Breast Cancer Surgery-Induced Immunomodulation

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Background and objectives: Surgical procedures can cause tumor cells to disseminate into the circulatory system. Although this spread of metastatic cells will be limited by immune activity, immunosuppression tends to be the main effect resulting from surgery. The objective of this study is to assess hormonal and immunological changes induced by breast cancer surgery.
Methods: Endocrine and immune responses to surgery were determined in 27 breast cancer patients. Blood samples were taken at 6 days and 1 day before surgery and 2 hr, 1 day, and 5 days after surgery. Changes in endocrine function, number of leucocytes and their subpopulations, enumerative immune expression, functional activity, and cytokine levels were determined.
Results: Breast cancer surgery induces a pro-inflammatory response and leucocytosis. Immunosuppression is indicated by decreased HLA-DR expression, decreased NKCA, and a Th2 response. A delayed Th1 response was also found 5 days after surgery. As no cortisol level change was observed, this hormone can be excluded as the mediator of surgery-related immunomodulation in breast cancer.
Conclusion: Although breast cancer surgery is classified as minor surgery the surgical procedure produces substantial immunomodulation.
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INTRODUCTION

For the majority of breast cancer patients, surgery provides the opportunity for cure. However, as breast cancer surgery involves physical manipulation of the tumor or its vasculature, this may induce tumor cells to shed into the circulation [1,2], which in turn may be harmful because of possible metastasis [1,3]. Surgery also induces activation of the hypothalamic pituitary adrenal (HPA) axis and the sympathetic adrenomedullary (SAM) system, and changes in immune function [4–7]. Activation of the HPA axis in major surgery is due to the release of the cytokines IL-1, IL-6, and tumor necrosis factor-α (TNF-α) by activated macrophages in the surgical wound [8]. Naito et al. [5] demonstrated that epidural blockade almost completely suppressed the response of ACTH and cortisol, which indicates that the neural input of the surgical wound is also an important factor in the activation of the HPA axis in surgery. Activation of the HPA axis was once thought to be the most prominent mediator of immunomodulation following surgery, but the role of the SAM system might be no less important. Both adrenaline and noradrenaline are secreted abundantly in the perioperative period and all lymphoid organs are richly innervated by sympathetic terminals [7].

Both adaptive and innate immunity arms of cell-mediated immunity have shown anti-metastatic activity in animals and humans. Natural killer cells (NKCs) are the instrument of the innate, cytotoxic immune response capable of recognizing and eliminating cells with down regulated MHC-I expression. Animal studies have shown that NKCs play an important role in limiting metastatic spread [9–11]. The level of NKC cytotoxicity has been demonstrated to be predictive of possible later recurrence in successfully treated malignant melanoma patients [12], breast cancer patients [13], and head and neck cancer [14],

although no convincing evidence for such a relationship was found in another study on melanoma patients [15]. Attention has primarily been focused on the role of NKCs, but monocytes and macrophages are also important in the host defense mechanism against tumor cells, as they present antigens to effector cells and thus play a central role in immune activation.

Several aspects of surgery are involved in immunosuppression, including tissue damage, anesthetic and analgesic drugs, hypothermia, blood loss, transfusion, pain and perioperative distress [3]. Major surgery (conventional thoracic or abdominal) induces an increment in cortisol level [5–7], catecholamine level [7], an acute phase response, a shift towards Th2 function [6], reduced relative expression of HLA-DR on monocytes [15], a decrease in NKC activity (NKCA) [16] and monocyte-mediated cytotoxicity [17], and a decrease in the number of cytotoxic T lymphocytes and T helper cells [7]. Pollock et al. [16] found that surgery caused a decrease in NKCA of about 50% of pre-operative values. Furthermore, there is an excessive release of growth factors needed for wound healing [3].

Although endocrine and immune responses have been extensively investigated in major surgery, the knowledge of these responses in breast cancer surgery, regarded as minor surgery, is limited. To date, eight studies have investigated the extent of immunomodulation in breast cancer surgery, none of which has investigated the hormonal response pattern and changes in monocyte cytotoxicity [18–25]. Four out of six studies found a decrease in NKCA [18–21], but this response was not found in two studies [24,25]. One study found an increase in the number of lymphocytes (total), B cells, CD4 and CD8 lymphocytes, and CD4/CD8 ratio, but no change in leucocytes (total) and NKCs at 7 days postsurgery [22]. Another study also reported an increase in lymphocytes [22], though a small-scaled study (N = 10) did not [21]. One study found an increase in IL-2, IL-6, and CRP [23], and one study found a decrease in interferon-γ (IFN-γ) [25]. Negative findings were reported with respect to changes in the number of large granular lymphocytes and lymphocyte proliferation response to PHA [18], and changes in IL-1β and TNF-α [23].

Because of the limited knowledge of endocrine and immune responses in breast cancer surgery, in our study we assessed a wide range of immune measures, including several laboratory parameters, in particular hemoglobin, C-reactive protein (CRP), several cytokines to determine the Th1 and Th2 response, expression of HLA-DR as a measure for antigen presenting capacity, the number of several lymphocyte subpopulations and cortisol. As we were particularly interested in the effect of breast cancer surgery on the innate immune response, we also investigated NKCA.

METHODS

Patients

New cases with clinically proven stages I–III breast cancer were recruited based on triple diagnostics (physical examination, mammography, and cytological or tru cut biopsy) were recruited.

Surgery involved lumpectomy/mastectomy with a sentinel node procedure or axillary lymph node dissection. Patients were discharged within 1 day after surgery, except those patients who underwent axillary lymph node dissection. The anesthesia, analgesia, and nausea protocol prescribed preoperative medication of 10 mg Normison and 1 g Paracetamol for all patients. Diprivan, Sufenta, Esmeron, and Oxygen/Sevoflurane were administered to induce narcosis and muscle relaxation. Postoperative medication consisted of 20 mg Piritramide, 1 g Paracetamol four times a day, and 4 mg Ondansetron. Exclusion criteria were (1) age >75 years old, (2) serious psychiatric disorder, (3) immune-related diseases, (4) other malignant tumors present or in patient's history, (5) chemotherapy or immunotherapy, (6) use of immunostimulating or depressing medication, or (7) use of non-steroid anti-inflammatory drugs.

The Medical Ethics Committee approved conducting this study and all participants gave their written informed consent. The physician researcher (M.B.) was not involved in treating the patients we assessed.

Hormonal and Immunological Measurements

Venipuncture. Sterile peripheral blood samples were collected by means of a vacutainer system 6 days and 1 day preoperatively, and 1–2 hr, 1 day, and 5 days postoperatively. We opted for two preoperative measurements, so as to ensure a more reliable interpretation of the postoperative changes. Measurements taken 6 days preoperatively were used as baseline values, because measurements taken at 1 day preoperatively were expected to be influenced by the stress of the upcoming surgery. Blood was taken between 08:30 and

09:30 pm, except for the 1–2 hr postoperative measurement. Patients lay supine for 15 min before blood withdrawal. Plasma and serum were separated from the blood and stored at −20°C. Batchwise measurement of the samples followed within 3 months. All samples were processed within 1.5 hr. For practical and financial reasons, the following immunological measures were not determined for the 1–2 hr postoperative measurement: cytokines, FACs analysis, and NKC cytotoxicity. Data from the following measures were available for all five time points: hemoglobin, CRP, white blood cell counts, cortisol, and HLA-DR.

C-reactive protein (CRP). Nephelometry was used to measure CRP (Behring Nephelometer II, Dade-Behring, Leusden, The Netherlands). The upper limit of the reference range was <5 mg/L. The inter-assay coefficient of variation was approximately 4%.

Leukocytes—Hemoglobin. Measurements were performed using the Sysmex NE-8000 analyzer (Sysmex, Kobe, Japan). The five-part leukocyte differentiation results are reported as neutrophils, lymphocytes, eosinophils, basophils, and monocytes.

Human leukocyte antigen-DR on monocytes (monocyte HLA-DR). Whole blood specimens were analyzed on fresh blood. HLA-DR expression on monocytes was assessed on a flow cytometer (Beckman Coulter, Mijdrecht, The Netherlands) with anti-CD14 PE (Beckman Coulter)/anti-HLA-DR FITC (Becton Dickinson) monoclonal antibodies. A sample of 100:l whole blood was stained with the monoclonal antibody mixture at room temperature in the dark for 15 min. Red cells were lysed with formic acid and stained cells were fixed with paraformaldehyde (PFA), using the TQ-prep system (Coulter Electronics, Hialeah, Florida). Data were expressed in MESF units. The relative expression (mean fluorescence) of HLA-DR positive cells versus HLA-DR negative cells was determined on CD14 positive cells.

Cortisol. Cortisol was determined by an automated chemoluminescence-immunoassay (LIA, Immulite; Diagnostic Products Corporation, Breda, The Netherlands). Reference ranges were 150–600 nmol/L and the intra-assay coefficient of variation was approximately 8%.

Peripheral blood mon onuclear cells (PBMC). Peripheral blood mononuclear cells were separated from the blood using a standard Ficoll–Hypaque gradient. The cells were washed, checked for viability (always >90%), counted and cryopreserved. Cryopreservation was achieved by resuspending the cells in RPMI, 10% FCS, 2 mM Glutamin, 100 U/ml Penicillin, 100 g/ml streptomycin, and then adding 10% dimethylsulfoxide immediately prior to cooling. Cups were placed overnight in a room temperature isopropanol bath in a −80°C freezer and cooled at the rate of 1°/min, and then transferred to the vapor phase of liquid nitrogen for storage.

The cells were thawed by following the protocol of quickly warming individual cups to 37°C in a warm bath, then rapidly diluting them into warm medium containing 20% FBS. The cells were washed, counted, and checked for viability (>90%), and then adjusted to the preferred cell concentrations for various experiments.

Cytokines stimulation and detection. In order to determine the capacity of PBMCs to produce the cytokines IL-1β, IL-6, IL-12, TNF-α, IL-10, IFN-γ, IL-2, and IL-4, PBMCs were thawed following the aforementioned protocol. To determine IL-6, IL-10, and IFN-γ production, supernatant was harvested by PBMC stimulation with 1 µg/ml phytohemaglutinin (PHA; Sigma L-4144, St Louis, MO) in wells (Greiner Cellstar) at 37°C, 5% CO_2, and 90% humidity for 24 hr. To determine IL-2 and IL-4, supernatant was harvested by PBMC stimulation with 10 µg/ml PHA in wells for 48 hr. To determine IL-1β, IL-6, and TNF-α, supernatant was harvested by PBMC stimulation with 0.1 ng/ml lipopolysaccharide (LPS; Difco 3120-25-0, Detroit, MI) in wells for 4 hr. To determine IL-12, supernatant was harvested by PBMC stimulation with 0.1 ng/ml LPS in wells for 24 hr. These concentrations were chosen based on pilot titration curves that showed

optimal stimulation (data not shown). Cell-free supernatant was harvested and stored at $-80°C$. Indirect enzyme linked immunosorbent assays (ELISAs; BIOSource, Etten Leur, the Netherlands) were conducted in 96-wells ELISA plates (Flatbottom; Nunc, Rochester, New York). The rinse buffer contained phosphate-buffered solution (PBS) with 0.5% bovine serum albumin (BSA; fraction V Boehringer, Mannheim, Germany) and Tween (0.05% v/v Riedl-de Haën, Seelze, Germany). The coating antibodies for IL-6, IL-10, IFN-γ, IL-2, IL-4, IL-1β, TNF-α, and IL-12 were 677B6A2, 945A5D11, 350B10G6, 419A7A3, 860A4B3, 508A7G8 + 508A4A2, 68B6A3 + 68B2B3, and A08E6E5, respectively. The detecting antibodies were 505E23C7, 945A5A10, 67F12A8, 297C16G2, 860F10F12, 508A3H12, 68B3C5, and A25C4B6, respectively. Standards were used as controls. Streptavidine-horseradish peroxidase in a dilution of 1:5,000 was used as an enhancement step, and in the final step substrate 3,3′,5,5′-tetramethylbenzidine was used (TMB; Sigma) to generate the colored reaction. To stop the reaction $2 N$ H_2SO_4 was used. Optical density (OD) was measured at 450 nm by use of a benchmark plate reader (Biorad, Hercules, CA). Different samples and different ELISAs taken from one patient were determined on the same day.

NKC activity (NKCA). K562 cells, target cells (T), cultured in RPMI, 10% FBS, 2 mM Glutamin, 100 U/ml Penicillin, 100 g/ml streptomycin, were harvested. Viability was always above 95% and 3×10^6 cells were labeled with 0.5 μM calcein-AM (1 mM stock, Eugen, OR) in the dark for 30 min at $37°C$. Labeled K562 cells were washed twice using Hank's buffer with 10% FCS. A sample concentration of 0.1×10^6 cells/ml was prepared. PBMCs effector cells (E) were thawed by following the aforementioned protocol. Viability was assessed by trypan blue exclusion. Sample results with viability >80% were included for analysis. The effector/target (E/T) cell ratio's 40:1 and 20:1 were tested. Tests were conducted in triplicate in 96-wells plates in 200:l/well in Hank's buffer, 10% FBS, without phenol red (Nunc). The spontaneous (minimum) release was measured by adding 100 μl medium to 100 μl labeled K562 cells. To measure the total (maximum) release, 100 μl 1% triton X (Triton X 100, Merck, Darmstadt, Germany) was added to 100 μl labeled K562 cells. Different E/T ratios were obtained by adding 100 μl different PBMC concentrations to 100 μl labeled K562 cells. The plates were centrifuged (at room temperature, 150 g) for 1 min, then incubated at $37°C$ and 5% CO_2 for 3.5 hr. A sample of 100:l of cell free supernatant was transferred to a 96-wells flat-bottomed plate (Greiner, Alphen a/d/Rijn, The Netherlands). Different samples of one patient were tested in the same experiment. Fluorescence was measured on Fluostar Galaxy (BMG Labtechnologies, Offenburg, Germany). The percentage cytotoxicity was calculated as follows:

TABLE I. Clinical Characteristics of the Study Sample (n = 27)

	Frequency	%
Stage of disease		
Stage I	12	44
Stage II A	7	26
Stage II B	5	19
Stage III A	1	4
Stage III C	2	7
Surgical procedures		
Lumpectomy and sentinel node procedure	16	59
Ablatio and sentinel node procedure	2	7
Breast conservative therapy	4	15
Modified radical mastecomy	5	19

TABLE II. Values of Hemoglobin (mmol/L), C-Reactive Protein (CRP; mg/L), Leukocytes and Their Subpopulations (10 E9/L), Cortisol (nmol/L), HLA-DR (HLA-DR+/CD4+), and Cytotoxicity of NKCs (%)

	Day −6	Day −1	2 hr postoperatively	Day +1	Day +5
Hemoglobin	8.43 (8.23–8.63)	8.36 (8.15–8.56)	7.52 (7.23–7.82)***###	7.68 (7.40–7.96)***	7.64 (7.35–7.94)***
CRP	3.38 (3.02–3.78)	3.67 (3.07–4.39)		7.89 (5.73–10.88)***###	5.26 (3.98–6.95)***##
Cortisol	526 (465–595)	510 (455–572)	362 (264–496)*	544 (458–646)###	499 (436–571)
Cortisol, corrected for hemodilution	519 (458–588)	507 (447–574)	381 (232–627)	592 (478–735)	539 (465–625)
Cortisol, corrected for circadian rhythm	525 (465–593)	511 (456–574)	583 (478–711)	544 (458–646)	499 (436–571)
HLA-DR	4.75 (4.04–5.57)	4.27 (3.71–4.92)	3.89 (3.16–4.79)**	3.89 (3.37–4.50)***	4.28 (3.65–5.00)*#
Leukocytes	6.23 (5.48–7.08)	6.36 (5.51–7.34)	8.22 (7.44–9.09)***###	8.31 (7.61–9.08)***	6.66 (5.88–7.54)###
Granulocytes	3.78 (3.22–4.44)	4.01 (3.33–4.83)	5.18 (4.57–5.87)***##	6.01 (5.37–6.72)***#	4.14 (3.56–4.81)###
Lymphocytes	1.63 (1.41–1.88)	1.56 (1.37–1.79)	2.05 (1.77–2.37)***###	1.47 (1.28–1.69)*###	1.61 (1.41–1.84)
Monocytes	0.42 (0.36–0.49)	0.41 (0.34–0.49)	0.60 (0.51–0.71)***###	0.47 (0.41–0.53)*##	0.46 (0.39–0.54)
Cytotoxicity					
NKC E/T ratio 40:1	23.7 (17.1–30.2)	22.3 (16.3–28.3)	22.9 (16.3–28.3)	17.7 (12.4–23.0)*#	22.9 (17.3–28.4)#
NKC E/T ratio 20:1	11.2 (7.5–14.8)	12.7 (8.4–17.0)		9.9 (6.3–13.6)##	13.3 (9.0–17.6)#

CRP and cytotoxicity were not measured 2 hr postoperatively. Data are expressed as mean with confidence intervals within brackets.
Significant change with respect to baseline (day −6): $*0.01 < P \leq 0.05$; $**0.001 < P \leq 0.01$; $***P \leq 0.001$.
Significant change with respect to previous measurement: $\#0.01 < P \leq 0.05$; $\#\#0.001 < P \leq 0.01$; $\#\#\#P \leq 0.001$.

$$\% \text{ Specific lysis} = \frac{[\text{Test fluorescence per minute} - \text{spontaneous fluorescence}]}{[\text{Maximum fluorescence per minute} - \text{spontaneous fluorescence}]} \times 100\%$$

Statistical Analysis

The data are expressed as means and confidence intervals. All data were logarithmically transformed to compensate for skewness. The repeated measures were analyzed with multilevel modeling (MLM), using the program MLwin version 1.0a. Using MLM to analyze the longitudinal data is preferred above the more traditional approaches, especially repeated measures MANOVA [26]. MLM is advised when data are missing, as in our study, because it uses all the information available, whereas traditional statistical procedures can only study one type of change at a time, either intraindividual change or interindividual change, MLM is a more powerful statistical tool, because both types of change can be assessed simultaneously in a single model.

Two sets of tests were conducted: (1) baseline values (day −6) were compared with all following measurements (days −1, +1, and +6) and (2) each measurement was compared with its previous measurement. The significance level was set at $P = 0.05$.

RESULTS

Of the 31 patients who were eligible for this study, 27 patients participated and 4 patients declined (see Table I for clinical characteristics of the participants). The disease's final pathological
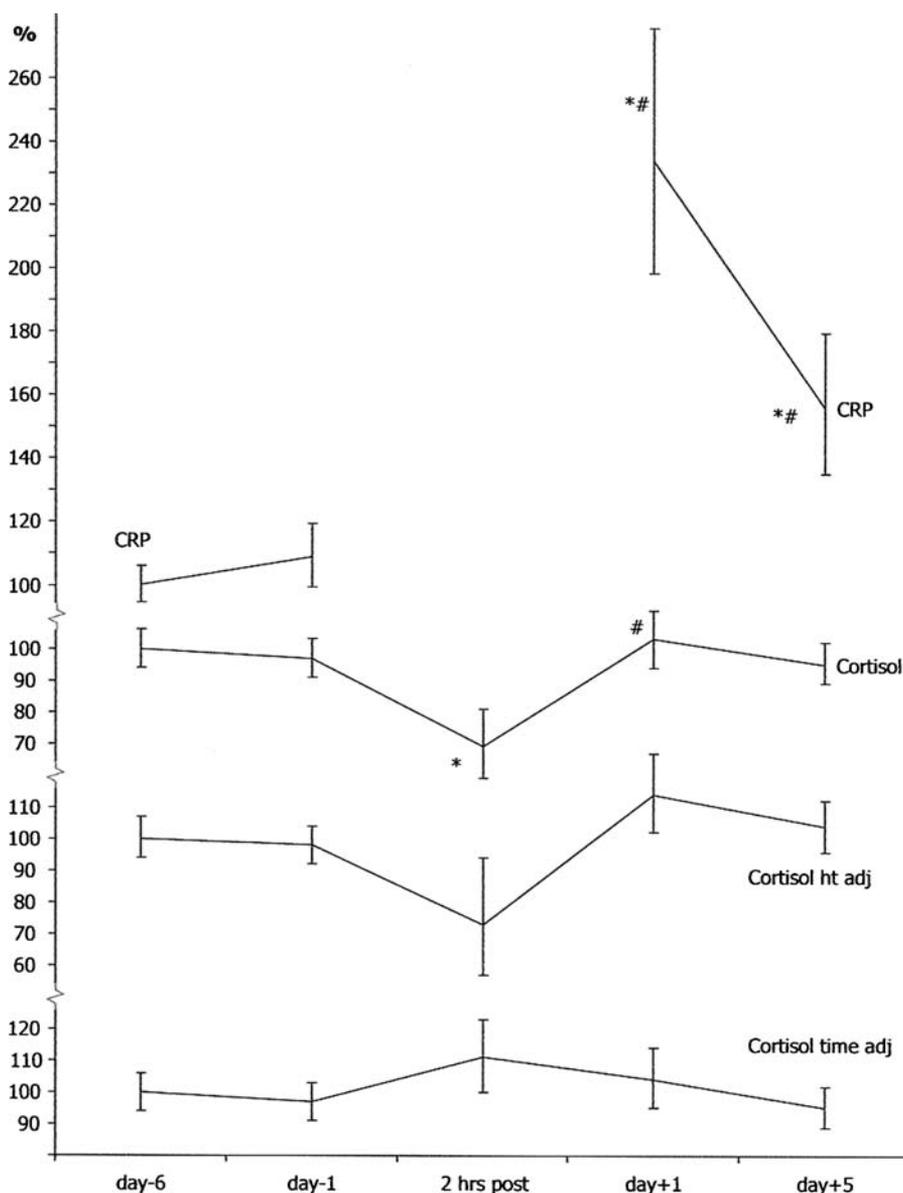

Fig. 1. Changes in CRP, cortisol, cortisol corrected for hemodilution (ht adj) and cortisol corrected for circadian rhythm (time adj) in the perisurgical period (means and SEMs; surgery at day = 0). Baseline levels set at 100%. CRP was not measured 2 hr postoperatively. *Significantly different from baseline; #significantly different from previous measurement ($P \leq 0.05$).

stage revealed that only three patients (11%) had two or more positive lymph nodes, indicating that the known tumor burden was low in the majority of patients. Only nine patients were treated by an axillary lymph node dissection. The baseline values of the hematological, endocrine, and immune parameters were within normal ranges.

Hematology

Hemoglobin decreased 2 hr postoperatively, which sustained throughout all postoperative measurements (see Table II).

C-reactive protein increased postoperatively, and showed a fall 5 days postoperatively although it still increased above baseline (see Table II and Fig. 1).

Cortisol

Serum cortisol showed a decrement 2 hr postoperatively. However, this decrease was no longer present 1 day postoperatively (see Table II and Fig. 1).

Mean relative *HLA-DR expression on monocytes* (HLA-DR+/ CD14+) decreased 2 hr postoperatively. This decrement persisted 1 day postoperatively, and increased thereafter but was still significantly lower than baseline at 5 days after surgery (see Table II and Fig. 2).
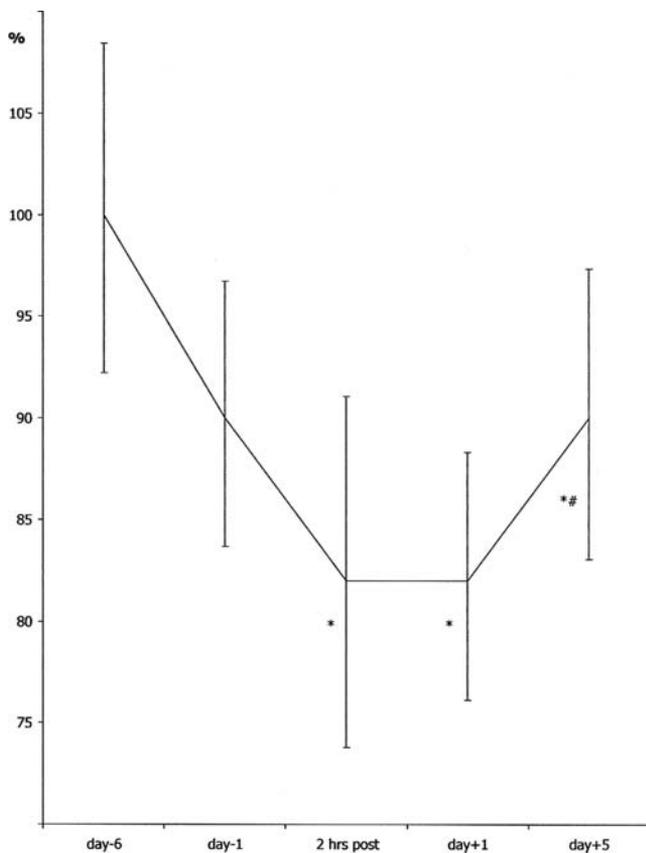

Fig. 2. Changes in human leukocyte antigen-DR on monocytes (HLA-DR) in the perisurgical period (means and SEMs; surgery at day = 0). Baseline levels set at 100%. *Significantly different from baseline; #significantly different from previous measurement ($P \leq 0.05$).

White Blood Cell Count

Leukocytes increased 2 hr postoperatively, and persisted at 1 day after surgery, but this increment was no longer present 5 days postoperatively (see Table II and Fig. 3). The increase in leukocytes was mainly due to the increment of granulocytes 2 hr postoperatively and 1 day postoperatively, but was no longer present 5 days postoperatively. Lymphocytes showed an increase in comparison to baseline values 2 hr postoperatively, but decreased to baseline levels 1 day postoperatively. Monocytes increased 2 hr postoperatively, decreased at 1 day postoperatively, but levels were still significantly higher than baseline. This increment was no longer present 5 days postoperatively. No change was observed in PBMC distribution ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD3^-16-56^+$, $CD14^+$). See Table III.

Cytokines

An increase in the capacity of PBMCs to produce the pro-inflammatory cytokines IL-1, IL-6 (LPS), and TNF-α was found 5 days postoperatively with respect to baseline values (see Table IV and Fig. 4). A Th1 response was found 5 days postoperatively as indicated by the increased production of IL-2 with respect to the previous measurement and TNF-α with respect to baseline level. An increase of Th2 cytokines IL-6 (PHA) and IL-10 was observed at the first postoperative measurement (day +1) with respect to the previous measurement (day −1).

Cytotoxicity

NKCA with E/T cell ratios 40:1 and 20:1 showed a lower level at the first postoperative day, but returned to baseline level after 5 days (see Table II and Fig. 5).

DISCUSSION

This study analyzed changes in a broad spectrum of immune measures, broader than in any previous study on breast cancer surgery. Immunomodulation resulting from breast cancer surgery was demonstrated in the form of a pro-inflammatory cytokine response, a delayed Th2 response, an increment of CRP, an increase in the number of leukocytes and their subpopulations granulocytes, lymphocytes, and monocytes, and a decrease in monocyte HLA-DR expression and NKCA. However, FACS analysis showed no changes in subsets in PBMC subsets.

CRP is one of the acute phase proteins and has the ability to recognize and bind to multiple biological substrates, thus mimicking the action of antibodies, activating the complement system and enhancing the activity of phagocytic cells [27]. Pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, activate the production of CRP. In line with several authors having found an increase in IL-6 and CRP in colorectal [28] and breast cancer surgery [23,28], we also found an increase in pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) and CRP. As the cytokine response was delayed with respect to the CRP response, these cytokines cannot have been the initiators of the CRP response in this study. The CRP response in our study was less profound than we found in laparoscopy [29].

The finding of the low CRP response in combination with the observation that the shift in Th1–Th2 balance is less pronounced than in major surgery leads to the conclusion that breast cancer surgery, with respect to its effect on immunomodulation, is a minor type of surgery. In major surgery, a postoperative decrease in Th1 response and an increase in Th2 response are commonly observed. We found the expected increment in Th2 response, but also observed an unexpected increase in Th1 cytokines (IL-2 and TNF-α) 5 days postoperatively. We propose two explanations for this unexpected finding. First, the
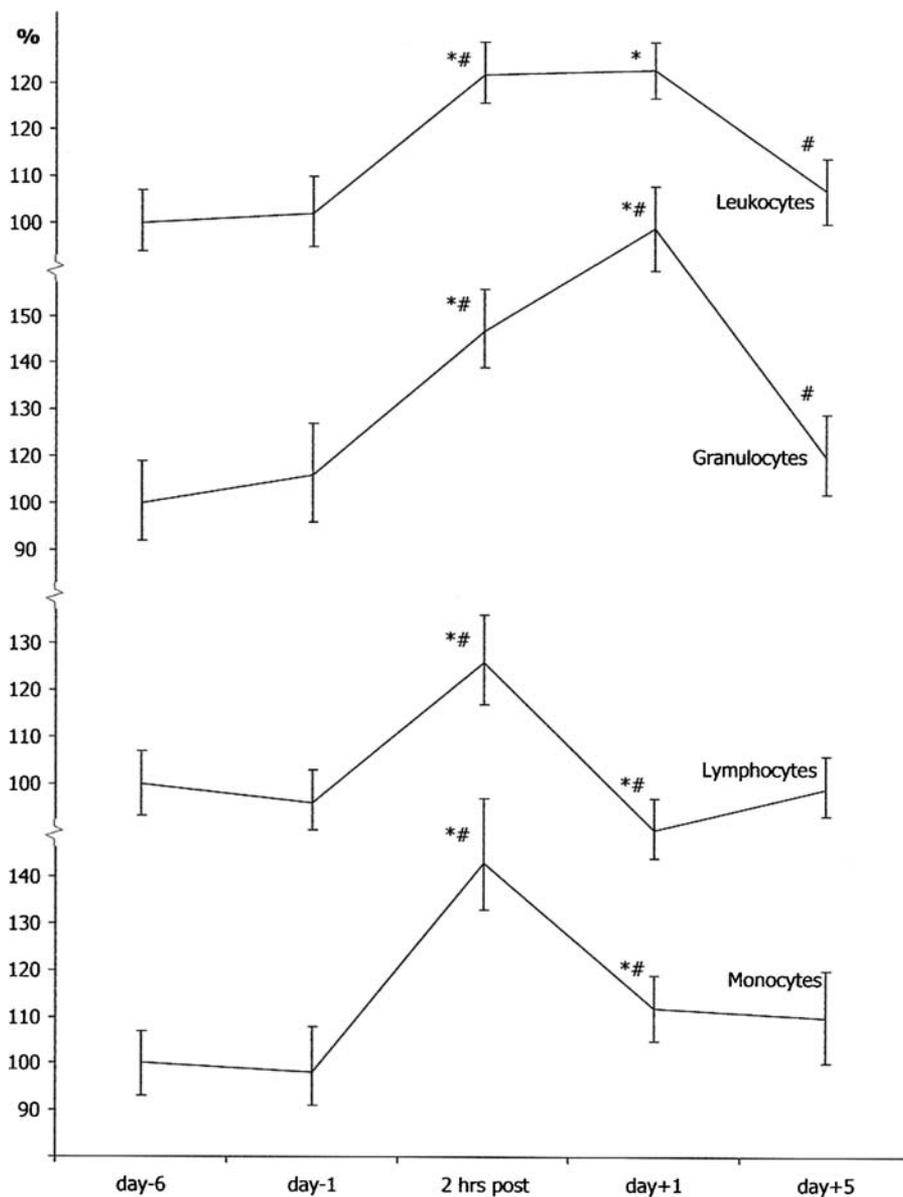
Fig. 3.   Changes in white blood cell counts in the perisurgical period (means and SEMs; surgery at day = 0). Baseline levels set at 100%. *Significantly different from baseline; #significantly different from previous measurement ($P \leq 0.05$).

increase in Th1 cytokines could be part of the wound healing process that is masked in major surgery by a stronger cortisol and/or catecholamines driven immunosuppressive effect. Second, another study that also found an increase in IL-2 after cancer surgery attributed this response to the removal of the inhibiting effect of cancer [6]. In

contrast to another breast cancer surgery study [25], we found no decrease in IFN-γ.

The number of leucocytes increased after surgery, both 2 hr postoperatively and 1 day postoperatively. This also applied to the subpopulations of granulocytes, lymphocytes, and monocytes,

TABLE III. Percentages of Lymphocytic Subsets (FACS Analysis of PBMCs)

	CD3+	CD4+	CD8+	CD19+	CD3/16/56+	CD14+
Day −6	71.4 (68.1−74.8)	50.1 (46.7−53.7)	29.0 (26.4−31.9)	12.2 (9.9−15.1)	11.0 (9.4−12.9)	15.5 (13.3−18.2)
Day −1	70.5 (66.6−74.6)	49.3 (45.6−53.2)	29.9 (27.5−32.4)	13.2 (11.2−15.6)	12.0 (10.3−13.9)	15.8 (13.0−19.4)
Day +1	71.5 (67.7−73.4)	50.7 (46.9−54.8)	29.1 (26.8−31.5)	13.3 (11.2−15.7)	11.0 (9.2−13.0)	16.8 (13.7−20.5)
Day +5	70.2 (66.3−74.2)	49.4 (45.6−53.5)	28.5 (25.8−31.5)	13.9 (11.9−16.3)	11.0 (9.5−12.8)	16.6 (13.9−19.9)

Data are expressed as mean with confidence intervals indicated in brackets.
Values were not significantly different with respect to day −6 or with respect to previous measurement.

TABLE IV. Cytokine Production of Peripheral Blood Mononuclear Cells

	Day −6	Day −1	Day +1	Day +5
Pro inflammatory cytokines				
IL-1	86 (48−157)	111 (73−169)	115 (77−173)	151 (92−249)*
IL-6 (LPS)	511 (337−776)	557 (392−791)	639 (458−892)	869 (642−1,175)**#
TNF-α	581 (406−832)	673 (491−921)	705 (519−958)	846 (633−1,132)**
Th1 cytokines				
IL-2	2,184 (1,578−3,022)	2,059 (1,521−2,787)	2,008 (1,460−2,762)	2,558 (1,835−3,567)#
TNF-α	581 (409−826)	673 (494−915)	705 (521−952)	846 (637−1,126)*
IFN-γ	2,540 (1,744−3,701)	2,305 (1,575−3,375)	2,473 (1,558−3,923)	2,805 (1,815−4,333)
IL-12	145 (106−199)	151 (105−218)	179 (142−225)	142 (101−202)
Th2 cytokines				
IL-6 (PHA)	13,643 (8,990−20,704)	15,306 (13,101−17,880)	18,845 (15,802−22,473)##	18,124 (14,872−22,087)
IL-10	2,652 (2,115−3,324)	2,248 (1,680−3,008)	2,816 (2,189−3,622)#	2,788 (2,096−3,707)
IL-4	70 (56−88)	69 (56−87)	70 (52−94)	73 (58−92)

Data are expressed in pg/ml as mean with confidence intervals indicated in brackets.
Significant change with respect to baseline (day −6): *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$.
Significant change with respect to previous measurement: #$0.01 < P \leq 0.05$; ##$0.001 < P \leq 0.01$.
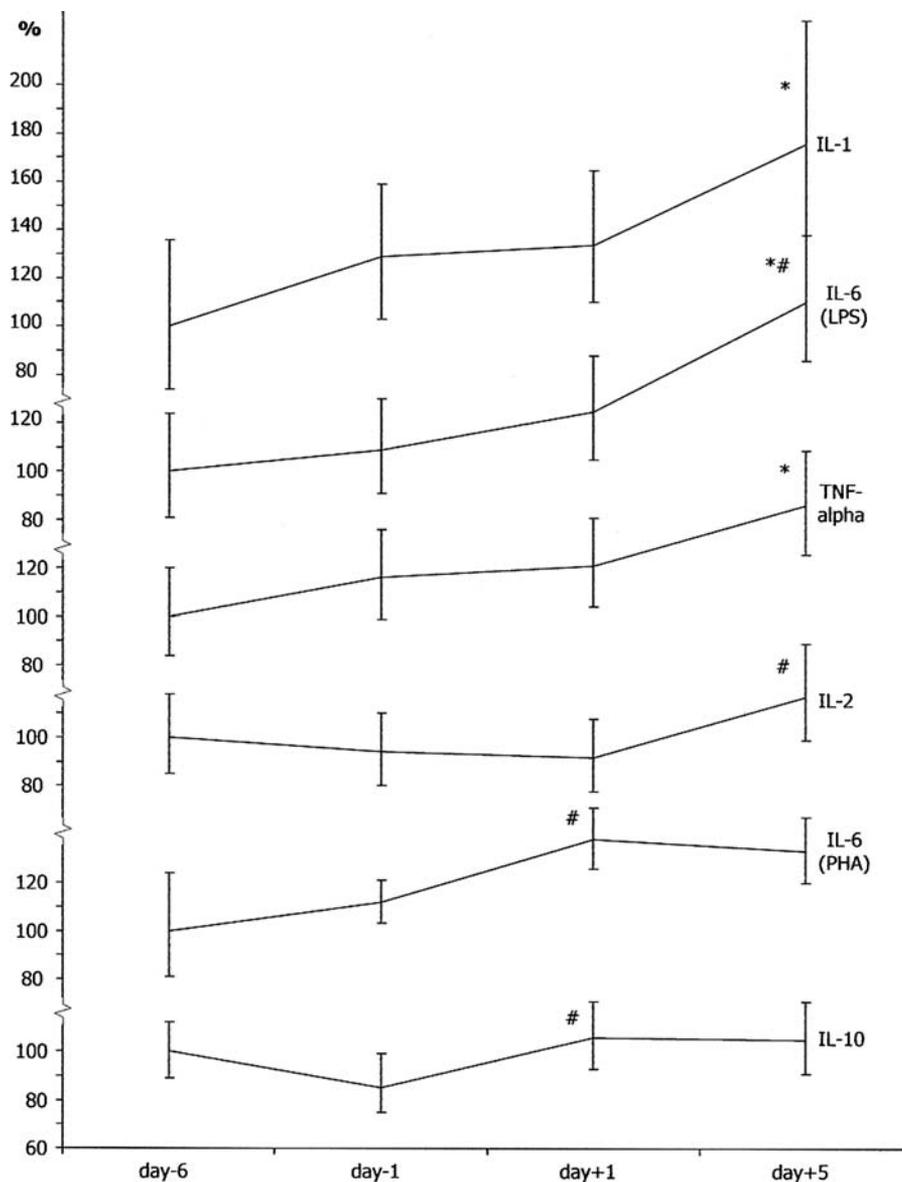

Fig. 4. Changes in cytokines in the perisurgical period (means and SEMs; surgery at day = 0). Baseline levels set at 100%. *Significantly different from baseline; #significantly different from previous measurement ($P \leq 0.05$).
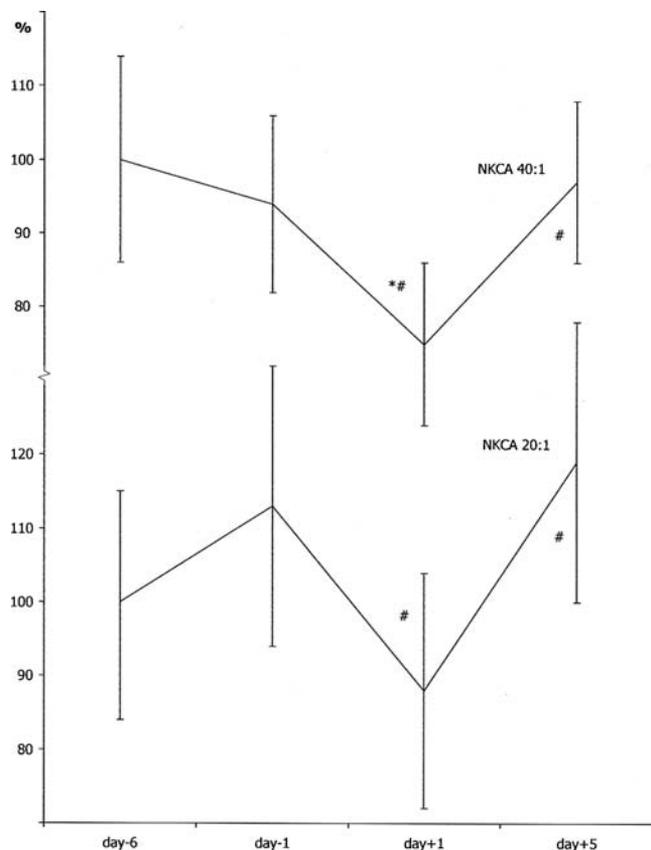
Fig. 5. Changes in cytotoxicity in the perisurgical period (means and SEMs; surgery at day = 0). Baseline levels set at 100%. *Significantly different from baseline; #significantly different from previous measurement ($P \leq 0.05$).

although the number of lymphocytes had already dropped below base level 1 day postoperatively. Another breast cancer surgery study also found an increase in lymphocytes, but no postoperative change in leucocytes [22].

Cortisol is an important mediator of surgery-related immunomodulation [5–7,30]. However, our study did not show an increment, but rather a decrement in cortisol level 2 hr postoperatively. There are two possible explanations for this anomaly: (1) dilution during surgery caused by the administration of intravenous fluids and medication, and (2) the sample was taken 2 hr postoperatively, whereas other measurements were taken between 08:30 and 09:30 pm. When a correction factor for hemodilution was applied (cortisol level divided by hematocrit), the decrement in cortisol was no longer significant (see Fig. 1 and Table II). We also applied a correction factor for the circadian rhythm of cortisol. The correction factor was derived from a gradient reflecting the decrease throughout the day. This gradient was calculated from two samples at 09:00 am and 16:00 pm 1 day preoperatively, which admittedly is rather a raw approach. After applying a correction factor for time of day, the cortisol levels 2 hr postoperatively even showed an (non-significant) increase (see Fig. 1 and Table II). This correction seems to suggest that time of day was the reason for the unexpected lower cortisol level 2 hr postoperatively. In any case, there is no reason to assume that a cortisol response occurred after breast cancer surgery, furthermore indicating that breast cancer surgery is a minor type of surgery.

On the other hand, we demonstrated a decrease in NKCA 1 day postoperatively, which was later restored 5 days postoperatively. Other

studies have shown that major surgery also reduces NKCA [16]. A reduction has also been found after breast cancer surgery, which lasted longer than in our study [18,25]. Empirical evidence suggests that surgery-related inhibition of NKCA is caused by impaired IFN-γ and IL-2 production, release of prostaglandin E2 (PGE2) from macrophages [2,31] or an increase in circulating levels of corticosteroids [32] or catecholamines [7,11,31]. We showed that IFN-γ and cortisol levels did not increase as a result of breast cancer surgery. An increment in IL-2 was found, although only at the 5th day after surgery. Catecholamines might be an important mediator or immunomodulation caused by breast cancer, but we did not assess the levels of these hormones.

The monocyte/macrophage has a strong inflammatory, phagocytic, and tumoricidal function and plays a central role in immune activation. These cells are important in the host defense mechanism, as they are capable of presenting antigens to effector cells and therefore play a central role in immune activation [33]. The major histocompatibility complex class II antigen-human leukocyte antigen-DR (HLA-DR)-expressed on the surface of monocytes/macrophages is critical in this situation. Surgery-induced immunosuppression depends on the degree of trauma, and HLA-DR expression is a valid parameter for this immune response [34]. Less invasive surgery, such as laparoscopic surgery, did not show a decrease [15] or showed a shorter lasting [35] decrease in HLA-DR expression in comparison to conventional surgery. We found that breast cancer surgery causes a significant increase of monocytes, but a decrease in HLA-DR expression on monocytes. The decrease in HLA-DR expression on monocytes is somewhat less than in laparoscopy [29].

In conclusion, although breast cancer surgery is considered minor surgery, the surgical procedure appears to induce marked immunomodulation as indicated by the lowered NKCA, lowered HLA-DR expression and the clear pro-inflammatory response (cytokines as well as CRP). Breast cancer surgery reduces NKCA 1 day postoperatively, but this response is already restored 5 days postoperatively. This short-lived response may be critical, however, as most tumor cells that enter the circulation are destroyed during the first 24 hr, mainly by NKCs [9]. A decrement in NKCA may allow more cancer cells to escape, which may lead to the development of metastatic foci in distant organs.

The clinical relevance of the knowledge about surgery-related immunomodulation has been discussed by Ben-Eliyahu [36] and van der Bij et al. [37]. During the immediate postoperative period, pre-existing micrometastases may grow out as a result of decreased levels of anti-angiogenic factors and increased levels of growth factors. This suggests that the later the primary tumor is removed, the less likely it is that the immune system is still effective, given that the selection pressure has promoted tumor escape mechanisms. Changes in surgical procedures also lead to higher or lower levels of immunosuppression, which have been shown to be associated with higher or lower rates of tumor recurrence. Blood transfusion and general anesthesia suppresses immune activity. Minimally invasive surgery is less immunosuppresive than larger operations, although Morrow [38] warns that the safety of such procedures for breast cancer has not yet been sufficiently proven. Finally, pharmacological and immunological interventions may prevent postoperative immunosuppression.

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