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Neutrophil extracellular trapping and angiogenesis biomarkers after intravenous or inhalation anaesthesia with or without intravenous lidocaine for breast cancer surgery: a prospective, randomised trial

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Abstract

Background: Experimental and, retrospective, clinical data indicate that anaesthetic technique might influence the risk of metastasis after cancer surgery. Neutrophil extracellular trapping (NETosis) is an immunological mechanism strongly linked with increased metastatic risk. Similarly, vascular endothelial growth factor A is linked to angiogenesis implicated in recurrence. Therefore, we investigated the effect of four anaesthetic techniques on NETosis and angiogenic factors expression in women undergoing breast cancer resection.

Methods: Women (n=120) undergoing primary breast tumour resection were randomly assigned to receive one of four anaesthetics: sevoflurane (S), sevoflurane plus i.v. lidocaine (SL), propofol (P), and propofol plus i.v. lidocaine (PL). Venous blood was collected before induction and 20–28 h after operation. Neutrophil myeloperoxidase and citrullinated histone H3, biomarkers of NETosis, and biomarkers of angiogenesis were measured by enzyme-linked immunosorbent assay.

Results: Patient characteristic data and perioperative management did not differ between study groups. The anaesthetic technique including lidocaine decreased expression of citrullinated histone H3 compared with no lidocaine (109 [23] *vs* 125 [22] ng ml^{-1}, P=0.01 for SL and S and 98 [14] *vs* 130 [32] mg ml^{-1}, P=0.007, for PL and P, respectively). Similarly, myeloperoxidase was decreased by lidocaine (8.5 [3.4] *vs* 10.8 [1.8] ng ml^{-1}, P=0.03 for SL and S and 8.6 [3.1] *vs* 11.6 [2.5] ng ml^{-1}, P=0.01 for PL and P, respectively). Lidocaine also decreased expression of matrix metalloproteinase 3 (MMP3) but not MMP9, whichever anaesthetic was used. Vascular endothelial growth factor A concentrations were not significantly influenced by the anaesthetic technique.

Conclusions: I.V. perioperative lidocaine decreased postoperative expression of NETosis and MMP3, regardless of general anaesthetic technique. This supports the hypothesis that i.v. lidocaine during cancer surgery of curative intent might reduce recurrence.

Clinical trial registration: NCT02839668.

Keywords: biomarker; breast cancer; lidocaine; neutrophil extracellular trapping; NETosis; propofol; recurrence; sevoflurane

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Editor's key points

- Micrometases are believed to occur during cancer surgery.
- Neutrophil extracellular trapping (NETosis) is an immunological mechanism strongly linked with increased metastatic risk.
- Lidocaine has analgesic and anti-inflammatory effects, and may also have specific anticancer properties.
- This study found that lidocaine reduced serum NETosis and other biomarkers of cancer dissemination.

Breast cancer is the greatest cause of cancer death among women, mostly attributable to recurrence and metastasis.[1] Surgery remains a mainstay of curative-intent treatment, along with chemo- and radiotherapy.[2] It has been hypothesised that various anaesthetics and analgesics might influence the risk of recurrence or metastasis.[3]

Signals from small translational studies[4,5] and larger retrospective clinical data[6] suggest an association between use of propofol-TIVA during tumour resection surgery and better recurrence-free survival among many tumour types, compared with use of volatile anaesthesia. In addition, the amide local anaesthetic lidocaine, used both as a local anaesthetic and as a systemic i.v. infusion for its analgesic and anti-inflammatory effects, has recently been shown to have specific anticancer properties, by interfering with cancer cell viability, migration, and apoptosis.[7,8]

The surgical stress response to tumour removal causes bloodstream release of a variety of pro-inflammatory cytokines and other molecules which may affect perioperative immune response and other conditions conducive to residual tumour cell survival that could later emerge as clinical recurrences or metastasis.[9] Limited data from small translational studies suggest that blood serum expression of some of these molecules (metalloproteinases, vascular endothelial growth factor [VEGF], epidermal growth factor, etc.), may be altered by anaesthetic-analgesic technique; however, their predictive value as biomarkers of metastatic risk remains uncertain.[10–12]

A promising new potential biomarker of metastatic risk has emerged recently. Neutrophil extracellular trapping (NETosis) is an immunologic response to tumour antigens or cells in blood, whereby the neutrophil degranulates on engagement with the tumour cell, 'trapping' it.[13,14] The process results in extrusion of neutrophil content into the circulation, including its DNA histones and metalloproteinases, which may be detected in serum. High levels of NETosis are associated with increased recurrence and metastasis in breast and other cancers.[15]

Whether anaesthetic or analgesic technique during cancer resection surgery modifies NETosis is unknown. The most promising anaesthetic-analgesic techniques, in terms of existing experimental and observational clinical evidence supporting a potential benefit in reducing recurrence after cancer resection surgery, are propofol-TIVA and perioperative systemic lidocaine infusion. Therefore, we tested the hypothesis that women undergoing primary breast tumour resection with these techniques have reduced postoperative serum expression of metastasis biomarkers, including NETosis, compared with women receiving sevoflurane anaesthesia without lidocaine.

Methods

After obtaining Institutional Review Board approval (No 54/14.03.2016) and written informed consent, 120 women with breast cancer undergoing surgery were enrolled into the study. The study was registered with ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/NCT02839668). Inclusion criteria were: patients aged 18–80 yr, ASA physical status 1–3, with breast cancer without disseminated disease. Exclusion criteria included any allergy to study substances, diabetes, chronic inflammatory diseases, neuropsychiatric diseases making the patient unable to give informed consent, incapacity of understanding study protocol or refusal to participate, and regular usage of corticosteroids or anti-inflammatory drugs.

Randomisation and masking

The patients were assigned to one of the four groups using a computer-generated randomisation process in a 1:1:1:1 ratio. The group allocation and patient study number were concealed in a sealed opaque envelope, which was opened after the patient had given their written, informed consent before surgery. The trial groups were: sevoflurane anaesthesia (S); sevoflurane anaesthesia plus i.v. lidocaine (SL), propofol-TIVA (P), and propofol-TIVA plus i.v. lidocaine (PL). While the involved anaesthetists were not masked to study group allocation, all the investigators involved in the postoperative follow-up, blood sampling, data analysis, and interpretation were unaware of the group allocation.

A saline infusion packaged identically to i.v. lidocaine was used as placebo control for the groups without lidocaine infusion. This was prepared and started after induction, by the anaesthetist and continued for 24 h after operation. The infusion rates mirrored lidocaine infusion rates.

Clinical protocol

During anaesthesia, all patients were monitored according to the Standards for Basic Anaesthetic Monitoring of the American Society of Anaesthesiologists. For anaesthetic induction in all groups, fentanyl 1–3 μg kg^{-1}, propofol 1.5–2 mg kg^{-1}, and atracurium 0.5 mg kg^{-1} were administered. In the sevoflurane groups (S and SL), anaesthesia was maintained with sevoflurane 1–1.5 MAC in 50/50 mixture of O_2/air, maintaining a bispectral index (BIS) of 45–55. For intraoperative analgesia, fentanyl 100 μg bolus was administered when necessary (defined as systolic arterial pressure or HR >20% higher than baseline). Neuromuscular antagonism was maintained with additional atracurium 10 mg boluses.

In TIVA groups (P and PL), anaesthesia was maintained with propofol-target controlled infusion C_e=4 μg ml^{-1} (Schnider model), adjusted during surgery in steps of 0.2 μg ml^{-1} to maintain BIS values between 45 and 55. Fentanyl and atracurium were administered as indicated at the discretion of the anaesthesiologist. Patient's lungs were ventilated using a 50-50 mixture of O_2 and air.

In the lidocaine groups, a bolus of lidocaine 1% (1.5 mg kg^{-1}) was administered during induction followed by a continuous infusion of lidocaine 2 mg kg^{-1} h^{-1} throughout the procedure and 1 mg kg^{-1} h^{-1} for 24 h after operation.[16] These dosing protocols result in plasma concentrations that are well below

toxic levels, and the dosing protocol is familiar to many anaesthesiologists.

At the end of surgery, neuromuscular antagonism was reversed by administration of neostigmine 2.5 mg and atropine 1 mg. Postoperative analgesia was standardised with acetaminophen 1 g every 8 h (with the first dose being administered intraoperatively) and rescue analgesia was tramadol 50 mg if VAS score was ≥4.We did not use NSAID for postoperative analgesia because of their potential interference with lidocaine's anti-inflammatory effects.[17]

Neutrophil extracellular traps/NETosis and VEGF assays

Blood samples (5 ml) were drawn from each patient before anaesthetic induction and 20–24 h after operation. The blood was collected into BD vaccutainer™ serum tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) by peripheral venous puncture from a different venous access site than the one used for drug administration. The blood was centrifuged within 1 h from collection, at 4000× rotations per minute for 15 min at room temperature and the resulting serum was stored in 2 ml aliquots at −80°C for further analysis using the enzyme-linked immunosorbent assay (ELISA) technique.

Two NETosis specific markers, myeloperoxidase (MPO) and citrullinated histone H3 (H3Cit) were measured. In addition, three other markers associated with cancer progression and metastasis were measured: the VEGF-A and the matrix metalloproteinase-3 and 9 (MMP-3, MMP-9). These measurements were made using commercially available ELISA kits for: MPO (Human MPO, MyBioSource, San Diego, CA, USA; assay range: 1.56–100 ng ml^{-1}, sensitivity: 0.938 ng ml^{-1}), H3Cit (Human H3Cit, MyBioSource, San Diego, CA, USA; sensitivity: 1.0 ng ml^{-1}), VEGF-A (Human VEGF-A, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; assay range: 23.4–1500 pg ml^{-1}, sensitivity: ≤5 pg ml^{-1}), MMP-3 (Human MMP-3, Bender MedSystems GmbH, Vienna, Austria; assay range: 2–28 ng ml^{-1}, sensitivity: 0.0005 ng ml^{-1}), and MMP-9 (Human MMP-9, Bender MedSystems; assay range: 0.23–15.0 ng ml^{-1}, sensitivity: 0.05 ng ml^{-1}) in accordance with manufacturer's instructions.

For the determination of MPO and H3Cit, we used the sandwich ELISA technique with the anti-MPO or H3Cit antibody precoated on the 96-well plates. The detection antibody used was a biotin conjugated anti-MPO antibody. This antibody reacts strongly with a horseradish peroxidase (HRP)-streptavidin conjugate (SABC solution). 3,3′,5,5′-Tetramethylbenzidine (TMB) was used as a substrate for HRP. Briefly, the serum samples were thawed and diluted with the sample dilution buffer to a final concentration of 1:100. Then, 0.1 ml aliquots of the test sample dilution were added into test sample wells. The plates were incubated for 90 min at 37°C, after which the excess content was discarded. The biotin conjugated detection antibody was added in 0.1 ml aliquots and incubated for 60 min at 37°C. The wells were washed three times with wash buffer before adding 0.1 ml aliquots of SABC working solution and being incubated at 37°C for 30 min. The TMB substrate was added in 90 μl aliquots to each well after they were previously washed five times with wash buffer, and the reaction was incubated for 30 min in dark at 37°C. The HRP-TMB reaction was terminated by adding 50 μl of an acidic stop solution, changing the colour of the solution from blue to yellow. The absorbance at 450 nm was read immediately using the ELISA TECAN Sunrise™ Microplate reader (Tecan Group Ltd., Grodig/Salzburg, Austria). A similar technique was used for the standard and control samples.

Serum concentrations of the VEGF-A, MMP-3, and MMP-9 concentrations were determined by similar protocols, as detailed above, according to manufacturer recommendations. The optical densities of the test samples were plotted against those of a group of standard samples of known concentration which represented the reference curve. The serum concentration of each factor was then determined from these curves. Intra-assay coefficient of variation for VEGF-A was 4.84%. H3Cit, MPO, MMP-3, and MMP-9 were not measured in duplicates because of financial constraints. The intra-assay coefficients of variation as given by the manufacturer are as follows: <10% for VEGF-A and H3Cit, 7.3% for MMP-9, 6.1% for MMP-3, and <9% for MPO. Minimal detectable doses calculated for our kits were as follows: 5.48 pg ml^{-1} for VEGF-A (lowest plasma concentration in our groups was 7.2 pg ml^{-1}), 0.055 ng ml^{-1} for MPO, 0.006 ng ml^{-1} for MMP3, and 0.038 ng ml^{-1} for MMP-9. For H3Cit the manufacturer declares no value for minimal detectable dose or range and does not recommend calculating sample values outside standard values.

Data management and statistics

Previous serum estimations of NETosis MPO values indicate typical values in the order of 10–15 ng ml^{-1} with standard deviation in the order of 3 ng ml^{-1}. Taking a 20% reduction of 2.0 ng ml^{-1} as being scientifically significant, and assuming a type I error of 0.05 and type 2 error of 0.1, then n=25 patients would be required in each group to have 90% power to detect this difference. We enrolled n=30 each group to allow for missing data.

Data were handled throughout in compliance with EU General Data Protection Regulations legislation, and uploaded onto an Excel file and imported into Graph Pad Prism TM v8 for analysis. All data were inspected for distribution. Normally distributed data were compared using analysis of variance with *post hoc* Bonferroni correction for differences between independent groups. Differences in serum marker values before and after anaesthesia and surgery within groups was undertaken using paired Student's *t*-tests. Values of $P<0.05$ were deemed statistically significant.

Results

We enrolled 120 patients between August 2016 and September 2019 (Fig. 1). Thirty patients were randomly assigned to one of each of the four study groups: sevoflurane anaesthesia (S), sevoflurane anaesthesia plus i.v. lidocaine (SL), propofol-TIVA (P), and propofol-TIVA plus i.v. lidocaine (PL). One patient was lost to follow-up (P group), as a result of early hospital discharge which prevented obtaining postoperative blood samples as per the study protocol.

The baseline subject characteristics, ASA physical status, type of surgery, and duration of anaesthesia were similar between study groups (Tables 1 and 2). All four groups had a similar number of patients having preoperative chemotherapy. Four and two subjects, respectively, of the SL and P groups had preoperative hormonal therapy. Also, the Nottingham prognostic index placed all four groups within the same 5-year survival category (85% survival probability). The total consumption of lidocaine and propofol was similar

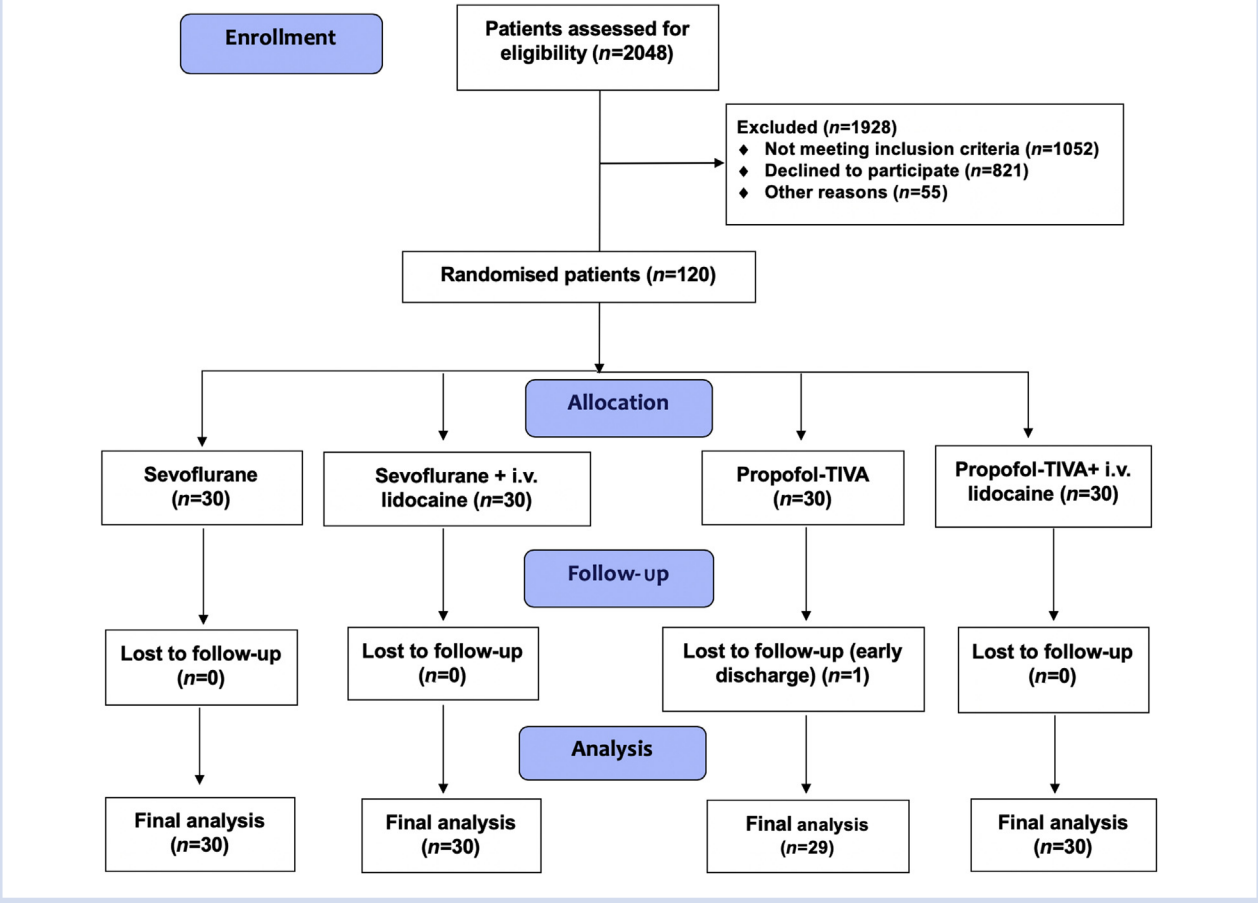

Fig 1. Consort trial profile of study subjects.

between respective study groups (Table 2). All four groups had similar consumption of intraoperative opioids (Table 2).

The reduction between the preoperative and postoperative H3Cit concentrations was statistically significant in the lidocaine groups (151 *vs* 109 ng ml^{-1}, P=0.001, in the SL group; 147 *vs* 98 ng ml^{-1}, P=0.0007, in the PL group, respectively) (Table 3 and Fig. 2). Lidocaine, in addition to either sevoflurane or propofol, significantly decreased the H3Cit expression when compared with no lidocaine (109 [23] *vs* 125 [22] ng ml^{-1}, P=0.01 for the SL *vs* S groups; and 98 [14] *vs* 130 [32] mg ml^{-1}, P=0.007 for the PL *vs* P groups, respectively). A similar reduction caused by lidocaine was noted for the postoperative concentrations of both the MPO (10.7 *vs* 8.5 ng ml^{-1}, P=0.03, in the SL group; and 13.3 *vs* 8.6 ng ml^{-1}, P=0.01, in the PL group, respectively) and the MMP-3 concentrations (8.8 *vs* 4.4 ng ml^{-1}, P=0.002, in the SL group; and 10.1 *vs* 4.1 ng ml^{-1}, P=0.001, in the PL group, respectively) (Table 3 and Fig. 3). Overall, lidocaine decreased the postoperative concentrations of H3Cit, MMP-3, and MPO, regardless of the main anaesthetic technique. Interestingly, although MMP-3 and MMP-9 are functionally interdependent, the change in the MMP-3 concentrations were not followed by a similar decrease of MMP-9 values. Rather than decreasing, the postoperative MMP-9 concentrations increased in all four groups, with higher postoperative concentrations in the lidocaine groups (241 *vs* 208 ng ml^{-1}, in the SL group; 203 *vs* 180 ng ml^{-1}, in the PL group, respectively).

The VEGF-A concentrations showed a notable decrease between the preoperative and postoperative concentrations in the propofol groups when compared with the sevoflurane groups (Table 3). The most substantial reduction was noted in the propofol group (232 *vs* 165 pg ml^{-1}). The addition of lidocaine significantly attenuated the reduction in the postoperative VEGF-A concentrations in both sevoflurane (25 *vs* 11 pg ml^{-1} mean difference between preoperative and postoperative concentrations in S and SL groups) and propofol groups (67 *vs* 34 pg ml^{-1} mean difference between preoperative and postoperative concentrations in P and PL groups).

Discussion

Our study evaluated, for the first time to our knowledge, the effect of the anaesthetic technique on serum NETosis markers (MPO and H3Cit) along with other markers contributing to cancer dissemination (MMP-3, MMP-9, VEGF-A) at 24 h after surgery. We found that the addition of lidocaine had an increased effect on NETosis. The result of lidocaine on attenuating the metastatic progression both *in vitro* and *in vivo*[18,19] may be explained by the effect of lidocaine on cancer cells cytoskeletal remodelling[20] and modulation of MMP-2 and Src tyrosine kinase[21] noted *in vitro* and in murine experimental models.[21] Lidocaine also seems to decrease VEGF-A, endothelial growth factor, tumour necrosis factor and interleukin-6

Table 1 Patient and breast tumour characteristics. All data shown are mean (standard deviation) or *n* (%). TCI, target controlled infusion.

Trial groups	Sevoflurane (S) (*n*=30)	Sevoflurane + lidocaine (SL) (*n*=30)	Propofol TIVA-TCI (P) (*n*=29)	Propofol TIVA-TCI+lidocaine (PL) (*n*=30)
Age (yr)	56.1 (32–80)	58.34 (42–72)	53.4 (35–77)	57.07 (38–77)
BMI (kg m^2)	27 (4.5)	28.5 (4.8)	26.8 (5.9)	27.5 (5.8)
ASA physical status (*n*, %)				
1	12 (40)	11 (37)	14 (48)	12 (40)
2	17 (56)	19 (63)	15 (52)	18 (60)
3	1 (3)	0	0	0
Preoperative treatment (*n*, %)				
Previous chemotherapy	18 (60)	15 (50)	16 (55)	15 (50)
Epirubicin+cyclophosphamide+docetaxel	16 (53)	12 (40)	13 (45)	13 (40)
Epirubicin+cyclophosphamide	1 (3)	0	1 (3)	0
Cyclophosphamide+methotrexate+fluorouracil	1 (3)	2 (7)	2 (7)	1 (3)
Doxorubicin+cyclophosphamide	0	1 (3)	0	0
Paclitaxel	0	0	0	1 (3)
Previous radiation (*n*, %)	0	0	1 (3)	0
Previous hormonal therapy (*n*, %)	1 (3)	4 (13)	2 (7)	0
Anastrazole	1 (3)	2 (7)		0
Trastuzumab (Herceptin)	0	1 (3)		0
Tamoxifen	0	1 (3)	2 (7)	0
Duration of anaesthesia (min)	74.6 (28.8)	79.1 (24.35)	71.5 (15)	76.7 (22.7)
Tumour site (*n*, %)				
Right	10 (33)	14 (47)	15 (52)	7 (23)
Left	20 (67)	15 (50)	14 (49)	22 (73)
Bilateral	0	1 (3)	0	1 (3)
TNM classification				
Pathology stage, tumour (*n*, %)				
Tx	2 (7)	0	0	0
Tis	2 (7)	1 (3)	0	4 (13)
T0	2 (7)	2 (7)	2 (7)	0
T1	11 (37)	14 (47)	15 (52)	11 (37)
T2	9 (30)	12 (40)	9 (31)	15 (50)
T3	2 (7)	1 (3)	2 (7)	0
T4	2 (7)	0	1 (3)	1 (3)
Pathology stage, nodes (*n*, %)				
Nx	2 (7)	1 (3)	0	4 (13)
N0	14 (47)	14 (47)	17 (59)	12 (40)
N1	7 (23)	6 (20)	6 (21)	10 (33)
N2	6 (20)	7 (23)	5 (17)	6 (20)
N3	1 (3)	2 (7)	1 (3)	0
Pathology stage, metastasis (*n*, %)				
Mx	23 (76)	20 (67)	25 (86)	25 (83)
M0	7 (23)	9 (30)	3 (10)	5 (17)
M1	0	1 (3)	1 (3)	0

(IL-6) concentrations.[18] Whether this might translate into a meaningful reduction of clinical tumour recurrence risk deserves further study.

Regarding VEGF-A, known to have a role in increasing vascular permeability, stimulating vascular endothelial proliferation, and tumour angiogenesis,[4] there are *in vitro* studies that report an increase in its concentrations under the influence of volatile anaesthetics,[5,6] and a decrease in similar factors (VEGF-C, a promoter of lymphangiogenesis) with propofol-based anaesthesia.[7,8] However, our data showed no significant difference in VEGF-A, although VEGF concentrations were lower in propofol groups. Based on the *in vitro* effect of amide-like local anaesthetics that show an antiproliferative effect on cancer cells[8] and decreased angiogenesis related or not to opioids *in vitro* (by activation of Src in endothelial cells)[22] and in animal studies,[23] we hypothesised that adding lidocaine to anaesthetic regimens would decrease VEGF-A, despite the use of fentanyl for intraoperative analgesia.

In fact, lidocaine had no significant effect on VEGF-A, which may be attributable either to an insufficient period of lidocaine infusion or the low postoperative dosage used (1 mg kg^{-1} h^{-1}). A differential effect of lidocaine depending on the tumour histology (oestrogen receptor-negative *vs* oestrogen receptor-positive) that differently express VEGF may also be implicated.

All subjects enrolled in this study received intraoperative opioids in similar amounts across all four groups. Opioids themselves have been implicated in tumour recurrence and are associated with an increase in angiogenesis-related factors. Regarding the impact of fentanyl on cancer progression, although there are controversies in the literature, recent studies showed that, at least for short-term exposure, there is no major clinical impact in tumour cells development and recurrence rate.[24,25]

Because no subjects received regional anaesthesia in this study, we provided some opioid analgesia to all patients. This may reduce the generalisability of our results to anaesthetic

Table 2 Surgical and anaesthesia characteristics. All data shown are mean (standard deviation) or *n* (%). BIS, a bispectral index.

Trial groups	Sevoflurane (S) (*n*=30)	Sevoflurane+lidocaine (SL) (*n*=30)	Propofol TIVA-TCI (P) (*n*=29)	Propofol TIVA-TCI+lidocaine (PL) (*n*=30)
Type of surgical intervention (*n*, %)				
Modified radical mastectomy	23 (77)	23 (77)	25 (86)	25 (83)
Quadrantectomy+lymph node removal	7 (23)	7 (23)	4 (14)	5 (17)
Postoperative tumour histology				
Invasive breast carcinoma NST (no special type)	21 (70)	27 (90)	22 (76)	22 (73)
Residual cancer burden zero	5 (17)	2 (7)	3 (10)	1 (3)
In situ ductal carcinoma	3 (10)	0	0	1 (3)
Invasive lobular carcinoma	0	1 (3)	0	2 (7)
Intraductal carcinoma	0	0	1 (3)	0
Micropapillary invasive carcinoma NST	0	0	1 (3)	0
Invasive cribriform carcinoma	0	0	1 (3)	1 (3)
Multifocal cribriform invasive breast carcinoma	0	0	0	1 (3)
Intracystic carcinoma	0	0	0	1 (3)
No available data	1 (3)	0	1 (3)	1 (3)
Intraoperative Fentanyl (mg)	0.25 (0.05)	0.23 (0.05)	0.24 (0.07)	0.27 (0.08)
Intraoperative crystalloids (L)	1 (0.23)	0.98 (0.36)	0.75 (0.32)	0.82 (0.28)
Intraoperative atracurium (mg)	36.55 (6.42)	38.83 (7.5)	39.66 (7.42)	41.5 (9.43)
Intraoperative propofol (mg)	114.41 (18.99)	123 (29.84)	685.82 (216.61)	739 (231.83)
Total lidocaine dose/24 h (mg)		1978.31 (381.34)		1995.12 (434.59)
Intraoperative BIS (mean [standard deviation])	48.1 (2.64)	48.48 (2.83)	48.7 (2.74)	47.17 (2.55)
Intraoperative HR	68.62 (8.52)	71.03 (9.08)	68.73 (8.31)	67.37 (8.39)
Intraoperative MAP (mmHg)	81.11 (5.92)	80.64 (7.66)	81.5 (6.27)	79.66 (6.71)
Nottingham Prognostic Index (mean [standard deviation])	3.2 (0.76)	2.8 (0.64)	2.9 (0.76)	2.8 (0.74)

Table 3 Serum biomarkers. Values shown are mean (standard deviation). H3Cit, citrullinated histone H3; MMP, matrix metalloproteinase; MPO, myeloperoxidase; TCI, target controlled infusion; VEGF-A, vascular endothelial growth factor-A. *P=0.001 for the preoperative *vs* postoperative H3Cit concentration change in the SL group. †P=0.0007 for the preoperative *vs* postoperative H3Cit concentration change in the PL group. ‡P=0.01 for the postoperative H3Cit concentrations comparison in the SL *vs* S group. ¶P=0.007 for the postoperative H3Cit concentrations comparison in the PL *vs* P group. §P=0.002 for the preoperative *vs* postoperative MMP3 concentrations change in the SL group. ||P=0.001 for the preoperative *vs* postoperative MMP3 concentration change in the PL group. #P=0.03 for the preoperative *vs* postoperative MPO concentration change in the SL group. **P=0.01 for the preoperative *vs* postoperative MPO concentration change in the PL group.

Trial groups		Sevoflurane (S) (*n*=30)	Sevoflurane+lidocaine (SL) (*n*=30)	Propofol TIVA-TCI (P) (*n*=29)	Propofol TIVA-TCI+lidocaine (PL) (*n*=30)	*P*
H3Cit (ng ml^{-1})	Preoperative	135 (28)	151 (51)	143 (49)	147 (47)	0.39
	Postoperative	125 (22)	109 (23)*	130 (32)	98 (14)†	0.001* 0.0007† 0.01‡ 0.007¶
VEGF-A (pg ml^{-1})	Preoperative	253 (204)	231 (210)	232 (209)	241 (159)	0.74
	Postoperative	228 (175)	220 (203)	165 (160)	207 (200)	0.21
MMP-3 (ng ml^{-1})	Preoperative	7.5 (5.0)	8.8 (5.5)	11.2 (7.4)	10.1 (6.1)	0.11
	Postoperative	6.2 (5.6)	4.4 (2.6)§	9.8 (6.6)	4.1 (2.8)||	0.002§ 0.001||
MMP-9 (ng ml^{-1})	Preoperative	210 (69)	208 (74)	216 (64)	180 (81)	0.52
	Postoperative	233 (79)	241 (73)	229 (77)	203 (75)	0.89
MPO (ng ml^{-1})	Preoperative	11.8 (2.5)	10.7 (1.9)	12.0 (3.1)	13.3 (3.0)	0.39
	Postoperative	10.8 (1.8)	8.5 (3.4)#	11.6 (2.5)	8.6 (3.1)**	0.03# 0.01**

techniques avoiding opioids. However, given that the first large randomised trial recently found no evidence of a difference between regional anaesthesia/analgesia and general anaesthesia with opioid analgesia[26] in terms of cancer-related outcomes, it is likely that opioids will continue to be used frequently in clinical practice.

Angiogenesis is likely to occur in the perioperative setting even without the presence of opioids. Our results showing an inhibitory effect of lidocaine on angiogenesis biomarkers are supported by recent *in vitro* evidence using a B16 melanoma mouse model.[23] However, opioids exert well documented effects on neutrophils (suppress migration, attenuate

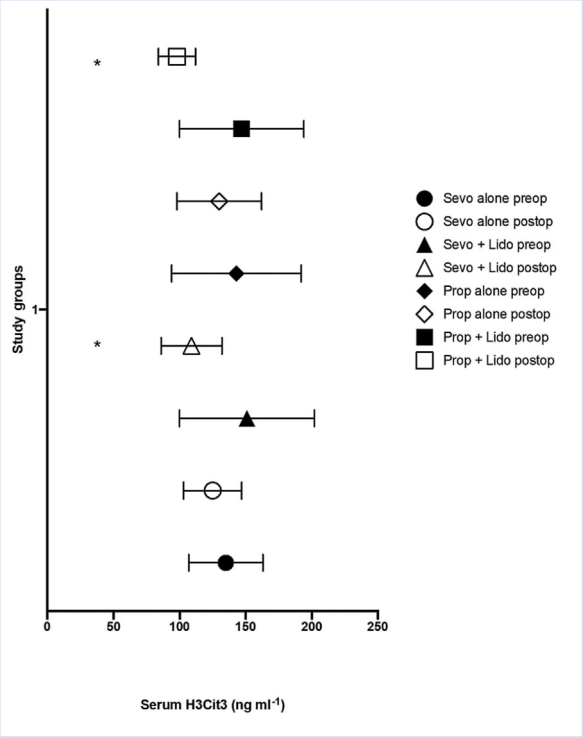

Fig 2. Citrullinated histone 3 (H3Cit): preoperative and postoperative serum concentrations in each anaesthetic group (represented as mean [standard deviation]). A decrease in the postoperative concentrations of H3Cit is seen in all patient groups. Lidocaine decreased H3Cit expression when combined with sevoflurane and propofol compared with either anaesthetic technique on their own. Lido, lidocaine; Prop, propofol; Sevo, sevoflurane.

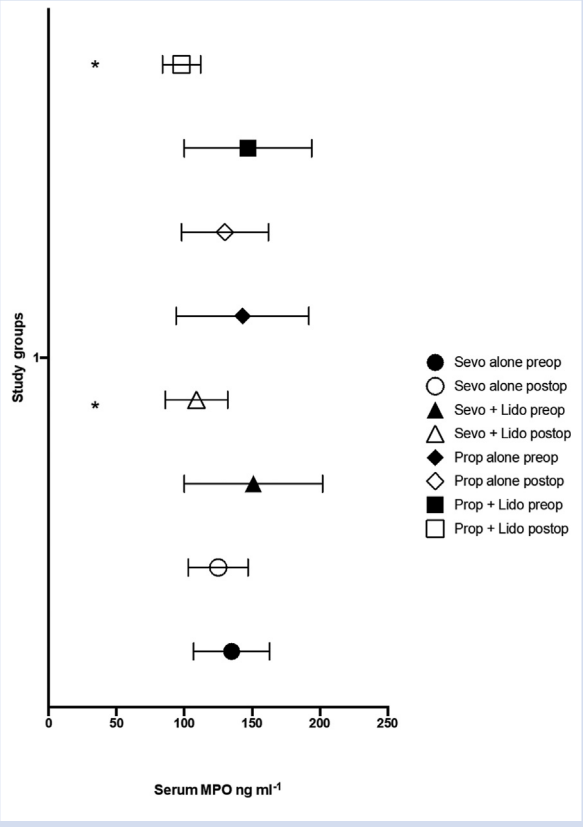

Fig 3. Myeloperoxidase (MPO): preoperative and postoperative serum concentrations in each anaesthetic group (represented as mean [standard deviation]). Lidocaine decreased MPO expression when combined with sevoflurane and propofol compared with either anaesthetic technique on their own. Lido, lidocaine; Prop, propofol; Sevo, sevoflurane.

activation). There is no evidence that opioids play a direct role in NETosis.

NSAIDs were not used in this trial. The exact mechanism that triggers NETosis has not been fully elucidated and it is possible that NSAIDs may have an effect on this process. Similarly, the role of NSAIDs in improving cancer outcomes is not fully known.[27]

In order to metastasise, cancer cells first lose their intercellular adhesion by changing their surface protein expression in the process of epithelial-mesenchymal transition (EMT). Degradation of the extracellular matrix under the influence of MMPs and urokinase plasminogen activator[8,10] is the next step. MMPs have been described as playing a role in the EMT process[28] and regulation of the extracellular matrix, by releasing biologically active proteins from their membrane-bound proform[29] and in the neovascularisation process.[30] MMP-3 function seems to activate MMP-9 which releases the biologically active form of VEGF-A and favours the EMT process, supporting the metastatic process. In our study, there was a reduction in postoperative values of MMP-3 in all groups, with the most significant reduction in the PL group. Lidocaine infusion associated with propofol anaesthesia accentuated the decrease in MMP-3 but did not correlate with the increase seen in the MMP-9 postoperative values, the most attenuated increase of which was seen in the propofol group. Though we expected a correlation between the MMP-3 and MMP-9 values, the lowest concentrations for MMP-3 did not determine the lowest concentration in MMP-9. These results support the ones reported by Deegan and colleagues[12] who found a decrease in MMP-3 and attenuated increase in MMP-9 in patients who received propofol/paravertebral anaesthesia compared with sevoflurane anaesthesia for breast cancer surgery.

Different stimuli (inflammatory or pathogens) release NETs from neutrophils in a process known as NETosis,[31] during which, nuclear histones become citrullinated, allowing chromatin decondensation. After NETosis, H3Cit can be detected at high concentrations in the bloodstream, rendering it a marker of cell death.[32] Attached to the net-like structures released during NETosis are granules of neutrophil elastase and MPO.[33] Studies investigating the relationship between NETs, H3Cit, and cancer plead for the association between high concentrations of H3Cit and MPO in patients with cancer compared with healthy individuals and an increase in mortality.[34,35] Although neutrophils have a role in cancer protection, the mechanism by which they facilitate metastasis is thought to be favouring cancer cell migration and extravasation.[13–15] In our study, both H3Cit and MPO were significantly decreased in lidocaine groups. This may be attributable to the known anti-

inflammatory effect of lidocaine, given that high concentrations of NETs are associated with inflammation in addition to cancer proliferation.[36] The relation between NETosis and the metastatic risk warrants further evaluation as it is not clear yet if NETosis is a true cause of, or the consequence of metastasis. Indeed, there are some data suggesting a positive association between components of NETs such as MPO and resistance in melanoma.[31]

This trial has several limitations which may preclude extrapolation to other sites where breast cancer surgery takes place. It is a single-centre trial with a standardised protocol for analgesia and anaesthesia. All subjects received intraoperative fentanyl and regional anaesthesia was not used. NSAIDs were omitted and BIS was used to titrate depth of anaesthesia. This standardisation was necessary to eliminate potential confounding variables, but may also reduce the extent to which our findings may be generalised to similar hospital settings. Because of the nonspecific nature of the initial screening for relevant cases, a large number of patients have been excluded from the trial after initial screening. Lidocaine infusions are an established part of many gery Enhanced Recovery After Surgery (ERAS) programmes. We chose to use a lidocaine dose that is in keeping with previous published studies.[16] However, our postoperative dose of lidocaine was at the lowest postoperative dose published in the literature. A direct effect of lidocaine on cancer cells or specimens was not directly measured. We can only hypothesise that a reduction in angiogenesis and NETosis markers could result in improved patient outcome, but we do not have long-term follow up data to demonstrate this. The results obtained support the future exploration of NETosis as a potential biomarker of metastasis in breast cancer.

Metastasis implies several steps: loosing intercellular adhesion, degrading of extracellular matrix under the influence of MMPs (which also determine the release of the proangiogenic factor VEGF-A), migration of cancer cells into the circulation, invading the target organs, and development of neovasculature for further tumoural growth (with implication of VEGF-A, MMP-2, -3, and -9, and other molecules).[12] Other factors recently recognised in promoting metastasis and tumoural progression are NETs (neutrophil extracellular traps—mesh-like structures associated with antimicrobial peptides such as neutrophil elastase) and MPO[34,37,38] known to be released in a process called NETosis.[39] Markers of NETosis such as MPO and H3Cit were associated with having a role in the metastatic process of breast cancer[40] and predicting a higher mortality rate in cancer patients.[35] The exact mechanisms by which NETosis facilitates metastatic development are still being elucidated. One theory is that the mesh-like network of NETosis acts as a scaffolding system in capillary beds that can trap circulating tumour cells at the time of surgery. In turn, NETs can then promote favourable local conditions—termed the 'pre-metastatic niche' that promote cell adhesion and finally metastatic spread.[41]

The possibility of modulating the factors responsible for metastasis and recurrence by the anaesthetic regimen represents an attractive intervention that may change the outcome of cancer patients. The general direction[4] is that propofol-based anaesthesia may influence these factors associated with angiogenesis and tumour progression (mainly VEGF-A and −C, IL-1β, MMP-3 and -9).[10,12] Propofol also has both anti-inflammatory properties (increases IL-10 and decreases IL-6)[29,42] and cellular modulator effects decreasing the neutrophil-lymphocyte ratio[43] which could translate into anticancer progression effects.

In conclusion, this trial found that the addition of lidocaine to either volatile sevoflurane or propofol i.v. anaesthesia reduced expression of NETosis (H3Cit and MPO) and MMP3, as markers of metastatic risk, compared with not adding lidocaine. The hypothesis that addition of lidocaine to a general anaesthetic technique may influence the risk of cancer recurrence warrants evaluation in a large randomised clinical trial.

Authors' contributions

Substantial contribution to study concept and design: EG, TT, RP, CIE, DF, DJB, DI
Substantial contribution to study design: CMM
Aquisition of data: EG, TT, RP
Drafting and revising critically the article: EG, CIE, DF, DJB, DI, CMM
Analysis and interpretation of data: CIE, DF, DJB, DI
Final approval of the version to be published: all authors

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Declarations of interest

DJB is an editorial board member of the *British Journal Anaesthesia*. All other authors declare that they have no conflict of interest.

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