



(RESEARCH ARTICLE)



## Effect of propofol and isoflurane on NK Cells: A pilot study on perioperative breast cancer patients from Eastern India

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### Abstract

**Introduction:** In onco-anesthesiology, the selection between intravenous and volatile anesthetics for maintaining NK cell response during cancer surgery has been an ambiguous area. In the absence of any such comparative Indian report, this pilot study aimed to investigate the effect of intravenous anesthetic, Propofol, and volatile anesthetic, Isoflurane on the NK cell-mediated immune-inflammatory response of perioperative breast cancer patients.

**Materials and Methods:** Treatment naive breast cancer patients (N, 50) selected for surgery were randomly subjected to either Isoflurane (N, 25) or Propofol (N, 25) anesthetic group. The peripheral blood samples were collected 1 day before surgery (Pre), 1 h after incision (Intra) and 48 h after surgery (Post). The blood samples were subjected to immunophenotyping, activation, degranulation, and cytokine analysis.

**Results:** The frequency of NK cell populations did not differ between the Isoflurane and Propofol groups irrespective of cancer stage II/III. NK cell degranulation marker CD107a and activation marker CD335 expression were significantly reduced in the postoperative period compared to the preoperative group in the Isoflurane group but Propofol did not inflict such alterations. Both Isoflurane/Propofol elicited similar trends in the cytokine profiles of interleukin (IL)-6, IL-15, interferon (IFN)- $\gamma$  and transforming growth factor (TGF)- $\beta$  levels. Isoflurane significantly increased IL-8 during the intraoperative period and matrix metalloproteinase (MMP)-9 during the postoperative period whereas no such changes were observed with Propofol.

**Conclusion:** This pilot study indicated that Propofol partially proved better over Isoflurane in maintaining the activation (CD335) and degranulation of NK cells (CD107a) and alleviating inflammation (IL-8 and MMP-9) during the perioperative period in the breast cancer patients.

**Keywords:** Propofol; Isoflurane; NK cell; Inflammation; Perioperative

### 1. Introduction

Breast cancer is the most commonly diagnosed cancer in developed [1] and developing countries and is the leading cause of cancer mortality among women [2]. According to GLOBOCAN 2022, breast cancer remains the second most prevalent cancer with an incidence rate of 11.5% [3].

Although surgical resection of tumors is the main treatment for breast cancer, postoperative metastasis and cancer recurrence continue to be the prime obstacles in cancer management. Postoperative immune activity and inflammatory response are very essential as a suppressed immune system leads to an aggravated metastatic progression of the

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remaining tumor and also delays in patient recovery [4]. Surgical stress and anesthetic exposure have been often reported to have a significant impact on the postoperative immune system [5]. Since the exact effect of the anesthetics on breast cancer patients is difficult to evaluate due to the presence of several factors like surgical stress, type of surgery, pain, and other perioperative drugs, therefore, more prospective clinical studies are needed with uniform treatment regimens [6,7]. Moreover, the choice of a better anesthetic agent for cancer surgery has been an ambiguous arena as several reports preferred intravenous anesthetics over volatile anesthetics in terms of immunosuppression [5] metastasis and patient survival [8] while others observed no significant difference between the two modes of anesthesia regarding the same outcomes [9].

Natural killer (NK) cells as a part of an effective innate immune system play a critical role in immunosurveillance against cancer cells [10,11]. NK cells are well known for their anti-tumor activity after cancer surgery [12,13]. There is limited research on the effect of anesthetic agents on NK cell response in perioperative cancer patients. At present, in India, there are no prospective studies on comparative analysis of Propofol and Isoflurane with reference to NK cell response in perioperative breast cancer patients.

With this background this pilot study was aimed to compare the effect of intravenous anesthetic, Propofol, and volatile anesthetic, Isoflurane on the CD56+NK cell-mediated immune response in perioperative breast cancer patients.

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## 2. Material and methods

### 2.1. Study setting

The pilot study was conducted at Chittaranjan National Cancer Institute (CNCI), a regional cancer center in Eastern India. The newly diagnosed breast cancer patients attending the Outpatient Department of Surgical Oncology at CNCI hospital who were advised for surgery were enrolled in the study. Participants were randomized into two treatment groups- Isoflurane (N, 25) and Propofol (N, 25), based on a computer-generated random number table. The Isoflurane group received volatile anesthesia with Isoflurane and Propofol group received intravenous anesthesia with Propofol. Prior written informed consent was obtained from every patient who participated in the study. The study was approved by the Institutional Ethics Committee [CNCI-IEC-DC-2019/11, dated- 9/9/2019] which adhered to the Indian Council of Medical Research's ethical guidelines for biomedical research on human participants (2017) and has been registered with the Clinical Trials Registry-India (CTRI/2020/11/02886 dated 3<sup>rd</sup> Nov 2020).

### 2.2. Inclusion and exclusion Criteria

The inclusion criteria were -1. Age between 18-65 yr (both inclusive); 2. Newly diagnosed breast cancer (unilateral) confirmed with histopathological diagnosis; 3. American Society of Anesthesiologists (ASA) I & ASA II; 4. T<sub>1</sub>N<sub>0</sub>, T<sub>2</sub>N<sub>0</sub>, T<sub>3</sub>N<sub>0</sub>, T<sub>1</sub>N<sub>1</sub>, T<sub>2</sub>N<sub>1</sub>, T<sub>3</sub>N<sub>1</sub>Mx; 5. Both male & female; 6. Modified radical mastectomy (MRM)/ breast conservation surgery (BCS); 7. Duration of surgery ≤ to 3 hrs. The exclusion criteria included- 1. Patient having any kind of anticancer treatment; 2. Active bleeding; 3. Recent history of massive blood transfusion; 4. Pregnant & lactating woman

### 2.3. Anesthetic management

All patients received an injection (inj) of Glycopyrrolate (Neon Laboratories Limited, Mumbai, India) 0.2 mg intravenous (iv) and inj Fentanyl (2 µg/kg, Verve Health Care LTD, New Delhi, India) before induction. For the Isoflurane (Swiss Parenterals Ltd. Ahmedabad, India) group induction with inj Thiopentone (Neon Laboratories Limited, Mumbai, INDIA), 3-5 µg/kg, was administered iv and titrated to loss of eyelash reflex. For the Propofol (Celon Laboratories Pvt Ltd, Telangana, India) group induction was achieved by Propofol infusion through a target-controlled infusion (TCI) pump with an effect site concentration of 8 µg/mL and was titrated to loss of verbal commands. The trachea was intubated after muscle relaxation with 0.1 mg/kg inj Vecuronium (Neon Laboratories Limited, Mumbai, India). Maintenance of anesthesia in the Isoflurane group was achieved by using Isoflurane 50 % in nitrous oxide, at concentrations to achieve monitored anesthesia care of 1.0. In the Propofol group, anesthesia was maintained with a TCI of Propofol, targeted iv at an effect-site concentration of 2-3 µg/ml. Both groups were monitored by standard monitoring parameters like electrocardiogram, end-tidal carbon dioxide, pulse oximetry, non-invasive blood pressure, and entropy. Muscle relaxation was maintained in both groups by using vecuronium (0.015 mg/kg) as needed. At the end of the surgery, muscle relaxation was reversed with inj. Neostigmine (0.05 mg/kg) and inj Glycopyrrolate (0.01 mg/kg). Postoperative analgesia was administered iv with inj Paracetamol (15 mg/kg) thrice daily and inj Tramadol 1 (mg/kg) if needed.

#### **2.4. Collection of peripheral blood and hematological analysis**

Venous blood samples (5 ml) were collected after obtaining informed consent, from an ante-cubital vein in 'vacutainer' tubes [BD, Franklin Lakes, NJ, USA] without anticoagulant. For serum, the blood was collected in vacutainers with K<sub>2</sub>EDTA. Blood samples were collected at different timepoints: 1 day before surgery (Pre), 1 h after surgical incision (Intra) and 48 h after surgery (Post).

The hematological parameters like total count and differential count were analyzed from the blood samples using an automated cell counter Mispa Count Plus [Agappe Diagnostics Ltd., Agappe Hills, Kerala, India] and by Labomed microscope Lx300 [Labomed Inc, Los Angeles, CA, USA] using Leishman staining.

#### **2.5. Flowcytometry for detection of lymphocyte subtypes**

Whole blood (100 µl) was added with a cocktail of antibodies (APC-Cy7-conjugated anti-human CD3, PE-conjugated CD56, and BV650-conjugated CD335) diluted in PBS and was incubated in the dark for 45 min, at 4°C. Thereafter the erythrocytes were lysed with 2 ml of 1×FACS lysing solution [BD, Franklin Lakes, NJ, USA]. Subsequently, samples were centrifuged at 200 g for 5 min. The residual leucocyte pellet of each sample was washed with ice-cold PBS containing 0.1% sodium azide, resuspended in PBS, and was analyzed using a flow cytometer [BD LSRFortessa cell analyzer, Franklin Lakes, NJ, USA] and the software BD FACS Diva 9.0.1. Data were analyzed in FlowJo version 10.8.1 [BD, Franklin Lakes, NJ, USA]. A biexponential transformation was applied to improve data visualization.

#### **2.6. Assessment of NK cytotoxicity by degranulation assay**

To determine the degranulation of NK cells in response to stimulation with tumor cells, whole blood (500 µl) was lysed with 1× FACS Lysing Solution and was centrifuged at 5000 rpm for 5 min. The leucocyte pellet was resuspended in PBS and subsequently incubated with K562 target cell at effector: target (1:1) ratio in 200 µl assay medium [(RPMI with 10% FBS and 1% penicillin/streptomycin) Invitrogen, Waltham, MA, USA] along with monoclonal antibodies [(FITC-conjugated anti-human CD3, PE-conjugated CD56 and BV-421 conjugated CD107a) BD, Franklin Lakes, NJ, USA] for 3h at 37°C in a humidified 5% CO<sub>2</sub> incubator. Thereafter, cells were centrifuged and the pellet was resuspended with PBS and 4% paraformaldehyde and kept at 4°C. Subsequently, cells were suspended in PBS and analyzed within 24 h, using three LASER (blue, red, and violet) DxFLEX flowcytometer [Beckman Coulter Life Sciences, Indianapolis, IN, USA] and software CytExpert for DxFLEX.

#### **2.7. Estimation of cytokines**

The serum concentration of interleukin (IL)-8, IL-6, IL-15, transforming growth factor (TGF)-β, interferon (IFN)-γ and tumor necrosis factor (TNF)-α levels were measured using commercially available ELISA kits [Ray Biotech, Norcross, GA, USA] according to manufacturer's protocol. The assays were read using an ELISA microplate reader [InfiniteM200, TECAN, Mannedorf, Switzerland] at 450 nm.

#### **2.8. Zymography**

The serum samples (protein content: 200µg) were shaken with gelatin sepharose 4B beads for 2 h at 4°C, washed with Tris-buffered saline with Tween-20 (0.02%) [TBST], and suspended in sample buffer [(Tris (1.2M), SDS (1.4M))] for 30 mins at 37°C. The extract was then subjected to zymography. The proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis under nonreducing conditions where the substrate gelatin was co-polymerized with acrylamide. Subsequently, the gel was washed with Triton® X-100, after which the enzymes partially renatured and recovered their activity. This was followed with the incubation of the gel in an appropriate activation buffer where the concentrated, renatured MMPs in the gel digested the substrate. Finally, the gel was stained with Coomassie Blue, and the MMPs were detected as clear bands against a blue background. Bands were analyzed using ImageJ Launcher (version 1.4.3.67).

#### **2.9. Statistical analysis**

To find the difference between Isoflurane and Propofol at three different time points, a Mixed Design Analysis of Variance was used. The design had two independent groups- 'between-subjects factors' (Isoflurane and Propofol) and 'within-subjects factors' (repeated measurements on each subject under each group at different time points- 'pre', 'intra', and 'post' perioperative conditions). The subjects of the Isoflurane group and the Propofol group were independent of each other. To compare the two independent groups, repeated-measures ANOVA with Bonferroni corrections was employed as a post hoc test to determine the statistical significance at each time point. The Chi-square test and Student's t-test were performed to compare the clinicopathological characteristics. Data were analyzed using

Statistical Package for Social Sciences (IBM SPSS, ver. 25.0, Chicago, IL, USA). For all of the tests, a significance level of 0.05 was considered.

### 3. Results

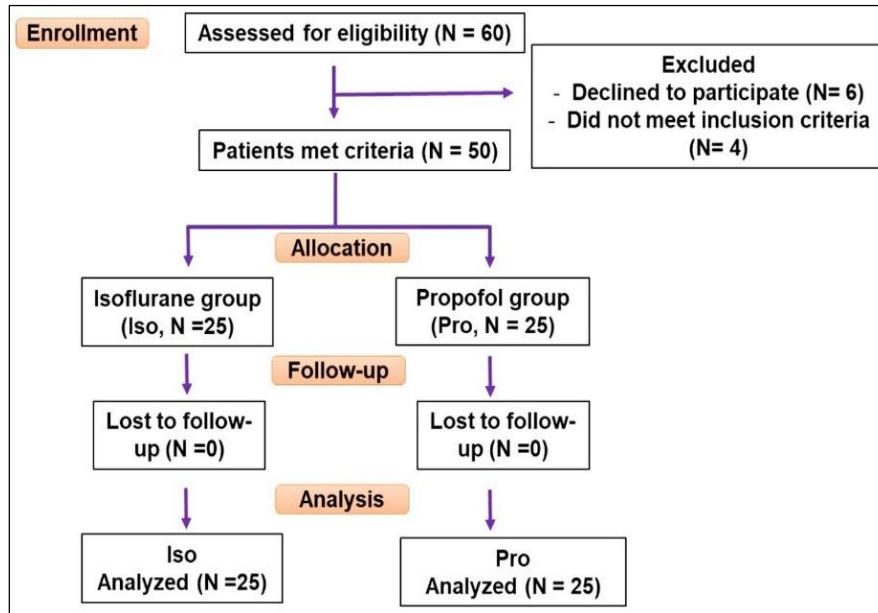
#### 3.1. Clinicopathological characteristics of breast cancer patients

The selection procedure of the patients was according to CONSORT guidelines (Figure 1). The clinicopathological details of the breast cancer patients are presented in Table 1. There was no difference in the type of surgery and analgesics used during the postoperative period between the two groups. No significant difference was found between the two groups of patients in terms of age, height, weight, duration of anesthesia, duration of surgery, and the postoperative numerical pain score of the patients. Overall the clinicopathological parameters showed no significant difference between the Isoflurane and the Propofol group. Additionally, the groups did not show any difference regarding the three-year overall survival. Moreover, we observed that in both the anesthetic groups the total count and differential count of the WBC were within the normal range (Table 2).

**Table 1** Clinicopathological characteristics of treatment naive breast cancer patients recruited for surgical resection in two different anesthetic groups- Isoflurane and Propofol

Characters	Character subtype	Anesthetic agent		p value
		Isoflurane (N, 25)	Propofol (N, 25)	
Age (yr)		48.56 ± 10.33	50.64 ± 8.80	0.447
Height (cm)		150.52 ± 4.23	150.39 ± 7.48	0.940
Weight (kg)		54.54 ± 9.8	54.00 ± 11.35	0.858
ASA (%)	ASA I	44	40	0.157
	ASA II	56	60	
Histopathology (%)	Invasive ductal carcinoma	60	60	0.199
	Ductal carcinoma in situ	8		
	Infiltrating ductal carcinoma	24	32	
	Mucinous carcinoma	8	4	
	Poorly differentiated carcinoma		4	
Hormone status (%)	ER+PR+HER2-	36	44	0.265
	ER-PR-HER2+	16	12	
	ER-PR-HER2-	12	16	
	ER+PR+HER2+	16	24	
	ER+PR-HER2-	8		
	ER+PR-HER2+	12	4	
Type of surgery (%)	MRM	88	68	0.157
	BCS	12	32	
Duration of surgery (min)		87.40 ± 35.65	92.12 ± 29.33	0.612
Duration of anesthesia (min)		107.00 ± 35.64	114.2 ± 32.10	0.457
Pain score (Number)		1.8 ± 0.71	1.44 ± 0.65	0.067
Stage (%)	Stage II	76	84	0.157
	Stage III	24	16	

\*Data represented as mean ±SD or percentage (%); Student's t-test and Chi-square test have been performed (as applicable to specific parameter) to compare the clinicopathological characteristics. [ER, Estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2 (Her2); MRM, Modified radical mastectomy; BCS, Breast-conserving surgery]



**Figure 1** CONSORT flow diagram for recruitment of the breast cancer patients in the study

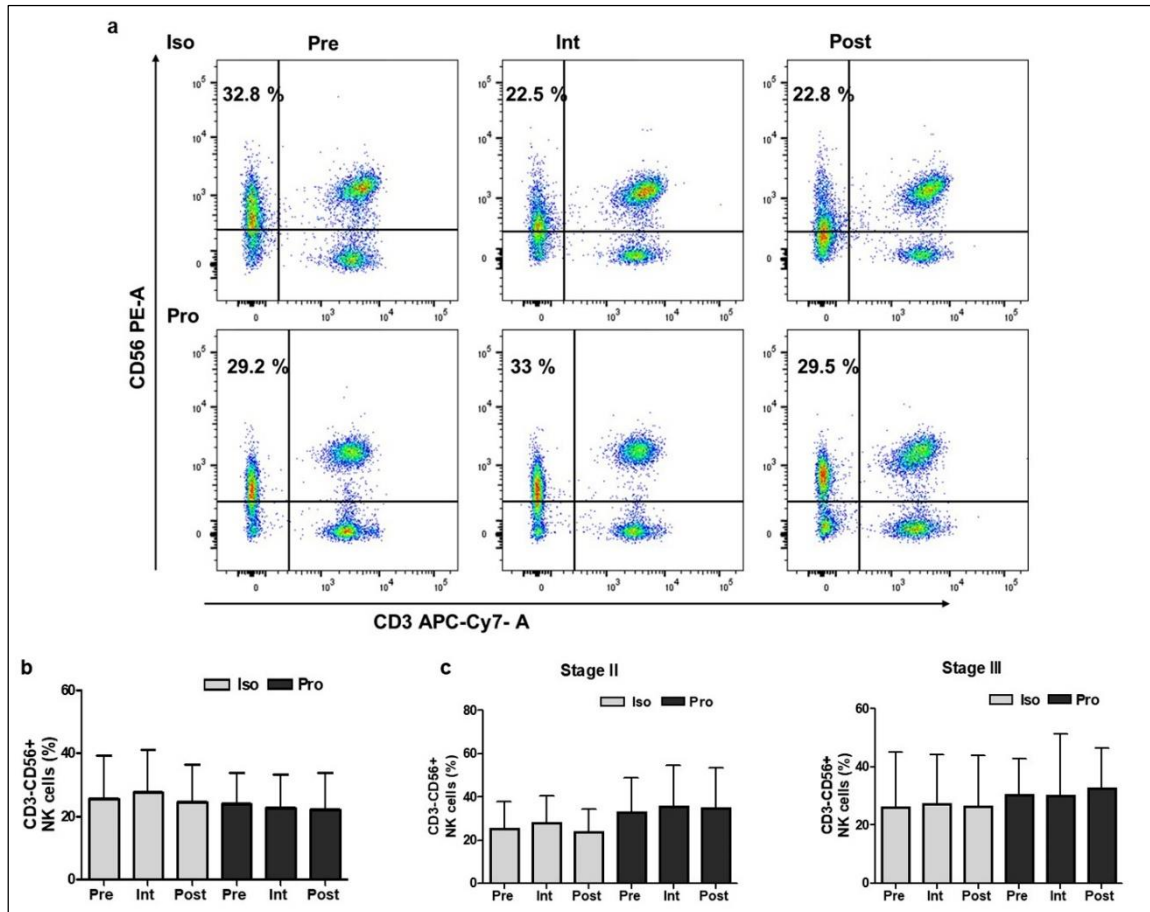
**Table 2** The total count and differential counts of WBCs in the two anesthetic groups- Isoflurane and Propofol at different time points

Parameters	Isoflurane			Propofol			Normal range
	Pre	Int	Post	Pre	Int	Post	
Total WBC	7940± 2208	7940± 1994	9250± 1528	6540±2356	5510±1765	9620±1325	4000-10000/ cu.mm
Neutrophils	61.10±12.71	63.80±10.34	70.80± 7.36	65.70±7.87	66.30±5.88	71.30±8.39	40- 80 %
Lymphocytes	34 ± 11.50	31.80±10.05	25.90±7.23	33±6.70	31.40±5.56	25.50±7.67	20- 40 %
Eosinophil	2.60 ±1.35	1.80±0.63	1.80 ±1.32	1.50±0.71	1.60 ±0.69	1.60 ±1.26	1-6 %
Monocytes	1.30 ±0.48	1.40±0.52	1.50±0.71	1.40±0.52	1.20±0.42	1.30±0.48	1- 10 %

\*Data represented as mean ±SD; Abbreviations: Pre: Preoperative; Int: Intraoperative; Post: Postoperative

### 3.2. Impact of Isoflurane/ Propofol on the frequency of NK cells

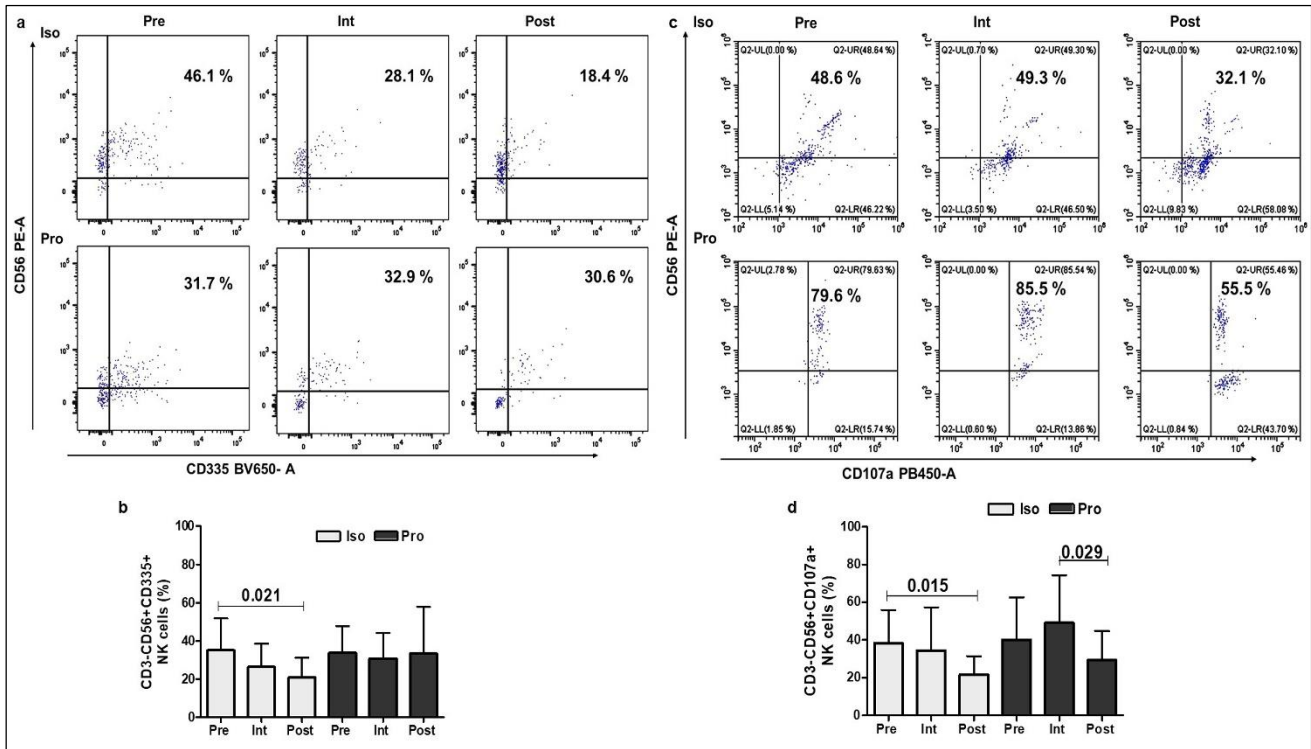
NK cells being an important part of the innate immune system, play an important role against tumor and viral infection. The CD3-CD56+ cell population acts as a marker of NK cells and CD3 is expressed in the human T lymphocyte population. No significant difference was found in the frequency of NK cells between the Isoflurane and Propofol group irrespective of cancer stage II/III of the breast cancer patients (Figure 2).



**Figure 2** Flowcytometric analysis of CD3-CD56+ cells (%) in the peripheral blood of the perioperative breast cancer patients administered with anesthetic agents, Iso or Pro. NK cell frequency in a representative female anesthetized with Iso/Pro (a); comparative effect of Iso/Pro on NK cells at different time points (b); and during different cancer stages (II/III) (c) of the breast cancer patients during the perioperative period. [The graphs were plotted based on the mean  $\pm$  SD; Iso: Isoflurane; Pro: Propofol; Pre: preoperative; Int: intraoperative; Post: postoperative]

### 3.3. Impact of Isoflurane/ Propofol on activation marker and degranulation activity of NK cells

For the analysis of NK cell functions, studies have shown a member of the natural cytotoxicity receptor family CD335 plays an important role during NK cell maturation. Engagement of this receptor on NK cells results in increased activation, cytokine production, and release of cytotoxic granules [14]. Another important marker for the degranulation of NK cells is lysosomal-associated membrane protein 1, CD107a. Expression of CD107a acts as a sensitive marker for the determination of NK cell degranulation activity [15]. So, we assessed the effect of Propofol and Isoflurane on degranulation of NK cells. We observed that NK activation with CD335 expression was significantly reduced in the postoperative period compared to the preoperative period in the Isoflurane group but was maintained at all time points in the Propofol group (Figure 3a and b). On a similar note, NK cell degranulation with CD107a expression was significantly decreased by Isoflurane during the postoperative period compared to the preoperative period. However, in the Propofol group, the NK cell pool with CD107a expression increased during the intraoperative period but came back to the baseline during the postoperative period (Figure 3c and d).

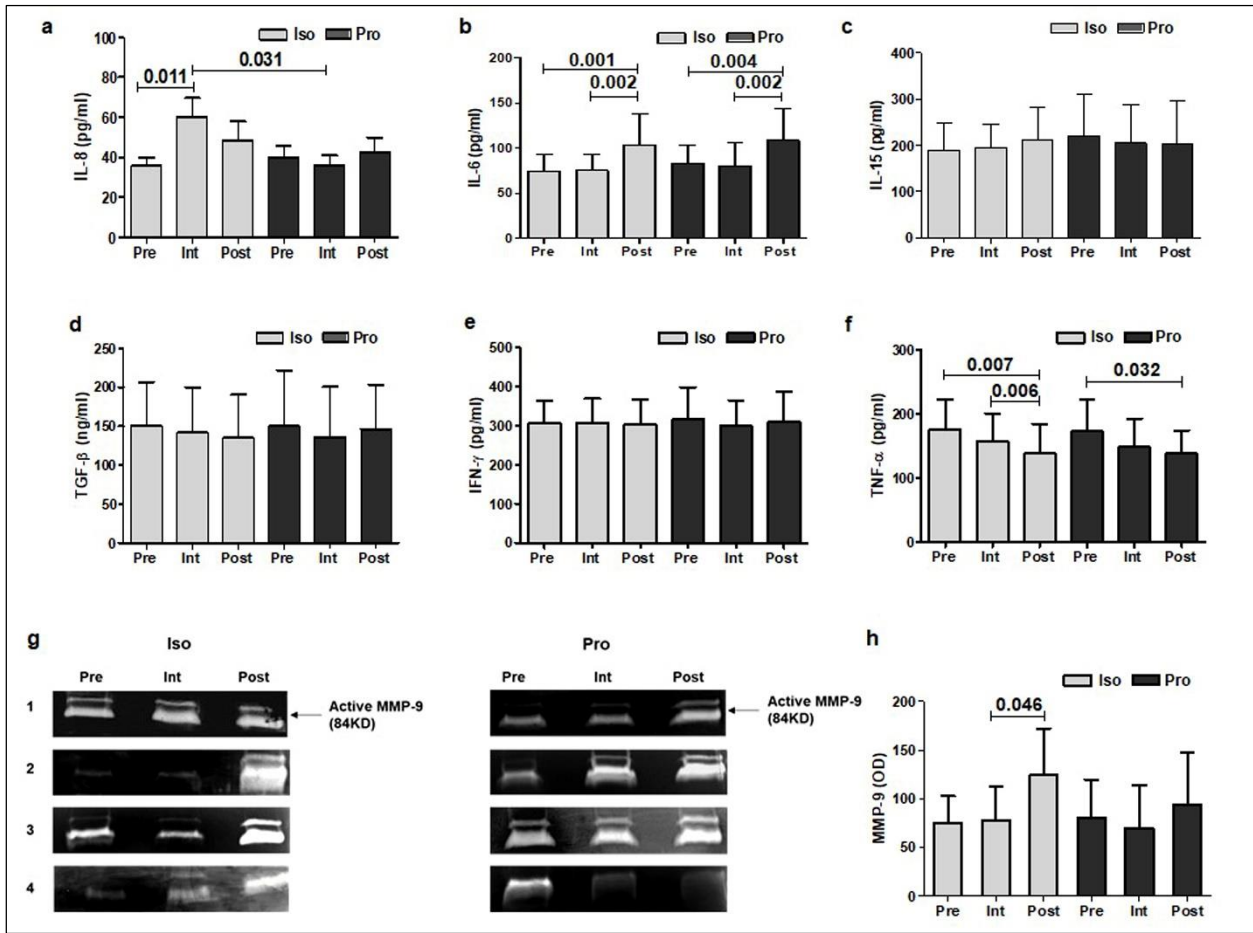


**Figure 3** Flowcytometric analysis of CD3-CD56+CD335+ cells and CD3-CD56+CD107a+ NK cells (%) in the peripheral blood of the perioperative breast cancer patients administered with anesthetic agents, Iso or Pro. NK cell natural cytotoxicity and activation marker (a) and degranulation marker (c) in a representative female anesthetized with Iso/Pro; comparative effect of Iso/Pro on NK cell activation (b) and degranulation (d) at different time points in the breast cancer patients. [The graphs were plotted based on the mean± SD; Iso: Isoflurane; Pro: Propofol; Pre: preoperative; Int: intraoperative; Post: postoperative]

### 3.4. Impact of Isoflurane/ Propofol on serum inflammatory mediators

Serum concentration of IL-8 (Figure 4a) significantly increased during the intraoperative period of Isoflurane. The IL-8 levels were also significantly high in the Isoflurane group compared to the Propofol group during the intraoperative period. Both Isoflurane and Propofol increased IL-6 (Figure 4b) levels significantly during the postoperative period. No significant difference was observed with IL-15 (Figure 4c), TGF-β (Figure 4d), and IFN-γ (Figure 4e) levels. TNF-α was found to be significantly reduced during the postoperative period compared to the preoperative period in both the groups (Figure 4e).

Matrix metalloproteinases (MMPs) play an important role in the migration and invasion of tumor cells. MMPs are zinc-containing endopeptidases which are responsible for the degradation of extracellular matrix proteins. MMP-9 (gelatinase B) activity was significantly increased during the postoperative period of the Isoflurane group but the same was not observed in the Propofol group (Figure 4g and h).



**Figure 4** Effect of Iso and Pro on the serum inflammatory markers in the peripheral blood of the breast cancer patients at different time points. Comparative effect of Iso /Pro on the serum inflammatory markers such as IL-8 (a), IL-6 (b), IL-15 (c), TGF-β (d), IFN-γ (e) and TNF-α (f) levels. MMP-9 activity as evidenced by representative zymography data (g) and mean band intensity (h) at different time points of the perioperative breast cancer patients. [The graphs were plotted as mean± SD; Iso: Isoflurane; Pro: Propofol; Pre: preoperative; Int: intraoperative; Post: postoperative]

#### 4. Discussion

The impact of intravenous and volatile anesthetics on the immune response of perioperative cancer patients is a major concern in relation to postoperative infection, cancer recurrence, and survival. NK cells play a critical role in controlling infection and immunosurveillance against tumor cells [10,11] after cancer surgery [12,13]. There is limited research on the effect of anesthetic agents on NK cell response in perioperative cancer patients. At present, in India, there are no prospective studies on the comparative analysis of Propofol and Isoflurane with reference to NK cell response in perioperative breast cancer patients. Moreover, the studies reported so far do not align in the same direction. Some studies proposed intravenous anesthetic Propofol as a better anesthetic agent over volatile anesthetic sevoflurane [16,17], some preferred volatile anesthetic, Desflurane over Propofol [18] while others observed no significant difference between the two anesthetics in terms of NK cell response in the perioperative cancer patients [9,19,20].

In the present study the anesthetic agents, Propofol and Isoflurane did not have any distinctive effect on the three-year overall survival of the breast cancer patients. However, the two anesthetics definitely differed in the perioperative NK cell-mediated immune response and inflammation in the breast cancer patients. NK cells might be impacted by the anesthetic agents during the perioperative period both in terms of frequency and activity. Therefore, we were interested in investigating the effect of Propofol and Isoflurane on both the aforementioned outcomes of NK cells in the perioperative breast cancer patients. We observed no significant difference regarding the NK cell frequency between Propofol and Isoflurane groups of the perioperative breast cancer patients. A pilot study conducted on the Russian population also found no significant differential effect between Propofol and Sevoflurane combined with epidural anesthesia on the frequency of NK cells and their subpopulations among perioperative kidney cancer patients [20].



However, we observed that compared to baseline, Isoflurane reduced the activation and the degranulation of NK cells in postoperative breast cancer patients. On the contrary, Propofol maintained the activation (CD335) and degranulation of NK cells (CD107a) even during the postoperative period. In congruence with our findings, other studies reported that Propofol proved better than Sevoflurane in promoting NK cell-mediated cytotoxicity in gastric cancer patients [21] and breast cancer patients [22].

The pro-inflammatory cytokines IL-6, IL-8, and TNF- $\alpha$  play a critical role in tumor progression [23]. Propofol has been reported to reduce IL-6, cyclooxygenase 2, IL-8, and tumor development in breast cancer cells and their xenograft [24]. In corroboration with these studies, we too observed that Isoflurane elevated levels of IL-8 during the intraoperative period but Propofol did not alter the IL-8 levels during the intra- or postoperative period. Both Isoflurane and Propofol were found to increase IL-6 levels significantly during the postoperative period. Secretion of IL-6 and IL-8 by tumor cells causes NK cell anergy [25,26]. So though IL-6 was elevated in both anesthetic groups, controlled levels of IL-8 during the postoperative indicated that NK cell anergy was minimized by Propofol but not by Isoflurane. TGF- $\beta$  is an immunosuppressive cytokine that can negatively influence NK cells [27] but in this study, we did not find any difference in TGF- $\beta$  levels between the two studied anesthetic groups. The cytokine IL-15 enhances the ability of NK cells and macrophages to mediate antibody-dependent cellular cytotoxicity [28]. Serum concentration of IL-15 did not show any significant difference between the Isoflurane and Propofol groups. This might have been due to the fact that the two anesthetics did not show any differential effect on NK cell frequency. Moreover, apart from NK cells, IL-15 secretion is also contributed by the monocytes whose differential count did not differ between the two groups. Another important inflammatory mediator and matrix remodeling component, MMP-9 plays a vital role in suppressing NK cell-mediated cytotoxicity [29]. A study on lung cancer patients revealed that those who underwent thoracoscopic surgery with sevoflurane had higher levels of MMP-9 than those who were anesthetized with Propofol [30]. On a similar note, the present study also observed that the postoperative Isoflurane group had significantly higher MMP-9 activity than the baseline activity but the Propofol group did not exhibit the same. Overall Propofol dampened the postoperative inflammatory changes better than Isoflurane in breast cancer patients.

Studies have shown that surgical stress and anesthetic exposure lead to cancer progression and recurrence [31]. Therefore, in this study, we have considered only a particular type of surgery for both groups in order to maintain the same condition. The two anesthetic groups were comparable in terms of the duration of surgery, duration of anesthesia, and pain score, which can have an additional effect on the immune system during the perioperative period. There was no use of opioids in our study, which are also known to inflict immunosuppressive effects. There was no adverse event related to anesthesia or surgery during the study period. However, there are some limitations in our study. Firstly, the sample size of the breast cancer patients is not large. Secondly, the effect of the anesthetics on the NK cell subtypes was not checked. Thirdly, we have not investigated the mechanism by which Propofol better maintained the NK cell response than Isoflurane. Despite the aforementioned limitations, we propose that the observations made in this study may give an important indication of the effect of Propofol and Isoflurane on NK cell response in perioperative breast cancer patients.

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## 5. Conclusion

Propofol proved better than Isoflurane in maintaining the activation (CD335) and degranulation of NK cells (CD107a) and alleviating inflammation (IL-8, MMP-9) during the perioperative period in breast cancer patients. However, clinical implications reflecting the beneficial effect of Propofol over Isoflurane regarding the maintenance of NK cell response during breast cancer surgery need validation from larger multicentric trials.

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## Compliance with ethical standards

### *Acknowledgments*

The authors are also grateful to the Director, Chittaranjan National Cancer Institute for providing the project grant, fellowship to PS, and all infrastructural facilities.

### *Disclosure of conflict of interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the reported work.

### *Statement of ethical approval*

The study was approved by the Institutional Ethics Committee [CNCI-IEC-DC-2019/11, dated- 9/9/2019] which adhered to the Indian Council of Medical Research's ethical guidelines for biomedical research on human participants (2017)

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study

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