suggested to present antigens which may elicit a CTL response (Van der Bruggen, Traversari, Chomez, Lurquin, De Plaen, Van den Eynde, 1991; Brasseur, Marchand, Van Wijck, Herin, Lethe, Chomez, Boon, 1992; Heike, Blachere, Srivastava, 1994; Tilkin, Lubin, Soussi, Lazar, Janin, Mathieu, Lefrere, Carlu, Roy, Kayibanda, Bellet, Guillet, Bressac-de Paillerets, 1995). In view of these observations we would like to pose the tempting hypothesis that the CD8 hyporesponsiveness to acute stress may not only reflect the inadequacy of these cells to migrate to damaged tissue sites but also to (metastatic) cancer sites. However, since it is still unknown what the contribution of peripheral blood cells is to immune system function, we can only state that we are dealing with a response pattern to an acute stressor that has no meaning in terms of protection against tumor growth.

Although, the task-induced changes in percentages of NK cells of breast cancer patients were essentially similar to those in controls, metastatic breast cancer patients showed a more pronounced increase in NK cell function. The mechanism for this phenomenon is also still unclear. It may be hypothesized that in an attempt to reduce the volume of metastatic tumor cells, the NK cells are already in an activated state in the

peripheral circulation and may have therefore been more active on a cell per cell basis in the in vitro NK cell assay.

Differences in the effects of ACTH on the immune reactivity to acute stress between metastatic breast cancer patients and healthy women.

Another important goal of the present study was to explore the relations between ACTH and cortisol levels and redistribution and function of peripheral blood cells in breast cancer patients. As described in Chapter 4 ACTH had a positive effect on CD4 cell percentages of node-positive breast cancer patients and healthy women at baseline and in response to the task. In contrast, in metastatic breast cancer patients ACTH had a negative effect on CD4 cell percentages, which might explain, in part, the greater decrease in CD4 percentages observed in this group. The background of this phenomenon will be very complex. However, in vitro studies have shown that ACTH can have either enhancing or inhibiting effects on immune cells depending on the level of ACTH as well as the metabolic status of the cell (Heijnen, Zijlstra, Kavelaars, Croiset, Ballieux, 1987; Kavelaars, Ballieux, Heijnen, 1990). Therefore the results may be explained by a possible difference in the ACTH levels and metabolic status of cells of patients with metastases versus patients with node-positive breast cancer, or healthy controls.

Methodological reflections

The present results seem to indicate that progression of breast cancer is associated with significant changes in various endocrine and immune measures in response to the speech task (see Chapter 2 and 3). For establishing causal inference we employed a repeated measures design which is combining within- and between-subjects factors. This design studied the effect of disease stage (no breast cancer, node-positive breast cancer, metastatic breast cancer) on the task-induced changes in cardiovasculair, endocrine and immune function over time. An important advantage of such a design is that a relatively small number of subjects is required. Despite the fact that the design seems adequate, the question remains whether drawing conclusions based on statistical inferences is justified. Although the healthy women were assumed to be representative for the target population, they, of course, did not receive endocrine treatment (tamoxifen). It is, however, unlikely that this would have biased our results because metastatic breast cancer patients showed different responses to the task than node-positive breast cancer patients, while both groups received tamoxifen. Moreover, the endocrine and immunological responses of node-positive breast cancer patients were essentially similar to those observed in healthy women. These observations may rule out the possibility that the differences in endocrine and immunological responses were due to tamoxifen administration. In addition, it has been shown that psychological distress and biobehavioral factors such as smoking, drinking and food intake can modulate the immune response (Holland, 1990). The results indicate that there were no differences in these factors between breast cancer patients and healthy women with the exception of alcohol consumption. Metastatic breast cancer patients consumed more alcohol than node-positive breast cancer patients. Because there were no differences in alcohol consumption between metastatic breast cancer patients and controls,

the changes in endocrine and immune measures measured at baseline and in response to the speech task observed in breast cancer patients with distant metastases can not be attributed to "alcoholic excess". In addition, breast cancer patients and controls had similar levels of psychological distress. Our group of breast cancer patients and controls showed a broad distribution of women experiencing mild to moderate levels of psychological distress. Since there were no differences in distress symptomatology between breast cancer patients and controls, we may rule out the possibility that the differences in endocrine and immunological responses were related to psychological distress.

Our results show that metastatic breast cancer patients has different endocrine and immunological values at baseline as compared to age-matched healthy women. It can be hypothesized that the differences in the redistribution and function of peripheral blood cells in response to the task observed in metastatic breast cancer patients as compared to those of healthy women are a reflection of these baseline differences. However, this is unlikely because there were significant task by group interactions for percentages of CD8 cells despite similar baseline values. This hypothesis could have been appropriately tested with a multivariate analysis of covariance (MANCOVA) model with baseline values as covariates in case that the repeated measures design consisted of not more than two measurements. In the case of more than two repeated measurements, as in the present study, this type of design would lose interpretability.

A limitation of the present study is the small number of patients. It therefore needs to be emphasized that the current study is exploratory. In statistical testing the sample size is always taken into account and a relatively small sample reduces the chance that statistical testing will be significant. A significant test based on a relatively small sample can be considered as relevant but the risk of less stable results is higher than in case of testing on large samples. In addition, in this study multiple statistical tests resulted in a high risk of inflation type 1 error. The rule of thumb is that for one out of twenty tested MANOVA's, only one is a product of chance. With regard to current findings six out of twenty two tested MANOVA'S were significant (see Chapter 2 and 3). Finally, the MANOVA's were carried out using raw scores instead of deltas (i.e. the arithmetic difference between a measure taken in response to a task and a measure taken at baseline), which has been suggested as appropriate for analyzing reactivity data (Llabre, Spitzer, Saab, Ironson, Schneiderman, 1991). The reason for using raw scores in the current study was that deltas are assumed to be unreliable due to the summation of measurements errors contained to both the baseline and the task levels which may have violated the internal validity of our design (Cronbach, Furby, 1970).

In Chapter 4 we explored the influence of ACTH and cortisol levels on redistribution and function of peripheral blood cells in breast cancer. Multiple regression was used to investigate whether changes in redistribution and function of peripheral blood cells in rest and in response to the task can be predicted from ACTH and cortisol on the one hand and from the health of the donor on the other. In vitro studies have shown that ACTH and cortisol can have a positive or negative effect on immune reactivity possibly dependent on the donor, which suggests that the relations between these neuroendocrine mediators and immunological parameters are non-bivariate of nature (Heijnen et al., 1987). Bivariate correlations would in this regard rather 'mask' than 'unmask' the relationships between these

parameters. Moreover, it appeared that the net effect of neuroendocrine mediators on immune reactivity is dependent on the metabolic status of the cell (Kavelaars et al., 1990). Thus, the health of the donor can be an effect modification factor. Possible emergence of this phenomenon has to be tested on plausibility. If there is one or more significant interaction terms, than the assumption of bivariate association is violated. The findings underline this assumption by showing that ACTH had a positive effect on distribution of T lymphocytes, but when the interaction between ACTH and the health of the donor was taken into account, it appeared that in healthy women and node-positive breast cancer patients ACTH values were positively related to T lymphocytes, while in patients with metastatic disease ACTH values were negatively related to T lymphocytes.

In accordance with the explorative nature of this study, the all possible regressions strategy was applied (Draper, Smith, 1981), which means that all possible regressions of Y on the X's are applied and tested. In any regression equation, each independent variable does or does not appear resulting in 2^p equations for p predictor variables including the interaction terms. In the current study the 5 predictors (i.e. main effects ACTH or cortisol and the dummy-coded variables X_1 and X_2 and the interactions X_1 by ACTH or X_1 by cortisol and X_2 by ACTH or X_2 by cortisol) resulted in 2^5 (=32) equations. A great advantage of this procedure is that nothing is missing. A disadvantage is that the acceptableness of the most plausible model may be reduced as compared to the saturated model. However, the model that turned out to be the most plausible with regard to the effect of ACTH on immune function (ACTH, X_1 and the interaction term ACTH by X_2) was most close to the saturated model (ACTH, X_1, X_2 and the interaction). These reflections are irrelevant to the prediction of distribution and function of cells by cortisol and the health status, beause only main terms were included.

Because in the present study the risk of multicollinearity was substantial due to high correlations between the independent variables (i.e., main and interaction terms), the variance inflation factor values had to be smaller than 4 for a model to be retained, which is more conservative than an upper limit of 10 which is generally accepted (Glantz, Slinker, 1990; Chatterjee, Price, 1991).

Considering the complexity of the three classes of outcome variables (i.e. cardiovascular, endocrine and immune) it is advisable for future studies to investigate the interrelations as well as the levels and variability of the different parameters in terms of statistical modelling in which the regression equations are solved simultaneously (Jöreskog, Sörbon, 1977; Jöreskog, 1993). This in combination with an optimal design could be the royal road to the understanding of the role of psycho-endocrine-immune interactions in breast cancer. In this regard it is important to note that the number of patients with statistical modelling need to be substantial, to all probability more than 200 on the average (Boomsma, 1983).

The effects of EEGP on psychological, endocrine and immune function of breast cancer

The studies described in the second part of the present thesis were designed to investigate the effectiveness of EEGP on psychological (Chapter 6), endocrine and immunological

measures (Chapter 6 and 7) in breast cancer patients. Special attention was given to the questions: 1. Does EEGP influence psychological and physiological functions of breast cancer patients? 2. Has the EEGP the same effect on all breast cancer patients or is there a differential sensitivity for EEGP with respect to psychological and physiological functions. In order to approach these two questions, we determined the effect of EEGP on baseline psychological, endocrine and immune functions as well as on the pattern of reactivity to an acute stressor before and after EEGP on the level of the cardiovascular, endocrine and immune system.

The effect of EEGP on psychological adjustment

Cumulative evidence suggests that psychosocial interventions have a consistent positive effect on psychological adjustment to disease-related and medical treatment related issues in cancer patients as well in patients with other life-treatening diseases (Andersen, 1992; Trijsburg, Van Knippenberg, Rijpma, 1992; Mulder, 1994). First, we investigated the effect of EEGP on psychological distress, coping and social support. It turned out that there were no significant main effects for the intervention on any of these measures. Secondly, we investigated the question whether EEGP is equally useful for patients with low distress as for those with high distress levels. The results of the present study (Chapter 6) seem to indicate that EEGP was most effective in those breast cancer patients presenting relatively high levels of psychological distress and frequently using maladaptive coping strategies for managing their disease. We found that selection of patients with high baseline distress levels, presumably being in need of professional help, in principle is feasable by applying psychological questionnaires. However, this method may not capture the specific difficulties that breast cancer patients have in various phases of their disease. Patients may experience severe psychosocial stressors (e.g., loss of roles and functional abilities and problems with social relationships) and psychosexual problems even years after surgery (Morris, Greer, White, 1977; Edgar, Rosberger, Nowlis, 1992; Schag, Ganz, Polinsky, Fred, Hirje, Petersen, 1993), but may not report high levels of psychological distress (Nelson, Friedman, Baer, Lane, Smith, 1994). Thus, it could be that these patients have benefitted from EEGP in ways that are not reflected in the dependent measures.

Another important issue is the speficity of the EEGP program in relation to other forms of therapy. It could be that some patients may benefit more from other intervention programs such as cognitive behavioral stress management. It is about time that the simple tests of the efficacy of psychosocial interventions on adjustment to cancer are replaced by more direct comparisons of different treatment modalities and to address which psychosocial interventions are most effective for which patients. In addition to the psychological distress values and coping styles as addressed in the present study, other factors such as disease-stage, type of surgery and of (adjuvant) treatment and sociodemographic variables may possibly modulate the efficacy of interventions need to be included in such studies. In addition to multiple procedural factors such as the number of sessions, timing of the intervention is another important issue that needs to be addressed.

The bulk of studies have investigated intervention-effects in cancer patients after surgery and/or adjuvant treatment. Resection of tumor tissue has been associated with

increasing levels of psychological stress. Cancer patients awaiting surgery have been shown to suffer high levels of anxiety (Jelicic, Bonke, Millar, 1993). In addition, the confrontation with a life-threatening diagnosis, surgery itself and post-operative pain may result in high levels of psychological distress during the post-operative period. Additional research might therefore also fruitfully address the question whether psychosocial interventions are more effective at the vulnerable time of admission to hospital for surgery than in the post-mastectomy period and during adjuvant treatment (Burton, Parker, Farrell, Bailey, Conneely, Booth, Elcombe, 1995).

The effect of EEGP on baseline endocrine and immune levels

The results of the present study (Chapter 7) have shown that progression of breast cancer is associated with alterations in hormones like cortisol, PRL and ACTH. EEGP had succeeded to reduce the basal cortisol and prolactin levels in those breast cancer patients displaying already high baseline levels of these hormones at study entry. In addition, EEGP had a stabilizing effect on the redistribution patterns of the lymphocytes expressing CD8 and CD16/56 markers. This findings may shed a favorable light on the application of EEGP for breast cancer patients because a lower percentage of NK cells in the peripheral blood has been associated with a better prognosis (Levy, Herberman, Whiteside, Sanzo, Lee, Kirkwood, 1990). It has been proposed that the percentage of these subsets in the peripheral blood is a reflection of the host response to the aggressiveness of the tumor process (Levy, Herberman, Lippman, D'Angelo, Lee, 1991). Consistent with this suggestion is the finding that node-positive breast cancer patients in this study had at baseline a significant higher NK cell percentage in the peripheral blood than their healthy counterparts with essentially similar levels of NKCA. These findings seem to indicate that a higher percentage of lymphocytes in the peripheral blood compartment is not necessarily associated with a better immune function.

Unlike some other research projects evaluating the effect of relaxation training and other types of psychosocial intervention programs on baseline endocrine and immune function of healthy subjects and cancer patients, we did not find increases in basal T or NK cell percentages in the EEGP group (Kiecolt-Glaser, Glaser, Strain, Stout, Tarr, Holliday, Speicher, 1986; Hall, Mumma, Longo, Dixon, 1992; McGrady, Conran, Dickey, Garman, Farris, Schumann-Brzezinkski, 1992; Schedlowski, Jung, Schimanski, Tewes, Schmoll, 1994; Fawzy, Kemeny, Fawzy, Elashoff, 1990). For example, Fawzy et al. (1990) found an increase in NK cells after participation in an intervention program among post-surgical melanoma patients. This study focussed, however, on early-stage cancer patients who had been curatively treated and may have actually been free of disease at the time of intervention, while a large number of patients in the present study were likely to be already suffering from a progressive disease at the moment of testing. Although a satisfactory explanation is lacking, the difference in findings can possibly be explained by the fact that the net outcome of an intervention program on immune function is determined by the level of immunosuppression associated with progression of the tumor and with the chronic psychological distress of having the disease.

The effect of EEGP on the reactivity to an acute stressor on the level of cardiovascular, endocrine and immune responses

Comparing the changes in HPA axis in response to the task pre-intervention with those post-intervention also suggest that EEGP may interfere with PRL release associated with mammary tumor progression. As described in Chapter 8, the task-induced PRL release after the 3 months intervention period exceeded those measured at study entry in patients assigned to the waiting-list control condition. In contrast, the increase in PRL in patients after EEGP was not larger.

With regard to the SNS system, there were no differences in the pre-post task-induced cardiovascular responses of patients in the EEGP and those in the WLC condition. The DBP, SBP and HR changes measured before and after the intervention period in patients assigned to EEGP and those in the WLC condition suggest a similar level of activation of the SNS system in both groups.

With regard to pre-post intervention task-induced changes in distribution of peripheral leukocytes, a similar pattern of findings emerged. The post-intervention NK cell response to the task of the waiting-list control group exceeded the NK cell response to the task at study entry. The lower NK cell percentages in response to the task obtained in patients after 3 months EEGP as compared to the NK cell percentages in response to the task at study entry do suggest a normalization of reactivity of the neuroendocrine system by EEGP. The mechanisms behind these observations are still unclear. A plausible theory might be that the intervention may down-regulate the activity of the neuroendocrine system thereby decreasing task-induced hormonal and immune responses. The clinical relevance of these results is unclear at present. EEGP seems to normalize the stress response and this can be important for the integrity of the immune system and the homeostasis of the body.

An interesting feature is that EEGP patients show increases in proliferation to PHA in reponse to the task after the experiment, while in WLC patients the acute stressor led to a decreased mean PHA-proliferation response. Research on the clinical importance of immune system variables in predicting disease progression has thus far been inconclusive (Burford-Mason, Gyte, Watkins, 1989). Diminished response to mitogen-stimulation has been assocated with an unfavorable prognosis in several studies (Burford-Mason et al., 1989; Seremet, Rudolf, Hrsak, Kastelan, 1993). However, more research is needed to show that increases in lymphocyte proliferative response can be regarded as a favorable effect of the EEGP program. Our results may suggest that EEGP may interfere with the capacity of T lymphocytes to react to neuroendocrine mediators induced by the speech task. Since it is plausible that this effect will occur after exposure to stressors in daily life, the EEGP may influence the net T cell activity.

Methodological reflections

In order to detect whether the effect of EEGP on psychological, endocrine and immunological outcome measures can be predicted by baseline values, multiple regression analyses were used. In these analyses the conditions to retain a model were similar to those used in analyses of the effect of ACTH and cortisol on lymphocyte distribution. A major

limitation of the present study was that the sample was too small to distinguish possible differences in EEGP effects between node-positive and metastic breast cancer patients. With regard to baseline immunological measures, this would be particularly of interest because the greatest degree of impairment of immunity was seen in patients with metastatic disease. However, including any more variables in the regression analyses would result in an unacceptable reduction of statistical power and in instable results, running a high risk of coincidental significant findings. Moreover, on the basis of the present findings the influence of aspecific factors such as social support provided by group members or attention or the quality of the therapist-patient relationship cannot be determined. Nor do we have any indication of which core ingredients of EEGP had a positive effect and which components did yield possible adverse reactions. It will be fruitful to investigate whether other types of intervention, for example, psychotherapy compared with social support which will not induce the depth of process and doesn't use the specific techniques offered in the group psychotherapy condition, are more or less effective in helping breast cancer patients to cope with the several psychosocial and psychosexual issues that they confront. Moreover, it will be important to examine the degree to which psychosocial variables such as life events, coping style, social support and other factors associated with adjustment such as type of adjuvant treatment, age, education and socio-economic status may explain differential treatment effects psychologically as well physiologically, and help to determine which patients are most likely to benefit from a particular type of psychological intervention. From a statistical-analytic perspective, it will be of utmost importance for future studies to focus not only the magnitude and the variability of psychological or physiological change but also on the interdependency of the variables in the study. An advantage of this procedure is running smaller risk of errors.

To measure the effect of EEGP on the reactivity to stress on the cardiovascular, endocrine and immune system, a repeated measures design was used. With respect to the evaluation of these analyses, similar considerations on the effect of the disease stage on task-induced changes in physiological parameters apply. Finally, because the same task is applied more than once, the phenomenon of habituation to the task may have influenced the responses to the task measured after the intervention. This is probably not the case in our experiment, as the cardiovascular changes to the task were essentially the same before and after the intervention.

Conclusions and future perspectives

Acute stress-induced endocrine and immune changes

We want to emphasize that due to small sample size the nature of this study is exploratory, and does not allow us to draw firm conclusions. Our research has to be viewed upon as a preliminary study for the evaluation of possible effects of psychotherapy on psychological and physiological function in breast cancer patients. Nevertheless, the results of the present study enriched our knowledge about the endocrine and immune responses in breast cancer patients. The results denote differences in the stress-induced responses of the neuroendocrine and the immune system between breast cancer patients and healthy women. The

alterations in the distribution and function of peripheral blood cells were most significant in metastatic breast cancer patients. Likewise, the task-induced changes in hormones belonging to the HPA axis of metastatic breast cancer patients differ from those observed in healthy women. In contrast, node-positive breast cancer patients show task-induced hormone endocrine changes more reflective of controls. Moreover, the results provided initial evidence that there were differences in the effect of hormones on immune reactivity between metastatic breast cancer patients on the one hand and node-positive breast cancer patients and healthy women on the other. The clinical relevance of these findings has yet to be established. In view of the putative stability of individual differences in cellular immune responses to acute stress, the stress-induced immune responses can be considered as a reliable marker of disease progression. In the future large scaled studies have to be initiated to examine the role of the acute stress-induced endocrine and immune changes in disease progression. In this line of research it is advisable to pay attention to the effect of catecholamines on β_2-adrenergic receptor function in this disease. This is important because these hormones are not only prime regulators of β_2-adrenergic receptors in lymphocytes, but are also assumed to participate in the process of growth and differentiation of the mammary gland (Draoui, VandeWalle, Hornez, Revillion, Lefebvre, 1991). Because carcinoma-derived mammary epithelial cells express β_2-adrenergic receptors, it may be likely that they are also subject to direct receptor-mediated adrenergic modulation (Draoui et al., 1991; Kinnard, Chelmicka-Schorr, Checinski, Jones, Arnason, 1986).

Additionally, the results of the present study have expanded our knowledge of the specific physiological parameters that may be sensitive to the influence of chronic stress (i.e. negative life experiences), but did not provide conclusive insights. We assume that real life human chronic stress may elicit other dimensions of the stress response than those observed in response to brief tasks. Laboratory tasks which mimic real-world stress such as confrontational role-play may serve as better probes for the relationship between negative life experiences and clinical outcome.

EEGP-effects

Despite the small number of patients and only one measurement after the 3 month intervention period, EEGP had a demonstrable effect on psychological, endocrine and immune function. These effects appeared to be most significant in the subgroup of breast cancer patients with high existing psychological distress and basal endocrine and immune levels. If replicated on a larger scale, the results may be relevant for the management of breast cancer. In this regard it will be of great importance to determine how these EEGP effects relates to clinical outcome. One of the interesting routes to investigate is whether EEGP will influence the formation of (micro)metastases possibly occurring directly after surgery (Baum, 1996). This may be 'the' critical period during which the immune system plays a pivotal role in eliminating tumor cells.

We want to conclude this thesis by expressing the hope that in the near future behavioral interventions will be implemented at the point of diagnosis of the disease and before surgery as an attempt to the improve quality of life of the patient and possibly to reduce surgical stress and concomittant endocrine and immune dysregulation. Hopefully

this will lead to a decrease of the incidence of metastases and extend the number of years of healthy living.

References

Andersen B (1992) Psychological interventions for cancer patients to enhance the quality of life. JCCP 66:552-568

Bachen EA, Manuck SB, Marsland AL, Cohen S, Malkoff, SB, Muldoon MF, Rabin BS (1992) Lymphocyte subset and cellular immune responses to a brief experimental stressor. Psychosom Med 54:673-679

Bhargava HN (1990) Opioid peptides, receptors, and immune function. NIDA-Res-Monogr 96:220-233

Benschop RJ, Nieuwenhuis EES, Tromp EAM, Godaert GLR, Ballieux RE, Doornen LJP van (1994) Effects of ß-adrenergic blockade on immunologic and cardiovascular changes induced by mental stress. Circulation 89:762-769

Benschop RJ, Brosschot JF, Godaert GLR, Smet MBM de, Geenen R, Olff M, Heijnen CJ, Ballieux RE (1994) Chronic stress affects immunologic but not cardiovascular responses to acute psychological sress in man. Am J Physiol 266:75-80

Baum M (1996) Does surgery disseminate or accelarate cancer? Lancet 347:260

Benschop RJ, Nieuwenhuis EES, Tromp EAM, Godaert GLR, Ballieux RE, Doornen LJP van (1994) Effects of ß-adrenergic blockade on immunologic and cardiovascular changes induced by mental stress. Circulation 89:762-769

Berger M, Bossert S, Krieg JC, Dirlich G, Ettmeier W, Schreiber W, Zeissen D von (1987) Interindividual differences in susceptibility of the cortisol system: an important factor for the degree of hypercortisolism in stress situations. Biol Psychiatry 22:1327-1339

Boomsma A (1983) On the robustness of LISRELL (maximum likelihood estimation) against small sample size and normality. Dissertation: Groningen

Brasseur F, Marchand M, Van Wijck R, Herin M, Lethe B, Chomez P, Boon T (1992) Human gene MAGE-1, which encodes for a tumor-rejection antigen, is expressed by some breast tumors. Int J Cancer 52:839-841

Bruggen P van der, Traversari C, Chomez P, Lurquin C, Plaen E de, Eynde B van den et al. (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 2:1643-1647

Burford-Mason A, Gyte GML, Watkins SM (1989) Phytohaemagglutinin responsiveness of peripheral lymphocytes and survival in patients with primary breast cancer. Br Canc Res Treat 13:243-250

Burton MV, Parker RW, Farrell WA, Bailey D, Conneely J, Booth S, Elcombe S (1995) A randomized controlled trial of preoperative psychological preparation for mastectomy. Psychooncology 4:1-19

Chambers DA, Cohen RL, Perlman RL (1993) Neuroimmune modulation: signal transduction and catecholamines. Neurochem Int 22:95-110

Chatterjee S, Price B (1991) Regression analysis by example. New York:Wiley

Cronbach LJ, Furby L (1970) How should we measure "change". Psych Bull 74:68-80

Dale DC (1980) Abnormalities of leukocytes. In: Harrison's Principles of Internal Medicine (Isselbacher KL, ed). New York:McGraw-Hill Book Company

Dimsdale JE, Moss J (1983) Plasma catecholamines in stress and exercise. JAMA 243:340-342

Draoui A, Vandewalle B, Hornez L, Revillion F, Lefebvre J (1991) Beta-andrenergic receptors in human breast cancer: identification, characterization and correlation with progesterone and estradiol receptors. Anticancer Res 11:677-680

Draper NR, Smith H (1981) Applied regression analysis. New York:Wiley

Edgar L, Rosberger Z, Nowlis D (1992) Coping with cancer during the first year after diagnosis. Assessment and intervention. Cancer 69:817-828

Eisenhofer G, Lambie D, Johnson R (1985) ß-adrenoceptor responsiveness and plasma catecholamines as determinants of cardiovascular reactivity to mental stress. Clin Sci 69:483-492

Fawzy F, Kemeny M, Fawzy N, Elashoff R (1990) A structured psychiatric intervention for cancer patients. II. Changes over time in immunological measures. Arch Gen Psychiatry 47:729-735

Fuchs BA, Albright JW, Albright JF (1988) Beta-adrenergic receptors on murine lymphocytes: density varies with cell maturity and lymphocyte subtype and is decreased after antigen administration. Cell Immunol 114:231-245

Glantz SA, Slinker BK (1990) Primer of applied regression and analysis of variance. New York:McGraw-Hill

Hall RH, Mumma GH, Longo S, Dixon R (1992) Voluntary immunomodulation: a preliminary report. Intern J Neuroscience 63:275-285

Heike M, Blachere NE, Srivastava (1994) Protective cellular immunity against a spontaneous mammary carcinoma from ras transgenic mice.Immunolobiol 1994:414-423

Heijnen CJ, Zijlstra, Kavelaars A, Croiset G, Ballieux RE (1987) Modulation of the immune response by POMC-derived peptides. I. Influence on proliferation of human lymphocytes. Brain Behav Immun 1:284-291

Herbert TB, Cohen S, Marsland AL, Bachen EA, Rabin BS, Muldoon MF, Manuck SB (1994) Cardiovascular reactivity and the course of immune response to an acute psychological stressor. Psychosom Med 56:337-344

Jelicic M, Bonke B, Millar (1993) Clinical note on the use of denial in patients undergoing surgery for breast cancer. Psychol Rep 72:952-954

Johnson HM, Torres BA (1994) Complementary peptides as probes to explore neuropeptide receptors on lymphocytes. Immunomethods 5:167-171

Jöreskog KG, Sörbon D (1977) Statistical models and methods for analysis of longitudinal data. In: (Aigner DJ, Goldberger AS, eds). Latent variables in socioeconomic models. Amsterdam:North-Holland

Jöreskog KG (1993) Testing statistical equation models. In: (Bollen KA, Long JS, eds). Testing statistical equation models. London:Sage

Kahn MM, Sansoni P, Silverman ED, Englemann EG, Melmon KL (1986) ß-adrenergic receptors on human suppressor, helper, and cytolytic lymphocytes. Biochem Parmacol 35:1137-1142

Kappel M, Tvede N, Galbo H, Haahr PM, Kjaer M, Linstow M, Klarlund K, Pederson BK (1991) Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. J Appl Physiol 70:2530-2534

Kavelaars A, Ballieux RE, Heijnen CJ (1990). Differential effects of ß-endorphin on cAMP levels in human peripheral blood mononuclear cells. Brain Behav Immun 4:171-179

Kiecolt-Glaser J, Glaser R, Strain E, Stout J, Tarr K, Holliday J, Speicher C (1986) Modulation of cellular immunity in medical students. J Behav Med 9:5-21

Kinnard RL, Chelmicka-Schorr E, Checinski ME, Jones KH, Arnason BG (1986) Sympathetic nervous system and glioma growth. Eur J Cancer Clin Oncol 22:501-503

Llabre MM, Spitzer SB, Saab PG, Ironson GH, Schneiderman N (1991) The reliability and specificity of delta versus residualized change as measures of cardiovascular reactivity to behavioral challenges. Psychophysiology 28:701-711

Landman RMA, Burgisser E, Wesp M, Buhler FR (1984) ß-adrenergic receptors are different in subpopulations of human circulating lymphocytes. J Recept Res 4:37-50

Larsson K (1985) Studies of sympathoadrenal reactivity and adrenoceptor function in bronchial asthma. Eur J Respir Dis Suppl 141:1-52

Leite-de-Moraes MC, Touraine P, Kelly PA, Kuttenn F, Dardenne M (1995) Prolactin receptor expression in lymphocytes from patients with hyperprolactinemia or acromegaly. J Endocrinol 147:353-359

Levy SM, Herberman RB, Whiteside T, Sanzo K, Lee J, Kirkwood J (1990) Perceived social support and tumor estrogen/progesterone receptor status as predictors of natural killer cell activity in breast cancer patients. Psychosom Med 52:73-85

Levy SM, Herberman RB, Lippman M, D'Angelo T, Lee J (1991) Immunological and psychosocial predictors of disease recurrence in patients with early stage breast cancer. Behav Med 67-75

Maisel AS, Folwer P, Rearden A, Motulsky HJ, Michel MC (1989) A new method for isolation of human lymphocyte subsets reveals differential regulation of ß-adrenergic receptors by terbutaline treatment. Clin Pharmacol Ther 46:429-439

Marsland AL, Manuck SB, Fazzari TV, Stewart CJ, Rabin BS (1995) Stability of individual differences in cellular immune responses to acute psychological stress. Psychosom Med 57:295-298

McGrady A, Conran P, Dickey D, Garman D, Farris E, Schumann-Brzezinski E (1992) The effects of biofeedback-assisted relaxation on cell mediated immunity, cortisol and white blood cell count in healthy adult subjects. J Behav Med 15:343-354

Mills PJ, Haeri SL, Dimsdale JE (1995) Temporal stability of acute stressor-induced changes in cellular immunity. Int J Psychophysiol 19:287-290

Morris T, Greer H, White P (1977) Psychological and social adjustment to mastectomy: A 2-year follow-up study. Cancer 40:2381-2387

Motulsky HJ, Cunningham EMS, Blasi A de, Insel PA (1986) Densensitization and redistribution of ß-adrenergic receptors on human mononuclear leukocytes. Am J Physiol 250:E583-590

Mulder CL (1994) Psycho-immunology and HIV infection. Biopsychosocial determinants of distress, immunological parameters, and disease progression in homosexual men infected with human immunodeficiency virus-I. Rotterdam:Dissertation

Mulligan HD, Tisdale MJ (1991) Metabolic substrate utilization by tumour and host tissues in cancer cachexia. Biochem J 227:321-326

Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom (1991) Immunological changes in young and old adults during brief laboratory stress. Psychosom Med 53:121-131

Nelson DV, Friedman LC, Baer PE, Lane M, Smith FE (1994) Subtypes of psychosocial adjustment to breast cancer. J Beh Med 17:127-141

Oskina IN, Tinnikov AA (1992) Interaction between cortisol and cortisol-binding protein in silver foxes. Comp Biochem Physiol 101:665-668

Ottoway CA, Husband AJ (1992) Central nervous system influences on lymphocyte migration. Brain Behav Immun 6:97-166

Saab PG, Liabre MM, Hurwitz BE, Frame CA, Reineke LJ, Fins AI, McCalla J, Cieply LK, Schneiderman N (1992) Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white americans. Psychophysiology 29:384-397

Sauer J, Polack E, Wikinski S, Holsboer F, Stalla GK, Arzt E (1995) The glucocorticoid sensitivity of lymphocytes changes according to the activity of the hypothalamic-pituitary-adrenocortical system. Psychoneuroendo 20:269-280

Scarpace PJ, Tumer N, Mader SL (1991) Beta-adrenergic function in aging. Basic mechanisms and clinical implications. Drugs Aging 1:116-129

Schag C, Ganz P, Polinsky M, Fred C, Hirje K, Petersen L (1993) Characteristics of women at risk for psychosocial distress in the year after breast cancer. J Clin Oncol 11:783-793

Schedlowski M, Wiechert D, Wagner TO, Tewes U (1992) Acute psychological stress increases plasma levels of cortisol, prolactin and TSH. Life Sci 50:1201-1205

Schedlowski M, Falk A, Rohne A, Wagner TD, Jacobs R, Tewes U, Schmidt RE (1993) Catecholamines induce alterations of distribution and activity of human natural killer (NK) cells. J Clin Immunol 13:344-351

Schedlowski M, Jung C, Schimanski G, Tewes U, Schmoll HJ (1994) Effects of behavioral intervention on plasma cortisol and lymphocytes in breast cancer patients: an exploratory study. Psychooncology 3:181-187

Schulte HM, Bamberger CM, Elsen H, Herrmann G, Bamberger AM, Barth J (1994) Systemic interleukin-1 alpha and interleukin-2 secretion in response to acute stress and to corticotropin-releasing hormone in humans. Eur J Clin Invest 24:773-777

Seremet M, Rudolf M, Hrsak M, Kastelan M (1993) Prognostic value of some parameters of cellular immunity in breast cancer patients. Acta Med Croat 46:9-13

Tischenkel NJ, Saab PG, Schneiderman N (1989) Cardiovascular and neurohormonal responses to behavioral challenge as a function of race and gender. Health Psychol 8:503-524

Tilkin AF, Lubin R, Soussi T, Lazar V, Janin N, Mathieu MC, Lefrere I, Carlu C, Roy M, Kayibanda M, Bellet D, Guillet JG, Bressac-de Paillerets (1995) Primary proliferative T cell response to wild-type p53 protein in patients with breast cancer. Eur J Immunol 25:1765-1769

Trijsburg R, Knippenberg F van, Rijpma S (1992) Effects of psychological treatment on cancer patients: A critical review. Psychosom Med 54:489-517

Weicker H, Werle E (1991) Interaction between hormones and the immune system. Int J Sports Med 1:S30-S37

Zakowski SG, McAllister CG, Deal M, Baum A (1992) Stress, reactivity and immune function in healthy men. Health Psych 11:223-232

APPENDICES

Appendix I

Depression and plasma cortisol responses to acute bicycle exercise

Introduction

In Chapter 3 we measured plasma cortisol levels in response to a speech task. In contrast to our expectations we did not observe an increase in plasma cortisol, but cortisol levels decreased in response to the speech task. Benschop et al. (1994) have observed the same phenomenon in a cohort of male teachers in response to an acute psychological stressor. These authors hypothesized that high levels of perceived stress and mood disturbances coincide with an attenuated cortisol response to acute stress. In our own study, we found that breast cancer patients and age-matched healthy women who displayed low BDI-depression scores were more likely to show increases in plasma cortisol in response to the speech task ($r = .74$, $p = < .05$; n=6) than those with high BDI-depression scores ($r = -.52$, $p = < .10$; n=8). These findings suggest that healthy women or those who have a systemic illness scoring high on depressive symptomatology may have an aberrant cortisol response in acute stress situations.

In line with these observations, we also observed only a modest or even no ACTH changes at all in response to the speech task. One may argue that the speech task activates preferentially the autonomic nervous system and is not strong enough to activate the hypothalamic pituitary adrenal (HPA) axis. It is well established that acute bicycle exercise activates the HPA-axis resulting in rapid increases in both plasma ACTH and cortisol concentrations (Rolandi, Reggiani, Franceschini, Bavastro, Messina Odaglia & Barreca, 1985; Hyppa, Aunola & Kuusela, 1986; O'Connor & Corrigan, 1987; Chearskul & Srichantaap, 1994). In order to investigate the HPA axis of our group of healthy women, we asked them to participate in a study evaluating the effect of physical stress on plasma cortisol changes.

Methods

Subjects and procedure

Thirteen healthy post-menopausal women (for details about subject recruitment and procedures see chapter 3) exercised on a bicycle ergometer at 80-100% of their VO2max until exhaustion. Blood samples were collected in the basal state (T1 and T2), at the end of exercise (T3), and 5 min (T4), 15 min (T5), and 30 min (T6) later.

Cortisol

The samples were collected in precooled EDTA-tubes, directly centrifuged and stored at -20 °C until analysis. Cortisol levels were determined using a fluorescence polarisation assay on a TDx analyser (Abbot, USA). The intra- and interassay coefficients of variation were both 6%.

Psychosocial measures

The Beck Depression Inventory was completed. For details about the questionnaire see chapter 6.

Statistical analyses

Paired *t* tests (two-tailed) were used to analyze differences in plasma cortisol levels between pre-exercise control values and those measured in response to strenuous exercise. In addition, raw difference scores were computed by subtracting the second baseline cortisol value (T2) from the one measured at T6. The second baseline value was used for data analysis because there was a high correlation between both baseline sampling points ($r = .88$, $p < .001$). The third post-exercise measurement (T6) was used because cortisol levels of all subjects reached their highest cortisol amplitude at that time point. Pearson correlations were performed between these raw difference scores and BDI-depression scores. The .10 probability level was adapted as a significance criterion in all tests.

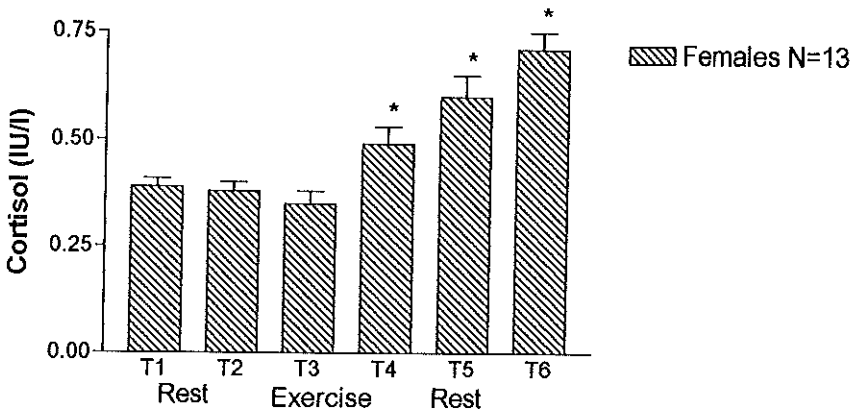

Figure 1. Changes in plasma cortisol in response to strenuous exercise

Results and discussion

In line with earlier established findings obtained in healthy men and pre-menopausal women, acute bicycle exercise elicited a strong HPA system response in post-menopausal women (Rolandi et al., 1985; Hyppa et al., 1986; O'Connor & Corrigan, 1987; Chearskul & Srichantaap, 1994). As can be seen in Figure 1, at 5 min post-exercise (T4) cortisol levels increased about 31% and about 62% at T5 10 min later. The highest amplitude of cortisol (93% increase) was reached at 30 min after termination of the exercise. The cortisol responses measured at 5 min post-exercise (T4) ($t(12)=3.18, p < .01$) and thereafter (T5 and T6) ($t(12)=4.65, p < .01$ and $t(12)=9.55, p < .001$) were significantly different from pre-exercise values.

According to our expectations, depression correlated negatively with the change in cortisol in response to acute bicycle exercise ($r=-.41, p < .10$; $n=13$). Although this finding is preliminary, it seems to implicate that post-menopausal healthy women who suffer from high levels of depression may have smaller increases in plasma cortisol in response to acute stress than those without depressive symptomatology.

It has been well established that some cases of depressive disorders produce sustained activation of the HPA-axis system resulting in elevated levels of cortisol in basal conditions (Van der Pompe et al., 1994). It is temping to speculate that lower plasma cortisol responses to acute bicycle exercise among healthy women are related to their elevated levels of plasma cortisol at baseline. Consistent with this assumption we found a negative correlation between basal cortisol levels and change in plasma cortisol measured after termination of the exercise ($r = -.64$, $p = .009$; n=13). These findings seem to indicate that persistent maximum activity of the HPA system may coincide with adrenal insufficiency in response to acute stress situations. Before drawing firm conclusions research on a larger scale is needed to replicate these findings.

References

Benschop RJ, Brosschot JF, Godaert GLR, de Smet MBM, Geenen R, Olff M, Heijnen CJ, Ballieux RE (1994). Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in man. Am J Physiol 266:75-80

Chearskul S, Srichantaap T (1994). Hormonal and metabolic responses to acute exercise in Thai women. J Med Assoc Thai 77:400-409

Hyppa MT, Aunola S, Kuusela V (1986). Psychoendocrine responses to bicycle exercise in healthy men. Int J Sports Med 7:89-93

O'Connor PJ, Corrigan DL (1987). Influence of short-term cycling on salivary cortisol levels. Med Sci Sports Exerc 19:224-228

Rolandi E, Reggiani E, Franceschini R, Bavastro G, Messina V, Odaglia G, Barreca T (1985). Comparison of pituitary responses to physical exercise in athletes and sedentary subjects. Horm Res 21:209-213

Van der Pompe G, Antoni MH, Mulder CL, Heijnen C, Goodkin, de Graeff A, Garssen B, de Vries MJ (1994). Psychoneuroimmunology and the course of breast cancer: an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psycho-oncology 3:271-288

Appendix IIa

Below the details of the calculation of the following regression model are provided:

$$Y = b_0 + b_1 . ACTH_{(T2,T3,T4, or\ T5)} + b_2 . X_1 + b_3 . ACTH_{(T2,T3,T4, or\ T5)} . X_2$$

In case of prediction of percentage of CD3 cells at baseline by ACTH and by the health status of the donor, the variables ($ACTH_{T2}$, X_1 and X_2), their unstandardized regression coefficients (b) and the constant value (b_0) constitute the following equation:

$$CD3\% = 67.38 + .92 . ACTH_{T2} + (-11.35 . X_1) + (-.63 . ACTH_{T2} . X_2)$$

X_1 is coded 2 for node-positive breast cancer patients and coded 1 for metastatic breast cancer patients and healthy women, and X_2 is coded 2 for metastatic breast cancer patients and coded 1 for node-positive breast cancer patients and healthy women. In our sample, plasma ACTH values vary between 10 ng/l and 50 ng/l.

Thus, node-positive breast cancer patients (X_1 coded 2 and X_2 coded 1), who had, for example, a plasma ACTH value of 50 ng/l, showed a higher percentage (± 59 %) of CD3 cells than metastatic breast cancer patients (X_1 coded 1 and X_2 coded 2), which was ± 39 % with similar levels of plasma ACTH. Healthy women (X_1 coded 1 and X_2 coded 1) with a plasma ACTH value of 50 ng/l, had a higher percentage of CD3 cells (± 71%) than both groups of breast cancer patients (see Figure 1).

Appendix IIb

The calculation of the regression model

$$Y = b_0 + b_1 . CORT_{(T2,T3,T4, or\ T5)} + b_2 . X_1 + b_3 . X_3$$

is as follows. Regarding the prediction of percentage of CD3 cells at baseline by cortisol and by the health status of the donor, the variables ($CORT_{T2}$, X_1 and X_2), their unstandardized regression coefficients (b) and the constant value (b_0) constitute the following equation:

$$CD3\ \% = 101.68 + 26.31 . CORT_{T2} + (-14.19 . X_1) + (-30.26 . X_2)$$

Plasma cortisol values in our sample of breast cancer patients and healthy women varied between .10 and 1.0 µmol/l. Thus, node-positive breast cancer patients (X_1 coded 2 and X_2 coded 1) with low levels of cortisol (.10 µmol/l), had ±46% of CD3 cells in their peripheral blood and metastatic breast cancer patients (X_1 coded 1 and X_2 coded 1) only ±30%. Healthy women (X_1 coded 1 and X_2 coded 1) had a higher percentage of CD3 cells (±60%) than both groups of breast cancer patients, of course with the same level of plasma cortisol (see Figure 2).

Appendix III

Calculation of the regression model

$$Y = b_0 + b_1.\text{CONDITION} + b_2.\text{CONDITION . BASELINE STATUS}$$

is as follows. In case of prediction of BDI post-treatment (PT) by the Condition and by BDI score at baseline, the variables (Condition and Baseline status [BS]), their unstandardized regression coefficients (b) and the constant value (b_0) constitute the following equation:

$$\text{PT BDI} = 6.21 + (-3.98 \text{ . CONDITION}) + .49 \text{ . CONDITION . BS}$$

Condition is coded 1 for EEGP and coded 2 for waiting-list controls (WLC). In our sample levels were between .00 and 28. Thus, breast cancer patients assigned to EEGP who had, for BDI-score of 28 before group assignment, had after participation in EEGP a score of ± 16. Breast cancer patients assigned to the WLC group with the same BDI-score at baseline, had after the 3 month follow-up, a BDI-score of 26. Thus, EEGP patients showed a reduction of 43%, while WLC patients displayed a negligible reduction of 7%.

Appendix IVa

Calculation of the regression model

$$Y = b_0 + b_1.\text{CONDITION} + b_2.\text{CONDITION.BASELINE STATUS}$$

is as follows. In case of prediction of percentage of CD8 cells post-treatment (PT) by the Condition and by percentage of CD8 at baseline, the variables (Condition and Baseline endocrine or immune status [BS]), their unstandardized regression coefficients (b) and the constant value (b_0) constitute the following equation:

$$\text{PT CD8\%} = 23.63 + (-9.25 \,.\, \text{Condition}) + (.43 \,.\, \text{Condition} \,.\, \text{BS})$$

Condition is coded 1 for EEGP and coded 2 for waiting-list controls. In our sample percentage of CD8 cells in the peripheral blood at baseline before group assignment varied in between 10 and 50.

Thus, breast cancer patients assigned to the EEGP group who had, for example, 50% CD8 cells in the peripheral blood before group assignment, had ± 36% CD8 cells after the conclusion of the intervention. In contrast, breast cancer patients assigned to the WLC also with 50% CD8 cells in the periphery had virtually the same level of CD8 cells (± 48%) after the intervention period.

Appendix IVb

With regard to the prediction of post-treatment PRL values the following model appeared to be most plausible:

$$Y = b_0 + b_1.\text{CONDITION} \,.\, \text{BS}$$

In case of the prediction of post-treatment PRL values by the Condition (EEGP or WLC) and by PRL value before group assignment, the variables (Condition and BS), their unstandardized regression coefficients (b) and the constant value (b_0) constitute the following equation:

$$\text{PT PRL} = .07 + .27 \,.\, \text{CONDITION} \,.\, \text{BS}$$

Plasma PRL values in our sample of breast cancer patients varied between .10 and .70 IU/l. Thus, breast cancer patients assigned to the EEGP group who had, for example, a PRL value of .70 IU/l before group assignment, had after the intervention a PRL value of ± .26 IU/l. Those breast cancer patients assigned to the WLC condition also with a PRL value of .70 IU/l had ± .45 IU/l after the intervention period.

Samenvatting

Uit onderzoek is naar voren gekomen dat psychosociale stressoren en stess reacties het beloop van borstkanker kunnen beinvloeden. Tot op heden is nog onbekend welke biologische mediatoren daarbij betrokken zijn. Er wordt verondersteld dat stress het beloop van borstkanker kan beinvloeden via het neuro-endocriene systeem en het immuunsysteem. In **hoofdstuk 1** zijn de onderzoeken beschreven die hebben laten zien dat psychosociale stressoren gepaard gaan met verhoging van bepaalde hormoonspiegels, zoals cortisol en catecholaminen, en met immuunsuppressie. Bovendien is gebleken dat bepaalde hormoonspiegels, zoals oestrogeen en prolactine, de activiteit van het immuunsysteem enerzijds en het beloop van borstkanker anderzijds kunnen beinvloeden. Om inzicht te krijgen in de immunologische reacties van borstkankerpatiënten op stress heeft men in het verleden psychosociale stressoren in verband gebracht met basale immunologische waarden. De huidige opvatting is dat men door het centrale zenuwstelsel te belasten met een kortdurende experimentele stressor betrouwbaarder informatie verkrijgt over de functie van het neuro-endocriene systeem en over de gevoeligheid van het immuunsysteem voor de hormonen dan door alleen basale waarden te meten. In **hoofdstuk 2** zijn naast de basale immunologische waarden, de effecten van een kortdurende experimentele stressor (spreektaak) beschreven voor een groep van 31 borstkankerpatiënten met okselkliermetastasen of metastasen op afstand. Uit de resultaten blijkt dat het beloop van borstkanker gepaard gaat met vermindering van het aantal immuuncellen. Bovendien werd gevonden dat de T-cel proliferatie en de NK-cel activiteit significant verminderd waren in vergelijking met die van gezonde vrouwen. Deze immuunsuppressie bleek het sterkst te zijn bij de groep patiënten met metastasen op afstand. In reactie op de spreektaak namen wij, evenals andere onderzoeken, bij onze groep borstkankerpatiënten en gezonde vrouwen een significante toename waar in het aantal natural killer (NK) en cytotoxische T (CD8) cellen. Deze beide soorten, respectievelijk niet-specifieke en specifieke, effector cellen worden geacht een belangrijke rol te spelen bij de bewaking van tumormetastasering. Eveneens werd vastgesteld dat er significante verschillen waren tussen borstkankerpatiënten met metastasen op afstand en gezonde vrouwen in de distributie en de functie van immuuncellen in reactie op de spreektaak. Zo namen wij bij patiënten met gemetastaseerde borstkanker nauwelijks veranderingen waar in het aantal CD8 cellen in het perifere bloed in reactie op de spreektaak, terwijl bij gezonde vrouwen een stijging van dat aantal optrad. Deze verschillen wijzen op de mogelijkheid dat de immuuncellen van borstkankerpatiënten minder gevoelig zijn geworden voor de hormonen die tijdens de spreektaak worden uitgescheiden. Dit zou veroorzaakt kunnen worden door persisterende blootstelling aan een overmaat van cortisol of catecholaminen. In overeenstemming met deze veronderstelling vonden wij, zoals beschreven in **hoofdstuk 3**, dat vrouwen met gemetastaseerde borstkanker significant hogere basale cortisolspiegels hadden dan gezonde vrouwen van vergelijkbare leeftijd. In hoeverre cortisolspiegels gemeten in rust de variabiliteit en de responscapaciteit van borstkankerpatiënten kunnen voorspellen dient nader te worden onderzocht.

In reactie op een kortdurende experimentele stressor wordt met name het sympathische zenuwstelsel (SZS) geactiveerd, waardoor de productie van catecholaminen toeneemt, die op hun beurt via ß-adrenerge receptoren de distributie van perifere immuuncellen veranderen. In **hoofdstuk 2** bleek de toename van NK en CD8

cellen samen te gaan met een toename van de bloeddruk en hartslag. Een duidelijke toename van de bloeddruk en hartslag wijst op activatie van het SZS. Naast het SZS, speelt de hypofyse bijnierschors as een belangrijke rol bij de aanpassing van het immuunsysteem aan stress. In **hoofdstuk 3** werd beschreven dat in reactie op de spreektaak de hypofyse bijnierschors as werd geactiveerd, met als gevolg een bescheiden, doch significante, toename van de ACTH spiegels. Bestudering van de afzonderlijke hormonale reacties van borstkankerpatiënten en gezonde vrouwen wees uit dat er geen veranderingen zijn waar te nemen in de ACTH spiegels bij vrouwen met metastasen op afstand, terwijl bij patiënten met alleen okselkliermetastasen en gezonde vrouwen een stijging van dat hormoon optrad. De mate van stijging in plasma ACTH in reactie op de spreektaak bleek negatief samen te hangen met de hoogte van de cortisolspiegels gemeten vóór blootstelling aan de taak. Dit suggereert dat een persisterende maximale activiteit van de hypofyse bijnierschors as samengaat met een onvoldoende toename van ACTH en cortisol in acute stress situaties. De matige stijging van de ACTH spiegels wijst op de mogelijkheid dat de speektaak niet sterk genoeg was om de hypofyse bijnierschors as voldoende te stimuleren. Gegevens uit de literatuur suggereren dat fysieke inspanning de hypofyse bijnierschors as stimuleert, met als gevolg een tijdelijke sterke toename van plasma ACTH and cortisol. In **Appendix I** werd de studie beschreven waarin de effecten van een fietstest op de hypofyse bijnierschors as van onze groep gezonde vrouwen werden onderzocht. Uit de resultaten blijkt dat de fietstest een sterke toename in plasma cortisol spiegels teweegbrengt en dat die toename afhankelijk is van het basale cortisol niveau. Bij post-menopausale vrouwen met relatief hogere cortisolspiegels is een kleinere toename in cortisol in reactie op de fietstest waar te nemen dan bij vrouwen met normale basale waarden. Bovendien bleek de mate van stijging in plasma cortisol in reactie op de spraaktaak én de fietsttest ook negatief samen te hangen met de mate van depressie. In de toekomst zal moeten worden onderzocht of de veranderingen in de neuro-endocriene respons én in de distributie van perifere cellen in reactie op acute stress de relatie tussen stress en het beloop van borstkanker kunnen verklaren.

Gegevens uit de literatuur suggereren dat het effect van ACTH en cortisol op de functionele activiteit van immuuncellen afhankelijk is van de (geestelijke) gezondheid. Bij proefpersonen met kenmerken van chronische stress werd waargenomen dat ACTH en cortisol de functionele activiteit van immuuncellen kunnen remmen. Bij gezonde proefpersonen werden deze effecten niet gevonden. Deze differentiele sensitiviteit van perifere immuuncellen zou worden veroorzaakt door het immuunsysteem langdurig bloot te stellen aan relatief hoge plasma cortisol- en catecholaminenspiegels. Zoals boven beschreven, namen wij in ons eigen onderzoek waar dat borstkankerpatiënten significant hogere cortisolspiegels hadden dan gezonde vrouwen. Deze redenering volgend, zou de invloed van ACTH en cortisol op de functionele activiteit van immuuncellen van borstkankerpatiënten kunnen verschillen van die van gezonde vrouwen. Deze hypothese werd onderzocht in **hoofdstuk 4**. Onderzocht werd of er verschillen zijn in de relaties tussen ACTH en cortisol enerzijds en de functie van immuuncellen anderzijds tussen borstkankerpatiënten en gezonde vrouwen. De resultaten ondersteunen de hypothese. Bij borstkankerpatiënten met metastasen op afstand bleek ACTH een negatieve invloed te hebben op de functionele activiteit van verschillende subtypen van immuuncellen met name T lymfocyten, terwijl bij gezonde vrouwen en borstkankerpatiënten met alleen okselkliermetastasen een positief of geen

effect werd gevonden. Cortisol bleek, in overeenstemming met de literatuur, negatief samen te hangen met de functionele activiteit van immuuncellen onafhankelijk van de gezondheid van de proefpersoon. De klinische relevantie van deze bevindingen is nog onbekend. Echter, ze doen vermoeden dat ACTH en cortisol kunnen bijdragen aan de immunologische kwetsbaarheid van borstkankerpatiënten. Bovendien suggereren de relaties tussen ACTH en cortisol enerzijds en het immuunsysteem anderzijds mechanismen waarlangs stress tumorprogressie kan beinvloeden. Opmerkelijk genoeg bleek het effect van ACTH niet alleen waarneembaar bij basale metingen, maar ook tijdens blootstelling aan de spreektaak. Dit wijst op de mogelijkheid dat er veranderingen kunnen optreden in de distributie en functie van perifere immuuncellen onder invloed van de hypofyse bijnierschors as.

Borstkankerpatiënten staan bloot aan verschillende bronnen van stress. Bovendien gaat het hebben van kanker vaak gepaard met angsten en depressieve stemmingsstoornissen. De huidige opvatting is dat de psychosociale stressoren bij het verdere verloop van de ziekte de immunologische kwetsbaarheid van borstkankerpatiënten kunnen vergroten en de progressie van de ziekte kunnen bevorderen. In het algemeen blijkt dat psychosociale begeleiding zoals relaxatietraining, wijziging en (of) verbetering van copingstijlen, en sociale ondersteuning, de psychische klachten van borstkankerpatiënten doet verminderen. Deze psychologische veranderingen blijken samen te gaan met een verbetering van de immuunfunctie. In **hoofdstuk 5** zijn in het kort de psychosociale stressoren beschreven die tijdens het verdere verloop van borstkanker kunnen optreden. Daarnaast is een overzicht gegeven van de onderzoeken naar de invloed van verschillende vormen van psychosociale begeleiding op het immuunsysteem. De onderzoeken in het tweede deel van het proefschrift hebben als doel de effectiviteit van een groepstherapie-programma te bestuderen. Dit programma is gebaseerd op de existentiële traditie. Dat is het faciliteren van de rouwverwerking door onder andere sociale steun te geven en copingstijlen te wijzigen die niet langer effectief blijken. Ten eerste werd onderzocht of groepstherapie het psychologisch en fysiologisch functioneren van borstkankerpatiënten kan beinvloeden; ten tweede werd onderzocht of alle borstkankerpatiënten gebaat zijn bij groepstherapie. Het effect van het groepstherapie-programma op het psychologisch functioneren is beschreven in **hoofdstuk 6**. Het bleek dat de patiënten die hadden deelgenomen aan het groepstherapie-programma minder psychische klachten hadden dan patiënten die aan de wachtlijst-controlegroep waren toegewezen. Dit effect bleek het sterkst bij patiënten met relatief veel psychische klachten. De patiënten met relatief weinig psychische klachten waren niet gebaat bij het groepstherapie-programma. Deze resultaten tonen aan dat selectie van patiënten op basis van psychische klachten zinvol is. Wat betreft het neuro-endocriene en het immuunsysteem werd eenzelfde patroon waargenomen. De resultaten in **hoofdstuk 7** laten zien dat groepstherapie een stabiliserende werking heeft op zowel het neuro-endocriene als het immuunsysteem van borstkankerpatiënten. Deelname aan het groepstherapie-programma bleek samen te gaan met een vermindering van plasma cortisol en prolactine. Dit effect bleek het meest duidelijk bij patiënten met relatief hoge hormoonspiegels. Deze bevindingen zijn van belang voor de behandeling van borstkanker omdat is gebleken dat zowel cortisol als prolactine niet alleen het immuunsysteem maar ook het beloop van borstkanker kunnen beinvloeden. Bovendien bleken borstkankerpatiënten na afloop van het groepstherapie-programma minder NK cellen te hebben dan de wachtlijst-controlegroep. Er wordt

verondersteld dat er een samenhang is tussen de aggressiviteit van de tumor en het percentage NK cellen in de perifere circulatie. Overeenkomstig deze veronderstelling, vonden wij in ons onderzoek dat borstkankerpatiënten meer NK cellen hadden in de perifere circulatie dan de gezonde vrouwen. Deze resultaten suggereren dat groepstherapie de responscapaciteit van borstkankerpatiënten kan veranderen. Dit hebben wij onderzocht in **hoofdstuk 8**. De belangrijkste uitkomst van deze studie was dat groepstherapie de responscapaciteit van NK cellen in reactie op de spreektaak deed verminderen in vergelijking met de wachtlijst-controlegroep. Bovendien hadden borstkankerpatiënten die hadden deelgenomen aan het groepstherapie-programma een betere T cel functie dan de wachtlijst-controle groep. Deze resultaten zijn mogelijk van belang voor de behandeling van borstkanker omdat een betere T cel functie in verband is gebracht met een kleinere kans op een recidief. In de toekomst zal moeten blijken in welke mate deze therapie-effecten invloed hebben op tumorprogressie -vooral die van metastasering.

SUMMARY

The impact of psychosocial stressors and psychological distress on progression of breast cancer comprises a growing literature. It has been hypothesized that psychosocial factors influence the course of breast cancer by modulating endocrine processes which are directly related to tumor growth or indirectly related by decreasing immunologic control over tumor development. In **chapter 1** we described that psychosocial stressors coincide with increases in "stress" hormones. Moreover, it has been shown that sex steroids such as estrogen may influence immunologic processes on the one hand and mammary tumor growth on the other. In order to get insight into the immunologic responses to stress several studies have explored the relationships between psychosocial stressors and basal immune responses. Initial data suggest that information on the organizational level of the neuro-endocrine system and the sensitivity of the immune system to neuro-endocrine mediators can better be obtained in response to an acute stressor than by only measuring baseline responses. In **chapter 2** we decribed, in addition to basal immune responses, the effect of an acute stressor on the distribution and function of peripheral blood cells in a group of breast cancer patients diagnosed with either axillary lymph node metastases or distant metastases. As a model for an acute stressor we used a standardized speech task. The results show that progression of breast cancer coincide with decreased levels of peripheral blood cells. In addition, it was found that natural killer (NK) cell activity and T cell function of breast cancer patients are decreased compared to healthy women. These immune decrements are progressive and related to the clinical stage, the least impairment being seen in node-positive breast cancer patients and the most in those with distant metastases. In line with earlier established findings obtained in healthy subjects, we found reliable increases in percentages of CD8 and NK cells in our group of breast cancer patients. These specific and non-specific effector cells are suggested to be responsible for the control of mammary tumor growth. It is an interesting phenomenon that we observed differences in redistribution of CD8 cell subtype in response to the speech task between breast cancer patients with distant metastases and healthy women. The CD8 cells of metastatic breast cancer patients did not change in response to the task. The nonresponsiveness of CD8 cells is in sharp contrast with the significant increases in CD8 cells found in women without breast cancer or with node-positive breast cancer. The non-responsiveness of CD8 cells for acute stress may be associated with a decreased sensitivity for hormones such as catecholamines or cortisol. It has been suggested that persistent elevated levels of hormones may result in a decreased sensitivity of ß-adrenergic receptors, which, in turn, may result in a low or no increase of immune cells in response to acute stress. Consistent with this assumption, we found that breast cancer patients with distant metastases had higher plasma cortisol levels at baseline than age-matched healthy women.

The speech task preferentially activates the autonomic nervous system resulting in rapid increases in plasma catecholamines. Catecholamines bring about changes in lymphocyte redistribution by binding to high affinity ß-adrenergic receptors expressed by lymphoid cells. As decribed in **chapter 2** the increases in NK and CD8 cells were accompanied by increases in blood pressure and heart rate levels in both breast cancer patients and healthy women. These increases in blood pressure and heart rate levels point to activation of the sympathetic nervous system (SNS). In addition to the SNS, the

hypothalamic-pituitary-adrenal (HPA-) axis has been suggested to be involved in the redistribution of immune cells. In **chapter 3** we measured plasma ACTH and cortisol levels in response to the speech task. A modest increase in plasma ACTH levels in response to the speech task was observed in breast cancer patients with node-positive breast cancer and healthy women, whereas the plasma ACTH levels of metastatic breast cancer patients did not change in response to the speech task. Interestingly, the change in plasma ACTH levels was negatively correlated with the basal cortisol levels. This seems to implicate that persistent maximum activity of the HPA-system may coincide with adrenal insufficiency in response to acute stress situations. One may argue that the speech task activates preferentially the autonomic nervous system and is not strong enough to activate the HPA-axis. It has been well established that acute bicycle exercise activates the HPA-axis resulting in rapid increases in both plasma ACTH and cortisol concentrations. In order to investigate the HPA-axis of our group of healthy women, we asked them to participate in a study evaluating the effect of physical stress on plasma cortisol changes. The results of this study, described in **appendix I**, clearly show that the acute bicycle exercise elicited a strong HPA-axis response in post-menopausal women, resulting in reliable increases in plasma cortisol levels. Moreover, we found a negative correlation between basal cortisol levels and change in plasma cortisol measured after termination of the exercise. Interestingly, we also found that increases in plasma cortisol in response to the speech task and the bicycle exercise correlated negatively with depressive symptomatology.

It has been emphasized that the relative contribution of ACTH and cortisol on distribution and function of peripheral blood cells may vary as a function of the (mental) health of the donor. Among patients diagnosed with major depression, ACTH was negatively correlated with distribution and function of peripheral blood cells, whereas among healthy controls, ACTH was unrelated to immune measures. It has been suggested that hyperstimulation of the immune system by catecholamines or cortisol may lead to a different metabolic status of the cells which implicates a differential sensitivity of T cell function for ACTH or cortisol. It is, therefore, tempting to speculate that there are differences between breast cancer patients and healthy women with respect to the influence of ACTH and cortisol on peripheral blood cells. This hypothesis was tested in **chapter 4**. In node-positive breast cancer patients and healthy women, ACTH had a modest positive effect on distribution and function of T lymphocytes. In contrast, in breast cancer patients with distant metastases ACTH had a negative effect on T lymphocyte percentages and function. Cortisol was, consistent with the literature, negatively correlated with the function of T lymphocytes independent of the health of the donor. The clinical importance of these findings is at present unknown. However, the results seem to implicate that both ACTH and cortisol may contribute to the inhibition of an effective host response against mammary tumor cells. Moreover, the relationships between ACTH and cortisol on the one hand and the immune system on the other suggest mechanisms along which stress may influence mammary tumor progression. Interestingly, the effect of ACTH occurred not only on the distribution and function of immune cells at baseline, but also during the speech task. These findings seem to indicate that in addition to the SNS, the hormones related to the HPA axis are involved in the recruitment of peripheral blood cells.

Breast cancer patients are facing serious psychological, physical and interpersonal problems. Consequently, a considerable fraction of these patients seem to suffer from a moderate to severe emotional morbidity. It has been suggested that psychosocial stressors may inhibit an effective host response in favor of mamary tumor progression. In general, it has been shown that psychosocial interventions including relaxation training, behavioral and cognitive techniques to build effective coping strategies and social support reduce psychological distress levels. Some studies reported that the psychological changes parallelled changes in immune function. It has been shown that participation in a psychosocial intervention program increases the number of NK cells of breast cancer patients. In **chapter 5** we described the results of studies investigating the effects of psychosocial interventions on psychological and biological functioning of cancer patients. The studies described in the second part of the thesis were designed to investigate the effectiveness of a grouppsychotherapy program on psychological, endocrine and immunological measures. This program is based on the principles of the experiential-existential therapy tradition. Special attention was given to the following questions: First, does this grouppsychotherapy progam influence psychological and physiological functions of breast cancer patients; second, has this grouppsychotherapy program the same effect on all breast cancer patients or is there a differential sensitivity for grouppsychotherapy with respect to psychological and physiological functions. The results in **chapter 6** show that the grouppsychotherapy program is most effective in those breast cancer patients presenting with elevated levels of depression; patients who entered the study with lower distress showed no benefits of the program. The findings of the present study seem to suggest that selection of patients with high baseline distress levels, presumably being in need of professional help, in principle is feasable by applying psychological questionnaires. With respect to the effect of the grouppsychotherapy program on the neuro-endocrine and the immune system, we found a similar pattern. The results in **chapter 7** show that the psychotherapy program had succeeded to reduce the basal cortisol and prolactin levels in those breast cancer patients displaying already high baseline levels of these hormones at study entry. These findings can be important for the management of breast cancer because prolactin as well as cortisol have been suggested to stimulate mammary tumor growth. In addition, the grouppsychotherapy program had a stabilizing effect on the redistribution patterns of NK and CD8 cells. This finding may shed a favorable light on the application of the grouppsychotherapy program for breast cancer patients because a lower percentage of NK cells has been associated with a better prognosis. It has been proposed that the percentage of these subsets in the peripheral blood is a reflection of the host response to the aggressiveness of the tumor process. Consistent with this suggestion is the finding that node-positive breast cancer patients in this study had at baseline higher NK cell percentages than their healthy counterparts. The results of this study suggest that grouppsychotherapy may alter the neuro-endocrine and immune reactivity of breast cancer patients. This hypothesis was tested in **chapter 8.** Breast cancer patients had a somewhat higher response to the speech task with respect to NK cell percentages than healthy women at the time before the intervention. The results show that patients in the grouppsychotherapy program had a lower reactivity. In contrast, the task-induced NK cell response of patients in the waiting-list control condition was of a similar magnitude as the NK cell response measured at baseline. The clinical relevance of these immune system changes remains to be established. In addition, grouppsychotherapy resulted in

an increase in T cell proliferation as compared with the waiting-list control condition. The clinical importance of immune system variables in predicting disease progression has thus far been inconclusive. However, a diminished response to mitogen-stimulation has been associated with an unfavorable prognosis in several studies. The results of the present study do justify studies investigating the effect of grouppsychotherapy on the reactivity to acute stress on the endocrine and immune system correlated with breast cancer recurrence and survival on a large scaled basis.

Curriculum Vitae

De schrijver van dit proefschrift werd geboren op 16 maart 1959 te Leiden. Na het behalen van het VWO-diploma, werd in 1984 begonnen met een studie Psychologie aan de Rijksuniversiteit te Leiden. Gedurende deze studie werd onderzoek verricht naar de effecten van stress, coping en sociale steun op het functioneren van managers. In oktober 1989 werd het diploma behaald. Vervolgens werkte ze een jaar bij de Vakgroep Klinische en Gezondheidspsychologie op het project De relatie tussen psychosociale stressoren en het beloop van de HIV-infectie. In april 1991 was zij werkzaam bij het Helen Dowling Instituut voor biopsychosociale geneeskunde op een project dat gefinancierd werd door de VSB-Foundation. Onder leiding van prof. dr M.J. de Vries (HDI) en dr J.J. Heijnen (Immunologie, Wilhelmina Kinderziekenhuis) werd het in dit proefschrift beschreven onderzoek verricht. Aansluitend is zij voor 6 maanden werkzaam geweest op het project "Neonatal stress and immune function" bij de afdeling Immunologie van het Wilhelmina Kinderziekenhuis. Thans is zij als post-doc verbonden aan de Vakgroep Experimentele & Arbeidspsychologie ten behoeve van het NWO-prioriteitsprogramma Acute psychische vermoeidheid in de arbeidssituatie en doet onder andere onderzoek naar de determinanten van de 'spill-over' van neuro-endocriene inspanningsverschijnselen uit de werksituatie naar herstelsituaties én naar de invloeden van 'stress' hormonen op het geheugen en cognitieve controle.

List of publications

Pompe G van der, Heus P de (1993) Work stress, social support and strains among male and female managers. Anxiety, Stress and Coping 6:215-229

Mulder CL, Pompe G van der, Spiegel D, Antoni MH, Vries MJ de (1992) Psychosocial factors and progression of breast cancer. A review of recent literature, methodological problems and future directions; Do psychosocial factors influence the course of breast cancer. Psycho-oncology 1:155-167

Pompe G van der, Antoni MH, Mulder CL, Heijnen CJ, Goodkin K, Graeff A de, Garssen B, Vries MJ de (1994) Psychoneuroimmunology and the course of breast cancer: an overview. The impact of psychosocial factors on progression of breast cancer through immune and endocrine mechanisms. Psycho-oncology 3:271-288

Pompe G van der, Antoni MH, Heijnen CJ (1996) Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. Psychoneuroendocrinology 21:361-374

Pompe G van der, Antoni MH, Duivenvoorden HJ, Heijnen CJ The relations of ACTH and cortisol levels with the distribution and function of peripheral blood cells in response to a behavioral challenge in breast cancer patients. Int J Behav Med, accepted for publication

Pompe G van der, Antoni MH, Visser A, Garssen B (1996) Adjustment to breast cancer: The psychobiological effects of psychosocial interventions. Pat Edu Coun 28:209-219

Pompe G van der, Antoni MH, Duivenvoorden HJ, Vries-Kragt K de, Pelgrim F, Vries MJ de. Prediction of psychological adjustment to breast cancer after participation in a group psychotherapy program. Br J Clin Psych, (prov) accepted for publication

Pompe G van der, Duivenvoorden JH, Antoni MH, Visser A, Heijnen CJ. The effect of a group psychotherapy program on endocrine and immune function in breast cancer patients. J Psychosom Res, in press

Pompe G van der. Neoadjuvant immunostimulation in oncologic surgery. An overview of the effects of preemptive medical and psychological therapy on circulating tumor cells. Psychoneuroimmunology: Stress, Mental Disorder and Health, in press

Pompe G van der, Antoni MH, Heijnen CJ. The effects of surgical stress and psychological stress on the immune function of operative cancer patients. Psych Health, accepted for publication

Pompe G van der (1996) Psychoneuro-immunologie en borstkanker. Ned Tijdschr Geneesk 140:2614-2617

Received grants

Effectiveness of group psychotherapy compared with group support in patients with early-stage breast cancer. A four-year study, funded by the Dutch Cancer Society (HDI-95-893)

Evaluation of an intervention model in breast cancer patients, funded by the Josephine Nefkens Stichting

DANKWOORD

Een aanzienlijk aantal mensen heeft bijgedragen aan de tot standkoming van dit proefschift. Een aantal mensen wil ik hiervoor in het bijzonder bedanken.

Allereerst wil ik dr. Cobi Heijnen bedanken. Beste Cobi, je bent mijn steun en toeverlaat geweest tijdens het onderzoek. Jouw intelligentie, creativiteit en bereidheid mee te denken zijn voor mij een bron van inspiratie geweest.

Prof. dr Marco de Vries ben ik zeer erkentelijk voor de mogelijkheid die hij mij heeft geboden om bij het Helen Dowling instituut mijn onderzoek uit te voeren. Beste Marco, je hebt het onderzoek aan mij toevertrouwd en mij gesteund bij de uitvoering ervan. Hiervoor ben ik je zeer dankbaar.

Klazien de Vries en Floor Pelgrim wil ik danken voor de wijze waarop zij de patienten hebben begeleid. Jullie betrokkenheid bij de patiënten hebben mij vaak ontroerd.

Van Hugo Duivenvoorden heb ik geleerd dat je dichterbij de waarheid kan komen door juist niet de begane paden te bewandelen. Beste Hugo, jouw intelligentie, loyaliteit en trouw zijn voor mij zeer belangrijk geweest.

I want to thank Dr. Michael Antoni for his enthusiasm for my work. Dear Michael, thank you very much for your support and inspiration during the writing of this thesis.

Verder wil ik Nelly Voogt en Marijke Tersteeg hartelijk danken voor hun assistentie bij de experimenten. Beste Nelly en Marijke, jullie nauwgezetheid, trouw en vriendschap zijn voor mij onontbeerlijk geweest.

Ook wil ik Pieta Bulthuis danken voor het werven van gelden voor het onderzoek. Beste Pieta, jouw loyaliteit en vriendschap heb ik zeer op prijs gesteld.

Dr. Adriaan Visser wil ik danken voor het commentaar op de manuscripten maar vooral voor de vele praktische tips.

Bij dit onderzoek zijn een aantal specialisten betrokken geweest. Dr. Alexander de Graeff (AZU, Utrecht), dr. Van der Vegt (Ziekenhuis Oudenrijn, Utrecht) en dr. Simonis (Het Groene Hart ziekenhuis, Gouda) wil ik in het bijzonder danken. De assistente van dr Simonis, Marianne Reijms en Anja de Kruif werkzaam bij het AZU wil ik danken voor hun hulp bij de speurtocht naar geschikte patiënten.

Een aantal collega's hebben mij tijdens het onderzoek gesteund. Beste Hans Schilder, Niels Mulder, Anneke Francke en Ellen Sanders ik heb jullie steun en vaak levendige discussie zeer gewaardeerd. Bovendien dank ik Cock Kuipers en Hanny Docx voor de gezelligheid en hun vriendschap.

Tenslotte wil ik mijn lieve partner Koos Bergsma bedanken. Koos, ik dank je voor alle "zaterdagochtenden" dat je naar me hebt willen luisteren. Jouw motto "Stick to your strategy" is onlosmakelijk met dit proefschrift verbonden.