

Effect of Anesthetic Technique on Serum Vascular Endothelial Growth Factor C and Transforming Growth Factor β in Women Undergoing Anesthesia and Surgery for Breast Cancer

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ABSTRACT

Background: In breast cancer, vascular endothelial growth factor C, transforming growth factor β , placental growth factor, and fibroblast growth factor (acidic and basic) promote angiogenesis and metastases. We tested the hypothesis that a propofol-paravertebral anesthetic (PPA) technique would attenuate postoperative changes in these angiogenic factors to a greater extent than balanced general anesthesia (GA) and morphine analgesia in women undergoing surgery for primary breast cancer.

Method: Forty women with primary breast cancer undergoing surgical excision were randomized to receive either standard GA or PPA technique. Venous blood was sampled before and at 24 h after surgery and serum analyzed. The primary endpoint was a preoperative *versus* postoperative change in vascular endothelial growth factor C and transforming growth factor β concentrations.

Results: Using a visual analog scale (median [25–75% interquartile range]), PPA patients (1 [0–2]) had less pain at 2 h ($P = 0.02$) than did GA patients (3 [2–5]). The mean postoperative change in vascular endothelial growth factor C

concentrations among GA patients was 733 *versus* 27 pg/ml for PPA patients (difference, 706 [97.5% CI, 280–1,130] pg/ml, $P = 0.001$). In contrast, the mean postoperative change in transforming growth factor β concentration among GA patients was -163 *versus* 146 pg/ml for PPA patients (difference, 309 [97.5% CI, -474 to -143] pg/ml, $P = 0.005$). Concentrations of placental growth factor and fibroblast growth factor, both acidic and basic, were undetectable in serum.

Conclusion: Anesthetic technique influences serum concentrations of factors associated with angiogenesis in primary breast cancer surgery.

What We Already Know about This Topic

- ❖ Vascular endothelium growth factor C (VEGF-C), transforming growth factor β (TGF- β), and others promote angiogenesis and metastases in breast cancer.

What This Article Tells Us That Is New

- ❖ In patients undergoing breast cancer surgery, postoperative VEGF-C serum levels were increased after standard general anesthesia (GA) compared with propofol plus paravertebral block anesthetic technique, whereas TGF- β levels decreased after GA. These changes are consistent with a possible protective effect against cancer recurrence and metastasis.

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BREAST cancer remains a leading cause of death among women and is second only to lung cancer as a cause of cancer mortality in western countries, most of which is attributable to recurrence and metastasis. Breast cancer also accounts for more new cases of cancer among women than any other cancer.¹ Initial treatment almost invariably involves surgical excision. However, tumor recurrence occurs in a significant number of patients. Even when the most experienced operator performs surgical resection, it is unavoidable that tumor cells are dispersed into the blood and lymphatic circulations.² The fate of this small burden of tumor cells depends on the balance between antimetastatic factors and the ability of the tumor to invade, propagate, and metastasize.³

Previous investigations have suggested that a number of factors in the perioperative period could promote metastases. These include surgery *per se* and its associated stress response,⁴ anesthesia *per se*,⁵ acute pain⁶ and opioid analgesics.⁷ Regional anesthesia has been consistently shown to attenuate the neuroendocrine response to surgery,^{8–9} and, therefore, perioperative immunosuppression. It may also reduce the amount of general anesthesia (GA) required intraoperatively, provides excellent analgesia, and reduces opioid consumption. Compared with GA, regional (spinal) anesthesia attenuated tumor metastasis in rats inoculated with a strain of breast adenocarcinoma.¹⁰ Recent retrospective analyses indicate that regional anesthesia (and analgesia) for breast¹¹ and prostate¹² cancer surgery is associated with a markedly reduced risk of tumor recurrence and metastasis. This finding has led to the generation of a hypothesis that an anesthetic technique consisting of paravertebral regional anesthesia with immune-friendly propofol paravertebral anesthesia (PPA) might reduce the incidence of metastases and recurrence in breast cancer, compared with standard volatile agent-opioid anesthesia and analgesia (GA). This hypothesis is currently being evaluated in a randomized controlled trial (NCT00418457).¹²

Angiogenesis, the process whereby a neoplastic tumor establishes its own blood supply from its host, is a necessary function of a primary cancer when the tumor begins to grow beyond a threshold size. Furthermore, metastasis is often associated with increased primary tumor growth.¹³ Once tumor dissemination has occurred, angiogenesis is paramount in the establishment of the secondary cancer before it becomes clinically apparent. It has been demonstrated that tumor tissue more than 2 mm diameter cannot survive without developing its own blood supply.¹⁴ Angiogenesis is stimulated and maintained by a number of molecular factors. Angiogenic mediators have been shown to drive breast cancer responses including vascular endothelial growth factor (VEGF), fibroblast growth factor acidic (aFGF) and fibroblast growth factor basic (bFGF), placental growth factor (PlGF), and transforming growth factor- β (TGF- β).^{15–19} In particular, VEGF has been shown to enhance growth of estrogen receptor positive breast cancer cells *in vivo*.²⁰ Bevacizumab, a monoclonal antibody inhibitor of VEGF, lengthens disease-free survival for women with metastatic breast cancer.²¹ The mechanism by which VEGF promotes tumor growth and metastases is believed to be through facilitating angiogenesis.²²

It is plausible that many of the perioperative factors affecting cancer's metastatic potential influence concentrations of angiogenic factors in breast cancer—and hence the extent of angiogenesis itself. Therefore, we investigated a hypothesis that, in women undergoing primary breast cancer surgery, an anesthetic technique consisting of paravertebral regional anesthesia and propofol-only GA would attenuate the expression of these angiogenesis-promoting molecular factors in breast cancer—to a greater extent than balanced GA with opioid analgesia.

Materials and Methods

Clinical Protocol

After obtaining approval from the Mater Misericordiae University Hospital Research Ethics Committee (Dublin, Ireland) and written informed consent, we enrolled 40 women aged 18–85 yr scheduled to undergo primary wide local excision of breast tissue or mastectomy and axillary node sampling or full clearance for confirmed breast cancer. Exclusion criteria included age, younger than 18 yr or older than 85 yr; American Society of Anesthesiology risk grade 4; any contraindication to administration of midazolam, fentanyl, propofol, sevoflurane, or morphine; or any contraindication to performance of paravertebral blockade. Other exclusion criteria were previous breast surgery (except diagnostic breast biopsy) and inflammatory breast cancer. This trial was conducted and data reported according to the CONSORT statement.²³

Patients were randomly assigned to receive combined PPA or GA. Patients were randomized using a secure Web-based system that used computer-generated random number allocation and automatically recorded the patient numbers and assignments. Randomization was done online as part of the randomization for the ongoing clinical trial. This is a computer-generated randomization process, equilibrated every 20 patients, and administered by the Outcomes Research Consortium at The Cleveland Clinic (Cleveland, Ohio), collaborators on the overall clinical trial. On entering the site and providing the investigator-specific user name and password, an invitation to “randomize patient” appears. Clicking this window yields an instruction to randomize the patient to one group or the other. Randomization occurred after a research nurse who visited the patient before surgery to outline the study obtained written informed consent. All patients were invited to read a patient information leaflet before deciding to consent to enrollment. Individuals who conducted the laboratory assays (outcome endpoints) were masked as to patient group allocation.

Before induction of GA, the PPA group received a catheter placed in the ipsilateral paravertebral space at the second or third thoracic vertebral space using a standard technique. A bolus dose of 20 ml 0.25% levobupivacaine was immediately administered *via* the paravertebral catheter over 5 min. Induction of GA was commenced using a propofol target-controlled infusion (Diprifusor; Graseby, London, United Kingdom). Patients spontaneously breathed an oxygen/air mixture through a laryngeal mask airway. GA was maintained with the target-controlled infusion of propofol. Postoperative analgesia was maintained with a continuous paravertebral infusion of 8–10 ml/hr 0.25% levobupivacaine. Paravertebral catheters were removed approximately 48 h after insertion. The need for rescue analgesia was determined by a 10-cm visual analog score (VAS). Rescue analgesia consisted of intravenous bolus 0.1 mg/kg morphine followed by additional doses at the same concentration, as required, every 3–4 h.

The GA group had induction of balanced GA with fentanyl 1–3 $\mu\text{g/kg}$ and propofol 2–3 mg/kg . Anesthesia was maintained with 1.0–1.5 minimum alveolar concentration sevoflurane with spontaneous respiration *via* a laryngeal mask airway. Intraoperative analgesia consisted of morphine 0.1–0.15 mg/kg . Patient-controlled analgesia using bolus 1 mg morphine, with a 6-min lockout time and a 4-h maximum dose of 30 mg , provided postoperative analgesia. All patients received 1 g of acetaminophen intravenously during surgery. Pain assessment was done using a VAS at 2 h after surgery by the anesthetist and again at 24 and 48 h after surgery by research nursing staff.

Growth Factor Measurement

Venous blood was sampled before and at 24 h after surgery. Samples were centrifuged at $4,000 \times g$ and the resulting serum was stored at -20°C for analysis. VEGF C, TGF- β 1, aFGF, bFGF, and PlGF were analyzed in the serum using the Quantikine Enzyme Immunoassay System (R&D Systems Europe, Abingdon, England, United Kingdom) in accordance with manufacturer instructions. Preoperative and postoperative concentrations of VEGF C, TGF- β 1, aFGF, bFGF, and PlGF angiogenesis factors were determined using patient serum from both study groups. Enzyme-linked immunosorbent assays were prepared for each angiogenic factor. The concentration of each angiogenic factor was determined by calculating its optical density of the enzyme-linked immunosorbent assays preparation at 540 nm using a spectrophotometer. Optical densities for a group of controls of known concentration were plotted against those of the study groups to create a standard curve. Serum concentration of each angiogenic factor was then determined from standard curves. The coefficients of variation and limits of quantification are listed below. Where the values were below the limit of quantification and where no concentration was obtained—as was the case for PlGF, aFGF, and bFGF—concentrations were recorded as undetectable. Per the manufacturer instructions, the minimal detectable dose were as follows: VEGF C, 4–48.4 pg/ml ; TGF β 1, 1.7–15.4 pg/ml ; aFGF, 1.19–13.9 pg/ml ; bFGF, less than 3 pg/ml ; and PlGF, less than 7 pg/ml .

Statistical Analysis

Although the VEGF, TGF data, and VAS pain scores were normally distributed, statistical advice suggested that non-parametric tests would be appropriate, especially given the large dispersion of data observed. Variables with heterogeneous variability across groups were analyzed with nonparametric tests. A P value of less than 0.05 was taken as statistically significant. The data were analyzed using Prism (version 5; GraphPad Software, Inc., La Jolla, CA).

The primary endpoint was defined *a priori* as the change in preoperative *versus* postoperative VEGF concentration. Changes in all other angiogenic cytokines and other outcomes were secondary endpoints. The primary outcome was held to a conservative type I error level. In this case, the

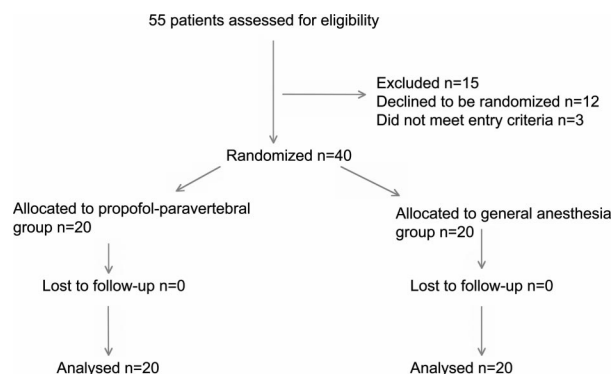


Fig. 1. CONSORT flow diagram. The progress of patients through the trial is shown. GA = general anesthesia.

primary outcome was assessed once, but there were two outcomes. Therefore, the α error level for the revised analysis was set at $P = 0.025$ (0.05 for each outcome).

Undertaking a prospective power analysis was difficult and fraught with potential pitfalls because the size of a “significant” change in postoperative VEGF is unknown. Further, ours was in effect a pilot study, as no previous reports have evaluated this question. We assumed that GA patients would increase their postoperative VEGF concentration by 50% and that PPA patients would demonstrate an attenuated response of 10%, which would nonetheless represent a remarkable numerical change between the groups. Available literature suggested a mean (SD) VEGF increase ranging from 500–750 (200) pg/ml ,^{24–28} accepting a type I error of 0.05 and a type II error of 0.1. Seventeen patients would be required to detect this difference between groups with a study power of 90%. Therefore, we enrolled 20 patients in each study group to allow for missing or unavailable data. Data were compared using an independent group t test for parametric data and a Mann–Whitney U test for nonparametric data. Categorical data were assessed by Fisher exact test. Data are expressed as median (25–75% interquartile range).

Results

Participants who were randomly assigned, received each intended treatment, and analyzed for the primary outcome are shown in the CONSORT flow diagram (fig. 1).²³ There were no significant differences between the two patient groups in terms of age, American Society of Anesthesiology grade, height, weight, or anesthetic and surgical factors (tables 1 and 2). There were no significant differences between the groups.

VAS pain scores at 2 h after operation were significantly lower in the PPA group (1 [0–2]) compared with the GA group (3 [1–5], $P = 0.02$). At 24 h after surgery, a similar difference remained (0 [0–1] *vs.* 2 [0–3], $P = 0.04$). However, at 48 h, there was no statistically significant difference between study groups (1 [0–3] *vs.* 2 [0–4], $P = 0.70$). Differences in VAS scores between the two groups were reflected in the amount of opioid given in the recovery room and at 24 h. Median morphine administered in the recovery room

Table 1. Patient Characteristics

Characteristic	General Anesthesia (n = 20)	Paravertebral (n = 20)	P Value
Age, yr	54 (52–60)	59 (53–74)	0.144
ASA risk classification	—	—	—
I	14 (70)	10 (50)	0.208
II	6 (30)	10 (50)	0.120
Height, cm	165 (159–167)	163 (155–170)	0.704
Weight, kg	69 (64–84)	80 (72–95)	0.077

All data are presented as No. (%) unless otherwise specified.
ASA = American Society of Anesthesiologists.

was 5 (1–10) mg *versus* 0 (0–1) mg at 2 h ($P = 0.005$) and 10 (5–17) mg *versus* 0 (0–2) mg at 24 h ($P < 0.001$) in the GA and PPA groups, respectively (table 3).

Median (interquartile range) serum concentrations of VEGF-C increased after surgery in patients receiving GA with 806 (502–981) pg/ml *versus* 1,385 (918–1,702) pg/ml ($P = 0.01$). In patients who underwent PPA, VEGF-C postoperative concentrations were unchanged 779 (440–985) pg/ml *versus* 775 (350–1,109) pg/ml ($P = 0.70$). On comparison of the PPA and GA patient groups, there was no statistically significant difference in serum concentrations of VEGF C before surgery. However, there was a significantly higher serum concentration in the GA patient group compared with the PPA group after surgery ($P = 0.015$), as shown in figure 2. The mean preoperative to postoperative change score for VEGF C GA patients was 733 pg/ml compared with mean change score of 27 pg/ml for PPA patients. Therefore the difference ($P = 0.001$) in mean change scores was 706 pg/ml (97.5% CI, 280–1,130).

Serum concentrations of TGF- β were decreased after surgery in patients who received the GA technique from 648

Table 3. Pain and Analgesia Data

Pain Measure	General Anesthesia (n = 20)	Paravertebral (n = 20)	P Value
Visual analog scale pain score	—	—	—
2 h	3 (3–5)	1 (0–2)	0.007
24 h	2 (1–4)	0 (0–2)	0.039
48 h	2 (1–4)	2 (0–4)	0.762
Morphine use, mg	—	—	—
Recovery	5 (1–10)	0 (0–2)	0.009
24 h	10 (5–17)	0 (0–2)	0.0001

All data are presented as median (25–75% interquartile range).

(523–746) pg/ml to 498 (360–660) pg/ml ($P = 0.04$). Among patients receiving PPA, concentrations increased 613 (337–850) pg/ml to 703 (555–864) pg/ml ($P = 0.04$). Although there was no difference between preoperative TGF- β serum concentrations of the study groups, postoperative serum concentrations in the PPA group were significantly higher than that of the GA group ($P = 0.02$), as shown in figure 3. The mean change score for TGF- β among GA patients was –163 pg/ml compared with that of PPA patients, 146 pg/ml. Therefore the difference in mean change scores was 309 pg/ml (97.5% CI, –474 to –143, $P = 0.005$).

The optical densities of the aFGF, bFGF, and PlGF serum samples approached that of our reference group despite achieving normal curves for our standard controls. This result indicates that the values obtained for these angiogenic factors were below detection limits in the serum.

Discussion

We have assessed the effects of anesthetic technique on serum concentrations of angiogenic factors associated with breast

Table 2. Anesthetic and Surgical Factors

Factor	General Anesthesia (n = 20)	Paravertebral (n = 20)	P Value
Duration of anesthesia, min median (25–75% interquartile range)	77.5 (70–90)	82.5 (71.25–100)	0.633
Margins after excision, mm median (25–75% interquartile range)	3 (1–5)	3 (1.5–9)	0.557
Mastectomy and axillary clearance	5 (25)	3 (15)	0.447
Wide local excision	15 (75)	17 (85)	0.447
Tumor size, mm median (25–75% interquartile range)	21 (10–29)	17 (13–23)	0.533
Nodes positive	10 (50)	6 (30)	0.208
Histological grade	—	—	0.851
Grade 1	4 (20)	3 (15)	—
Grade 2	9 (45)	10 (50)	—
Grade 3	7 (35)	7 (35)	—
Positive receptors	—	—	—
Estrogen	16 (80)	18 (90)	0.432
Progesterone	15 (75)	15 (75)	0.941
HER2/neu	4 (20)	2 (10)	0.393

All data are presented as No. (%) unless otherwise specified. Comparisons were derived from independent samples *t* test, Mann-Whitney U test, or Fisher exact test, as appropriate.

HER2/neu = human epidermal growth factor receptor 2.

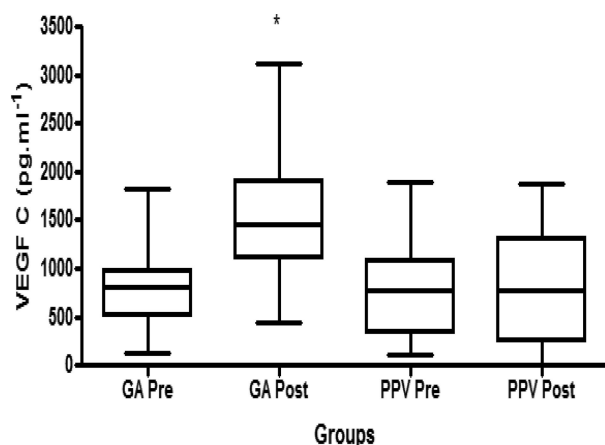


Fig. 2. Median (interquartile range) vascular endothelial growth factor C (VEGF-C) concentrations are shown. * $P = 0.01$, higher general anesthesia (GA) postoperative values versus GA preoperative and propofol-paravertebral (PPV) postoperative values. Horizontal line denotes median values, box borders refer to interquartile range, whiskers indicate range of values.

cancer by analyzing serum obtained from patients randomly assigned to receive either sevoflurane GA with opioid analgesia or combined GA and regional anesthesia for primary breast cancer surgery. The principal findings were that VEGF C concentrations are increased after surgery in the GA group but remain unchanged in the PPA group. In contrast, TGF- β 1 concentrations were increased after surgery in the PPA group compared with the GA group.

Experimental and retrospective clinical evidence suggests that although some anesthetics and analgesics impair perioperative immune function (*e.g.*, volatile agents and opioids), others (*e.g.*, propofol) do not—and may, in fact, inhibit cancer cells.²⁹ Furthermore, regional anesthesia is thought to be beneficial in that it attenuates the stress response, preserves

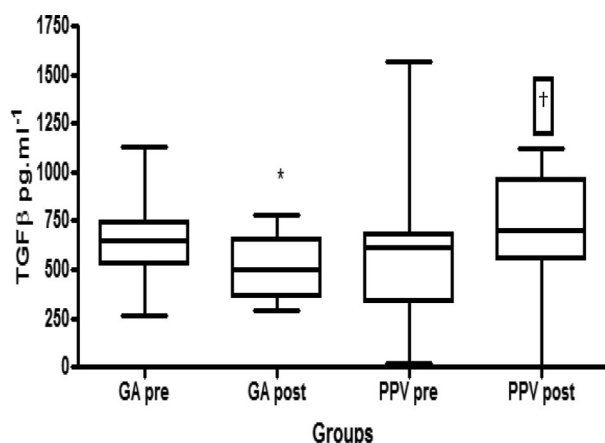


Fig. 3. Median (interquartile range) transforming growth factor (TGF) β concentrations are shown. * $P = 0.04$, lower general anesthesia (GA) postoperative values versus GA preoperative values. † $P = 0.04$, higher postoperative propofol-paravertebral (PPV) values versus GA postoperative values. Horizontal line denotes median values, box borders refer to interquartile range, whiskers indicate range of values.

perioperative immune function, and reduces opioid reliance.¹⁰ Although propofol was given to patients receiving standard GA as an induction agent, its effects would have been observed in 5 min or less, because anesthesia was maintained throughout surgery with sevoflurane. In addition, we were determined that the anesthetic techniques used in our experimental model would be realistic and easily applicable to the “real world” if a benefit was subsequently described from clinical studies.

VEGF C has been shown to be overexpressed in breast cancers. Previous studies have shown that VEGF C is an important factor in promoting angiogenesis in breast cancer and in aiding the dissemination of cancer cells into the systemic circulation. VEGF C may also induce paracrine signaling between breast cancer cells and the endothelium, altering the permeability of lymphatic and blood vessels. Breast cancer seems to upregulate the expression of VEGF C in the endothelium of neovascularized blood vessels of the cancer.^{16,17,20} VEGF-C and its receptor are augmented in breast cancer and may be important in promoting angiogenesis and micrometastases. VEGF and VEGF C have also been demonstrated to be specific angiogenic and lymphangiogenic factors¹⁶ which, when secreted by breast cancer cells, increase metastatic potential by stimulating blood vessel growth.²⁴ In breast cancer, the lymphatic system is important as a mechanism of early dissemination of micrometastases. This finding underlies the negative impact on prognosis of lymph node involvement by cancer cells. VEGF-C may be important in inducing tumor lymphangiogenesis and seeding cancer cells into the lymphatic microcirculation. Overexpression and intratumoral augmentation of lymphangiogenesis by VEGF-C may be an important mechanism in micrometastases to regional lymph nodes and the lungs *via* the lymphatic system.²⁴

Studies have also shown that VEGF C may convert breast cancers with low metastatic potential to more aggressive behavior.²⁵ Therefore, if anesthetic technique can decrease VEGF C concentrations, it may also reduce metastatic risk in breast cancer by reducing tumor angiogenesis. The mechanism by which anesthetic technique could alter VEGF and TGF concentrations is unknown. VEGF may be secreted from host cells in the body including platelets, muscle cells, and tumor-associated stromal cells.^{22,26,27} Perhaps the use of regional anesthesia and propofol alters immunologic or surgical stress responses in a way that renders the serum milieu less conducive to angiogenic factor production.

It would have been interesting to study patients without breast cancer who were also undergoing anesthesia and breast surgery. Available evidence, however, indicates that patients undergoing surgery for benign breast disease have similar concentrations of VEGF to that observed in cancer patients.^{22,26,27} We focused on the basic question of whether anesthetic technique could affect differences in VEGF C in patients with breast cancer undergoing primary breast cancer surgery. Further studies may elucidate the roles of surgery and cancer in postoperative VEGF C serum concentrations.

Although it is most unlikely that increased VEGF for several hours may change the prognosis of breast cancer, the observation that anesthetic technique may alter it at all suggests the possibility of creating of serum conditions conducive to promoting or resisting cancer micrometastases released after surgery.

In a clinical prospective observational study with up to 8-yr follow-up, preoperative VEGF and postoperative change in VEGF did not predict disease-free survival in breast cancer.²⁷ All patients received similar, standard anesthetic technique. The difference in magnitude of measured VEGF concentrations between our observations (nanograms per milliliter *vs.* picograms per milliliter) reflects the wide variation in the literature in relation to serum VEGF C concentrations associated with breast carcinoma. Variations may be the result of measurement techniques used. Our study demonstrated a doubling of VEGF C concentrations from baseline in the GA group and these concentrations in other studies were indeed associated with metastatic disease.^{20,26,27}

Serum VEGF C concentrations from 90 to 150 pg/ml have also been demonstrated in studies of metastatic colorectal carcinoma, although serum VEGF C concentrations associated with cancer vary widely in the literature.²⁸ Thus, it is plausible that an increase of VEGF C concentrations from 700 to 1,400 pg/ml could have a negative impact on the natural history of breast cancer.

An experimental study has shown that a peripheral μ opioid antagonist, methylnaltrexone, inhibits VEGF-induced angiogenic proliferation and migration in cancer cells.³⁰ Further clinical studies could compare the effect of different analgesic strategies (*e.g.*, opioids, nonsteroidal antiinflammatory drugs) on postoperative VEGF and TGF β concentrations.

aFGF and bFGF are also potent stimulators of angiogenesis and have an almost equipotent mitogenic effect on endothelium.^{18,31} PlGF is also a potent angiogenic factor that seems to have prognostic value in breast cancer and also has prognostic significance in other cancers, including lung cancer and gastric cancer.^{31,32}

TGF- β can act as a potent angiogenic factor, but this attribute depends on the presence and concentration of other cytokines in the local environment as well as TGF- β concentrations present. In breast cancer, augmentation of TGF- β expression is associated with an increased potential for invasion and metastasis.^{33,34}

The role of TGF- β in breast cancer is complex. Under normal conditions, it suppresses cell proliferation, induces differentiation, or promotes apoptosis.³⁵ TGF- β is also a potent promoter of angiogenesis *in vivo* by stimulating cell-adhesion molecules and by affecting the production of proteolytic enzymes.³⁶ In cancer cells, studies have described the mutation of the TGF-signaling pathway of cell cycle regulation resulting in a cell line that is insensitive to TGF- β regulation. After cancer cells become resistant to TGF- β , cancer cells and surrounding stromal cells increase their production of TGF- β . This increased production of TGF- β may in-

crease the metastatic potential of breast cancer cells by increasing angiogenesis and by increasing their proteolytic activity.^{34–36}

However, in early breast cancers, TGF- β may have an inhibitory role on tissue growth when its expression may instead promote resistance to tumor metastasis.^{34–36} This finding is in contrast to TGF- β 's role in advanced breast cancer, where it stimulates growth and metastasis. Our cancers were indeed early. Therefore, our observation of increased TGF- β after PPA and reduced concentrations after GA could also be consistent with the hypothesis that the PPA anesthetic technique might reduce the risk of metastasis in early breast cancer by modulating the serum concentration of TGF- β in a manner conducive to metastatic resistance.

Therefore, TGF- β responses in breast cancer are far more complex than VEGF, and may increase or decrease after surgery depending on a number of factors, including nodal involvement. The significance of our finding of decreased TGF- β concentrations after a PPA technique is unknown, but the fact that anesthetic technique itself seems to modulate this known angiogenic marker seems worthy of note and further study to correlate to longer term, actual clinical outcomes, which will be possible in the context of the ongoing randomized clinical trial.

We were unable to detect significant serum concentrations of aFGF, bFGF, or PlGF in serum samples. The apparently low concentrations of these angiogenic factors may reflect dilution in the serum *in vivo*. Previous studies in relation to breast cancer focused on concentrations of these angiogenic factors in breast cancer tissue. A bFGF serum value of 1.0 pg/ml has previously been reported,³⁷ within the limits of detection using our test method.

In a recent study, we demonstrated that serum from patients randomized to receive the same two distinct anesthetic regimens as described in the current study differentially affected breast cancer cell migration *in vitro*, PPV patient serum inhibiting breast cancer cell migration to a greater extent than that of GA patients.³⁸ It is plausible that altered serum angiogenic factors as observed in the current study could influence angiogenesis, but this theory remains to be investigated.

Our results should be interpreted cautiously. It is possible that our findings could be attributable to chance, and the scale of the absolute value changes is relatively modest. Serum concentrations of the markers evaluated are significantly diluted compared with breast cancer tissue concentrations. As a recent review has highlighted,³⁹ a number of perioperative factors may influence metastatic progression or resistance, including β -blocker, cyclooxygenase-2, and statin therapy, as well as inadvertent mild hypothermia, which we were unable to control for and could theoretically influence our findings. None of our patients received a blood transfusion during the study period. However, this was a randomized, controlled trial, with a relatively homogenous study population: all women with proven early breast cancer and differing only in anesthetic technique received. The study

was not designed to attribute observed changes in VEGF and TGF- β to one anesthetic drug. Instead, we sought to test the hypothesis that distinct anesthetic techniques packaged together could make a difference as an important surrogate risk factor for metastasis, namely, angiogenesis-promoting factor concentrations. The current findings are one “snapshot” of angiogenic factor concentrations in postoperative time. Whether our observations persist over a longer time and whether that would alter the prognostic significance of our observations is unknown.

In conclusion, in this randomized, controlled clinical trial measuring serum markers of breast cancer growth and angiogenesis in women with primary breast cancer, we found that GA increased postoperative serum concentrations of VEGF C but reduced TGF- β compared with propofol-paravertebral anesthesia, a result that is consistent with the hypothesis that anesthetic technique may influence breast cancer outcome. This clinical question can only be definitively addressed by a large, multicenter, randomized clinical trial with breast cancer recurrence and metastasis as the primary outcome.

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