ANESTHESIOLOGY

Anesthesia and Circulating Tumor Cells in Primary Breast Cancer Patients

A Randomized Controlled Trial

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Anesthesia may contribute to the distant spread of cancer during surgical treatment
- The presence of circulating tumor cells has been independently associated with both a higher risk of disease recurrence and reduced survival in both nonmetastatic and metastatic breast cancer

What This Article Tells Us That Is New

- The hypothesis that postoperative circulating tumor cell counts would be higher in primary breast cancer patients receiving sevoflurane anesthesia than in those receiving intravenous anesthesia with propofol was tested in a randomized controlled trial of 210 patients
- The type of anesthesia did not affect circulating tumor cell counts over time (median circulating tumor cell count/7.5 ml blood [interquartile range]: for propofol, 1 [0 to 4] at end of surgery (0 h), 1 [0 to 2] at 48 h, and 0 [0 to 1] at 72 h; and for sevoflurane, 1 [0 to 4] at 0 h, 0 [0 to 2] at 48 h, and 1 [0 to 2] at 72 h; rate ratio, 1.27 [95% CI, 0.95 to 1.71])

ABSTRACT

Background: The effect of anesthetic drugs on cancer outcomes remains unclear. This trial aimed to assess postoperative circulating tumor cell counts—an independent prognostic factor for breast cancer—to determine how anesthesia may indirectly affect prognosis. It was hypothesized that patients receiving sevoflurane would have higher postoperative tumor cell counts.

Methods: The parallel, randomized controlled trial was conducted in two centers in Switzerland. Patients aged 18 to 85 yr without metastases and scheduled for primary breast cancer surgery were eligible. The patients were randomly assigned to either sevoflurane or propofol anesthesia. The patients and outcome assessors were blinded. The primary outcome was circulating tumor cell counts over time, assessed at three time points postoperatively (0, 48, and 72 h) by the CellSearch assay. Secondary outcomes included maximal circulating tumor cells value, positivity (cutoff: at least 1 and at least 5 tumor cells/7.5 ml blood), and the association between natural killer cell activity and tumor cell counts. This trial was registered with ClinicalTrials.gov (NCT02005770).

Results: Between March 2014 and April 2018, 210 participants were enrolled, assigned to sevoflurane (n = 107) or propofol (n = 103) anesthesia, and eventually included in the analysis. Anesthesia type did not affect circulating tumor cell counts over time (median circulating tumor cell count [interquartile range]; for propofol: 1 [0 to 4] at 0 h, 1 [0 to 2] at 48 h, and 0 [0 to 1] at 72 h; and for sevoflurane: 1 [0 to 4] at 0 h, 0 [0 to 2] at 48 h, and 1 [0 to 2] at 72 h; rate ratio, 1.27 [95% CI, 0.95 to 1.71]; $P = 0.103$) or positivity. In one secondary analysis, administrating sevoflurane led to a significant increase in maximal tumor cell counts postoperatively. There was no association between natural killer cell activity and circulating tumor cell counts.

Conclusions: In this randomized controlled trial investigating the effect of anesthesia on an independent prognostic factor for breast cancer, there was no difference between sevoflurane and propofol with respect to circulating tumor cell counts over time.

(ANESTHESIOLOGY 2020; 133:548–58)

Breast cancer represents a major health issue: with more than 2 million new cases worldwide,[1] it is the most frequently diagnosed tumor and the leading cause of cancer deaths in women.[2] Despite primary treatment, between 6% of patients with localized tumors and 22% with nodal extension will face recurrence at 5 yr.[3]

This article is featured in "This Month in Anesthesiology," page 1A. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site. This article has a visual abstract available in the online version. Part of the work presented in this article has been presented as abstract A2182 at the American Society of Anesthesiologists Annual Meeting in Chicago, Illinois, October 24, 2016.

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Most patients diagnosed with breast cancer undergo surgical treatment. There have been increasing concerns, however, that the perioperative period would promote tumor spreading, either directly (*i.e.*, through tumor manipulation), or indirectly, because systemic inflammation may affect immune responses against tumor cells.[4] Evidence also suggests that anesthesia itself may contribute to distant spread: anesthetic drugs seem to interfere directly with tumor cell biology and to decrease natural killer cells cytotoxic activity, which plays a critical role in tumor cell destruction and tumor growth.[5,6]

Although these effects have been well documented in preclinical studies, their relevance in the clinical setting is still matter of debate: intravenous anesthesia has been suggested to result in better survival rates compared with inhalational anesthesia, but evidence was mostly driven by retrospective analyses, which are prone to important methodologic limitations.[7–14] Conflicting findings also emerged from a few randomized controlled trials suggesting no effect on survival, but sample sizes were small, follow-up duration was short, and multiple interventions were evaluated without an adequate control group.[15–17]

Large, well designed randomized controlled trial are thus needed to clarify the effect of anesthetic drugs on cancer prognosis, but long follow-up periods often undermine the feasibility of such studies. To overcome this issue, the use of biologic markers as surrogates for prognosis may represent a valuable approach.[18] Among others, the presence of circulating tumor cells in the peripheral blood has been identified as a particularly promising indicator.[19] Hematogenous dissemination seems to occur long before clinical or radiological signs of metastases develop,[20] which places circulating tumor cells at an ideal location in the causal pathway leading to distant disease.[21] There is also increasing evidence that circulating tumor cells are independently associated with a higher risk of disease recurrence and with reduced survival, both in nonmetastatic and metastatic breast cancer.[22,23] In this respect, circulating tumor cell monitoring may represent a promising approach to better understand the effect of anesthesia on tumor behavior during the perioperative period.

Therefore, we conducted a randomized controlled trial to evaluate the effect of intravenous (*i.e.*, propofol) *versus* inhalational (*i.e.*, sevoflurane) anesthesia on postoperative circulating tumor cell counts in primary breast cancer patients. A superiority design was used to test the hypothesis that postoperative circulating tumor cell counts would be higher in patients receiving sevoflurane. The association between immune cell responses (*i.e.*, natural killer cell cytotoxic activity) and circulating tumor cell counts was assessed in an exploratory *in vitro* study nested within this trial.

Materials and Methods

We used the Consolidated Standards of Reporting Trials recommendations for the reporting of randomized trials.[24] This trial was approved by the local ethical committee (Zurich, Switzerland, registration number PB_2016-01791) and was registered with ClinicalTrials.gov (NCT02005770, https://clinicaltrials.gov/ct2/show/NCT02005770, principal investigator: Beatrice Beck-Schimmer, registration date: December 9, 2013). The study protocol is available on ClinicalTrials.gov.

Trial Design and Participants

This was a parallel-group, randomized, controlled trial conducted at a university hospital (University Hospital of Zurich) and a private clinic (Hirslanden Group, Zurich) in Switzerland. Patients were considered eligible if they were aged 18 to 85 yr, diagnosed with primary preinvasive and invasive breast cancer without distant metastases (stage 0 to III) and scheduled for surgery with or without axillary node dissection. Patients were excluded if they met one of the following criteria: preoperative chemotherapy, possible immune impairment (*i.e.*, autoimmune disease, human immunodeficiency virus, other active cancer, American Society of Anesthesiologists (ASA; Schaumburg, Illinois) Physical Status IV or V), immunosuppressive or chronic opioid therapy, secondary surgery (*e.g.*, for recurrence, reconstruction), or surgery performed under general anesthesia with concomitant regional anesthesia (*i.e.*, epidural catheter, paravertebral blockade, wound infiltration with local anesthetics). Those with a known or suspected hypersensitivity or allergy to anesthetics were considered ineligible. Patients were approached on the day before surgery by research staff, who evaluated eligibility, obtained written informed consent, and enrolled the participants.

Randomization and Blinding

Randomization was performed by research staff using a secure Internet-based system (www.randomizer.at; accessed April 10, 2018) that stratified patients according to their ASA status and ensured concealment of random allocation. The patients were randomly assigned in a 1:1 ratio to either intravenous anesthesia (propofol group) or inhalational anesthesia (sevoflurane group). Patients remained blinded to their assignment group (standardized induction in both groups), as was the study personnel involved in circulating tumor cell measurements (*i.e.*, outcome assessors did not have access to patient charts).

Procedures

Anesthesia induction was standardized in both groups using fentanyl (2 to 3 μg/kg), thiopental (4 to 6 mg/kg), and rocuronium (0.6 mg/kg). Patients requiring a rapid sequence induction received 0.9 mg/kg rocuronium instead of 0.6 mg/kg. Further administration of fentanyl during surgery followed a standardized protocol (*i.e.*, 2 μg/kg; total amount, 5 to 10 μg/kg). In the propofol group, anesthesia was maintained using a target-controlled infusion device providing an intravenous propofol dose adjusted to keep

Bispectral Index values between 40 and 60; in the sevoflurane group, sevoflurane was provided to keep Bispectral Index values between 40 and 60. Postoperative nausea and vomiting prophylaxis and perioperative analgesia followed standardized protocols that were applied until hospital discharge.

Outcome

The primary outcome was the number of circulating tumor cells assessed postoperatively by the CellSearch assay (Menarini Silicon Biosystems Inc., USA). Based on immunomagnetic separation, this detection technique uses a magnetic field to isolate ferrofluid-labeled tumor cells of epithelial origin, such as breast cancer cells.[25] This standardized procedure uses antibodies directed against a common molecular signature displayed by circulating tumor cells in breast cancer patients (*i.e.*, the "EpCAM+/CK+/DAPI+/CD45−" signature, where EpCAM indicates epithelial cell adhesion molecule, CK indicates cytokeratin, and DAPI indicates 4′,6-diamidino-2-phenylindole). After staining of the isolated cells, circulating tumor cell identification was confirmed by two independent, specifically trained laboratory technicians that were masked to treatment assignment. Identification of circulating tumor cells followed a predefined set of criteria (*i.e.*, morphological features, compatible staining pattern).

Peripheral blood was collected at four different time points, *i.e.*, before the induction of anesthesia (baseline), after surgery but before extubation (0 h), on day 2 (48 h), and on day 3 (72 h) postoperatively. The last measurement was initially planned on day 4 but was rescheduled to day 3 in January 2016 to avoid data loss due to early hospital discharge. This was the only change made to the original trial design.

Secondary outcomes were defined as the maximal circulating tumor cell count value at any time point after surgery (0, 48, and 72 h); circulating tumor cell counts as a binary outcome (using two different cutoff values, *i.e.*, at least 1 and at least 5 circulating tumor cells/7.5 ml blood); and the association between natural killer cell activity and circulating tumor cell counts (see also "Additional Analyses"). Initially, only a cutoff value of a least 5 circulating tumor cells/7.5 ml blood was considered. We added the threshold of a minimum of 1 cell at the time of analysis, because evidence suggested that values as low as 1 circulating tumor cell/7.5 ml blood were associated with poorer prognosis in primary breast cancer patients.[22] No other changes were made to primary/secondary outcomes definitions over the study period.

Statistical Analyses

Sample size calculation was performed using a method accounting for repeated measurements of count data over time.[26] Because evidence on the effect of intravenous or inhalational anesthesia on circulating tumor cell counts was nonexistent, we adopted a conservative approach and assumed that the expected effect size (Cohen's d) between groups would be small (0.3). Thus, assuming a within-subject correlation of circulating tumor cell counts over time of 0.4 and a dropout rate of 10%, we estimated that a total of 232 patients would be required (209 patients without dropout) to detect a difference between groups corresponding to an effect size of 0.3, with a power of 80%, at a significance level of 5% (two-sided). Because the dropout rate was particularly low, the trial ended after enrolling 217 patients.

All analyses were based on intention to treat. Continuous data were expressed as means and standard deviations or as medians and interquartile ranges if distributions were skewed. The primary analysis used a mixed Poisson model with random intercept per patient to account for repeated measurements over time and thus correlated observations within subjects. We opted for this approach because the Poisson model is appropriate for count data (primary outcome of circulating tumor cell counts). The results of the Poisson models are presented as rate ratios, denoting the comparison of circulating tumor cell counts between the two groups. To avoid assuming a linear development of circulating tumor cells over time, time was alternatively included as a factor variable in our model. We also explored the effect of anesthetics on the maximal circulating tumor cell count value at any time point after surgery in additional Poisson models (0, 48, and 72 h).

Because circulating tumor cell detection is usually reported as a binary outcome (*i.e.*, positive *vs.* negative endpoint using a cutoff value of at least 1 or at least 5 circulating tumor cells/7.5 ml blood), circulating tumor cell count data were dichotomized and further assessed using a mixed logistic regression model with random intercept per patient. Finally, models were adjusted to account for tumor-related and perioperative factors presumed to affect circulating tumor cell counts (*i.e.*, tumor size, tumor type, and overall opioids consumption, all preplanned).

All statistical analyses were conducted in R, version 3.6.1. Two-sided tests were performed, and a level of significance of 0.05 was used.

Additional Analyses

Because of the interplay between natural killer cell cytotoxic activity and tumor growth, we also assessed natural killer cell activity (*i.e.*, apoptosis rate induced in tumor cells) in a preplanned, exploratory, *in vitro* study nested within this trial. Natural killer cell–induced apoptosis was evaluated in a subgroup of patients randomly selected from the study data set. For each patient, natural killer cell activity was assessed at a single, predefined time point, *i.e.*, when circulating tumor cell counts reached their maximal value. The association between natural killer cell–induced apoptosis rate and circulating tumor cell count was then assessed using linear regression analysis.

Natural killer cell–induced apoptosis rate and necrosis rate were determined *in vitro* by measuring target cell killing of the K562 tumor cell line (human chronic myelogenous leukemia, ATCC, CCL-243).[27,28] Patients blood samples were collected in EDTA-coated vials. Buffy coats (Blutspende Zürich, Switzerland) were used as controls. Peripheral blood mononuclear cells of both patient samples and buffy coats were isolated by Ficoll–Hypaque density gradient centrifugation and stored in liquid nitrogen. For determination of natural killer cell activity, peripheral blood mononuclear cells were thawed and coincubated with K562 for 24 h at 37°C with 5% CO_2 in 10% human serum/RPMI medium. An effector (natural killer cells)–to–target cell (K562 cells) ratio of 1:1 was used. All cells were then washed in phosphate-buffered saline and stained in 2% bovine serum albumin in phosphate-buffered saline for 25 min at 4°C using the following panel: CD3-APC (lymphocyte staining; Biolegend, United Kingdom), dilution of 1:100; CD 56-PE (natural killer cells staining; Biolegend), dilution 1:100; and CD16-FITC (FcγRIIIA staining, which is essential for cellular cytotoxicity, expressed on the surface of a subset of monocytes; Biolegend), dilution 1:200. After a washing step in annexin V binding buffer, the cells were simultaneously stained with annexin–PerCPCy5.5 for staining of apoptotic cells (Biolegend) at a dilution of 1:20 and Zombie-NIR for staining of necrotic cells (Biolegend) at a dilution of 1:500.

Zombie-NIR–stained K562 boiled for 5 min at 80°C or annexin V–stained apoptotic K562 and treated for 24 h with 10 mM benzamide were used as positive controls for cytotoxicity. Unstained K562, unstained patient peripheral blood mononuclear cells, and unstained peripheral blood mononuclear cells from buffy coats served as negative controls. Cell analysis was performed using the spectral analyzer SP6800 (Sony Biotechnology, United Kingdom).[29]

Results

Between March 10, 2014, and April 10, 2018, 586 patients were assessed for eligibility (fig. 1). Of 217 enrolled participants, seven patients withdrew consent after randomization. We eventually included 210 patients in the intention-to-treat analysis (sevoflurane group: n = 107, propofol group: n = 103).

Baseline characteristics are presented in table 1. Demographic and clinical data were balanced between treatment groups. Most participants were middle-aged, modestly morbid patients with an early-stage tumor. Baseline circulating tumor cell counts and positivity (using a cutoff value of at least 1 and at least 5 circulating tumor cells/7.5 ml blood) were similar in both allocation groups. Table 2 depicts the intra- and postoperative characteristics, which were well balanced between groups.

The evolution of circulating tumor cell counts over time is illustrated in figure 2, table 3, and Supplemental Digital Content figure 1, which depicts predicted tumor cell counts using the estimates from the Poisson model, including a linear time variable and baseline circulating tumor cell counts (http://links.lww.com/ALN/C415). Administrating sevoflurane *versus* propofol did not affect the primary outcome of circulating tumor cell counts over time (rate ratio, 1.27 [95% CI, 0.95 to 1.71]; $P = 0.103$). This was the case, regardless of whether time was considered as a linear or a factor variable, or whether an interaction term between time and anesthesia was introduced. However, when we explored the effect of anesthetics on the maximal circulating tumor cells value at any time point after surgery, administrating inhalational anesthesia (*i.e.*, sevoflurane) led to a significant increase in maximal circulating tumor cell counts postoperatively (sevoflurane *vs.* propofol: rate ratio, 1.36 [95% CI, 1.18 to 1.56]; $P < 0.0001$; *i.e.*, the maximum number of circulating tumor cells increased by a factor of 1.36 (or 36%) when sevoflurane was used compared with propofol).

When circulating tumor cells were analyzed as a binary outcome over time, the type of anesthesia did not have any effect on circulating tumor cell positivity, regardless of the cutoff value considered (cutoff value of at least 1 circulating tumor cell/7.5 ml blood: sevoflurane *vs.* propofol odds ratio, 1.21 [95% CI, 0.84 to 1.74]; $P = 0.309$; cutoff value of at least 5 circulating tumor cells/7.5 ml blood: sevoflurane *vs.* propofol odds ratio, 1.59 [95% CI, 0.86 to 3.01]; $P = 0.139$). Similar results were obtained when time was considered as a factor variable, and there was no evidence for an interaction between treatment and time.

We performed predefined analyses to explore whether tumor-related and perioperative factors modified the effect of anesthetics on circulating tumor cell counts. Models adjusted for tumor type (DCIS, luminal A, luminal B, triple negative, HER2 positive, other) and tumor size (Tis, T1, T2, T3, T4) did not reveal any relevant effect modification on circulating tumor cell counts over time or positivity (regardless of the cutoff value considered). Similarly, adjusting for opioid consumption did not yield any effect modification. In the exploratory models, however, the effect of inhalational anesthesia on maximal postoperative circulating tumor cells values remained robust (sevoflurane *vs.* propofol rate ratio, 1.26 [95% CI, 1.09 to 1.47]; $P = 0.002$; adjustment for tumor type, size, and opioid consumption).

Exploratory *in vitro* analyses were conducted in a subgroup of 60 patients randomly selected from the study data set (30 in the sevoflurane group and 30 in the propofol group). Similar natural killer cell–induced apoptosis rates were found in both treatment groups (mean apoptosis rate, for sevoflurane group, 34.7%; for propofol group, 35.7%). Overall, the necrosis rate of K562 tumor cells was less than 1%. Linear regression yielded no evidence for an association between apoptosis rates and maximal circulating tumor cell counts (regression coefficient, −0.077; 95% CI, −0.33 to

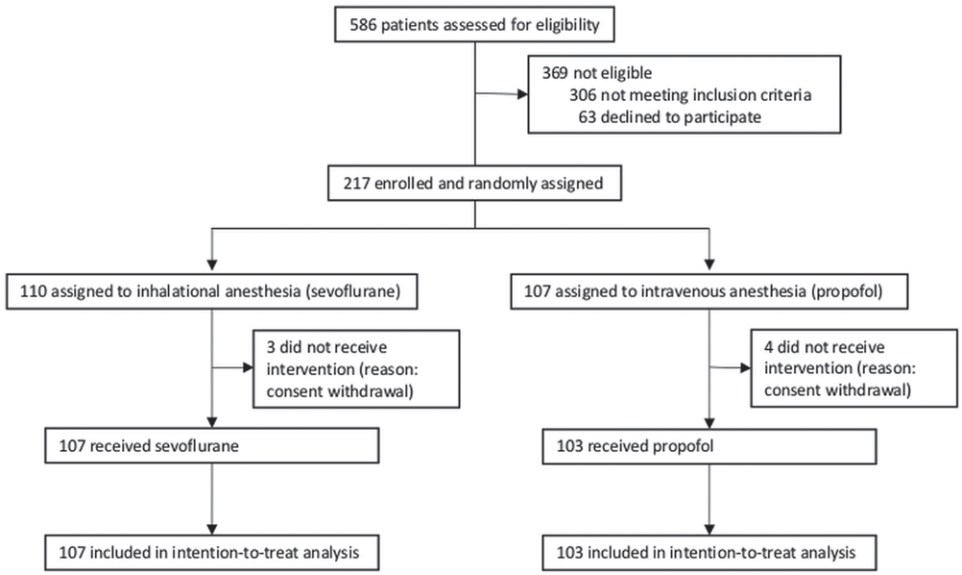

Fig. 1. Flow diagram.

0.17; fig. 3). This was the case, regardless of treatment group assignment or whether an interaction term between anesthesia type and natural killer cell activity was introduced.

Discussion

In this randomized controlled trial including 210 participants undergoing surgery for primary breast cancer, the type of anesthesia did not seem to affect circulating tumor cell counts over time or circulating tumor cell positivity. In one secondary analysis, there was a 36% increase in the maximal number of postoperative circulating tumor cells in patients receiving inhalational anesthesia. Additional *in vitro* analyses in a random selection of 60 patients did not reveal any evidence for an association between natural killer cell–induced apoptosis rates and maximal circulating tumor cell counts.

This trial investigated the effect of anesthesia on perioperative circulating tumor cell counts, an independent prognostic factor for breast cancer. In contrast to previously published randomized trials,[15–17] our study was larger and had an adequate control group, and the issue of long follow-up periods was mitigated by using a prognostic factor.

In our trial, circulating tumor cell counts at baseline were higher than those reported in previous studies. Several reasons may account for this discrepancy. First, all of our patients underwent sentinel lymph node localization 18 to 24 h before baseline circulating tumor cell assessment, and we cannot formally exclude that an injection in the vicinity of the tumor would not lead to any circulating tumor cells release. Second, approximately 30% of our patients had wire-guided localization of the tumor, which implies direct manipulation of the tumor shortly before circulating tumor cell assessment.

Because the identification of circulating tumor cells with the CellSearch assay may imply some degree of subjectivity (*i.e.*, images of potential tumor cell candidates are displayed to trained laboratory technicians and assessed following predefined criteria), we verified all samples with at least 5 tumor cells/7.5 ml blood using the automated software ACCEPT (Supplemental Digital Content fig. 2, illustrating the flow chart of the validation analysis; http://links.lww.com/ALN/C415).[30] Overall, the comparison showed a good correlation (Supplemental Digital Content fig. 3 illustrates the correlation between these two methods; http://links.lww.com/ALN/C415). Compared to the ACCEPT software, there was an overestimation of circulating tumor cell counts by 1.66 units with human assessment (Supplemental Digital Content fig. 4 illustrates the agreement between these two methods; http://links.lww.com/ALN/C415). However, in this validation analysis, only samples with high tumor cell counts were considered. This may bias the results toward an overestimation of the difference in means. In other words, if all samples, *i.e.*, including those with 0 to 4 tumor cells/7.5 ml blood, had been included, the difference in means of 1.66 units would have likely been smaller. Second, the overestimation of 1.66 units was nondifferential, *i.e.*, applied to both groups, regardless of treatment assignment.

Apart from one secondary analysis, our findings contrast with numerous previously published studies suggesting better outcomes with the use of intravenous anesthesia. The potential reasons for this disparity are two-fold. First, clinical

Table 1. Baseline Characteristics

	Sevoflurane (n = 107)	Propofol (n = 103)
Age, yr	59 ± 13	59 ± 12
Body mass index, kg/m^2	26.7 ± 6.1	26.2 ± 5.6
ASA class		
I	29 (27.1)	25 (24.3)
II	73 (68.2)	73 (70.9)
III	5 (4.7)	5 (4.8)
Tumor size		
Carcinoma *in situ*	9 (8.4)	5 (4.9)
T1, < 2 cm	55 (51.4)	55 (53.4)
T2, 2–5 cm	38 (35.5)	33 (32.0)
T3, > 5 cm	3 (2.8)	7 (6.9)
T4, any size, growing into the chest wall or skin	1 (0.9)	2 (1.9)
Not reported	1 (0.9)	1 (1.0)
Pathologic nodal status		
N0, node-negative	65 (60.7)	62 (60.2)
N1, 1–3 lymph nodes	29 (27.1)	22 (21.4)
N2, 4–9 lymph nodes	4 (3.7)	6 (5.8)
N3, >10 lymph nodes or infra-/supraclavicular	1 (0.9)	2 (1.9)
Not reported	8 (7.5)	11 (10.7)
Receptors		
Estrogen receptor –/Progesterone receptor –	11 (10.3)	12 (11.7)
Estrogen receptor +/Progesterone receptor –	9 (8.4)	7 (6.8)
Estrogen receptor –/Progesterone receptor +	0 (0)	0 (0)
Estrogen receptor +/Progesterone receptor +	85 (79.4)	76 (73.8)
Human epidermal growth factor 2 receptor + (immunohistochemistry score 3+)	7 (6.5)	14 (13.6)
Tumor type*		
Ductal carcinoma *in situ*	8 (7.5)	4 (3.9)
Luminal A	58 (54.2)	58 (56.3)
Luminal B	19 (17.8)	12 (11.7)
Triple negative	5 (4.7)	6 (5.8)
Human epidermal growth factor 2 receptor status positive (fluorescence *in situ* hybridization)	9 (8.4)	16 (15.5)
Other	2 (1.9)	3 (2.9)
Not reported	6 (5.6)	4 (3.9)
Surgery type		
Lumpectomy with lymph node resection	76 (71.0)	70 (68.0)
Lumpectomy without lymph node resection	6 (5.6)	5 (4.8)
Quadrantectomy with lymph node resection	5 (4.7)	3 (2.9)
Quadrantectomy without lymph node resection	0 (0.0)	1 (1.0)
Modified radical mastectomy	3 (2.8)	6 (5.8)
Radical mastectomy	13 (12.2)	14 (13.6)
Other	4 (3.7)	4 (3.9)
Circulating tumor cells		
Number, median [interquartile range]	1 [0–3]	1 [0–3]
Circulating tumor cell positivity		
Cutoff value: ≥ 1 cell/7.5 ml blood	73 (69.5)	68 (68.7)
Cutoff value: ≥ 5 cells/7.5 ml blood	18 (17.1)	15 (15.2)

The data are means ± SD or n (%), unless otherwise specified.

*Based on guidelines from the European Group on Tumor Markers.

ASA, American Society of Anesthesiologists.

studies reporting on cancer outcomes were based on retrospective data analyses,[7–14] which are prone to bias and confounding. Second, evidence of a protective effect associated with propofol was partly driven by *in vitro* studies,[31–35] which may not reflect the delicate interplay between immune and tumor cells observed *in vivo*. Our findings, however, are consistent with a recently published, large, randomized controlled trial addressing the effect of regional *versus* general anesthesia on breast cancer recurrence.[36] Although this trial was not specifically designed to compare inhalational with intravenous anesthesia, most patients allocated to general anesthesia received sevoflurane, whereas those allocated to regional anesthesia received propofol. In line with our study, this trial failed to show any difference in cancer outcomes.

Our results, however, need to be interpreted with caution. First, we assumed circulating tumor cell counts would be an appropriate prognostic factor to measure the impact of anesthesia on the risk of tumor recurrence, but we did

Table 2. Intra- and Postoperative Characteristics

	Sevoflurane (n = 107)	Propofol (n = 103)
Duration of anesthesia, min	163 ± 78	167 ± 50
BIS value, median [interquartile range]	43 [40–48]	36 [30–40]
Core temperature, °C	36.2 ± 0.5	36.2 ± 0.4
Fentanyl, mg, median [interquartile range]	0.4 [0.4–0.5]	0.5 [0.4–0.6]
Morphine PACU intravenous, mg, median [interquartile range]	0.0 [0.0–4.0]	0.0 [0.0–2.5]
NSAID administration		
Intraoperative	13 (12.2)	9 (8.7)
Postoperative	95 (88.8)	92 (89.3)
Intraoperative radiotherapy	70 (65.4)	71 (68.9)

The data are means ± SD or n (%), unless otherwise specified.
BIS, Bispectral Index; NSAID, nonsteroidal antiinflammatory drugs; PACU, postanesthesia care unit.

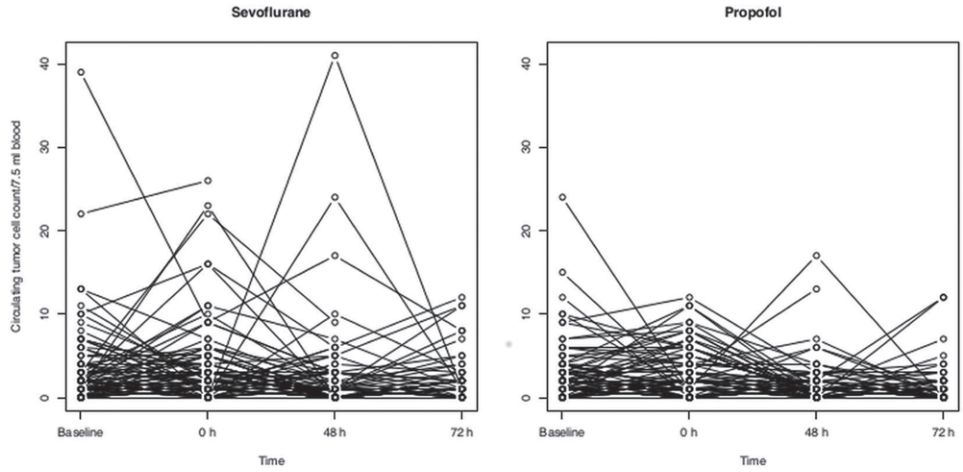

Fig. 2. Evolution of circulating tumor cell counts over time.

Table 3. Perioperative Circulating Tumor Cell Counts

Time Point	Allocation Group	Number of Patients	Minimum	Median	Interquartile Range	Maximum
Baseline	Sevoflurane	105	0	1	[0–3]	39
	Propofol	99	0	1	[0–3]	24
0 h	Sevoflurane	107	0	1	[0–4]	26
	Propofol	100	0	1	[0–4]	12
48 h	Sevoflurane	100	0	0	[0–2]	41
	Propofol	94	0	1	[0–2]	17
72 h	Sevoflurane	81	0	1	[0–2]	12
	Propofol	79	0	0	[0–1]	12

not perform a long-term outcome analysis to confirm this assumption. Although many oncological markers seem to be ideally placed in the causal pathway leading to distant disease, several other factors will eventually be needed to result in metastatic spread, and uncertainty regarding the ability of these prognostic factors to predict "hard endpoints" must be acknowledged.[37] A second concern is that the exact meaning of circulating tumor cell changes in the perioperative period remains unclear. In studies investigating the predictive validity of circulating tumor cells changes

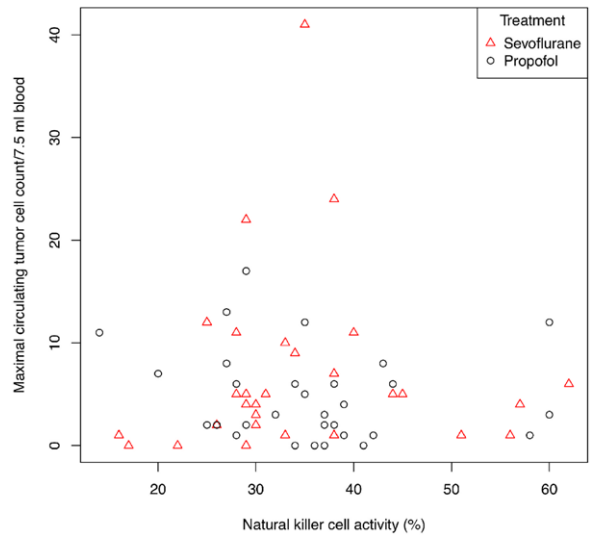

Fig. 3. Scatter plot of natural killer cell activity and maximal circulating tumor cell counts, by treatment.

in primary and metastatic breast cancer, patients converting from "positive" to "negative" status were found to have longer progression-free survival and overall survival than those with a persisting "positive" status.[23,38–42] However, circulating tumor cell detection was performed over many weeks or months, and there is no firm evidence that these findings also apply to the immediate and rather short perioperative period.

Other limitations are inherent to the CellSearch assay itself. Although the pattern EpCAM+/CK+/DAPI+/CD45− is a widely accepted molecular circulating tumor cell signature, other combinations may also occur: it has been argued, for instance, that 7.8 to 10.3% of breast cancers might lack EpCAM expression.[43,44] Further skepticism has been partly related to the fact that for a given tumor, a variety of circulating tumor cells phenotypes seems to exist.[45] Thus, in some patients included in our study, the ability to detect circulating tumor cells might have been hampered by the technique used. Finally, the *in vitro* analysis was performed in a sample of 60 patients only, thereby limiting our ability to fully assess the association between natural killer cell–induced apoptosis rates and circulating tumor cell counts. The risk of other sources of bias (such as selection, performance, attrition, and detection bias) was deemed low.

In this randomized controlled trial, we investigated the effect of anesthesia on an independent prognostic factor in primary breast cancer patients. There was no difference in circulating tumor cell counts over time or circulating tumor cell positivity between patients receiving sevoflurane and patients receiving propofol. One secondary analysis suggested a favorable effect of propofol on maximal postoperative circulating tumor cell values. Trials collecting long-term outcomes (NCT02786329, NCT03034096, NCT01975064, and NCT02660411) will bring further evidence regarding the possible effects of anesthesia during cancer surgery.

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Competing Interests

Dr. Schläpfer received travel support from Baxter (Volketswil, Switzerland; unrelated to the study). Dr. Dedes received honoraria and consultancies from Roche (Basel, Switzerland), Novartis (Basel, Switzerland), AstraZeneca (Baar, Switzerland), Amgen (Rotkreuz, Switzerland), Tesaro (Zug, Switzerland), PharmaMar (Berlin, Germany), and Daiichi (Thalwil, Switzerland; unrelated to the study). Dr. Tausch received consultancies from Roche (not related to this work). Dr. Beck-Schimmer received a grant from Baxter AG (Deerfield, Illinois; not related to this work), was a participant of an advisory board meeting of Baxter AG (not related to this topic), and received a speaker's fee from Abbvie (Baar, Switzerland; topic: "Pro/cons of volatile anesthetics") for a grand round talk in a Swiss Hospital. Dr. Beck-Schimmer also holds Patent 20140100278 for injectable formulation for treatment and protection of patients having an inflammatory reaction or an ischemia-reperfusion event (April 10, 2014) with M. Urner, L. K. Limbach, I. K. Herrmann, and W. J. Stark (applied as Patent Cooperation Treaty internationally, July 2009). The other authors declare no competing interests.

Reproducible Science

Full protocol available at: beatrice.beckschimmer@uzh.ch. Raw data available at: beatrice.beckschimmer@uzh.ch.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Labeling Coca Extract: After Ridding Red-eye, the Bottle Sheds Its Own Reds

When cocaine was discovered to be a topical anesthetic for the eye, physicians learned that this local anesthetic "got the red out" by vasoconstricting inflamed vessels in bloodshot eyes. Ironically, clinicians today recognize cocaine addicts by the latter's reddened eyes, which suffer from not only "rebound redness" but also hyperdynamic cardiovascular states. The 4 fluid ounce bottle of coca extract (*above*) was labeled with red ink by Parke, Davis & Company. Unfortunately, reds as a color rank lowest in visible light energy. So, for us to see the red letters, they must absorb more energetic non-red visible light (and ultraviolet) rays, all of which degrade the molecular bonds of red inks. Consequently, museums that display such bottles actually risk having red labels bleach entirely white in the ambient light. *Caveat curator!* (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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