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Report

Does subjective burden of early breast cancer and its treatment affect immune measures during adjuvant therapy?

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Summary

Psychosocial factors have been described as affecting cellular immune measures in healthy subjects. In patients with early breast cancer we explored bi-directional psycho-immune effects to determine whether subjective burden has an impact on immune measures, and vice versa.

Patients ($n = 239$) operated for early breast cancer and randomized into International Breast Cancer Study Group (IBCSG) adjuvant clinical trials were assessed immediately before the beginning of adjuvant treatment (baseline) and 3 and 6 months thereafter, at the beginning of the corresponding treatment cycle. Cellular immune measures (leukocytes, lymphocytes, lymphocyte subset counts), markers of activation of the cellular immune system (β_2-microglobulin, soluble interleukin–2 receptor serum levels), and self-report subjective burden (global indicators of physical well-being, mood, coping effort) were assessed concurrently. The relationship between subjective burden and gradients of immune measures was investigated with regression analyses controlling for adjuvant treatment. There was a pattern of small negative associations between all variables assessing subjective burden before the beginning of adjuvant therapy with the gradients of the markers of activation of the cellular immune system and NK cell counts. In particular, better mood predicted a decline in the course of β_2-microglobulin and IL-2r at months 3 and 6. The gradient of β_2-microglobulin was associated with mood and coping effort at month 3. However, the effect sizes were very small. In conclusion, in this explorative investigation, there was an indication for subjective burden affecting and being affected by markers of activation of the cellular immune system during the first 3 and 6 months of adjuvant therapy. The question of clinical significance remains unanswered. These associations have to be investigated with refined assessment tools and schedules.

Introduction

Psychosocial factors, particularly distress, have been shown to be associated with changes in a number of immune measures in healthy subjects [1]. However, very few studies have investigated the influence of psychosocial factors on immunity in cancer patients and the importance of this relationship for the course of the disease [2, 3].

The exact role of the immune response in breast cancer and the best way to assess it are issues of continuing debate. Patients with breast cancer

showed changes in peripheral lymphocyte counts, skin testing, in vitro blastogenesis, and assays of T- and B-cell competence in advanced disease [4–8]. Natural Killer (NK) cell counts, β_2-microglobulin, and soluble interleukin-2 receptor (sIL-2r) serum levels in patients with early stage breast cancer were associated with prognostic factors after surgery, but before adjuvant treatment [9]. NK cells are considered to play an important role in the immune response to neoplasia in general, and specifically to breast cancer [10, 11].

The potential interaction between psychosocial factors and immunity is of particular importance in the tolerability of chemotherapy. Chemotherapy may increase psychological distress and delays patients' adaptation after diagnosis and surgery for breast cancer [12]. Distress is hypothesized to have a negative effect on immunity in these patients [13]. On the other hand, the immuno-suppressive effects of adjuvant chemotherapies are well documented.

Present knowledge of the interaction between immune measures and psychosocial factors in early breast cancer is mainly derived from cross-sectional studies, or studies with a short follow-up or with a limited number of patients. Chronically stressed individuals show the same pattern of immune changes as patients with progression of breast cancer [13]. Perceived good social support, specifically attachment, was associated with higher leukocyte and granulocyte counts, while social integration was negatively associated with proportion of NK cells of lymphocytes 3 months after chemotherapy [14]. There was no association between psychosocial factors and functional immune assays in this study. This relationship may be overridden by the biological factors affecting the haematopoetic recovery process. Preoperative intrusive anxiety and anxious preoccupation were inversely correlated with the preoperative number of lymphocytes, B, T total and T4 lymphocytes, and depression with B and T4 lymphocytes in early stage breast cancer patients [15]. Preoperative depression had an inverse effect on the total number of lymphocytes, T total, and T4 lymphocytes 7 days after surgery. Prepostoperative changes in immune measures were decreased by depression and increased by intrusion [15]. More self-reported intrusive and avoidant thoughts and behavior related to cancer after surgery for breast cancer were associated with lower proliferative responses of peripheral blood lymphocytes to mitogens and to a monoclonal antibody to the T cell receptor [16]. This form of distress was also related to lower NK cell lysis [16]. Baseline NK cell activity in women with breast cancer was accounted for by patient adjustment, lack of social support, and fatigue/depression symptoms [17]. NK cell activity 3 months later was explained by baseline level of NK cell activity, fatigue/depression symptoms and lack of social support. Tumor burden was related to NK activity but neither radiation nor chemotherapy showed an effect [18].

Although there is some evidence of an association between psychosocial factors and immunity in patients with breast cancer, its nature is not clear. This association may be bi-directional. We designed a longitudinal study in patients receiving adjuvant therapy within randomized clinical trials to explore the association between subjective burden and immune measures. We hypothesized subjective burden before the beginning of adjuvant therapy to have an effect on immune measures on and off adjuvant treatment at months 3 and 6, and the course of immune measures to have an effect on subjective burden at these time points. The assumption was that possible psycho-immune relationships during adjuvant treatment would be best detected when the immune system had recovered from the noxious effects of chemotherapy, i.e. immediately before a new cycle of chemotherapy, and that only a sustained change of immune measures would likely be of clinical relevance.

In order to be able to assess a large number of patients, enumerative assays were used in the present study rather than the more time-consuming functional assays. The choice of peripheral enumerative immune measures will not allow us to clarify possible immune mechanisms. However, individual differences in enumerative cellular immune responses to psychological stress in healthy subjects have been shown to be reproducible, indicating that enumerative immune measures are suitable to investigate psycho-immune relationships [1, 19]. In addition to cellular immune counts, β_2-microglobulin and sIL-2r serum levels were assessed as markers of a nonspecific activation of the cellular immune system. The longitudinal design of our study allowed the calculation of a gradient over time describing the course of

each immune measure during the first 3 and 6 months of adjuvant treatment. Each patient was her own control and the assessments were performed at the same time of the day for each patient.

Patient and methods

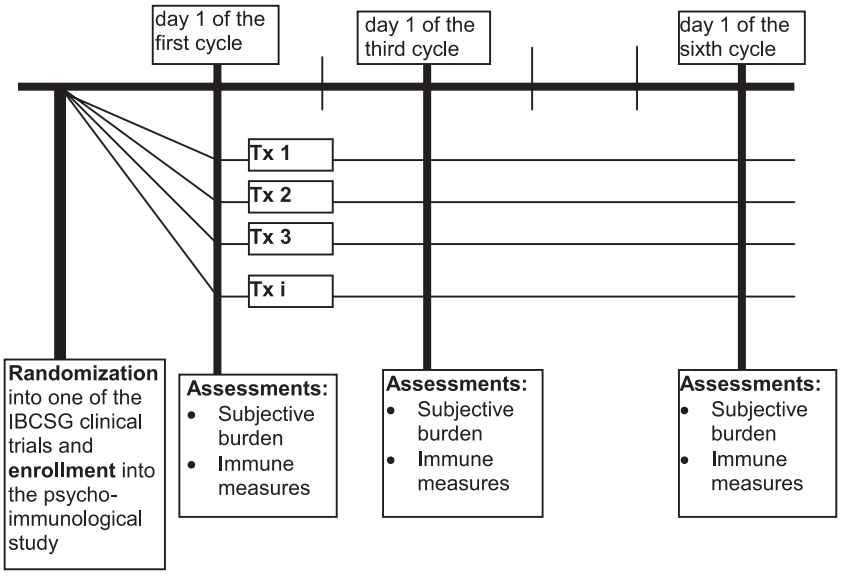
At six Swiss centers, 239 patients with stage I and II breast cancer (see Table 1) were enrolled in this explorative study between July 1991 and July 1997 immediately after being randomized into one of six International Breast Cancer Study Group (IBCSG) adjuvant therapy trials (Trials VI, VII, VIII, IX, 13–93, 14–93). The randomization took place within 6 weeks of primary surgery with a median of 28.2 days [9]. The trials investigated different regimens of chemotherapy and/or endocrine therapy according to menopausal status; eligibility criteria of two closed trials have been published elsewhere [20, 21]. They were similar for the subsequent trials. Briefly: adequate marrow, renal and hepatic function, no previous or concomitant malignancy (except basal or squamous carcinoma of skin, or adequately treated *in situ* carcinoma of the cervix), no prior adjuvant therapy for breast cancer, no psychiatric or addictive disorder to prevent informed consent, no systemic disease which would prevent the patient from undergoing any of the treatment options, and no pregnancy. Patients were excluded from the present study if they were affected by an immune disorder, by a systemic disease, or were receiving other than the study medication interfering with the immune measures of interest.

Table 1. Patient characteristics ($n = 239$)

	Median (range)	*n* (%)
Age	52.6 (25.1–76.3)	
Menopausal status		
Premenopausal		117 (49%)
Postmenopausal		122 (51%)
Type of surgery		
Total mastectomy		112 (46.8%)
Breast conserving		123 (51.5%)
Unknown/missing		4 (1.7%)
Nodal status		
Negative		97 (41%)
Positive		142 (59%)
Estrogen receptor-status		
Negative (ER < 10 fm/g cytosol protein)		79 (33%)
Positive (ER > 10 fm/g cytosol protein)		157 (66%)
Unknown/missing		3 (1%)
Adjuvant therapy		
	Month 3	Month 6
Endocrine therapy yes	61	91
Endocrine therapy no	178	148
Chemotherapy yes	188	85
Chemotherapy no	51	154

Note: Every patient received adjuvant treatment according to the IBCSG adjuvant therapy trials VI, VII, VIII, IX, 13–93 or 14–93 investigating different regimens of chemotherapy and/or endocrine therapy. The definition of menopausal status as a dichotomous variable changed across trial generation (i.e., age cut off 52 years, premenopausal *versus* peri-/postmenopausal) to (i.e., age cut off 55 years, pre-/perimenopausal vs. postmenopausal).

The psychological and immune assessments of patients were performed immediately before the first adjuvant treatment (baseline), and immediately before the month 3 and 6 infusions, or at corresponding time points if the patient was receiving endocrine treatment (see Figure 1). The time of blood draw was recorded. The protocol required a follow-up blood draw taken within 2 h of the time the baseline was assessed, in order to control for diurnal variation of immune measures. According to the IBCSG protocols, patients received either adjuvant chemotherapy, endocrine therapy or chemoendocrine therapy. Chemotherapy consisted of CMF (cyclophosphamide 100 mg/m^2 orally days 1–14, methotrexate 40 mg/m^2 iv days 1 and 8, 5-fluorouracil 600 mg/m^2 iv days 1 and 8; repeated every 28 days for 3–6 cycles), AC/EC (doxorubicin 60 mg/m^2 or epirubicin 90 mg/m^2 iv and cyclophosphamide 600 mg/m^2 iv day 1; repeated every 21 days for four cycles), or a sequence of both. Endocrine treatment consisted of tamoxifen (20 mg daily), toremifen 60 mg daily, or goserelin (3.6 mg s.c. q 28). Patients with less than total mastectomy received radiation therapy



Notes:

day 1:	Day 1 of a treatment cycle or a corresponding time point if the patients receives endocrine treatment
Tx1-i:	One of the 6 International Breast Study Group (IBCSG) adjuvant therapy trials (Trials VI, VII, VIII, IX, 13-93, 14-93)
Assessments:	Assessments were performed at the first day of a treatment cycle before treatment was given
Subjective burden:	Subjective burden was assessed with 3 LASA scales for physical well-being, mood and coping effort and a disease specific questionnaire FBK-SAKK
Immune measures:	Immune cell counts, β_2-microglobulin and soluble interleukin-2 receptor

Figure 1. Study Design.

according to the guidelines of each participating institution.

Immune cell counts

Leukocytes and lymphocytes (cells/mm^3) were counted in a SYSMEX K-1000 CELL COUNTER (Digitana AG, Horgen CH). Lymphocyte subsets were assessed with flow cytometry (FACSAN-Cytometer, Becton Dickinson, Heidelberg, GFR with a SIMULSET Test Kit, BD, Heidelberg and the corresponding software) at baseline (day 1, before start of adjuvant treatment). Thus, lymphocytes were tested for the following markers: lymphocytes and monocytes: CD45/CD14; negative control: mouse lgG1/mouse lgG2; T-cells/B-cells: CD3/CD19; T4-cells: CD3/CD4; T8-cells: CD3/CD8; CD4/CD8; natural killer-cells (NK): CD3/CD16 and CD56; activated T-cells (AT): CD3/anti HLA-DR. The results of the subpopulations were assessed as percent values of total lymphocytes and converted into counts (cells/mm^3) to calculate a gradient over time ((immune count at 3 or 6 months – immune count before first adjuvant treatment)/time since first adjuvant treatment) for the current evaluation. A negative gradient indicates a decrease in the immune measure over the period of interest. Leukocytes, lymphocytes and lymphocyte subset counts are summarized as immune cell counts.

β_2-microglobulin and soluble interleukin-2 receptor serum levels

β_2-microglobulin (μg/l) and soluble interleukin-2 receptor (sIL-2r) serum levels (pmol/l) are referred to as markers of activation of the immune system. β_2-microglobulin is part of the HLA class I molecule which is critical in the recognition of intracellular antigens by the immune system and is

present in virtually all human nucleated cells. The catabolism depends on normal kidney filtration and excretion. The soluble interleukin-2 receptor can be considered to reflect the response to interleukin-2, which is a cytokine with several effects such as activating cytotoxic T cells and NK cells, stimulating the proliferation and differentiation of T cells and co-stimulating the proliferation of B cells and antibody secretion. Gradients over time were calculated (serum level at 3 or 6 months – serum level before first adjuvant treatment)/time since first adjuvant treatment) for the current evaluation.

β_2-microglobulin serum level was assessed with an IMX-β_2-microglobulin test (Abbott) with a microparticle-EIA technique. Briefly, microparticles coated with monoclonal mouse-anti-β_2-microglobulin-antibody are incubated with the samples. After washing, the antigen-antibody-complex reacts with alkaline phosphatase-conjugated monoclonal mouse-antibody to sandwich aggregate. After addition of 4-methylumbelliferyl-phosphatase as a substrate, the fluorescent product is measured. The test has a sensitivity of 5 μg/l.

Soluble interleukin-2 receptor (sIL-2r) serum level was assessed with a sandwich-EIA-test technique (Immunotech SA). Briefly, monoclonal anti-sIL-2-r antibody against epitope 1 of IL-2r binds IL2r in sample sera. Addition of alkaline phosphatase conjugated anti-sIL-2r antibody against epitope 2 completes the sandwich. The amount of enzyme-conjugate is measured by adding paranitrophenyl phosphate as substrate. The sensitivity of the test is 5 pmol/l. The assay was performed on batches of frozen samples. Due to technical problems the results of the assays of several batches were not available, resulting in a smaller n. β_2-microglobulin and sIL-2r serum levels are summarized as immune measures.

Contextual factors of the immune assessments

A face valid questionnaire developed by the authors and filled out by the oncology nurse or the physician assessed several medical or behavioral conditions, such as the consumption of concomitant medication, a history of infection in the previous 6 weeks, the presence of allergies, chronic disorders unrelated to cancer or the immune system, change in sleeping habits or in physical activity. This information was used to control the eligibility of patients for the present study also during the follow up assessments.

Psychosocial assessment

Subjective burden was assessed by two questionnaires. First, as part of the standard assessment in all IBCSG trials included in this project, patients responded to three linear analogue self-assessment (LASA) indicators of components of quality of life. These were *global indicators* for physical well-being, mood, and coping effort (PACIS) designed to reflect the individual meaning and importance of various factors [22]. Concurrent validity [23], test-retest-reliability [24], and responsiveness to chemotherapy [25] have previously been documented. Their clinical relevance has been shown in both early [12] and advanced disease [26]. Their sensitivity to performance status [27], disease recurrence [12], and tumor response in advanced disease [28], and their prognostic value for survival [29–31] have been demonstrated.

Second, for this project only, patients filled out a disease-specific questionnaire (FBK-SAKK). The FBK-SAKK is based on the FBK and contains single- and multi-item scales about specific *disease- and treatment related problems* which patients are requested to rate according to their occurrence and subjective burden in an ordered six point categorical scale (0–5) [32]. For the present analysis, we selected scales of physical performance, anxiety, sleep disturbance and hair loss. There was no indication of a substantial association between these measures and the investigated immune measures. These results are not shown in this paper for the sake of brevity.

Statistics

This exploratory analysis with comprehensive assessments of factors of influence and responses was based on the following overarching hypotheses. (1) Subjective burden before the beginning of adjuvant treatment influences gradients of immune measures between the beginning and the third and/or the sixth cycle of adjuvant therapy. This hypothesis is derived from findings of studies on the effect of distress on immunological measures in healthy subjects [1]. (2) Gradients of immune

measures will affect subjective burden at the third and/or sixth cycle of adjuvant treatment.

Following the two overarching hypotheses, we looked for patterns of effect, effect sizes and their significance. These patterns can be considered in two directions, first on a temporal scale, comparing the gradients of immune measures from the beginning of adjuvant therapy to the third cycle with those from beginning to the sixth cycle with respect to the different measures of subjective burden. Secondly, patterns can be considered across the independent psychosocial variables. Besides direction and size of the effects, statistical significance is of particular interest when it appears with the same endpoints. Comparison of patterns can be considered an assessment of the repeatability of the finding (although within the same study).

For analysis of the global indicators we used the square roots of the scores, because this transformation approximated a normal distribution and was effective in stabilizing the variances [12]. Simple (univariate) linear regressions were used to explore possible associations of psychosocial measures with gradients of immune measures and vice versa, and to confirm responsiveness of both immune and psychosocial measures to chemotherapy. In order to control for treatment (chemotherapy yes/no, endocrine treatment yes/no) multiple (multivariate) linear regressions were performed where appropriate. Type of primary surgery and planned radiation therapy were stratification factors in all the IBCSG studies and were therefore not controlled for also statistically. In early breast cancer NK-cell counts, β_2-microglobulin and sIL-2r serum levels before the beginning of chemotherapy were not associated with tumor size and number of positive lymph nodes, but with prognostic positive factors such as ER positive tumor, older age and menopausal status [9]. Time since surgery was positively correlated with β_2-microglobulin [9]. Type of surgery was associated with T8-cell counts, β_2-microglobulin and sIL-2r serum levels [9]. The longitudinal design of our study allowed the calculation of a gradient over time describing the course of each immune measure for every patient during the first 3 and 6 months of adjuvant treatment and reduced the interference of confounding factors. Hence, the regression models did not include age, menopausal status, type of surgery, diurnal variability, treatment for other underlying diseases, and physical activity.

Due to the explorative nature of the study, the sizes of clinically relevant differences could not be identified in advance. Hence, no formal power calculation was performed. All *p*-values were two-tailed. To take multiple evaluations into account, the significance level of 0.01 was used. The statistical evaluations were performed with different versions of S-plus (for Unix, 2000 and 6.0).

Results

The patient characteristics are described in Table 1. There were no substantial correlations between cellular immune measures (leukocytes, lymphocytes, lymphocyte subset counts) and the markers of activation of the cellular immune system (β_2-microglobulin, soluble interleukin-2 receptor serum levels) with the measures of subjective burden (global indicators of physical well-being, mood, coping effort) at baseline.

Results are presented in detail for the gradients of the two markers of activation of the cellular immune system (serum levels of β_2-microglobulin (μg/l) and soluble interleukin-2 receptor (sIL-2r)) because, to our knowledge, they have never been reported in this context and showed the most consistent pattern of findings, and for the gradients of NK-cell counts because of the existing reports of the role they are considered to play in the immune response to breast cancer [10, 11]. The results of the other cellular immune measures (counts of leukocytes, lymphocytes, T-, B-, T4-, T8-, activated T-cells) will be summarized.

Effect of chemotherapy on the course of immune measures

The effect of chemotherapy on the gradients of immune measures was investigated to confirm their responsiveness to cytotoxic effects.

β_2-microglobulin and IL-2r showed an increase over time (positive gradients), with a marginally significant ($0.01 \leq p$-value < 0.05) change only between 0 and 6 months for β_2-microglobulin (Table 2). On the contrary, all cellular immune counts dropped (negative gradients) with the exception of activated T cells (data not shown). Between baseline and month 3 significant (p-value < 0.01) deterioration was observed for leukocyte and B cell counts. Between baseline and month 6 significant deterioration was

Table 2. Chemotherapy effect on the course of immune measures

Outcome variable (gradient)	Parameter estimates of the chemotherapy effect (±SE)					
	0–3 months	*n*	*p*	0–6 months	*n*	*p*
β_2-microglobulin	1.15 (±0.91)	210	0.21	0.84 (±0.32)	208	0.01
IL-2r	0.07 (±0.07)	151	0.38	0.05 (±0.03)	137	0.07
NK cells	−0.76 (±0.50)	207	0.13	−0.69 (±0.18)	202	<0.001

Note: Gradients of immune measures were the outcome variables in simple linear regressions with chemotherapy as the predictor. The parameter estimate describes the change of the gradients of immune measures for having chemotherapy or not, e.g. β_2-microglobulin increases on average 1.15 μg/l per day during the first three cycles of chemotherapy compared to patients not having chemotherapy. Chemotherapy had a significant negative effect on the gradients of leucocytes, lymphocytes, T cells, B-cells and T4 cells, but not on T8 cells and activated T cells (data not shown).

Table 3. Effects of chemotherapy on subjective burden at months 3 and 6

Outcome variable (transformed)	Parameter estimate of chemotherapy effect (±SE)					
	month 3	*n*	*p*	month 6	*n*	*p*
Physical well-being	−0.57 (0.3)	232	0.06	−0.62 (0.20)	221	0.003
Mood	0.06 (0.29)	234	0.82	−0.24 (0.22)	222	0.26
Coping effort	−0.55(0.36)	229	0.13	−0.65 (0.25)	221	0.01

Note: The square-root transformed subjective burden indicators at months 3 or 6 were the outcome variables of simple linear regressions with chemotherapy as the predictor. Higher scores (transformed full scale range: 0–10) indicate less subjective burden. The parameter estimate describes the change of the transformed subjective burden score for having chemotherapy or not, e.g., at month 3 physical well-being is on average 0.57 lower (in transformed scale) in patients with chemotherapy compared to those without.

observed for leukocyte, lymphocyte, B cell and NK cell counts, and marginally significant deterioration for T cell and T4 cell counts. That is, most immune cell counts decreased substantially in the first 6 months of chemotherapy. In summary, adjuvant chemotherapy affected the gradients of immune cell counts, β_2-microglobulin and IL-2r between baseline and months 3 and 6 in the expected directions.

The effect of adjuvant chemotherapy on subjective burden assessed with three indicators was investigated to confirm their responsiveness to adjuvant treatment (Table 3). At 6 months the negative effect of adjuvant chemotherapy was significant on physical well-being score and marginally significant on coping effort score. That is, adjuvant chemotherapy gave patients burden in physical well-being and coping effort at 6 months.

Effect of subjective burden at baseline on the course of immune measures

For the first hypothesis investigating the possible effect of subjective burden at baseline on the course of immune measures, gradients of immune measures were the outcome variable in multiple linear regressions, including as predictor variables the respective baseline subjective burden indicator and type of treatment (chemotherapy yes/no, endocrine treatment yes/no). The values of the multiple *R*-squared of the regressions were mostly below 5%, indicating that the overall effect size is small, even when the known effect of chemotherapy on immune measures was taken into account.

There was a consistent pattern of small negative associations over time between baseline subjective burden indicators and the gradients of β_2-microglobulin, IL-2r and NK cell counts from baseline to months 3 and 6. That is, less subjective burden [higher score] before treatment was associated with greater reduction rates, as compared to baseline values, for activation of the cellular immune system and NK cell counts before the third and sixth cycles of treatment. The association between baseline mood and gradient of β_2-microglobulin at month 3 was statistically significant. Marginally significant associations were observed between baseline physical well-being and gradient

Table 4. Effects of baseline subjective burden on the gradients of immune measures

Predictor variable (transformed)	Outcome variable (gradient)	Parameter estimates of baseline subjective burden scores effect (± SE)					
		0–3 months	*p*	*n*	0–6 months	*p*	*n*
Physical well-being	β_2-microglobulin	−0.17 (0.29)	0.56	203	−0.14 (0.12)	0.23	205
	IL-2r	−0.05 (0.02)	0.03	149	−0.01 (0.01)	0.21	135
	NK cells	−0.03 (0.16)	0.85	203	−0.08 (0.07)	0.21	199
Mood	β_2-microglobulin	−0.73 (0.23)	0.002	206	−0.23 (0.09)	0.015	205
	IL-2r	−0.04 (0.02)	0.07	149	−0.02 (0.009)	0.04	135
	NK cells	−0.17 (0.13)	0.18	203	−0.09 (0.05)	0.1	199
Coping effort	β_2-microglobulin	−0.27 (0.19)	0.17	207	−0.04 (0.08)	0.59	206
	IL-2r	−0.01 (0.017)	0.48	149	−0.005 (0.007)	0.43	135
	NK cells	0.14 (0.11)	0.19	204	−0.08 (0.04)	0.054	200

Note: Gradients of immune measures were the outcome variable in multiple linear regressions including the respective transformed subjective burden indicator and type of treatment (chemotherapy yes/no, endocrine therapy yes/no) as predictors. Higher scores (transformed full scale range: 0–10) indicate less subjective burden. The parameter estimate describes the change of the gradient of immune measure per day for every point increase of the transformed baseline subjective burden score of interest, e.g., soluble IL-2r decreases on average 0.05 pmol/l per day during the first three months for every point increase on the transformed baseline physical well-being indicator.

of IL-2r at month 3, between baseline mood and gradients of β_2-microglobulin as well as IL-2r at month 6 (see Table 4).

On the other hand, the patterns of associations between baseline subjective burden indicators and gradients of other immune cell counts were not consistent: there was a pattern of small positive associations of baseline physical well-being and coping effort with the gradients of immune cell counts at month 3, and a pattern of small negative associations of baseline mood and coping effort with the gradients of immune cell counts at month 6 (not shown).

Effect of the course of immune measures on subjective burden at month 3 or 6

The second hypothesis that the course of immune measures during adjuvant treatment predicts subjective burden at month 3 or 6 was also investigated using multiple linear regressions. The values of the multiple R^2 of the regressions were again mostly below 5%, indicating that the overall effect size is small, even when the known effect of chemotherapy on subjective burden was taken into account. There was a consistent pattern of negative associations of the gradients of β_2-microglobulin, IL-2r and NK-cell counts with subjective burden at month 3. That is, declining gradients of β_2-microglobulin, IL-2r and NK-cells were associated with less subjective burden at month 3. The associations of β_2-microglobulin with mood and coping effort were marginally significant. There was no consistent pattern between the gradients of the marker of activation of the cellular immune system or NK cell counts and subjective burden at month 6 (Table 5).

There was no consistent pattern of associations between the gradients of the other cellular immune measures and subjective burden at month 3 or 6. Marginally significant associations were observed between the gradient of B-cell counts and physical well-being ($n = 203$; regression coefficient $= 0.13$; $p = 0.01$) as well as coping effort scores at month 3 ($n = 201$; regression coefficient $= 0.14$; $p = 0.02$). That is, increasing gradients of B cell counts were associated with less subjective burden.

Discussion

In studies with a cross-sectional design or with a short follow-up the evaluation of a causal relationship is difficult. An immune-psycho relationship may be mistakenly interpreted as a psycho-immune relationship. We therefore investigated both directions. In this explorative study in 239 patients with stage I and II breast cancer receiving adjuvant therapy according to the protocols of 6

Table 5. Effects of the gradients of immune measures on subjective burden at months 3 and 6

Predictor variable (transformed)	Outcome variable (gradient)	Parameter estimates of the effect of gradients of immune measures (±SE)					
		0–3 months	*p*	*n*	0–6 months	*p*	*n*
β_2-microglobulin	Physical well-being	−0.03 (0.024)	0.22	207	−0.02 (0.048)	0.67	197
	Mood	−0.05 (0.024)	0.04	209	−0.002 (0.05)	0.97	199
	Coping effort	−0.06 (0.03)	0.03	205	0.01 (0.06)	0.83	197
IL-2r	Physical well-being	−0.46 (0.35)	0.20	149	0.03 (0.87)	0.97	131
	Mood	−0.19 (0.37)	0.60	150	0.89 (0.92)	0.33	132
	Coping effort	−0.40 (0.46)	0.38	147	−0.43 (1.03)	0.68	131
NK cells	Physical well-being	−0.01 (0.046)	0.81	204	0.04 (0.09)	0.66	192
	Mood	−0.08 (0.045)	0.07	206	−0.08 (0.10)	0.40	194
	Coping effort	−0.01 (0.055)	0.84	202	0.08 (0.11)	0.49	193

Note: Transformed subjective burden indicators at months 3 or 6 were the outcome variables in multiple linear regressions including the respective gradients of immune measures and type of treatment (chemotherapy yes/no, endocrine therapy yes/no) as predictors. Higher scores (transformed full scale range: 0–10) indicate less subjective burden. The parameter estimate describes the change of the subjective burden score, in transformed scale, for every unit increase of the gradient of immune measure of interest, e.g., physical well-being at month 3 is on average 0.03 points lower for every increase of 1.0 μg/l per day of β_2-microglobulin.

IBCSG trials a pattern of a possible psycho-immune relationship was found: subjective burden before adjuvant therapy predicts a decline in the course of β_2-microglobulin and IL-2r, two markers of activation of the cellular immune system, as well as of NK cell counts in the first 3 and 6 months of therapy.

Effect of subjective burden at baseline on the course of immune measures

There were small negative associations over time between all variables assessing subjective burden before the beginning of adjuvant therapy with the (0–3)- and (0–6)-gradients of the markers of activation of the cellular immune system and NK cell counts. This was mostly expressed in the prediction of the gradients of β_2-microglobulin and IL-2r between baseline and months 3 and 6 by mood, and (0–3) – gradients of IL-2r by physical well-being. It is the first time, to our knowledge, that these two markers of activation of the immune system have been reported in connection with a psycho-immune relationship. These individual results could be due to chance owing to the large number of comparisons performed. However, these findings are noteworthy because they are consistent within the pattern of negative associations.

The negative associations unexpectedly indicate that less subjective burden [higher score] at baseline was associated with a large decline of β_2-microglobulin, IL-2r and NK cell counts between the beginning and the third/sixth cycle of adjuvant therapy. Associations between psychological distress and lower immunological measures have been previously reported [33]. Our findings suggest that mood, physical well-being, or coping effort indicating less subjective burden are associated with lower values of immune measures. It is conceivable that the duration of the stressor may be related to different directions of the psycho-immune association, as described for the effect of acute (laboratory stressor of less than half an hour) or chronic stress on immunological measures [1]. We used enumerative immune measures, whereas in some of the studies functional assays were used which may have a different sensitivity to the effect of psychosocial factors. In healthy humans, changes in enumerative measures and functional assays of the immune system have both been reliably associated with distress [1]. It was usually the chronic stressor that was associated with lower immune measures. Most studies had a smaller sample [34], a cross-sectional design, or only a very short follow-up of the immune measures of a few weeks. A further problem in considering long-term psycho-immune associations consists in the possibility of

habituation to the stressor, or the possibility of a floor and bottom effect of such association. In our study the effect size of some gradients of the immune measures decreased over time, thus supporting this possibility.

Our study consisted of a follow-up over the first 3 and 6 months of adjuvant therapy. The effect of chemotherapy or radiation therapy on the immune system or the acute distress associated with the application of the drugs may interfere with the evaluation of a long-term psycho-immune association. The schedule of radiation therapy for patients with breast conserving surgery was not standardized in these trials limiting the possibility of controlling for its interfering effects particularly in those patients receiving endocrine treatment only or three cycles of CMF. In future studies, psycho-immune relationships should be investigated over short and long periods of time, in order to limit the interference of unidentified factors and to be able to detect changes in the direction of the effect by distinguishing short-term from long-term psycho-immune association.

The (0–6)-gradients of the other cellular immune counts showed the same pattern of small negative associations with baseline subjective burden. Probably due to lack of statistical power these associations are not statistically significant. It is noteworthy that these findings contradict those of previous studies with much smaller samples in which a relationship between distress and cellular immune counts was reported [13,15,18,35].

As a reference comparison we evaluated the known impact of chemotherapy on immune measures and subjective burden. The residual negative effect on the immune measures after recovery from the previous cycle of chemotherapy immediately before a new cycle was only slightly larger than that of the psycho-immune association. The effect of chemotherapy on subjective burden assessed with global quality of life indicators was in the expected direction, indicating a detrimental effect on physical well-being and coping effort [25].

The effects of adjuvant treatment on immune measures and on psychosocial factors may override an interaction between psychosocial factors and immune measures [29]. In another study perceived attachment significantly predicted white blood cell levels in women after, but not during adjuvant chemotherapy for breast cancer [14]. Higher NK cell activity at the 15th month follow-up predicted disease-free survival, and psychosocial factors at follow-up predicted time to recurrent disease [35].

Effect of the course of immune measures on subjective burden at month 3 or 6

There was a pattern of negative associations between the (0–3)-gradients of β_2-microglobulin, IL-2r, and NK-cell counts and all subjective burden indicators at month 3. There was some indication of positive associations over time between the (0–3) – gradients of immune cell counts and physical well-being and coping effort. Since the immune-psycho association is partially in the same direction as the psycho-immune association this raises the possibility that subjective burden and immunological measures may be affected by a third factor not identified and assessed in this study, e.g. endocrine factors [36].

The interpretation of our findings is limited by the unresolved underlying problem of how changes in specific immune measures relate to what is called the immune system or immunity in patients with breast cancer, since the immune system consists of a complex network involving a great number of elements that may interact or have very different and even contradictory functions in different stages of the disease. It is therefore impossible to say whether the pattern of small decline of immune measures associated with less subjective burden at baseline has any clinical or prognostic significance. The use of different immune measures may have shown different results.

In summary, the effect size of the relationship between subjective burden and immune measures was very small. The question of clinical significance remains unanswered. A pattern of associations was found between subjective burden before adjuvant therapy predicting a decline in the course of β_2-microglobulin and IL-2r, two markers of activation of the cellular immune system, and of NK cell counts in the first 3 and 6 months of therapy. These associations have to be prospectively investigated with refined assessment tools and schedules. We believe that our findings are of interest given the frequently postulated pathway between psychological distress and immunity and the paucity of available data in early breast cancer.

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