Surgery Today (2023) 53:1305–1316
https://doi.org/10.1007/s00595-023-02700-1

ORIGINAL ARTICLE

An observational study of the impact of immediate breast reconstruction on perioperative inflammatory cytokines

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Received: 20 February 2023 / Accepted: 21 March 2023 / Published online: 22 May 2023

Abstract

Purpose Perioperative inflammatory cytokines may be related to cancer proliferation, although few studies have investigated this issue in patients undergoing breast reconstruction surgery.

Methods We conducted a prospective study of patients scheduled for mastectomy only, mastectomy plus deep inferior epigastric perforator flap reconstruction (DIEP), or mastectomy plus tissue expander reconstruction (TE), with or without axial dissection (Ax), for primary breast cancer. Blood samples were collected for analysis of serum IL-6 and VEGF preoperatively, then within 24 h postoperatively (POD 1), and 4–6 days postoperatively (POD 4–6). We investigated the difference in serum cytokine levels over time for each surgical procedure and the difference in serum cytokine levels among the procedures at the three measurement time points.

Results There were 120 patients included in the final analysis. Serum IL-6 was significantly higher than the preoperative level on POD 1 in patients who underwent mastectomy only, DIEP, or TE and Ax (+), with higher values persisting on POD 4–6 except in those who underwent DIEP. IL-6 was significantly higher after DIEP than after mastectomy only on POD 1, but no differences were observed at POD 4–6. VEGF did not differ significantly among the surgical procedures at any time.

Conclusions The increase in IL-6 was short term and immediate breast reconstruction is considered a safe procedure.

Keywords Breast reconstruction · Inflammatory cytokines · Interleukin-6 · Vascular endothelial growth factor

Introduction

While surgical resection is important in the treatment of many cancers, including breast cancer, inflammatory cytokines associated with wound healing may cause the activation and proliferation of dormant cancer cells [1–3]. Among these cytokines, interleukin-6 (IL-6) has been studied extensively, as an inflammatory cytokine released from various cells and a sensitive marker of tissue damage [4]. IL-6 affects cell proliferation, survival, differentiation, migration, invasion, metastasis, angiogenesis, and metabolism, as well as cancer onset [5]. Elevated serum IL-6 has been associated with cancer metastasis and survival in colon, renal cell, and breast cancer patients [6–9].

There are several reports on the relationship of vascular endothelial growth factor (VEGF) with cancer. VEGF promotes the proliferation and migration of vascular endothelial cells during wound healing by increasing vascular permeability and facilitating the local migration of serum proteins and vascular endothelial cells [10]. However,

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VEGF-mediated angiogenesis also plays a role in increasing the blood supply to tumors and accelerating metastasis and the invasion of malignant tumor cells [11]. Thus, higher serum VEGF levels are associated with recurrent metastases and a poorer prognosis of colorectal, lung, ovarian, and breast cancers [12–15]. Basic fibroblast growth factor (bFGF) is another angiogenesis-promoting factor involved in cell differentiation and proliferation, and is expressed in many solid tumors [16]. A relationship between the serum bFGF level and cancer progression has been reported in ovarian and breast cancer [17, 18].

Despite these findings on the relationships between the cytokines involved in wound healing and various cancer types, there is a paucity of literature investigating differences in perioperative cytokine levels. Breast reconstruction following mastectomy has become an important surgical procedure in recent years because of the high survival rate of breast cancer patients and the importance of reconstruction to improving quality of life. However, this technique is more invasive than mastectomy only. Moreover, axillary dissection performed on patients with lymph node metastases is considered to be more invasive than sentinel node biopsy. Hence, more inflammatory cytokines are likely to be released in the perioperative period of breast reconstruction following mastectomy and axillary dissection; yet, there are few reports on perioperative cytokine levels in breast cancer, including the extent to which cytokines increase in any given surgery and how long the increase in concentration persists. Previous meta-analyses comparing the prognosis of mastectomy and breast reconstruction following mastectomy have shown no difference [19–21]; however, the literature we reviewed includes some studies with short observation periods, so the long-term local and distant recurrence rates remain unclear. Therefore, it would be meaningful to establish whether invasive breast cancer surgery affects cancer recurrence by investigating changes in perioperative cytokine levels. In this study, we investigated perioperative serum levels of IL-6, VEGF and bFGF, prospectively, in patients with breast cancer undergoing mastectomy only, mastectomy plus deep inferior epigastric perforator flap reconstruction (DIEP), or mastectomy plus tissue expander reconstruction (TE), with or without axial dissection (Ax).

Patients and methods

Study design

We conducted a single-center, prospective, observational study to identify perioperative trends in the inflammatory cytokines: IL-6, VEGF, and bFGF, which have been suggested to be associated with cancer proliferation. The criteria for eligibility were as follows: women aged ≥ 20 years diagnosed with primary breast cancer at the Breast Cancer Treatment and Reconstruction Center, Okayama University Hospital; surgery scheduled as mastectomy only, immediate breast reconstruction following mastectomy with deep inferior epigastric perforator flap (DIEP), or immediate tissue expander breast reconstruction following mastectomy (TE); and written consent to participate in the study. Exclusion criteria were as follows: scheduled breast conservation surgery, delayed reconstruction, history of preoperative radiotherapy, recurrent breast cancer, cStage IV breast cancer, bilateral breast cancer, inflammatory breast cancer, and infectious disease.

Survey

Clinicopathological information for patients and tumors (age, body mass index [BMI], menopausal status, hormonal receptor status, lymph node status, clinical tumor stage, pathological tumor stage, nuclear grade), and short-term complications were obtained from electronic medical records. Online Resource 1 defines each parameter.

Assay of serum IL-6, VEGF, and bFGF

Blood samples were collected preoperatively, then within 24 h postoperatively (POD 1), and 4–6 days postoperatively (POD 4–6). Blood (7 ml) was centrifuged at 3000g for 3 min immediately after collection, and the supernatant was stored at – 80 °C in the Biobank of Okayama University Hospital. Serum cytokines were analyzed using a Bio-Plex 200 system that uses multiplex technology (Luminex Trading, Inc., Japan) with 5.6-μm diameter beads stained with two fluorescent dyes, each in a 10-step ratio, to enable simultaneous detection of up to 100 different analytes. Beads are conjugated with a reagent specific to a particular bioassay. The technology makes use of multiple assays whereby one antibody to a specific analyte is attached to a set of beads of the same color and the second antibody is used to quantify the bound antigen. The use of different colored beads enables the simultaneous detection of many analytes in the same sample. IL-6, VEGF, and bFGF were detected using the Bio-Plex Pro Human Cytokine 27-plex Assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Analysis was performed according to the protocol of the assay kit. To ensure the reliability of specimens, cytokine levels were measured in duplicate in 96-well microtiter plates. Actual values were obtained by drawing standard curves and analyzing the data using Bio-Plex Manager software.

Outcomes

The primary endpoint was the difference in serum cytokine levels over time for each procedure. Secondary endpoints

were differences in serum cytokine levels in patients with vs. those without short-term complications; in patients who underwent different surgical procedures; and in patients with vs. those without axillary dissection; and differences in serum cytokine levels over time in patients with vs. those without axillary dissection.

Statistical analysis

The patients were divided into three groups: those who underwent mastectomy only, those who underwent mastectomy with DIEP reconstruction, and those who underwent mastectomy with TE reconstruction. Continuous variables were compared among the groups using analysis of variance, and categorical variables were compared by Pearson chi-square test. To investigate whether preoperative status affected the preoperative cytokine levels, the levels were compared using an independent t-test or ANOVA for age ($\leq 50, > 50$), BMI ($\leq 25, > 25$ kg/m^2), estrogen receptor (ER) expression (yes, no), human epidermal receptor 2 [HER-2] expression (yes, no), nuclear grade (1, 2, 3), pStage (in situ, advanced), and neoadjuvant chemotherapy (yes, no). Complications were defined as those of grade III or above in the Clavien–Dindo classification, developing between the day of surgery and postoperative day 7 [22]. An independent t-test was used to evaluate the difference in mean serum cytokine levels between patients with and those without complications. Next, to investigate differences in serum cytokines over time for each surgical procedure, patients were stratified according to the type of procedure and whether axillary dissection was performed. Then, differences in cytokine levels were evaluated on POD 1 and POD 4–6 by a paired sample t-test, using the preoperative level for comparison. Differences in cytokine levels among the DIEP, TE, and mastectomy only groups at each time point were compared using an independent t-test. The same method was used to compare cytokine levels in patients with (Ax (+)) and those without (Ax (–)) axillary dissection at each time point. All data were analyzed using SPSS ver. 24 and $p < 0.05$ was considered to indicate a significant difference.

Results

Subjects

Initially, 140 patients were enrolled in the study between December, 2017 and December, 2019, including 67 who underwent mastectomy only, 34 who also underwent DIEP reconstruction, and 39 who also underwent TE reconstruction. Among these patients, consent was withdrawn by 3 (mastectomy alone 2, DIEP 1); 13 had insufficient data for analysis (mastectomy alone 10, DIEP 1, TE 2); one DIEP patient had her breast cancer surgery cancelled; and 3 patients originally scheduled to undergo TE underwent different surgical procedures that were outside the inclusion criteria. Thus, 20 patients were excluded from this analysis. Moreover, the surgical procedure was changed after enrollment in 6 patients (2 from DIEP to TE, 1 from DIEP to mastectomy only, and 3 from TE to mastectomy only). Finally, 120 patients (mastectomy only 59, DIEP 28, and TE 33) (Ax (+) 84, Ax (–) 36) were included in the study (Fig. 1). None of the groups included patients with a history of collagen or inflammatory bowel disease.

Table 1 summarizes the patients' characteristics. The mean ages were 58.0 years for the mastectomy only group, 48.9 years for the DIEP group, and 42.4 years for the TE group, with patients undergoing reconstruction being significantly younger. The BMI was 23.5, 21.9, and 20.9 kg/m^2 in the respective groups, being significantly lower in those undergoing reconstruction. There were also significantly more premenopausal patients in the reconstruction group (21 mastectomy vs. 19 DIEP and 24 TE). The DIEP group patients had significantly longer surgeries. There were no

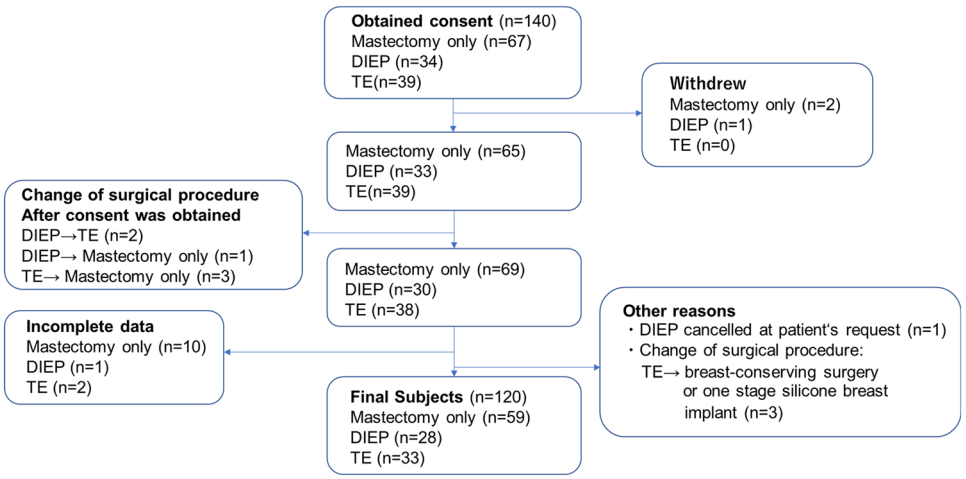


Fig. 1 Schematic diagram of patient selection. Of the 140 patients originally enrolled, 120 were the subjects of the final analysis

Table 1 Demographic information on the three groups of mastectomy patients according to their surgical procedure

Characteristics	Number of patients (%)			*p* value
	Mastectomy only ($n=59$)	DIEP ($n=28$)	TE ($n=33$)	
Age at diagnosis (years)				
Mean	58.0	48.9	42.4	< 0.001[a]
Range	34–87	28–69	23–61	
Interquartile range	47.0–69.0	45.3–51.8	36.5–48.0	
Missing	0	0	0	
Body mass index (kg/m^2)				
Mean	23.5	21.9	20.9	0.003[a]
Range	17–35	18–29	17–31	
Interquartile range	21.1–25.7	20.3–24.2	19.3–22.4	
Missing	0	0	0	
Pre-menopause				< 0.001[b]
No	37 (62.7)	9 (32.1)	8 (24.2)	
Yes	21 (35.6)	19 (67.9)	24 (72.7)	
Missing data	1 (1.7)	0 (0)	1 (3.0)	
Clinical tumor size				0.245
0	12 (20.3)	12 (42.9)	10 (30.3)	
1	16 (27.1)	4 (14.3)	10 (30.3)	
2	22 (37.3)	11 (39.3)	13 (39.4)	
3	6 (10.2)	1 (3.6)	0 (0)	
4	1 (1.7)	0 (0)	0 (0)	
Missing data	2 (3.4)	0 (0)	0 (0)	
Positive lymph node status				0.152
No	43 (72.9)	21 (75.0)	32 (97.0)	
Yes	14 (23.7)	7 (25.0)	1 (3.0)	
Missing data	2 (3.4)	0 (0)	0 (0)	
Pathological tumor stage				0.310
0	11 (18.6)	9 (32.1)	9 (27.3)	
1	27 (45.8)	13 (46.4)	12 36.4)	
2	14 (23.7)	6 (21.4)	11 (33.3)	
3	6 (10.2)	0 (0)	1 (3.0)	
Missing data	1 (1.7)	0 (0)	0 (0)	
Nuclear grade				0.778
1	23 (39.0)	6 (21.4)	18 (54.5)	
2	10 (16.9)	3 (10.7)	4 (12.1)	
3	8 (13.6)	3 (10.7)	4 (12.1)	
Missing data	18 (30.5)	16 (57.1)	7 (21.2)	
Estrogen receptor status				0.442
Negative	13 (22.0)	6 (21.4)	3 (9.1)	
Positive	45 (76.3)	22 (78.6)	30 (90.9)	
Missing data	1(1.7)	0 (0)	0 (0)	
Human epidermal growth factor receptor 2 status				0.402
Negative	43 (72.9)	18 (78.3)	26 (78.8)	
Positive	12 (20.3)	5 (17.9)	3 (9.1)	
Missing data	4 (6.8)	5 (17.9)	4 (12.19	
Duration of surgery (min)				< 0.001[a]
Mean	92.5	524.4	187.6	
Missing data	0	0	0	

DIEP breast reconstruction with a deep inferior epigastric perforator flap immediately following mastectomy; *TE* tissue expander breast reconstruction immediately following mastectomy

[a]Analysis of variance

[b]Pearson chi-square test

significant differences in tumor size, lymph node metastasis, or other cancer progression factors among the procedures, and no significant differences in biological characteristics such as nuclear grade or expression of ER and HER-2. Of the three inflammatory cytokines measured, bFGF was excluded from the analysis because many cases were below the assay sensitivity and only 16 had data obtained at all three time points. In addition, one patient with a clearly abnormal preoperative serum IL-6 level of 4743.09 pg/dl was excluded from the analysis. The mean preoperative serum IL-6 and VEGF levels did not differ significantly with age, ER expression, HER-2 expression, nuclear grade, BMI, or pathologic

Table 2 Clinicopathologic data and mean preoperative levels of serum cytokines

Parameter	*n* (%)	IL-6 (pg/ml) Mean (SD)	*p* value	VEGF (pg/ml) Mean (SD)	*p* value
Age (years)			0.742[a]		0.125[a]
≤ 50	68 (56.7)	1.5 (3.8)		48.8 (83.9)	
> 50	51 (42.5)	1.3 (1.3)		30.7 (41.0)	
Body mass index (kg/m^2)			0.508[a]		0.392[a]
≤ 25	90 (75.0)	1.3 (3.4)		38.0 (64.4)	
> 25	29 (24.2)	1.8 (1.0)		50.7 (83.0)	
Estrogen receptor status			0.506[a]		0.375[b]
No	22 (18.3)	1.1 (0.7)		53.3 (83.4)	
Yes	96 (80.0)	1.5 (3.3)		38.7 (66.0)	
Human epidermal growth factor receptor 2 status			0.755[a]		0.802[b]
No	86 (71.7)	1.5 (3.5)		39.6 (65.6)	
Yes	20 (16.7)	1.2 (0.8)		44.0 (84.8)	
Nuclear grade			0.422[b]		0.991[b]
1	46 (38.3)	1.2 (1.3)		41.0 (65.1)	
2	17 (14.2)	1.2 (1.2)		39.5 (89.6)	
3	15 (12.5)	1.7 (2.0)		38.6 (45.1)	
pStage			0.345[a]		0.141[b]
In situ	33 (27.5)	1.9 (5.3)		63.7 (102.6)	
Advanced	84 (70.0)	1.3 (1.4)		33.8 (53.1)	
Neoadjuvant chemotherapy			0.681[a]		0.555[b]
No	101 (84.2)	1.5 (3.2)		42.7 (73.7)	
Yes	18 (15.0)	1.2 (0.8)		32.1 (35.2)	

SD standard deviation, *IL-6* interleukin-6, *VEGF* vascular endothelial growth factor

[a]Analysis of variance

[b]Pearson chi-square test

Table 3 Mean cytokine levels at different time points in the three groups of mastectomy patients according to the surgical procedure

Time point	Mastectomy only	DIEP	TE	Ax (–)	Ax (+)
IL-6					
Preoperative	1.4 (1.5)	1.9 (5.7)	1.1 (0.9)	57.4 (514.3)	1.0 (0.8)
POD1	7.3 (7.1)	35.2 (25.4)	10.0 (8.8)	12.9 (15.0)	18.6 (23.6)
POD4-6	3.8 (4.9)	4.1 (7.2)	2.8 (4.3)	3.6 (5.8)	3.6 (4.3)
VEGF					
Preoperative	37.1 (50.2)	27.2 (75.1)	60.5 (89.5)	48.3 (76.6)	23.0 (41.4)
POD1	42.6 (49.1)	68.2 (130.7)	450.1 (2164.8)	54.2 (61.0)	407.4 (2073.8)
POD4-6	46.6 (65.7)	64.2 (125.4)	96.6 (185.8)	73.4 (129.4)	42.8 (108.8)

Data are expressed as means (pg/ml) (standard deviation)

DIEP breast reconstruction with a deep inferior epigastric perforator flap immediately following mastectomy, *TE* tissue expander breast reconstruction immediately following mastectomy, *IL-6* interleukin-6, *VEGF* vascular endothelial growth factor, *POD 1* postoperative day 1, *POD 4–6* postoperative days 4–6

progression or neoadjuvant chemotherapy (Table 2). Table 3 shows the IL-6 and VEGF levels measured preoperatively and on POD 1 and POD 4–6 categorized by type of surgery and axillary dissection.

Differences in serum cytokine levels in patients with vs. those without short-term complications

Short-term complications developed in two patients who underwent mastectomy only (both hematoma) and two patients who underwent DIEP (hematoma and total flap necrosis). Revision surgery was performed on the day of surgery or on POD 1 for these patients. There were no complications in the TE patients. The serum IL-6 levels on POD 1 and POD 4–6 in the mastectomy only group were 7.1 pg/ml (Standard deviation (SD) 7.1) and 3.8 pg/ml (SD 5.0) in those without complications and 12.6 pg/ml (SD 9.2) and 4.0 pg/ml (SD 0.2) in those with complications. These levels in the DIEP group were 37.0 pg/ml (SD 25.4) and 4.4 pg/ml (SD 7.4) in those without complications and 12.4 pg/ml (SD 10.6) and 0.4 pg/ml (SD 0.3) in those with complications, respectively. Similarly, the serum VEGF levels on POD 1 and POD 4–6 in the mastectomy only group were 41.3 pg/ml (SD 47.0) and 44.4 pg/ml (SD 65.7) in those without complications and 80.6 pg/ml (SD 114.0) and 111.4 pg/ml (SD 16.1) in those with complications. These levels in the DIEP group were 69.1 pg/ml (SD 136.0) and 68.7 pg/ml (SD 129.2) in those without complications and 56.6 pg/ml (SD 9.93) and 5.4 pg/ml (SD 5.3) in those with complications, respectively. There were no significant differences in serum cytokine levels for each surgical procedure in patients with vs. those without complications (Table 4).

Differences in serum cytokines for each surgical procedure

In the mastectomy only group, the serum IL-6 levels were 1.4 pg/ml (SD 1.5), 7.3 pg/ml (SD 7.1), and 3.8 pg/ml (SD 4.9) preoperatively, and on POD 1 and POD 4–6, respectively, being significantly higher than the preoperative level on POD 1 ($p < 0.001$) and POD 4–6 ($p < 0.001$). Conversely, in the DIEP group, the respective levels were 1.9 pg/ml (SD 5.7), 35.2 pg/ml (SD 25.4), and 4.1 pg/ml (SD 7.2), being significantly higher than the preoperative level on POD 1 ($p < 0.001$), but then decreasing and not differing significantly from the preoperative level on POD 4–6 ($p = 0.211$). In the TE group, the respective levels were 1.1 pg/ml (SD 0.9), 10.0 pg/ml (SD 8.8), and 2.8 pg/ml (SD 4.3), being significantly higher than the preoperative level on POD 1 ($p < 0.001$) and POD 4–6 ($p = 0.021$), although, as with the mastectomy only group, there was a trend for a decrease over time (Fig. 2). The serum VEGF levels on POD 1 and POD 4–6 did not differ significantly from the preoperative levels in the mastectomy only, DIEP, or TE groups (Fig. 2).

Differences in serum cytokines over time in patients with vs. those without axillary dissection

In the Ax (–) patients, there was no significant difference in serum IL-6 from the preoperative levels on POD 1 ($p = 0.428$) or POD 4–6 ($p = 0.338$). In the Ax (+) patients,

Table 4 Differences in cytokine levels in patients with vs. those without short-term complications after each surgical procedure

	No complications POD1	Complications POD1	*p* value
IL-6			
Mastectomy	7.1 (7.1)	12.6 (9.2)	0.293
DIEP	37.0 (25.4)	12.4 (10.6)	0.192
VEGF			
Mastectomy	41.3 (47.0)	80.6 (114.0)	0.711
DIEP	69.1 (136.0)	56.6 (9.93)	0.899
	No complications POD4–6	Complications POD4–6	*p* value
IL-6			
Mastectomy	3.8 (5.0)	4.0 (0.2)	0.950
DIEP	4.4 (7.4)	0.4 (0.3)	0.460
VEGF			
Mastectomy	44.4 (65.7)	111.4 (16.1)	0.158
DIEP	68.7 (129.2)	5.4 (5.3)	0.501

Data are expressed as means (pg/ml) (standard deviation)

DIEP breast reconstruction with a deep inferior epigastric perforator flap immediately following mastectomy, *IL-6* interleukin-6, *VEGF* vascular endothelial growth factor, *POD 1* postoperative day 1, *POD 4–6* postoperative day 4–6

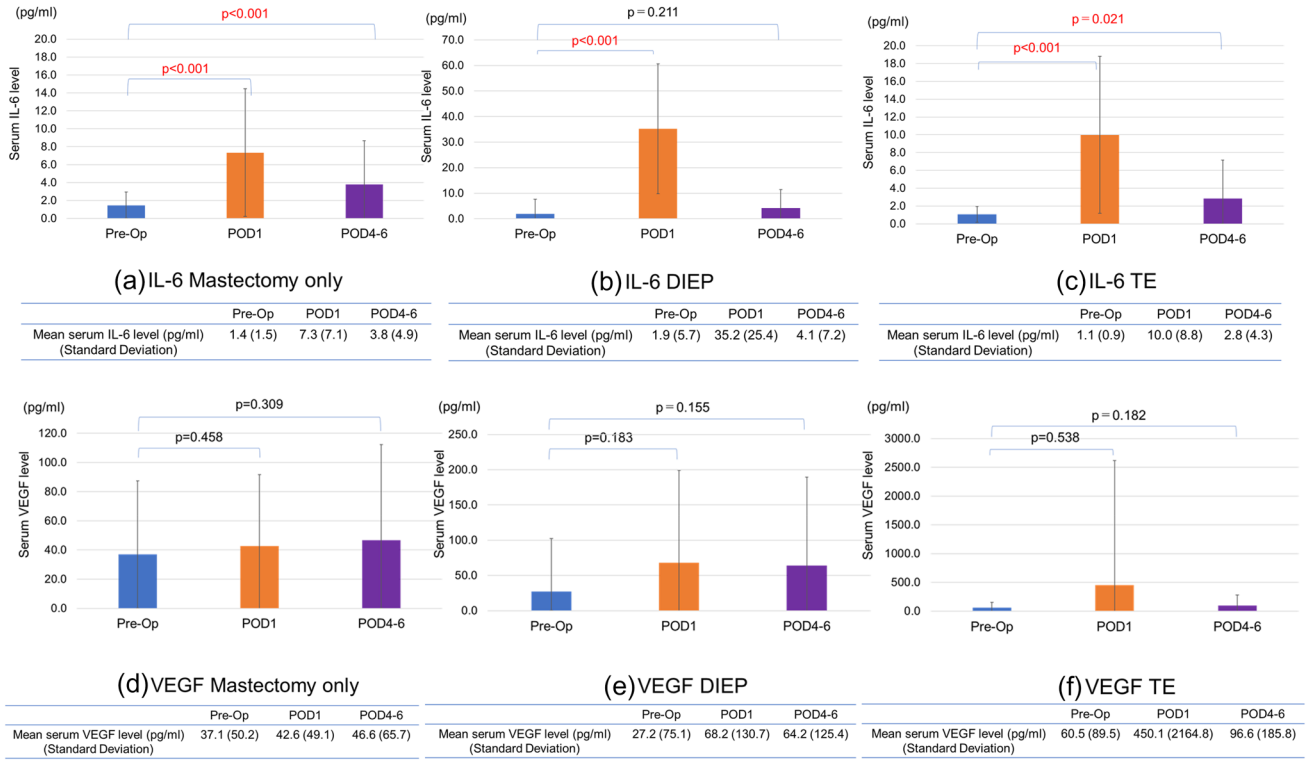


Fig. 2 Differences in serum interleukin 6 (IL-6) (**a**–**c**) and vascular endothelial growth factor (VEGF) (**d**–**f**) levels over time for each surgical procedure. IL-6 levels were significantly higher than the preoperative levels on postoperative day (POD) 1 after mastectomy with deep inferior epigastric perforator reconstruction (DIEP), and on POD1 and POD 4–6 after mastectomy only and after mastectomy with tissue expander reconstruction (TE). There was no significant difference in VEGF from the preoperative levels at any time point

the IL-6 level of 18.6 pg/ml (SD 23.6) on POD1 was significantly higher than the preoperative level of 1.0 pg/ml (SD 0.8; $p < 0.001$) and remained significantly higher at 3.6 pg/ml (SD 4.3) on POD4-6 ($p = 0.001$), although there was a trend of decrease over time (Fig. 3). In the Ax (–) patients, the VEGF did not change significantly from the preoperative level of 48.3 pg/ml (SD 76.6) to the level on POD 1 of 54.2 pg/ml (SD 61.0) ($p = 0.471$); however, it showed an increasing trend from 73.4 pg/ml (SD 129.4) on POD 4–6 ($p = 0.051$). In the Ax (+) patients, there was no significant difference in serum VEGF from the preoperative level on POD 1 ($p = 0.125$) or POD 4–6 ($p = 0.309$) (Fig. 3).

Comparison of cytokine levels among surgical procedures at each time point

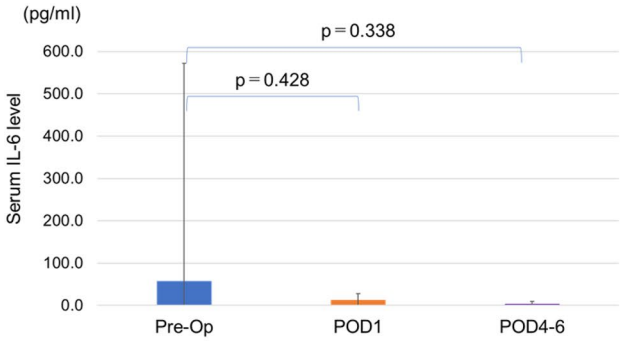
The preoperative serum IL-6 levels did not differ significantly between the mastectomy only group and the DIEP ($p = 0.585$) or TE ($p = 0.202$) groups, but they were significantly higher on POD 1 in the DIEP patients than in the mastectomy only group (35.2 pg/ml (SD 25.4) vs. 7.3 pg/ml (SD 7.1), $p < 0.001$). In contrast, there was no significant difference in IL-6 levels on POD 1 between the mastectomy only group and the TE group ($p = 0.120$). Moreover, there was no significant difference in serum IL-6 on POD 4–6 between the mastectomy only group and the DIEP ($p = 0.780$) or TE groups ($p = 0.358$) (Fig. 4). VEGF did not differ significantly between the mastectomy only group and the DIEP or TE groups at any time point (preoperative: DIEP $p = 0.468$, TE $p = 0.114$; POD1: DIEP $p = 0.190$, TE $p = 0.150$; POD4-6: DIEP $p = 0.393$, TE $p = 0.064$) (Fig. 4).

Comparison of cytokine levels in patients with vs. those without axillary dissection at each time point

There was no significant difference in IL-6 or VEGF between the Ax (+) and Ax (–) patients at any time point (preoperative, POD 1, POD 4–6: IL-6: $p = 0.519$, $p = 0.114$, $p = 0.994$; VEGF $p = 0.072$, $p = 0.321$, $p = 0.222$) (Fig. 4).

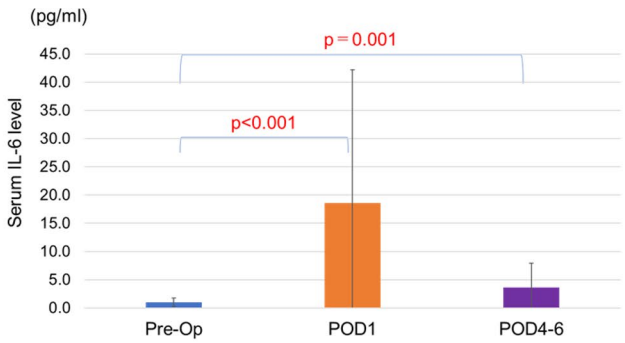
Discussion

Based on reports that the levels of inflammatory cytokines may be related to cancer proliferation, the goal of this study was to examine these levels immediately after breast reconstruction. We found that preoperative serum cytokine levels were unaffected by age, body shape, hormone receptor



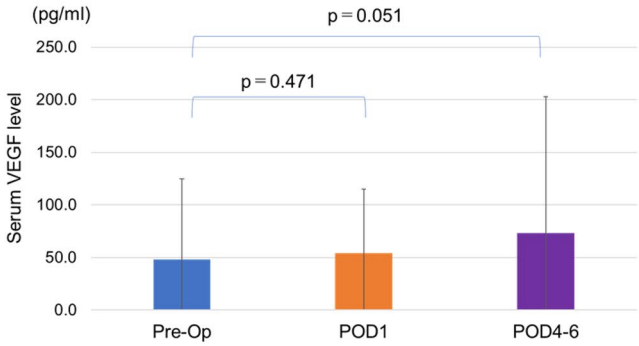
(a) IL-6 No axillary dissection

	Pre-Op	POD1	POD4-6
Mean serum IL-6 level (pg/ml) (Standard Deviation)	57.4 (514.3)	12.9 (15.0)	3.6 (5.8)



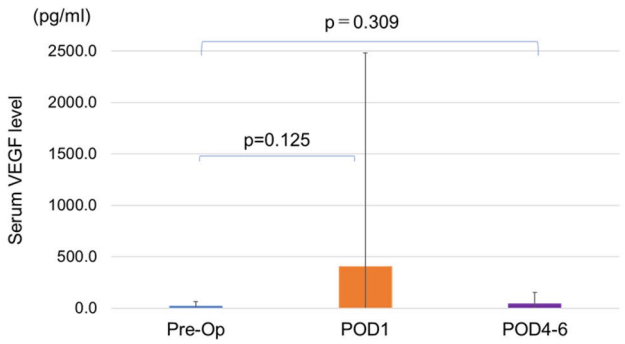
(b) IL-6 Axillary dissection

	Pre-Op	POD1	POD4-6
Mean serum IL-6 level (pg/ml) (Standard Deviation)	1.0 (0.8)	18.6 (23.6)	3.6 (4.3)



(c) VEGF No axillary dissection

	Pre-Op	POD1	POD4-6
Mean serum VEGF level (pg/ml) (Standard Deviation)	48.3 (76.6)	54.2 (61.0)	73.4 (129.4)



(d) VEGF Axillary dissection

	Pre-Op	POD1	POD4-6
Mean serum VEGF level (pg/ml) (Standard Deviation)	23.0 (41.4)	407.4 (2073.8)	42.8 (108.8)

Fig. 3 Differences in serum IL-6 (**a**, **b**) and VEGF (**c**, **d**) levels over time in patients without axillary resection (Ax (–)) vs. those with axillary resection (Ax (+)). IL-6 levels were significantly higher than preoperative levels in the Ax (+) patients on POD1 and POD 4–6. The VEGF level in the Ax (–) patients tended to higher than the preoperative level on POD4-6

status, pathologic progression, or preoperative chemotherapy; and that the serum cytokine levels on POD 1 and POD 4–6 were unaffected by short-term complications. IL-6 increased significantly over time on POD 1 after all three surgical procedures of mastectomy only, DIEP, and TE, with prolongation of these higher levels on POD 4–6 after mastectomy only and TE. There was no significant variation in IL-6 in Ax (–) patients, while Ax (+) patients showed a similar trend to TE patients. A comparison of IL-6 among the different surgeries at each time point showed significantly higher levels after DIEP than after mastectomy only on POD 1, but there were no differences among the groups on POD 4–6. There were also no significant differences between the Ax (+) and Ax (–) patients at any time points. Serum VEGF showed no significant variation at any time point in the mastectomy only, DIEP, TE, or Ax (+) patients, however, the serum level tended to be higher than the preoperative level on POD 4–6 in the Ax (–) patients. There were no significant variations in VEGF among the surgical procedures at any time point.

Serum IL-6 levels have been studied widely in patients with cancer and in healthy controls and have been discussed in relation to cancer proliferation. For example, patients with colorectal cancer were found to have higher serum IL-6 levels than healthy controls, and an elevated IL-6 level is associated with increased tumor stage and size, and metastasis and poorer survival [6]. In renal cell carcinoma patients, a high postoperative IL-6 level is a strong predictor of recurrence, and IL-6 also predicts disease-specific survival and overall survival (OS) [7]. An elevated IL-6 level in pretreated patients with recurrent breast cancer indicates a poor prognosis [8] and a high serum IL-6 in patients with metastatic breast cancer receiving palliative chemotherapy is a poor prognostic factor for OS [9].

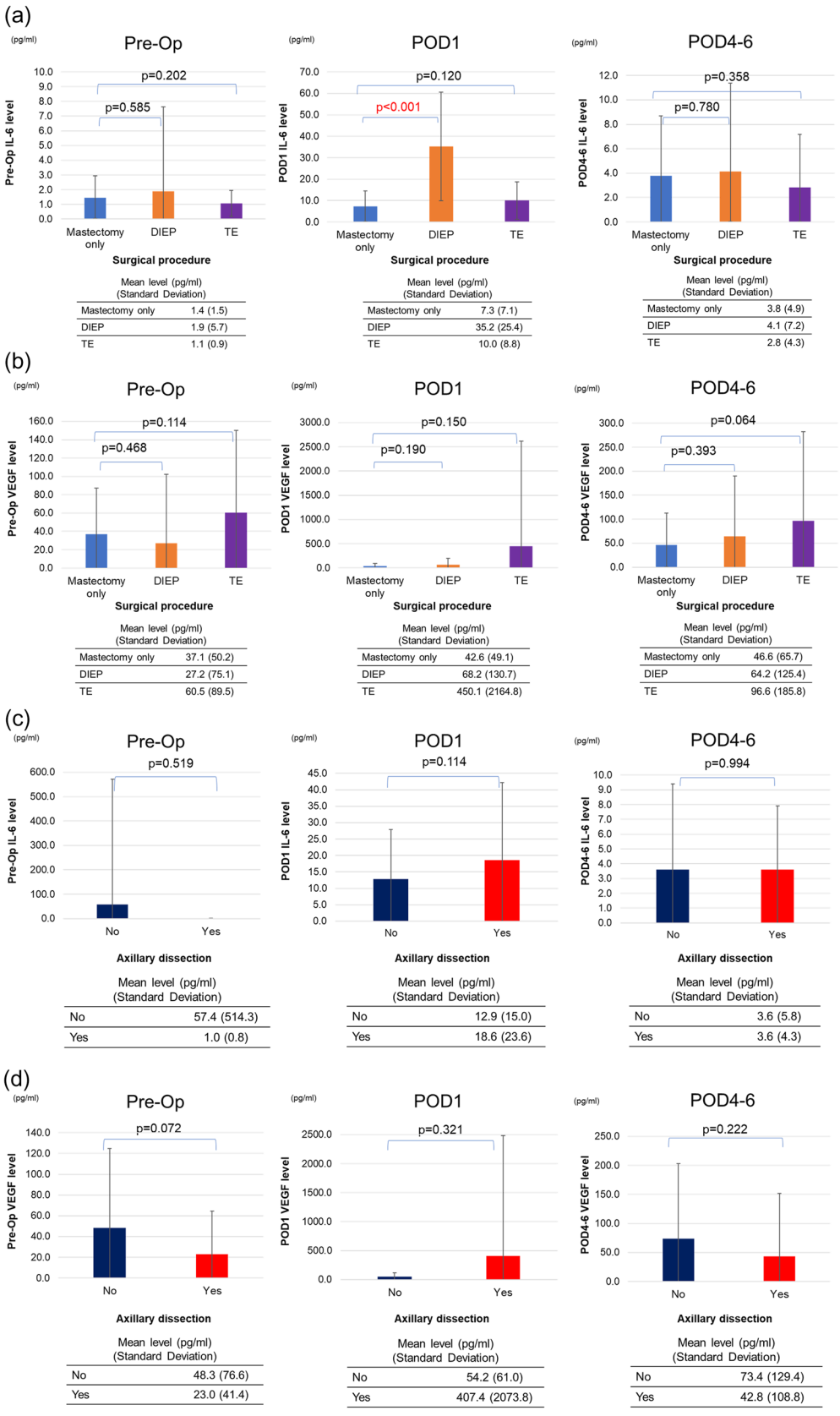


Fig. 4 Comparison of serum cytokine levels among the surgical procedures. **a** IL-6 in the three procedures. **b** VEGF in the three procedures. **c** IL-6 in patients with vs. those without axillary dissection. **d** VEGF in patients with vs. those without axillary dissection

Regarding the primary endpoint of this study, the differences over time showed serum IL-6 levels significantly higher on POD1 and POD4-6 than the preoperative levels in the patients who underwent mastectomy only. The reason for this is unclear, although the mastectomy only group included more elderly patients than the other groups. Pietrobon et al. suggested that immunosenescence associated with age may be caused by a failure to resolve inflammation, since many regulatory factors are deficient in older people [23]. Thus, IL-6 metabolism in the mastectomy only patients, who tended to be older, may have been more prone to delay than in the other groups. Further studies are needed to investigate this hypothesis.

The significantly higher serum IL-6 levels on POD 1 than preoperatively in the DIEP group was likely to have been caused by the high levels of cytokines released immediately after surgery related to the longer operative time and extensive invasion. However, wound healing progressed over the next few days and the serum IL-6 level decreased quickly. Conversely, as with the mastectomy only group, IL-6 levels in the TE group were significantly higher on POD 1 and this persisted until POD 4–6. Maira et al. found significantly higher C-reactive protein levels 2 months postoperatively in breast implant patients than in those treated by abdominal liposuction ($p < 0.001$) [24]. Our results similarly suggest that artificial reconstruction tends to cause more prolonged inflammation. The trend over time for IL-6 in the Ax (+) patients was similar to that in TE group. In a study of perioperative serum IL-6 levels in thyroid cancer patients undergoing cervical lymph node dissection, Geng et al. found that they increased on POD 1, then decreased from POD 3, until recovering to normal on POD 5 [25]. Our results followed a different course, which may be due to the serum IL-6 level differing depending on the site and extent of lymph node resection. In our study, dissection of the axillary lymph nodes may have caused lymphatic obstruction and prolonged wound fluid cytokine drainage to the general circulation, and this possibility needs further investigation. IL-6 was still significantly elevated on POD 4–6 in the mastectomy only, TE, and Ax (+) patients; however, the mean IL-6 levels of 3.8 pg/ml (SD 4.9), 2.8 pg/ml (SD 4.3) and 3.6 pg/ml (SD 4.3) were similar to the median level of 3.14 pg/ml found by Noman et al. [26], (compared with 53.98 pg/ml in patients with operable breast cancer in the same study). Thus, the IL-6 elevation was prolonged in the mastectomy only, TE and Ax (+) patients, although this may not have affected future cancer growth remarkably.

The serum IL-6 levels did not change significantly from the preoperative levels between the mastectomy only and DIEP groups, but it was significantly higher in the DIEP group than in the mastectomy only group on POD 1. Zhang et al. also found no significant difference in preoperative serum IL-6 levels of patients undergoing laparoscopic-assisted vs. open radical gastrectomy for gastric cancer; however, on POD 1, the IL-6 level was significantly higher after open radical gastrectomy than after laparoscopic-assisted surgery, but there was no significant difference between the two groups on POD 3. They concluded that the difference in IL-6 levels on POD 1 was due to laparoscopic-assisted surgery being less invasive than open radical gastrectomy [27]. IL-6 showed a similar trend in our patients, and the higher level on POD 1 after DIEP was presumably due to greater surgical invasiveness than mastectomy only. However, there was no significant difference on POD 4–6, which suggests that the effect of the surgical invasiveness of DIEP was temporary. Moreover, the serum IL-6 levels were not significantly higher in the Ax (+) patients than in the Ax (–) patients at any time, suggesting that dissection did not affect the serum IL-6 levels.

VEGF has been associated with shorter survival and recurrence in a variety of cancers. For example, high VEGF levels tended to be associated with worse OS for colorectal cancer patients [12] and the number of metastases increased significantly with increasing serum VEGF in hematogenous lung metastasis model mice [13]. A meta-analysis of ovarian cancer showed that increased serum VEGF shortened OS [14]. In breast cancer, there have also been reports of significantly higher levels of serum VEGF in patients with malignant tumors than in those with benign tumors, with increased serum VEGF observed with increasing grades of malignancy. However, the VEGF levels did not differ significantly in our mastectomy only, DIEP, TE, and Ax (+) patients at any time point, and there were no differences among the surgical methods. These results suggest that IL-6 is a more sensitive indicator of perioperative surgical invasion than VEGF. However, the VEGF level in our Ax (–) patients tended to be higher than the preoperative level on POD 4–6. In a study of perioperative serum VEGF in patients with breast cancer, Curigliano et al. found that VEGF was lower than the preoperative level 24 and 48 h postoperatively in patients treated with a transverse rectus abdominis myocutaneous (TRAM) flap, then it increased again on POD 5. It was hypothesized that this trend was due to the consumption of platelets necessary for VEGF production in the early stages of wound healing, resulting in a decrease in VEGF, which then increased again as the wound healed [28]. The cause of re-elevation in our Ax (-) patients was unclear, although it is possible that it was due to this platelet effect.

Limitations

The limitations of this study include its single-center design and the relatively small number of subjects. Moreover, measurement of bFGF was not possible in many samples so these

results could not be analyzed. The cause of this difficulty is uncertain, although the narrow working range of the Bio-Plex 200 (assay working range: 3.26 3341) may be one of the reasons. As for TE, although later surgery for replacement with silicone breast implant is necessary to complete the reconstruction, the differences in cytokine levels during this second surgery have not been evaluated. Finally, the direct relationship between serum cytokine levels and prognosis was not investigated, and this relationship requires clarification in a future study.

Conclusion

The current prospective study investigated early postoperative differences in inflammatory cytokine levels in breast surgery and evaluated the extent and duration of elevated levels for each procedure. Our findings suggest that serum IL-6 is a more sensitive marker of surgical invasion than serum VEGF in breast cancer patients. However, by POD 4–6, IL-6 had decreased to the same level as that in healthy subjects for all surgical procedures, with and without axillary dissection, and a comparison of cytokine levels among surgical procedures also showed no significant difference at this time point. These findings suggest that long-term elevation of serum IL-6 caused by the surgical invasiveness of breast reconstruction is extremely unlikely and that breast reconstruction can be performed safely immediately after mastectomy. However, future studies are needed to investigate and confirm the direct relationship between cytokine levels and prognosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00595-023-02700-1.

Funding Japan Society for the Promotion of Science, JP 17K17017, 22K10526.

Declarations

Conflict of interest Yuko Mukai received a research grand from JSPS KAKENHI Grant Numbers JP 17K17017 and 22K10526. The other authors declare no financial interests in relation to the content of this article.

Ethical approval This study was approved by the Ethics Committee of Okayama University Hospital (approval no: 1711–029). All procedures involving human participants were performed in accordance with the ethical standards of institutional and national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all participants in the study.

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