British Journal of Anaesthesia **113** (S1): i56–i62 (2014)
Advance Access publication 9 July 2014 · doi:10.1093/bja/aeu200

BJA

Effect of anaesthetic technique on the natural killer cell anti-tumour activity of serum from women undergoing breast cancer surgery: a pilot study

A. Buckley[1*], S. McQuaid[2], P. Johnson[2] and D. J. Buggy[1,3,4]

[1] Department of Anaesthesia, Mater Misericordiae University Hospital, Dublin, Ireland
[2] Department of Immunology, Dublin City University, Dublin, Ireland
[3] School of Medicine & Medical Science, University College Dublin, Dublin, Ireland
[4] Outcomes Research Consortium, Cleveland Clinic, Cleveland, OH, USA

* Corresponding author. E-mail: buckleyaisling@hotmail.com

Editor's key points

- Anaesthetic technique might influence metastasis after cancer surgery.
- A pilot study compared the effects of serum from breast cancer resection patients receiving different anaesthetics.
- Serum from subjects receiving propofol/paravertebral block resulted in greater NK cell cytotoxicity than serum from subjects receiving sevoflurane/opioid.
- Clinical studies of the impact of anaesthetic technique on immune suppression and metastasis are indicated.

Background. Animal models and retrospective clinical data suggest that certain anaesthetic techniques can attenuate immunosuppression and minimize metastasis after cancer surgery. Natural killer (NK) T cells are a critical component of the anti-tumour immune response. We investigated the effect of serum from women undergoing primary breast cancer surgery, randomized to propofol–paravertebral block (PPA) or sevoflurane–opioid (GA) anaesthetic techniques, on healthy human donor NK cell function and cytotoxicity against oestrogen and progesterone receptor-positive breast cancer cells (HCC1500).

Methods. Ten subjects who donated serum before operation and 24 h after operation in an ongoing randomized prospective trial (NCT 00418457) were randomly selected. Serum from PPA (n=5) and GA (n=5) subjects was co-cultured with HCC1500 and healthy primary NK cells. NK cell activating receptors (NKp30, NKp44, NKp46, 2b4, CD16, NKG2D), cytokine production, NK CD107a expression, and cytotoxicity towards HCC1500 were examined.

Results. Serum from PPA subjects did not alter normal NK marker expression or secretion of cytokines. Serum from GA subjects reduced NK cell activating receptor CD16 [from mean (SEM), 82 (2)% to 50 (4)%, P=0.001], IL-10 [from 1700 (80) to 1200 (92) pg ml^{-1}, P=0.001], and IL-1β [from 68 (12) to 19 (4) pg ml^{-1}, P=0.01]. An increase in NK cell CD107a [23 (2)% to 37(3)%, P=0.007] and apoptosis of HCC1500 [11 (1)% to 21 (2)%, P=0.0001] was observed with PPA serum, but not GA serum, treated NK cells.

Conclusion. Serum from women with breast cancer undergoing surgical excision who were randomized to receive a PPA anaesthetic technique led to greater human donor NK cell cytotoxicity *in vitro* compared with serum from women who received GA.

Clinical trial registration. NCT 041857.

Keywords: anaesthesia, general; anaesthesia regional; breast neoplasms; immune cells, natural killer cells; immunity, innate

Accepted for publication: 18 March 2014

The perioperative period might be a critical time in primary cancer surgery because various factors can influence whether inadvertently displaced tumour cells become established metastases or are eliminated by the immune system.[1] Modifiable factors that could influence perioperative immunosuppression are of ongoing research interest. Previous retrospective clinical studies of both breast and prostate cancer surgery have suggested an association between anaesthetic technique and cancer recurrence.[2–6] However, other retrospective trials on various forms of cancer have shown no such benefit.[7–9] Definitive evidence of a causal link is awaited from an ongoing prospective trial (NCT 00418457).[10] Animal studies using an inoculation model of breast cancer have suggested a potential protective effect of certain anaesthetic drugs and techniques against metastatic spread in cancer surgery, especially regional anaesthetic techniques and propofol.[11 12] The mechanism underpinning any benefit of these techniques remains unclear but preservation of innate immune function and a direct anti-inflammatory effect of local anaesthetics or other anaesthetic drugs have been hypothesized.[13–20]

Natural killer (NK) cells are CD3$^-$ CD56$^+$ lymphocytes and play an important role in the innate immune response against

tumour cells. Their main function is recognition and killing of virus infected cells or tumour cells. Patients with low levels of NK cell activity are predisposed to tumour progression.[21]

Our group has previously used patient serum as a tool to evaluate the effect of anaesthetic technique on cancer cell biology. Serum was obtained from breast cancer patients randomized in an ongoing prospective trial to receive two distinct anaesthetic techniques, and its effects on breast cancer cell biology *in vitro* have been evaluated.[18 19]

The potential effect of serum from patients who received different anaesthetic techniques on healthy human NK cells *in vitro* has not been investigated. In this pilot study, we examined the effect of serum from subjects randomized in the ongoing clinical trial on anti-tumour activity of healthy human NK cells towards an oestrogen and progesterone receptor-positive breast cancer cell line (HCC1500).

Methods

Clinical protocol

Primary breast cancer surgery patients aged 18–85 yr were randomized in an ongoing trial (NCT 00418457) to either propofol and paravertebral block (PPA) or sevoflurane and opioid (GA) anaesthetic techniques, using a secure web-based randomization system according to a table of random numbers. Additional consent was obtained retrospectively by telephone contact from subjects randomly selected to participate in this add on serum study. Exclusion criteria were inflammatory breast cancer, prior breast cancer surgery, other cancer, ASA status $\geq$IV, contraindications to paravertebral block or midazolam, propofol, sevoflurane, fentanyl, or morphine. Written informed consent and Research Ethics Committee approval were obtained. In the PPA group, a paravertebral catheter was placed using a landmark technique and 20 ml of 0.25% levobupivacaine was administered. Induction and maintenance of anaesthesia was with propofol target-controlled infusion to 4 μg ml^{-1} and oxygen/air. Paravertebral catheter infusion of 8–10 ml h^{-1} 0.25% levobupivacaine was continued for 24 h after operation. Rescue analgesia, if indicated, was given for verbal pain response score $>$3 and consisted of a paravertebral bolus 0.25% levobupivacaine 10 ml. If this did not alleviate pain in $<$15 min, morphine 0.1 mg kg^{-1} i.v. was administered.

The GA group received 1–3 μg kg^{-1} fentanyl, 2–4 mg kg^{-1} propofol, and maintenance with sevoflurane and oxygen/air to maintain arterial pressure and heart rate within 20% of preoperative baseline values. I.V. morphine was titrated to a respiratory rate of 12–14 bpm. Postoperative analgesia was provided as patient-controlled analgesia morphine or bolus i.m. morphine 0.1 mg kg^{-1} for 24 h. Blood samples were obtained from all patients before operation and 24 h after operation, centrifuged, and stored at -80°C. Serum from PPA (n=5) and GA subjects (n=5) was randomly selected from among $>$100 patients already randomized to the long-term clinical trial.

Buffy coat isolation

Healthy donor blood was supplied by the Irish Blood Transfusion Service, St James Hospital, Dublin, Ireland. This was treated with 5% ethylenediaminetetraacetic acid and diluted (1:2) with hank's balanced salt solution (HBSS) (Bio-Sciences, Oxford, UK) supplemented with 1% fetal calf serum (FCS) and 10 mM hydroxyethyl piperazineethanesulfonic acid (HEPES). Treated blood was layered over the density gradient medium Lymphoprep (STEMCELL Technologies, Grenoble, France) and centrifuged at 400 *g* (Hettich Universal 320R, DJB Labcare Ltd, Buckinghamshire, UK) for 25 min. The interface cells were collected and washed once in HBSS and suspended in RPMI 1640 medium (Bio-Sciences) supplemented with 10% FCS, 10 mM HEPES, 2 mM L-glutamate, 100 U penicillin ml^{-1}, and 100 μg streptomycin ml^{-1}.

NK cell isolation

CD56^{+} cells were purified from peripheral blood mononuclear cells, using magnetic-activated cell sorting Anti-CD56 conjugated microbeads (Miltenyi Biotec, Surrey, UK) according to the manufacturer's protocol. Flow cytometry routinely determined a purity of $>$95% for NK cells.

Tumour cell culture

The human oestrogen, and progesterone receptor-positive primary breast cancer cell line HCC1500, was cultured in ATCC-formulated RPMI-1640 Medium supplemented with 10% FCS. Media were renewed every 3–5 days and subcultivation was performed using the trypsin method.

Cell co-culture in serum samples

RPMI-1640 medium was supplemented with 10% of sample serums. Ten per cent of serum concentration was chosen as this was shown to be the optimum serum concentration for *in vitro* cell culture.[18] NK and human cancer cells (HCCs) were resuspended in this supplemented medium before coculture at a ratio of 1:10 for 24 h.

Enzyme-linked immunosorbent assay

Supernatants were retrieved after 24 h co-incubation with healthy CD 56 NK cells and HCC1500 cell line. ELISA kits (R&D Systems, Abingdon, UK) were used to quantify levels of IL-8, IL-10, IL-2, IFN, and IL-1β according to the manufacturer's protocol.

NK cell receptor and CD107a expression

Activating receptor expression was determined by flow cytometry. After 24-h culture, NK cells were stained with fluorescence conjugated antibodies for the detection of CD56, NKp30, NKp44, NKp46, 2b4, CD16, or NKG2D expression. NK cells expressing each particular receptor were analysed by FACSCalibur™ Flow Cytometer and CellQuest software (BD Bio-sciences, Oxford, UK). CD107a expression was for NK cells cultured alone or co-cultured with HCC cells in subject serum-supplemented medium.

Apoptosis assay

HCC cells were cultured either alone or with NK cells in medium supplemented with subject serum. After 24 h, cells were stained with FITC-Annexin V and propidium iodide using FITC-Annexin V Apoptosis Detection Kit I (BD Pharmingen, Oxford, UK) according to the manufacturer's protocol with analysis by flow cytometry.

Statistical analysis

Normally distributed data were analysed using parametric tests and non-parametric tests were applied to data not normally distributed. There were 10 serum samples (n=5 each anaesthetic technique), and three healthy donor NK sets, with each experiment carried out in triplicate. Statistics were performed using GraphPad Prism™ V5 for windows (GraphPad Software, San Diego, CA, USA). Data were compared using unpaired t tests for parametric data and the Mann–Whitney U-test for non-parametric data. Data are expressed as mean (SEM).

Results

Ten subjects were randomly selected as a subgroup of an ongoing prospective randomized trial (NCT 00418457). Both groups were balanced regarding weight, height, oestrogen and progesterone receptor status, and ASA physical status (Table 1). Two subjects in GA and three in PPA had node positivity. All subjects underwent wide local excision and axillary clearance apart from one subject who had mastectomy PPA. One subject in PPA received 5 mg morphine as rescue analgesia (Table 1).

PPA serum maintained normal NK cell cytokines IL-1β and IL-10 function after operation [preoperative to postoperative; IL-1β: 61 (10) to 66 (13) pg ml^{-1}, P=0.5; IL-10: 1800 (71) to 1800 (85) pg ml^{-1}, P=0.9]. However, there was a reduction after operation in IL-1β [68 (12) to 19 (4) pg ml^{-1}, P=0.01] and IL-10 [1700 (80) to 1200 (92) pg ml^{-1}, P=0.001] expression in NK cells treated with GA serum. No significant reduction was seen in IL-8 [260 (8) to 240 (9) pg ml^{-1}, P=0.1 PPA; 260 (10) to 250 (8) pg ml^{-1}, P=0.6 GA], IFN-γ [1910 (88) to 2040 (130) pg ml^{-1}, P=0.2 PPA; 2010 (133) to 2080 (102) pg ml^{-1}, P=0.5 GA], and IL-2 [560 (40) to 580 (54) pg ml^{-1}, P=0.7 PPA; 590 (86) to 550 (56) pg ml^{-1}, P=0.7 GA] expression with either anaesthetic technique (Supplementary Fig. S1).

PPA serum maintained normal NK cell CD16 activating receptor expression [mean (SEM) preoperative to postoperative; 87 (1)% to 85 (2)%, P=0.3]. However, there was a significant reduction after operation in CD16 expression [82 (2)% to 50 (4)%, P=0.001] in GA serum-treated NK cells (Fig. 1A and B). No reduction was seen in NKp30 [35 (1)% to 33 (2)%, P=0.6 PPA; 40 (2)% to 40 (2)%, P=0.8 GA], NKp44 [35 (2)% to 36 (2)%, P=0.7 PPA; 37 (1)% to 32 (2)%, P=0.06 GA], NKp46 [47 (4)% to 48 (4)%, P=0.5 PPA; 51 (5)% to 49 (2)%, P=0.4 GA], 2b4 [31 (1)% to 29 (1)%, P=0.4 PPA; 30 (1)% to 34 (1)%, P=0.01 GA], or NKG2D marker [47 (4)% to 45 (4)%, P=0.4 PPA; 43 (4)% to 41 (3)%, P=0.4 GA] expression following either anaesthetic technique (Supplementary Fig. S2).

Serum from subjects receiving PPA caused an increase in NK cell expression of CD107a when co-incubated with HCC tumour cells [23 (2)% to 37 (3)%, P=0.007]. GA serum did not increase the percentage of NK cells expressing CD107a when exposed to HCC cells [15 (1)% to 17 (2)%, P=0.3] (Fig. 2A and B). This effect was reproduced against three different healthy NK donors.

NK cells conditioned with serum from PPA subjects exhibited strong cytotoxicity by inducing apoptosis in HCC cells [HCC; 11 (1)% *vs* 21 (2)%, P=0.0001]. However, NK cells cultured in GA serum displayed a marked impairment in their ability to induce apoptosis in HCC cells [12 (2)% to 12 (2)%, P=0.9] (Fig. 3A and B). This effect was reproduced across three different healthy NK cell donors.

Table 1 Subject characteristics and breast cancer staging. Data presented as median (interquartile range) or number (%)

	GA (sevoflurane–opioid)	PPA (propofol–paravertebral block)
Height (cm)	155 (152–162)	165 (159–174)
Weight (kg)	69 (52–73.6)	79 (55–94)
ASA I	3 (60%)	2 (40%)
ASA II	2 (40%)	3 (60%)
Mastectomy	0	1
WLE	5	4
Node positive	2 (40%)	3 (60%)
Oestrogen receptor positive	5 (100%)	5 (100%)
Progesterone receptor positive	5 (100%)	5 (100%)
HER positive	3 (60%)	2 (40%)
Morphine (mg)	10 (0–17)	0 (0–2.5)

Discussion

Serum from women with breast cancer who had been randomized to receive propofol–paravertebral anaesthesia for their primary breast cancer surgery maintained healthy donor NK anti-tumour cell activity compared with serum from women receiving a sevoflurane–opioid anaesthetic technique.

In the perioperative period, a brief profound inflammatory stress response occurs that is conducive to tumour progression through suppression of cell-mediated immunity.[20 21] The stress response is characterized by sympathetic nervous system mediated β-adrenergic stimulation with release of catecholamines and prostaglandins and activation of the hypothalamic–pituitary axis with glucocorticoid production. A reduction in number of NK cells occurs and the ratio of TH1/TH2 cells is shifted towards pro-inflammatory TH2 development with an increase in plasma concentrations of TH2 cytokines.[20]

Laparotomy increases animal corticosterone levels with a simultaneous decrease in NK cytotoxicity, whereas dexamethasone reduces NK cell cytotoxicity and expression of cytolytic enzyme granzyme A.[21 22] Cytokines IL-1β, IL-6, IL-8,

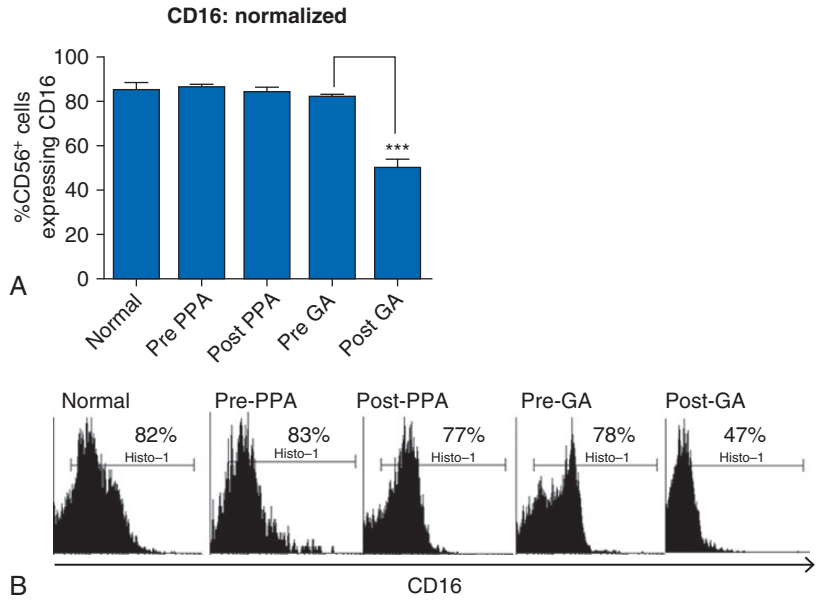

Fig 1 NK receptor expression on $CD56^+$ cells cultured in subject serum-supplemented media. The percentage of $CD56^+$ cells expressing CD16 after 24 h culture in serum-supplemented media for normal healthy control, and pre and postoperative (post) PPA or GA study subjects was determined by surface staining and flow cytometry. (A) Mean normalized percentages of $CD56^+$ cells expressing each receptor for 10 study serum samples and three healthy NK cell donors with standard error represented by error bars. Each experiment was carried out in triplicate. Statistical analysis was performed using the Newman–Keuls one-way ANOVA and compared either pre- and postoperative PPA groups or pre- and postoperative GA groups (***$P \leq 0.001$). (B) Results from one replicate from one donor/serum set.

and IL-12 are down-regulated by glucocorticoids with up-regulation of IL-10 and the TH2 response.[23]

NK cells express β adrenergic receptors.[24] Upon activation, an increase in cAMP and protein kinase A results in decreased NK cytotoxicity.[25] This effect is prevented by β adrenergic receptor block.[12] Suppression of NK cell cytotoxicity was demonstrated in rats undergoing laparotomy through splenic nerve sympathetic activation via brain opioid receptor activation. This effect was antagonized by naloxone.[24] Opioids have conflicting immunomodulatory effects. Fentanyl depressed NK cell function in a rat laparotomy model, while tramadol prevented metastasis in animal surgery and increased postoperative NK activity in cancer patients.[1 20] Pain also suppresses cell-mediated immunity.[20] This effect is attenuated by opioid analgesia in animal pain models.[26] The increased cytotoxic response of PPA-treated NK cells exposed to tumour cells in our study, which was not seen in the GA group, could result from an immunomodulatory effect of opioids or from reduced pain with the use of regional anaesthesia.

Anaesthetic agents including ketamine, thiopental, and volatile agents reduce NK cell activity and increase breast cancer metastasis in animal models, while propofol did not suppress NK cell cytotoxicity.[12] Propofol suppresses tumour growth in a mouse model with preservation of *in vitro* cytotoxic T lymphocyte activity.[27] Propofol also reduces PGE_2 production *in vitro*,[28] which is a known mediator of pain and inflammation that inhibits NK cell cytotoxicity through EP2 and EP4 receptors.[29] This attribute of propofol compared with volatile agents is consistent with the increased cytotoxicity of PPA serum-treated NK cells noted in our pilot study.

Regional anaesthesia through afferent sensory and efferent sympathetic block attenuates the stress response to surgery.[2] Spinal anaesthesia preserves NK cell function and TH1/TH2 balance[30] and might also reduce opioid and intraoperative volatile requirements. In animal studies, spinal anaesthesia is associated with preserved NK cell function and reduced lung metastasis after laparotomy.[11] However, a meta-analysis found no association between regional anaesthesia and NK cell function, but was limited by number and heterogeneity of existing studies.[31] Our findings build upon the scarce existing literature evaluating potential mechanisms for effects of anaesthetic technique on metastasis in primary cancer surgery.

We observed decreased IL-10 expression in GA serum-treated NK cells. IL-10 is produced by Type 2 helper T cells and is involved in inhibition of pro-inflammatory cytokines and down-regulation of cell-mediated immunity.[30 32] Previous *in vitro* studies of breast and ovarian cancer found increases in serum IL-10 with use of regional anaesthetic techniques.[33 34] IL-10 inhibits tumour metastasis through an NK-dependent mechanism.[33] Breast cancer surgery patients receiving sevoflurane and opioid displayed significantly lower serum IL10/IFN-γ ratio than those receiving propofol and paravertebral.[32] This is in keeping with our findings; however, our study was unique in that we measured cytokines from supernatants retrieved after co-incubation of NK cells and HCC1500 tumour

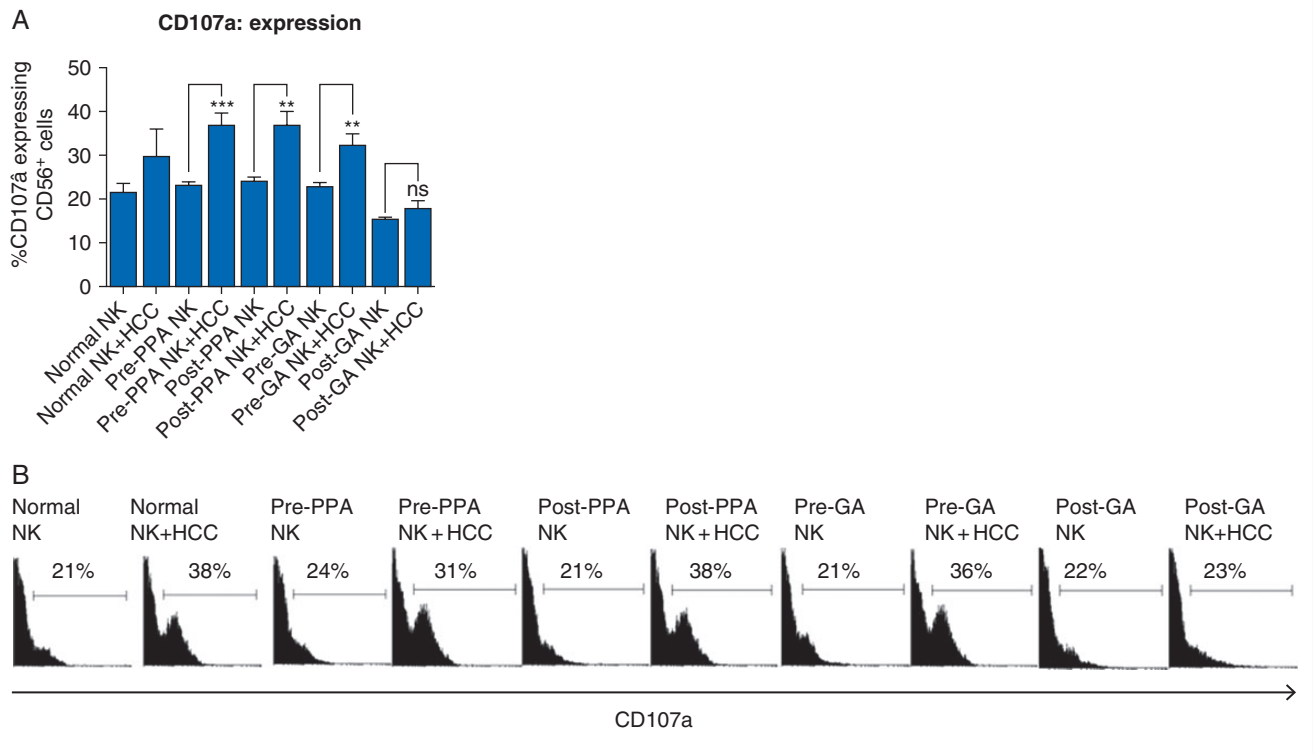

Fig 2 CD107a expression on $CD56^+$ cells cultured in subject serum-supplemented media. The percentage of $CD56^+$ cells expressing CD107a after 24 h culture in serum-supplemented media, either alone or with HCC tumour cells, for normal healthy control, and pre- and postoperative (post) PPA or GA study subject was determined by surface staining and flow cytometry. (A) The mean normalized percentage of $CD56^+$ cells expressing CD107a for 10 study serum samples and three healthy NK cell donors with standard error represented by error bars. Each experiment was carried out in triplicate. Statistical analysis was performed using the Newman–Keuls one-way ANOVA and compared NK cells cultured alone with NK cells cultured with HCC cells, for each serum type (**$P \leq 0.01$, ***$P \leq 0.001$). (B) Representative results from one replicate from one donor/serum set.

cells in serum rather than directly from patient serum in order to look at the effect of NK cell-related cytokine production.

We found a decrease in the IL-1β expression by GA serum-treated NK cells. IL-1β is a mediator of the acute phase response resulting in elevated prostaglandin levels, tachycardia, and accelerated catabolism.[35] It has been shown to facilitate nociception during neuropathic and inflammatory pain.[36] IL-1β stimulates T and B cells, prostaglandin production, myelopoiesis, and adrenocorticotropic hormone (ACTH) release.[37] Laparoscopy compared with open laparotomy recto-sigmoid carcinoma excision resulted in significantly lower elevation in IL-1β.[38] A study of breast cancer patients' serum found reduced serum IL-1β after operation with PPA anaesthesia compared with GA.[33] Another clinical study of ovarian cancer showed attenuated increase in serum IL-1β with a regional anaesthetic technique.[34] However, sedation with propofol–ketamine did not modify plasma concentrations of IL-1β, IL-2, IL-6, IL-10, IL-8, IL-12p70, or IFN-γ or the T helper cell 1/2 ratio in children with leukaemia undergoing bone marrow aspiration.[39] Isoflurane suppressed glial cell IL-1β up-regulation and plasma ACTH but not plasma IL-1β in intraperitoneal lipopolysaccharide inoculated mice.[35] Our findings of reduced IL-1β expression by the GA group differs from those of previous studies; however, results might vary between studies depending on stimulation methods and cell type.

NK cells express activating receptors that are involved in tumour lysis and control of metastatic spread[22] including NKp30, NKp44, NKp46, 2b4, CD16, and NKG2D.[29] NKp30, NKp44, and NKp46 are natural cytotoxicity receptors expressed mainly on NK cells. CD16 is an important NK activating receptor involved in direct tumour cell lysis, antibody-dependent cellular cytotoxicity, cytokine production, and post-activation NK cell apoptotic death.[40] When NK cells become cytotoxic, they degranulate and express the surface protein CD 107a. The expression of this protein correlates with target tumour cell death.[22] The reduction in CD16 and failure to increase CD107a NK receptor expression in response to HCC1500 in GA serum correlated with functional changes in NK cell cytotoxicity seen through the effects of the GA serum on HCC1500 apoptosis.

Caution should be exercised in interpreting these data and extrapolating implications to the clinical scenario. Healthy NK cells from donors could exhibit different immunological behaviour compared with NK cells of women with breast cancer. The scope of cytokine function and interactions is large with alterations occurring in inflammation, pain, surgery, and trauma,

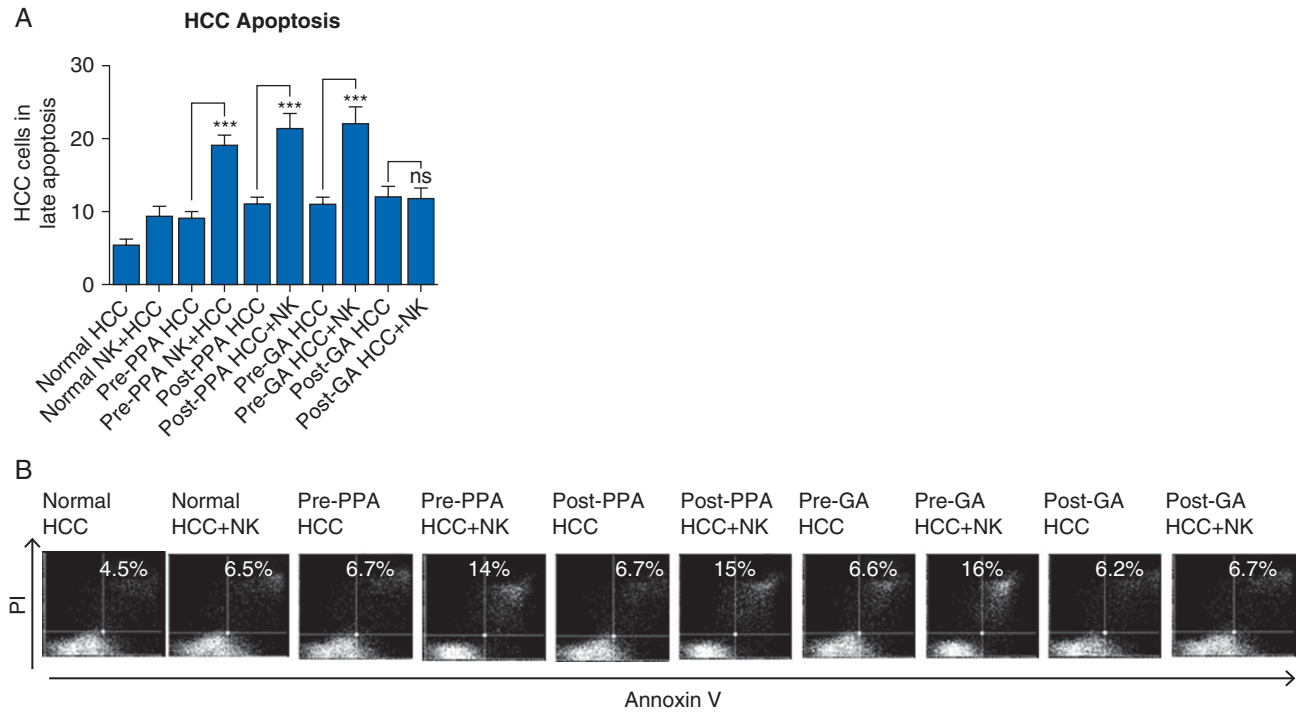

Fig 3 NK cell induced apoptosis in HCC cells when cultured in subject serum-supplemented media. The percentage of HCC cells in late apoptosis after 24 h culture in serum-supplemented media, either alone, or with $CD56^+$ NK cells, for normal healthy control, and pre- and postoperative (post) PPA or GA study subjects was determined by dual staining with FITC-Annexin V and propidium iodide. The percentage of cells in late apoptosis was determined by flow cytometry (upper right histogram quadrant). (A) The mean normalized percentage of dead HCC cells for 10 study serum samples and three healthy NK cell donors with standard error represented by error bars. Each experiment was carried out in triplicate. Statistical analysis was performed using the Newman–Keuls one-way ANOVA and compared HCC cells cultured alone with HCC cells cultured with $CD56^+$ cells for each serum type (***$P \leq 0.001$). (B) Representative results from one replicate from one donor/serum sample with the upper right quadrant showing cells in late apoptosis.

which might behave differently *in vitro*. NK cells play an important role in the cancer immune response.[20] The findings of our study are in keeping with the hypothesis that regional anaesthesia and avoidance of volatile agents and opioids could contribute to reduced perioperative residual disease. Further studies to examine the influence of anaesthetic technique on NK cells from cancer patients are warranted.

Authors' contributions

A.B., S.McQ., P.J., and D.J.B.: conception, design, analysis, interpretation of data, and manuscript preparation; A.B., S.McQ., and D.J.B.: acquisition of data.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Acknowledgement

The authors thank Elizabeth Coghlan, Clinical Research Nurse for collection of subject data.

Declaration of interest

D.J.B. is an Editorial Board member of *BJA*.

Funding

This study was funded by The Sisk Healthcare Foundation and by the Division of Anaesthesia, Mater University Hospital, Dublin.

References

1 Heaney A, Buggy DJ. Can anaesthetic and analgesic techniques affect cancer recurrence or metastasis? *Br J Anaesth* 2012; **109**(Suppl. 1): i17–28

2 Biki B, Mascha E, Moriarty DC, Fitzpatrick JM, Sessler DI, Buggy DJ. Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. *Anesthesiology* 2008; **109**: 180–7

3 Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology* 2006; **105**: 660–4

4 Lin L, Liu C, Tan H, Ouyang H, Zhang Y, Zeng W. Anaesthetic technique may affect prognosis for ovarian serous adenocarcinoma: a retrospective analysis. *Br J Anaesth* 2011; **106**: 814–22

5 Day A, Smith R, Jourdan I, Fawcett W, Scott M, Rockall T. Retrospective analysis of the effect of postoperative analgesia on survival in

patients after laparoscopic resection of colorectal cancer. *Br J Anaesth* 2012; **109**: 185–90
6 Ismail H, Ho KM, Narayan K, Kondalsamy-Chennakesavan S. Effect of neuraxial anaesthesia on tumour progression in cervical cancer patients treated with brachytherapy: a retrospective cohort study. *Br J Anaesth* 2010; **105**: 145–9
7 Gottschalk A, Ford JG, Regelin CC, *et al.* Association between epidural analgesia and cancer recurrence after colorectal cancer surgery. *Anesthesiology* 2010; **113**: 27–34
8 Gupta A, Bjornsson A, Fredriksson M, Hallbook O, Eintrei C. Reduction in mortality after epidural anaesthesia and analgesia in patients undergoing rectal but not colonic cancer surgery: a retrospective analysis of data from 655 patients in central Sweden. *Br J Anaesth* 2011; **107**: 164–70
9 Gottschalk A, Brodner G, Van Aken HK, Ellger B, Althaus S, Schulze HJ. Can regional anaesthesia for lymph-node dissection improve the prognosis in malignant melanoma? *Br J Anaesth* 2012; **109**: 253–9
10 Sessler DI, Ben-Eliyahu S, Mascha EJ, Parat MO, Buggy DJ. Can regional analgesia reduce the risk of recurrence after breast cancer? Methodology of a multicenter randomized trial. *Contemp Clin Trials* 2008; **29**: 517–26
11 Bar-Yosef S, Melamed R, Page GG, Shakhar G, Shakhar K, Ben-Eliyahu S. Attenuation of the tumor-promoting effect of surgery by spinal blockade in rats. *Anesthesiology* 2001; **94**: 1066–73
12 Melamed R, Bar-Yosef S, Shakhar G, Shakhar K, Ben-Eliyahu S. Suppression of natural killer cell activity and promotion of tumor metastasis by ketamine, thiopental, and halothane, but not by propofol: mediating mechanisms and prophylactic measures. *Anesth Analg* 2003; **97**: 1331–9
13 Votta-Velis EG, Piegeler T, Minshall RD, *et al.* Regional anaesthesia and cancer metastases: the implication of local anaesthetics. *Acta Anaesthesiol Scand* 2013; **57**: 1211–29
14 Ecimovic P, McHugh B, Murray D, Doran P, Buggy DJ. Effects of sevoflurane on breast cancer cell function in vitro. *Anticancer Res* 2013; **33**: 4255–60
15 Benzonana LL, Perry NJ, Watts HR, *et al.* Isoflurane, a commonly used volatile anesthetic, enhances renal cancer growth and malignant potential via the hypoxia-inducible factor cellular signaling pathway in vitro. *Anesthesiology* 2013; **119**: 593–605
16 Gupta K, Kshirsagar S, Chang L, *et al.* Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. *Cancer Res* 2002; **62**: 4491–8
17 Lennon FE, Mirzapoiazova T, Mambetsariev B, Salgia R, Moss J, Singleton PA. Overexpression of the mu-opioid receptor in human non-small cell lung cancer promotes Akt and mTOR activation, tumor growth, and metastasis. *Anesthesiology* 2012; **116**: 857–67
18 Deegan CA, Murray D, Doran P, Ecimovic P, Moriarty DC, Buggy DJ. Effect of anaesthetic technique on oestrogen receptor-negative breast cancer cell function in vitro. *Br J Anaesth* 2009; **103**: 685–90
19 Jaura A, Flood G, Gallagher H, Buggy DJ. Serum from women with breast cancer receiving distinct anaesthetic techniques on apoptosis in ER negative breast cancer cells *in vitro*. *Br J Anaesth* 2014 (in press)
20 Snyder GL, Greenberg S. Effect of anaesthetic technique and other perioperative factors on cancer recurrence. *Br J Anaesth* 2010; **105**: 106–15
21 Shakhar G, Blumenfeld B. Glucocorticoid involvement in suppression of NK activity following surgery in rats. *J. Neuroimmunol* 2003; **138**: 83–91
22 Viel S, Charrier E, Marçais A, *et al.* Monitoring NK cell activity in patients with hematological malignancies. *Oncoimmunology* 2013; **2**: e26011
23 Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. *Ann N Y Acad Sci* 2004; **1024**: 138–46
24 Katafuchi T, Take S, Hori T. Roles of sympathetic nervous system in the suppression of cytotoxicity of splenic natural killer cells in the rat. *J Physiol* 1993; **465**: 343–57
25 Raskovalova T, Lokshin A, Huang X, Jackson EK, Gorelik E. Adenosine-mediated inhibition of cytotoxic activity and cytokine production by IL-2/NKp46-activated NK cells: involvement of protein kinase A isozyme I (PKA I). *Immunol Res* 2006; **36**: 91–9
26 Page GG, Blakely WP, Ben-Eliyahu S. Evidence that postoperative pain is a mediator of the tumor-promoting effects of surgery in rats. *Pain* 2001; **90**: 191–9
27 Kushida A, Inada T, Shingu K. Enhancement of antitumor immunity after propofol treatment in mice. *Immunopharmacol Immunotoxicol* 2007; **29**: 477–86
28 Inada T, Kubo K, Shingu K. Possible link between cyclooxygenase-inhibiting and antitumor properties of propofol. *J Anesth* 2011; **25**: 569–75
29 Martinet L, Jean C, Dietrich G, Fournie JJ, Poupot R. PGE2 inhibits natural killer and gamma delta T cell cytotoxicity triggered by NKR and TCR through a camp-mediated PKA type I-dependent signaling. *Biochem Pharmacol* 2010; **80**: 838–45
30 Wada H, Seki S, Takahashi T, *et al.* Combined spinal and general anesthesia attenuates liver metastasis by preserving TH1/TH2 cytokine balance. *Anesthesiology* 2007; **106**: 499–506
31 Conrick-Martin I, Kell MR, Buggy DJ. Meta-analysis of the effect of central neuraxial regional anesthesia compared with general anesthesia on postoperative natural killer T lymphocyte function. *J Clin Anesth* 2012; **24**: 3–7
32 Sultan SS. Paravertebral block can attenuate cytokine response when it replaces general anesthesia for cancer breast surgeries. *Saudi J Anaesth* 2013; **7**: 373–7
33 Deegan CA, Murray D, Doran P, *et al.* Anesthetic technique and the cytokine and matrix metalloproteinase response to primary breast cancer surgery. *Reg Anesth Pain Med* 2010; **35**: 490–5
34 Dong H, Zhang Y, Xi H. The effects of epidural anaesthesia and analgesia on natural killer cell cytotoxicity and cytokine response in patients with epithelial ovarian cancer undergoing radical resection. *J Int Med Res* 2012; **40**: 1822–9
35 Tanaka T, Kai S, Matsuyama T, Adachi T, Fukuda K, Hirota K. General anesthetics inhibit LPS-induced IL-1β expression in glial cells. *PLoS One* 2013; **8**: e8290
36 Zhang RX, Liu B, Li A, *et al.* Interleukin 1beta facilitates bone cancer pain in rats by enhancing NMDA receptor NR-1 subunit phosphorylation. *Neuroscience* 2008; **154**: 1533–8
37 Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009; **27**: 519–50
38 Leung KL, Lai PB, Ho RL, *et al.* Systemic cytokine response after laparoscopic-assisted resection of rectosigmoid carcinoma: a prospective randomized trial. *Ann Surg* 2000; **231**: 506–11
39 Bertolizio G, Stucchi R, Sahillioglu E, *et al.* The effects of propofol and ketamine on the cytokine levels of children with acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2013; **35**: e296–300
40 Inge L, Virginie MR, Thiry M, Delvenne P, Jacobs N. Natural killer cells: role in local tumor growth and metastasis. *Biologics* 2012; **6**: 73–82

Handling editor: H. C. Hemmings