Original Article

Comparison of desflurane and propofol in the speed and the quality of emergence from anesthesia in patients undergoing lung cancer surgery—a prospective, randomized study

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Background: Anesthesia with desflurane or propofol enables rapid emergence. In patients undergoing lung cancer surgery, however, the speed of emergence from desflurane, but not from propofol, may be affected by the deteriorated postoperative respiratory function. We prospectively compared the speed and quality of emergence between desflurane and propofol.

Methods: We conducted a parallel study. Eighty patients scheduled for lung cancer surgery were randomly allocated to Desflurane group (Group D) and Propofol group (Group P). Combined general and epidural anesthesia was performed in the identical way except for the anesthetic.

Results: There was no significant difference between the groups in the time to awakening, extubation, or orientation. However, emergence agitation (EA) occurred more frequently in Group D than in Group P (20/40 *vs.* 4/40, P<0.001). Numbers of patients not achieving full scores in respiration and circulation components of the modified Aldrete score 5 min after extubation were more in Group D (4/40 *vs.* 0/40, P=0.040; and 8/40 *vs.* 2/40, P=0.043, respectively). More patients required antiemetics during postoperative 24 hours in Group D (15/40 *vs.* 7/40, P=0.045).

Conclusions: Desflurane was not inferior to propofol in the speed of emergence from anesthesia after lung cancer surgery, but it was slightly inferior to propofol in the quality of emergence.

Trial Registration: UMIN-CTR identifier: UMIN000009221.

Keywords: Desflurane; emergence; lung cancer; postoperative nausea and vomiting (PONV); propofol

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Introduction

In patients undergoing lung cancer surgery, deterioration of postoperative lung function is inevitable because of a reduced lung volume after surgery and atelectasis e.g., due to inadequate coughing from postoperative pain (1). In such patients, rapid emergence from anesthesia and adequate postoperative pain control are crucial to achieve early recovery of postoperative respiratory function. To achieve such goals in patients undergoing lung cancer surgery, general anesthesia with desflurane or propofol and remifentanil, combined with thoracic epidural anesthesia, seems a reasonable choice (2), since desflurane and propofol enable rapid emergence from anesthesia (3-13), adequate intraoperative analgesia provided by remifentanil and/or epidural anesthesia reduces doses of anesthetics required for anesthesia (14,15), ultrashort-acting remifentanil does not cause postoperative respiratory depression (16), and epidural anesthesia reduces doses of opioids required for

intra- and post-operative pain control while providing adequate postoperative analgesia without causing respiratory depression (2).

Desflurane is characterized by more rapid emergence from anesthesia compared to other inhalational anesthetics (3-7). However, when desflurane and propofol are compared, studies report variable results, including more rapid emergence from desflurane anesthesia (8-10), more rapid emergence from propofol anesthesia (11,12), and no significant difference between both anesthetics (13). The results may be variable depending on various factors such as patients' demography, surgical procedures, co-used opioids and/or nitrous oxide, and doses and durations of anesthetic administration (4-6,8-13,17).

When desflurane is applied to lung cancer surgery, emergence from anesthesia may be affected by deteriorated postoperative respiratory function because desflurane is eliminated primarily via the lungs (6,18), unlike propofol. To date, however, no study has compared, between desflurane and propofol, recovery from anesthesia for lung surgery, although one previous study compared, among desflurane, sevoflurane, and isoflurane, recovery from anesthesia for lung surgery (7).

This prospective, randomized study was conducted to compare, between desflurane and propofol, the speed and the quality of emergence from anesthesia in patients undergoing lung cancer surgery. We present the following article in accordance with the CONSORT reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/rc).

Methods

Patients

Prior to the study, the trial protocol was approved by the Institutional Review Board of Juntendo University Hospital (No. 12-097, date: 2012/10/19), and registered at UMIN Center (identifier: UMIN000009221, date: 2012/10/30). The trial was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participants.

Included were American Society of Anesthesiologists (ASA) physical status I or II patients aged 20–75 years, who were scheduled for lung cancer surgery less extensive than pneumonectomy. Excluded were patients with any of the following disorders; severe cardiac disease corresponding to the New York Heart Association Classification more than II, severe respiratory dysfunction defined as the percent predicted vital capacity or percent predicted forced expiratory volume in one second less than 50%, pulmonary hypertension with mean pulmonary arterial pressure more than 30 mmHg, active inflammation, a history of treatment with steroids and/or immunosuppressive agents within 3 months prior to surgery, severe cognitive impairment, interstitial pneumonia or any contraindication for epidural anesthesia. These exclusion criteria were based on the following assumptions: severe cardiac dysfunction and severe pulmonary hypertension might affect propofol elimination through possible hepatic congestion, severe respiratory dysfunction might affect elimination of desflurane via the lungs, and uses of steroids and/or immunosuppressive agents might affect postoperative courses such as developments of postoperative nausea/vomiting (PONV) and postoperative infection.

Eighty patients scheduled for lung cancer surgery between December, 2013 and March, 2014 in Juntendo University Hospital were enrolled. This was a parallel study and the allocation ratio was 1:1. Patients were divided into the desflurane group (Group D, n=40) and the propofol group (Group P, n=40) in a randomized manner using the envelope method. The patients did not know the allocated group. After the participants gave written informed consent on the day before surgery, they were randomized into each group by using the envelope method, in the sequence of registration. Forty pairs of cards, indicating either 'propofol' or 'desflurane' and put in 40 pairs of envelopes sealed subsequently, had been prepared and shuffled by one anesthesiologist in advance immediately after approval by the IRB.

Anesthesia management

General anesthesia combined with thoracic epidural anesthesia was performed in the identical way in both groups, except for the use of desflurane or propofol. Monitors during anesthesia included 3-lead electrocardiogram, blood pressure, percutaneous oxygen saturation (SpO_2), end-tidal carbon dioxide tension ($PETCO_2$), body temperature, the Bispectral Index (BIS), and a muscle relaxation monitor (TOF-Watch, ORGANON Ireland LTD, Dublin, Ireland). A left-sided double-lumen tube (DLT) was used for one lung ventilation (OLV). Control ventilation during OLV was achieved with pressure-controlled ventilation employing a peak pressure, 15–20 cmH_2O; positive end-expiratory pressure, 4–6 cmH_2O; and respiratory rate, 10–14/min; to maintain $ETCO_2$

between 35 and 40 mmHg. Blood pressure was maintained at ±20% of the baseline value measured immediately before induction of general anesthesia. Ephedrine and/or phenylephrine were used to treat hypotension, if required.

Epidural anesthesia

In both groups, an epidural catheter was inserted via the T6-7 intervertebral space. The effect of epidural analgesia was confirmed with loss of cold sensation 5 min after injection of 2% lidocaine (2 mL). After induction of general anesthesia and before surgery, a combination of 0.25% levobupivacaine (4 mL), fentanyl (50 μg), and morphine (1–2 mg) was injected into the epidural space. A disposable infusion pump (Baxter In-fusor® BB30-LV4, Baxter Healthcare, Deerfield, IL, USA) was filled with 0.25% levobupivacaine (144 mL) and morphine (3–6 mg). Continuous epidural infusion for intra- and post-operative analgesia was started at a rate of 3 mL/h during surgery, following epidural injection of 0.25% levobupivacaine (4 mL).

General anesthesia

In Group D, general anesthesia was induced with fentanyl (50 μg), remifentanil (0.3 μg/kg/min), and propofol (1–2 mg/kg). Tracheal intubation with a DLT was facilitated with rocuronium (0.8–1.0 mg/kg). General anesthesia was maintained with desflurane and remifentanil. Rocuronium was added whenever the first twitch or the first and second twitches in response to train-of-four (TOF) stimulation were detected until the beginning of chest closure. Intraoperative analgesia was achieved with low-dose remifentanil (0.05–0.2 μg/kg/min) and thoracic epidural analgesia. Inspired concentration of desflurane was adjusted to maintain BIS between 40 and 60 during surgery and BIS around 60 before the end of surgery. Remifentanil infusion was discontinued at the end of surgery. Desflurane was discontinued immediately before repositioning into the supine position. Finally, muscle relaxation was reversed with sugammadex (2–4 mg/kg), as required depending on the response to TOF stimulation.

In Group P, general anesthesia was induced with fentanyl (50 μg), remifentanil (0.3 μg/kg/min), and target-controlled infusion (TCI) of propofol (2–3 μg/mL) using a TCI pump (Terufusion TE-371, Terumo, Tokyo, Japan). Tracheal intubation with a DLT was facilitated with rocuronium (0.8–1.0 mg/kg). General anesthesia was maintained with TCI-propofol and remifentanil. Rocuronium was added, as mentioned above. Intraoperative analgesia was achieved with low-dose remifentanil (0.05–0.2 μg/kg/min) and thoracic epidural analgesia. Target concentration of TCI-propofol was adjusted to maintain BIS between 40 and 60 during surgery and BIS around 60 before the end of surgery. Remifentnil infusion was discontinued at the end of surgery. TCI-propofol was discontinued immediately before repositioning into the supine position. Finally, muscle relaxation was reversed with sugammadex (2–4 mg/kg), as mentioned above.

The speed and the quality of emergence from general anesthesia

The primary endpoint was the speed of emergence from anesthesia in lung cancer surgery patients, and the secondary endpoint was the quality of it. Briefly, measuring time with a stopwatch was started just when desflurane or propofol was discontinued. Times from discontinuation of an anesthetic to awakening, extubation, and orientation were measured. The modified Aldrete score consisting of five components, including patient activity, respiration, blood pressure, consciousness, and SpO_2, was measured (19). Occurrences of emergence agitation (EA) and PONV also were noted.

The time from discontinuation of an anesthetic to awakening defined as eye opening in response to voice was measured by calling the patient's name at least every 1 minute. The time to extubation was measured until patients were extubated when they met the extubation criteria including clear consciousness, sufficient respiration defined as the minute ventilation volume more than 8 mL/kg/min, and systolic blood pressure more than 100 mmHg. The time to orientation was measured by questioning the patient about the name and birthday every 1minute after extubation. The modified Aldrete score was assessed every 5 min after extubation until it reached the full score.

EA was noted if it occurred from the point of extubation to the point of 60 min after the end of surgery. The presence of EA was determined according to the Richmond Agitation and Sedation Scale (RASS) (20), a 10-point scale with 4 levels of anxiety/agitation, one level denoting a calm and alert state patient, and 5 levels of sedation. EA was defined as a RASS score ≥+1. PONV, if any, occurring immediately after anesthesia also was recorded. Data on numbers of patients who experienced PONV and

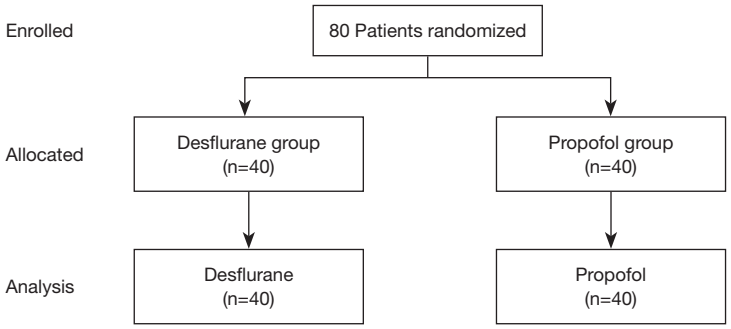

Figure 1 Flow chart of patients included in this study.

who required an antiemetic metoclopramide during 24 postoperative hours were collected from medical records.

Statistical analysis

Sample size calculation based on previous data revealed that at least more than 15 patients per group would be required to detect a 7.4-min difference in the time to extubation based on the SD value of 5.3 min (21). Considering multiple endpoints set in the present study, however, we increased the sample size to 40 patients per group. Data are shown as mean ± SD (range) or number (%) according to data types. Comparisons between groups were performed with unpaired the *t*-test and the chi-square test accordingly. P<0.05 was considered statistically significant, except for a comparison of the modified Aldrete score and its components between groups, which were measured three times in both groups, and for which P<0.0083 was considered statistically significant based on the Bonferroni correction for six possible intra- as well as inter-group comparisons. Statistical analysis was performed using SPSS 25.0 (SPSS, Chicago, IL, USA).

Results

Eighty patients scheduled for lung cancer surgery between December, 2013 and March, 2014 in Juntendo University Hospital were enrolled. All of eighty patients enrolled completed the study (*Figure 1*). Patients' demographic, anesthetic, and surgical characteristics are shown in *Table 1*. These data did not differ between Group D and Group P, except for surgical procedures; more patients in Group P underwent lung resection more extensive than partial resection (i.e., lobectomy or segmentectomy), compared with those in Group D (lobectomy/segmentectomy/partial resection, 25/7/8 *vs.* 23/17/0, P=0.002).

Inspired desflurane concentration to maintain BIS between 40 and 60 during surgery and BIS around 60 before the end of surgery were 3.43%±0.60% and 3.29%±0.70%, respectively. Target concentration of propofol to maintain BIS at the same levels were 2.22±0.29 and 2.00±0.47 μg/mL, respectively.

Data related to the speed and the quality of emergence from anesthesia are shown in *Table 2*. There was no significant difference between the groups in the time to awakening, extubation, or orientation. EA occurred in 24 patients in the total cohort, albeit for brief periods of time (141±96 s). EA occurred more frequently in Group D than in Group P (20/40 *vs.* 4/40, P<0.001). All patients in both groups recorded the full Aldrete score within 15 min after extubation. However, the number of patients who did not achieve the full Aldrete score 5 min after extubation was more in Group D than in Group P (12/40 *vs.* 2/40, P=0.003); numbers of patients who did not achieve the full score in respiration and circulation components of the Aldrete score at 5 min tended to be more, albeit insignificantly, in Group D than in Group P (12/40 *vs.* 2/40, P=0.040; and 8/40 *vs.* 2/40, P=0.043, respectively). None of patients in Group D or Group P complained of pain or required rescue analgesics during the 60-min immediate postoperative observation period. Numbers of patients who experienced PONV immediately during the observational period and during postoperative 24 hours were not different between Group D and Group P (3/40 *vs.* 1/40, P=0.305; and 15/40 *vs.* 10/50, P=0.228, respectively). However, the number of patients who required antiemetic metoclopramide during postoperative 24 hours was more in Group D than in Group P (15/40 *vs.* 7/40, P=0.045).

Any important harms and adverse events did not occur in all participants.

Table 1 Patients' demographic, anesthetic, and surgical data

Variables	Desflurane (n=40)	Propofol (n=40)	P values
Demography			
Sex (M/F)	21 (52.5)/19 (47.5)	24 [60]/16 [40]	0.499
Age (years)	64.5±9.7 [43–79]	63.2±6.6 [44–74]	0.407
Height (cm)	161.2±9.7 [145–184]	163.2±8.5 [146–181]	0.371
Weight (kg)	60.2±14.0 (36.7–101)	61.3±11.5 (39–86.7)	0.269
Body mass index (kg/m^2)	23.1±4.4 (13.9–39.9)	23.0±3.3 (16.0–29.4)	0.504
Coexisting disease and habit			
Cardiovascular disease	10 [25]	12 [30]	0.617
Respiratory disease	4 [10]	4 [10]	1.000
Neurological disease	1 (2.5)	0 (0)	0.314
Metabolic disease	7 (17.5)	8 [20]	0.775
Renal disease	1 (2.5)	0 (0)	0.314
Smoking habit	24 [60]	21 (52.5)	0.499
Respiratory function			
%VC (%)	104.1±12.5 (78.1–146.1)	104.5±14 (72.9–145)	0.883
FEV $_1$/FVC (%)	73.1±8.0 (54.8–92.6)	72.7±7.7 (55.7–85.3)	0.831
%FEV$_1$ (%)	94.6±16.4 (65.1–142.2)	91.4±15.4 (58.7–115.1)	0.371
%DLCO (%)	72.2±16.4 (27.9–109.3)	68±15.5 (41.4–110.3)	0.269
PaO_2 (mmHg)	86.4±11.7 (65.4–115.3)	84.9±9.7 (70.8–121.2)	0.504
Baseline hemodynamics			
Heart rate (bpm)	60.3±10.7 [54–81]	63.0±9.3 [48–89]	0.249
Mean blood pressure (mmHg)	73.2±14.17 [54–105]	72.3±13.4 [51–99]	0.474
Anesthesia and Surgery			
Surgery time (min)	137.4±51.9 [65–249]	131.8±40.1 [67–249]	0.598
Surgical sides (right/left)	28 [70]/12 [30]	24 [60]/16 [40]	0.348
Surgical procedures (L/S/P)	25 (62.5)/7 (17.5)/8 (20.0)	23 (57.5)/17 (52.5)/0 (0)	0.002
Anesthesia time (min)	189.5±54.2 [111–294]	181.0±44.6 [110–316]	0.445
One lung ventilation time (min)	124.0±52.8 [41–241]	114.8±37.4 [55–206]	0.375
Fluid infusion (mL)	1,039.0±325.8 [550–1,800]	1,017.1±266.9 [610–1,660]	0.743
Urine output (mL)	157.5±116.4 [30–620]	176.1±172.0 [20–930]	0.574
Bleeding (mL)	49.6±96.6 [1–465]	51.4±124.9 [5–790]	0.943

Data are shown as mean ± SD (range) or number (%). FEV1/FVC, the ratio of the forced expiratory volume in one second to the forced vital capacity; L, lobectomy; P, partial resection; PaO2, partial pressure of oxygen of arterial blood; %DLCO, percent predicted diffusing capacity of the lung for carbon monoxide; %FEV1, percent predicted forced expiratory volume in one second; %VC, percent predicted vital capacity; S, segmentectomy.

Table 2 The speed and the quality of emergence from anesthesia, and the quality of life after anesthesia

Variables	Desflurane (n=40)	Propofol (n=40)	P values
Time from to awakening (s)	252.3±156.3 [78–717]	269.5±142.9 [55–775]	0.607
Time to extubation (s)	342.3±162.0 [115–784]	355.2±158.3 [113–813]	0.720
Time to orientation (s)	450.8±198.3 [194–1,013]	475.4±209.5 [149–900]	0.591
Emergence agitation	20 (50.0)	4 (10.0)	<0.001
PONV immediately after anesthesia	3 (7.5)	1 (2.5)	0.305
PONV during postoperative 24 h	15 (37.5)	10 (25.0)	0.228
Antiemetic drug use during postoperative 24 h	15 (37.5)	7 (17.5)	0.045
Modified Aldrete score at 5 min <10	12 (30.0)	2 (5.0)	0.003
Activity score at 5 min <2	0 (0)	1 (2.5)	0.314
Respiration score at 5 min <2	4 (10.0)	0 (0)	0.040
Circulation score at 5 min <2	8 (20.0)	2 (5.0)	0.043
Consciousness score at 5 min <2	2 (5.0)	0 (0)	0.152
SpO_2 score at 5 min <2	0 (0)	0 (0)	1.000
Modified Aldrete score at 10 min <10	2 (5.0)	1 (2.5)	0.556
Activity score at 10 min <2	0 (0)	1 (2.5)	0.314
Respiration score at 10 min <2	0 (0)	0 (0)	1.000
Circulation score at 10 min <2	2 (5.0)	1 (2.5)	0.556
Consciousness score at 10 min <2	0 (0)	0 (0)	1.000
SpO_2 score at 10 min <2	0 (0)	0 (0)	1.000
Modified Aldrete score at 15 min <10	0 (0)	0 (0)	1.000
Activity score at 15 min <2	0 (0)	0 (0)	1.000
Respiration score at 15 min <2	0 (0)	0 (0)	1.000
Circulation score at 15 min <2	0 (0)	0 (0)	1.000
Consciousness score at 15 min <2	0 (0)	0 (0)	1.000
SpO_2 score at 15 min <2	0 (0)	0 (0)	1.000

Data are shown as mean ± SD (range) or number (%). Time (in seconds) from discontinuation of desflurane or propofol to awakening, extubation, and orientation are shown. In addition, numbers of patients who experienced emergence agitation, who experienced postoperative nausea and vomiting (PONV) immediately after anesthesia and within 24 hours postoperatively, who required antiemetics within 24 hours postoperatively, and who did not achieve full scores in the modified Aldrete scoring system (full score =10) and in its five components (full score =2 for each) at 5, 10, and 15 minutes after extubation are shown. SpO2, percutaneous oxygen saturation.

Discussion

Recently, an increasing number of aged patients undergo lung cancer surgery (22), although old age is a risk factor for postoperative morbidity and mortality (23). Rapid emergence from anesthesia is essential to achieve early recoveries of adequate respiration and protective airway reflexes, which are closely associated with patients' safety (5,7). Desflurane is suited to anesthesia for high-risk patients, including aged and obese patients and patients undergoing long-lasting surgery, primarily because it enables rapid emergence from anesthesia independent of such risk factors (4-6). Propofol also is characterized by rapid emergence (11-13). Our study is the first one that

compared emergence profiles between desflurane and propofol in patients undergoing lung surgery.

In our study, the time from discontinuation of an anesthetic to awakening, extubation, or orientation did not differ significantly between desflurane and propofol. These results partly agreed with a previous meta-analysis showing that the time to awakening or orientation did not differ between inhalational anesthetics and propofol (10), but partly disagreed with this meta-analysis showing that the time to extubation was longer after propofol (10). In the present study, doses of desflurane and propofol before the end of surgery could be lowered considerably under the strict BIS monitoring, by achieving sufficient intraoperative analgesia with remifentanil and epidural anesthesia (14,15). Such relatively low anesthetic doses might contribute to the present data showing no difference between anesthetics not only in the time to awakening or orientation but also in the time to extubation.

In clinical practice, not only the speed of emergence but also the quality of emergence is important. In the present study, the incidence of EA was higher after desflurane than after propofol. A previous study in adults reported that the incidence of EA did not differ among desflurane, sevoflurane, and propofol (13). In that study, however, nitrous oxide co-used with all three anesthetics might provoke EA even after propofol anesthesia (13). While EA occurs often after inhalational anesthesia in children (2), a meta-analysis reported that the conversion from inhalational anesthetics to propofol reduces the incidence of EA in children (24). Our present results seemed in agreement with such previous data (24).

A previous study comparing emergence and recovery from anesthesia for lung surgery among desflurane, sevoflurane, and isoflurane showed that times to awakening and extubation were the shortest after desflurane, and the Aldrete score 15 min after extubation was the highest after desflurane (7). Further, a previous meta-analysis showed that the time to respiratory recovery was longer after propofol anesthesia than inhalational anesthesia (10). In the present study, however, numbers of patients who could not achieve the full score in the Aldrete scoring system and in its circulation and respiration components 5 min after extubation were significantly more or tended to be more after desflurane than after propofol, despite that patients anesthetized with desflurane underwent less extensive lung resection than those anesthetized with propofol. Although all patients in both groups achieved full Aldrete scores within 15 minutes, it seemed plausible that a slight delay in recovery of an adequate cardiorespiratory status after desflurane anesthesia might reflect the delayed elimination of desflurane via the lungs after extubation due to deteriorated pulmonary function after lung surgery (1). Further, relatively low doses of propofol used in the present study might facilitate early recovery of adequate respiration even after propofol anesthesia.

There was no difference in the incidence of PONV between the anesthetics immediately after anesthesia or during postoperative 24 hours. However, significantly more patients required antiemetics after desflurane than after propofol, suggesting that more patients experienced severe PONV requiring treatment after desflurane. These results seemed in line with a previous meta-analysis reporting that the incidence of PONV was higher and patients' satisfaction was lower after inhalational anesthesia, compared with propofol anesthesia (10).

This study had some limitations. Desflurane and propofol were used in relatively low doses, which might hamper detection of a possible difference in the speed of emergence. Further, we did not examine the effects of anesthetics on postoperative respiratory function, although postoperative respiratory function can remain impaired for hours or a day even after anesthesia for non-lung surgery (8,23,25). Further studies are required to evaluate effects of anesthetics on postoperative respiratory function after lung cancer surgery.

Conclusions

The time to awakening, extubation, or orientation did not differ between desflurane and propofol in patients undergoing lung cancer surgery. However, transient EA and a slight delay in the recovery of an adequate cardiorespiratory status occurred more frequently after desflurane than after propofol. Further, more patients required antiemetics within 24 hours after desflurane. Our data indicated that desflurane was not inferior to propofol in the speed of emergence from anesthesia, but slightly inferior to propofol in the quality of emergence and the quality of life after emergence.

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Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/rc

Trial Protocol: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/tp

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2635/coif). The authors report that the study was supported by competitive funds from Baxter Healthcare, which were supplied to Juntendo University. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The trial protocol was approved by the Institutional Review Board of Juntendo University Hospital (No. 12-097, date: 2012/10/19), and registered at UMIN Center (identifier: UMIN000009221, date: 2012/10/30). The trial was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participants.

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