STROBE Statement - checklist of items included in reports of observational studies

Completed checklist for PJPS submission | Study type: exploratory prospective comparative observational cohort study

Item	No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract				
Title and abstract	1(a)	Indicate the study's design with a commonly used term in the title or the abstract.	p.1	The abstract identifies the study as an exploratory prospective comparative observational study comparing sevoflurane-based and propofol-based anesthesia in patients undergoing breast cancer surgery.
Title and abstract	1(b)	Provide in the abstract an informative and balanced summary of what was done and what was found.	p.1	The abstract summarizes the 38 included patients, anesthetic exposure groups, tumor/adjacent tissue collection, RT-qPCR and Western blot assays, postoperative recovery outcomes, follow-up outcomes, and the main molecular and clinical findings.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported.	pp.1-2	The introduction explains the clinical context of breast cancer surgery, the possible influence of anesthetic choice on immune/inflammatory regulation, and the biological rationale for examining RYR2/CALML5 and calcium-signaling pathways.
Objectives	3	State specific objectives, including any prespecified hypotheses.	p.2	The objective is to compare the effects of propofol-based and sevoflurane-based anesthesia on RYR2/CALML5 expression and to relate these molecular changes to postoperative recovery, pain, and short-term oncologic outcomes.
Methods				
Study design	4	Present key elements of study design early in the paper.	p.2	The manuscript presents the design early as an exploratory prospective comparative observational study with two anesthetic exposure groups: propofol-based anesthesia and sevoflurane-based anesthesia.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.	p.2	The study was conducted at the Affiliated Tumor Hospital of Xinjiang Medical University. Recruitment occurred from September 2024 to March 2025, intraoperative tissue collection was performed during surgery, and patients were followed for 6 months.
Participants	6(a)	Cohort study - Give eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	pp.2-3	Eligible participants were women aged 18-65 years, ASA I-III, with biopsy-confirmed breast cancer scheduled for surgery. Exclusion criteria included major organ disease, hepatic/renal dysfunction, severe systemic disease, uncontrolled blood pressure or glucose, concurrent malignancy, previous chemotherapy/radiotherapy, and recent calcium-channel-active drugs or supplements. Follow-up assessed incision pain and recurrence/metastasis at 3 and 6 months.
Participants	6(b)	Cohort study - For matched studies, give matching criteria and number of exposed and unexposed participants.	p.3	Not applicable. This was not a matched study. Patients were allocated in a 1:1 ratio to the propofol-based anesthesia group or the sevoflurane-based anesthesia group, with 19 patients in each group.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	pp.3-5	The exposure was anesthetic regimen. Outcomes included RYR2/CALML5 mRNA and protein expression, serum calcium, 24-hour NRS pain score, QoR-15 score, 3- and 6-month incision pain, recurrence, and metastasis. Baseline, clinicopathological, and perioperative variables were defined.

Data sources/measurement	8	For each variable of interest, give sources of data and details of methods of assessment. Describe comparability of assessment methods if there is more than one group.	pp.3-6	Data sources included clinical records, intraoperative tumor and adjacent tissue samples, RT-qPCR, Western blotting, serum calcium measurement, NRS-11 pain assessment, and the validated Chinese QoR-15. Assessment methods were applied comparably in both anesthetic groups.
Bias	9	Describe any efforts to address potential sources of bias.	pp.2-5	Potential bias was addressed through predefined inclusion/exclusion criteria, balanced 1:1 group allocation, standardized anesthesia and intraoperative monitoring, BIS control, standardized tissue handling, exclusion of calcium-channel-active agents, and triplicate molecular assays.
Study size	10	Explain how the study size was arrived at.	p.3	The sample size was feasibility-based for this exploratory mechanistic study. A total of 38 patients were included, consistent with prior mechanistic studies enrolling approximately 20-40 subjects.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	p.6	Normality was assessed using the Shapiro-Wilk test. Normally distributed data were reported as mean +/- SD; non-normally distributed data were reported as median (P25, P75). NRS categories were defined as mild (1-3), moderate (4-6), and severe (>=7), with NRS >=4 regarded as clinically meaningful pain.
Statistical methods	12(a)	Describe all statistical methods, including those used to control for confounding.	p.6	Analyses were performed using SPSS 26.0. Depending on distribution and variable type, t tests, Mann-Whitney U tests, Wilcoxon tests, chi-square tests, Fisher exact tests, effect sizes, and repeated-measures methods were used. Analyses were mainly unadjusted because of the exploratory sample size; baseline comparability was assessed.
Statistical methods	12(b)	Describe any methods used to examine subgroups and interactions.	p.6; p.12	No formal subgroup or interaction analyses were performed. The absence of formal interaction/sensitivity analyses is acknowledged as a limitation of the exploratory design.
Statistical methods	12(c)	Explain how missing data were addressed.	p.3; pp.7-10	Available tissue and outcome numbers were reported in the relevant tables and the participant flow diagram. Analyses used available cases for each outcome; no imputation was performed because of the exploratory design and small number of missing observations.
Statistical methods	12(d)	Cohort study - If applicable, explain how loss to follow-up was addressed.	p.3; Fig. 1	One patient in each group was lost to follow-up at 3 months. No additional loss occurred between 3 and 6 months. Follow-up numbers and analysis numbers were reported in the flow diagram and outcome tables.
Statistical methods	12(e)	Describe any sensitivity analyses.	p.12	No formal sensitivity analysis was performed. This is stated as a limitation, and the findings are interpreted cautiously as exploratory results requiring confirmation in larger multicenter studies.
Results				
Participants	13(a)	Report numbers of individuals at each stage of study - e.g., potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed.	p.3; Fig. 1	Fifty-six patients were assessed for eligibility; 18 were excluded before group assignment. Thirty-eight patients were included and assigned equally to the two anesthetic groups. Follow-up and analysed numbers were reported in the flow diagram and tables.
Participants	13(b)	Give reasons for non-participation at each stage.	p.3; Fig. 1	Reasons for exclusion were reported: 11 patients did not meet eligibility criteria, 5 declined participation, and 2 were excluded for other reasons.

Participants	13(c)	Consider use of a flow diagram.	p.3; Fig. 1	A participant flow diagram was included as Figure 1 to show screening, exclusion, allocation, follow-up, and analysis numbers.
Descriptive data	14(a)	Give characteristics of study participants and information on exposures and potential confounders.	pp.7-8; Table 1	Table 1 reports demographic, clinicopathological, and perioperative characteristics of the participants. No significant baseline differences were found between groups.
Descriptive data	14(b)	Indicate number of participants with missing data for each variable of interest.	pp.7-10; Tables 2-7	Numbers available for molecular assays, serum calcium, recovery outcomes, pain outcomes, recurrence, and metastasis were reported in the relevant tables. Follow-up loss was one patient in each group at 3 months, with no additional loss by 6 months.
Descriptive data	14(c)	Cohort study - Summarise follow-up time.	p.3; Table 7	Patients were followed for 6 months after surgery. Incision pain was assessed at 3 and 6 months, and recurrence/metastasis outcomes were summarized over the 6-month follow-up period.
Outcome data	15	Cohort study - Report numbers of outcome events or summary measures over time.	pp.8-10; Tables 2-7	Outcome data included RYR2/CALML5 mRNA and protein expression, serum calcium, NRS scores, QoR-15 scores, 3- and 6-month incision pain, recurrence, and metastasis. Results were presented as summary measures or as n (%), as appropriate.
Main results	16(a)	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision. Make clear which confounders were adjusted for and why.	pp.8-10	Unadjusted estimates were reported. For example, CALML5 mRNA expression was higher in the sevoflurane group than in the propofol group (2.053 +/- 0.480 vs 1.428 +/- 0.618, P<0.001), whereas RYR2 mRNA showed a non-significant upward trend (P=0.083). Western blot differences were significant for both markers (P=0.001). No multivariable adjustment was performed because of the exploratory sample size.
Main results	16(b)	Report category boundaries when continuous variables were categorized.	p.6	The NRS pain score categories were defined as 1-3 for mild pain, 4-6 for moderate pain, and >=7 for severe pain. NRS >=4 was defined as clinically meaningful pain.
Main results	16(c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.	pp.9-10; Table 7	Absolute event rates were reported as n (%) for 3- and 6-month incision pain, postoperative recurrence, and postoperative metastasis.
Other analyses	17	Report other analyses done - e.g., analyses of subgroups and interactions, and sensitivity analyses.	pp.8-10; p.12	Other analyses included tumor versus adjacent normal tissue comparisons and perioperative serum calcium comparisons. No formal subgroup, interaction, or sensitivity analyses were performed.
Discussion				
Key results	18	Summarise key results with reference to study objectives.	pp.10-11	The discussion summarizes that sevoflurane anesthesia was associated with higher CALML5 expression and altered calcium-signaling markers, but postoperative pain, QoR-15, incision pain, recurrence, and metastasis did not differ significantly between the anesthetic groups during short-term follow-up.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	p.12	Limitations discussed include small sample size, single-center design, limited subtype representation, short 6-month follow-up, lack of formal sensitivity analysis, and possible confounding from adjuvant therapy. These limitations may reduce precision and limit causal interpretation.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	pp.10-12	The interpretation is cautious: anesthetic choice may modulate calcium-dependent signaling, but the observed molecular divergence did not translate into significant short-term clinical divergence. Larger studies with longer follow-up are recommended.

Generalisability	21	Discuss the generalisability of the study results.	p.12	External validity is limited; larger multicenter studies with longer follow-up are recommended.
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.	p.13	The manuscript states that the study received no funding and no industry support, commercial funding, or external sponsorship.

Note: Page numbers should be checked against the final revised manuscript before submission. If the manuscript pagination changes, update this checklist accordingly.