Prospective Clinical Research Protocol

1. Research title, stage, plan identification number, and history of enactment and revision, etc.

Study Title:

A double-blind, randomized, parallel design study to compare the recovery after prolonged surgery between sugammadex group and neostigmine group in post COVID-19 patients

Research Phase: Phase 4

Plan identification number: Protocol No. MIN_MSD_2023

Revision history: Version 1. 5

2. Researcher's affiliation, position, and name

Principal investigator: Too Jae Min, Professor, Department of Anesthesiology and Pain Medicine, Ansan Hospital, Korea University

Co-investigator: Sangmin Yoon, Medical doctor, Department of Anesthesiology and Pain Medicine, Ansan Hospital, Korea University

3. Place and duration of the study

Conducted by : Korea University Ansan Hospital

Address: 123, Jeokgeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do

Period: Medical Research Review Board (IRB) approval date (2023.4.4) - 2024.12.31

4. Research necessity and background

Post-COVID-19 conditions are a novel and poorly understood clinical disease with life-affecting ramifications, and recent studies have shown that patients have worsened their autonomic nervous system after COVID-19 and are potentially at risk of autonomic dysregulation/autonomic neuropathy.

Currently, most patients undergoing general anesthesia are using neuromuscular blockers, and the residual effect of neuromuscular block delays the recovery of autonomic functions of the body after surgery, and causes problems such as deterioration of bladder and bowel function after surgery. Therefore, reverse premise is used for postoperative muscle relaxation recovery, and clinically, sugammadex and neostigmine have been used a lot. Sugammadex generally provides rapid recovery, including recovery from muscle relaxation after surgery, compared to neostigmine, but postoperative outcomes have been reported with limited coverage in postoperative patients. Therefore, in this study, we

expect that sugammadex will help restore bowel function and lung function more than neostigmine after surgery in patients who have recovered from COVID-19 infection.

5. Risk/Benefit Analysis

Currently, most patients undergoing general anesthesia are using neuromuscular blockers, and reverse agents are used to restore muscle relaxation after surgery. Clinically sugammadex and neostigmine have been widely used (both of which are standard treatments). This study compares the results of the two drugs used in clinical trials and there is no additional risk from clinical trials, except for general side effects caused by general anesthesia. However, there is a risk of personal information exposure, and this will be minimized through encryption and access restrictions to protect personal information. There is no direct benefit to subjects from the trial, and the information from this study may be used to help with future clinical applications.

6. Research Objectives

To compare the effects of sugammadex on postoperative recovery in patients with COVID-19 infection and regular abdominal surgery within one year of treatment. In particular, we want to check the occurrence of dysuria after alcohol.

7. Number of target subjects and basis for calculation

In this study, based on the results of the previous paper reported by Han et al., the target number of subjects was calculated as follows (Jiwon Han, Ah-Young Oh, Yong-Tae Jeon, Bon-Wook Koo, Bo Young Kim, Donghyun Kim, Insung Hwang. Quality of Recovery after Laparoscopic Cholecystectomy Following Neuromuscular Blockade Reversal with Neostigmine or Sugammadex: A Prospective, Randomized, Controlled Trial. J. Clin. Med. 2021, 10, 938. https://doi.org/10.3390/ jcm10050938).

The primary variable was based on the frequency of dysuria (6/38 vs 1/39) and the number of samples was checked using the G*Power program. The allocation ratio for both patients was 1, significance 0.05, and power 0. 9, set as awo-tailed test and expected the dropout rate to be more than 10%, and calculated the number of test subjects as 112 per group, a total of 224.

8. Selection/Exclusion Criteria

Inclusion criteria

American anesthesiology physical status I-III patients between the ages of 20 and 70 who are

scheduled to have regular abdominal surgery that is expected to take more than one hour and have been diagnosed positive for COVID-19 in the past year and have received oxygen therapy.

Regular surgery is a concept that contrasts with emergency surgery and refers to surgery in which sufficient pre-examination and treatment are performed before the start of surgery.

(ASA I: healthy patients, II: patients with mild illness but no impact on daily life, III: patients with illness that affects daily life)

Exclusion criteria

1. Patients currently infected with COVID-19.
2. 1 Patients under 9 years of age or over 70 years of age
3. Patients who are planning to use a heart-lung machine during surgery (e.g., open-heart surgery, heart transplantation, lung transplantation, etc.)
4. Patients who are expected to undergo surgery within one hour or are scheduled to leave the hospital within 48 hours after surgery
5. Those who show active lesions on lung imaging images during preoperative screening or who are expected to be difficult to remove intubation immediately because endotracheal intubation maintenance is required for postoperative breathing management
6. Those who are hypersensitive to Benzodiazepines, propofol, r emifentanil, f entanyl citrate, rocuronium bromide, sugammadex, flumazenil, and other anesthetics
7. Those with myasthenia gravis or myasthenia gravis syndrome
8. Those with cognitive impairment who cannot understand the instructions and consent manual of this study
9. Other cases where the researcher determines that he or she is not suitable for participating in this study

Suspension and Elimination Criteria

If the surgical plan is changed and is not performed, the study will be discontinued and dropped.

9. Investigational Drug Information and Management

Trial drug: Sugammadex is used for the reversal of neuromuscular blockade induced by rocuronium or becuronium in patients 2 years of age and older, and is a colorless transparent vial containing a colorless to light yellow transparent solution, and the storage method of the drug is as follows. 1) Store at 2~30°C. Do not freeze. 2) Store in its original packaging to maintain shading. Vials removed from the packaging can be stored outside for up to 5 days.

Treaty: Korean neostigmine methyl sulfate injection (neostigmine) is used for the purpose of antagonizing the action of non-depolarizing muscle relaxants and is stored in room temperature shading in yellow vials containing a colorless transparent solution.
Management in this study: The drug is routinely applied in actual clinical practice and is stored and administered in accordance with the department policy of the Department of Anesthesiology and Pain Medicine along with other anesthetic drugs.

10. Recruitment method and consent procedure

10.1 How to recruit candidates

- Recruitment is performed for patients who are scheduled to undergo general anesthesia for surgery. After explaining the contents of the study and the criteria for participation, the researcher receives the subject's consent form only for those who voluntarily wish to participate in the study. After that, the eligibility for participation in the study is determined and this study is carried out on those who are judged eligible through the selection/exclusion criteria.

10.2. How to obtain a consent form

- The principal investigator and the person in charge of the study shall verbally fully explain the contents and risks of the experiment to those who are willing to participate voluntarily before the clinical trial, and obtain written consent.
- The researcher may express his or her intention to drop out at any time and announce that there is no penalty.
- After sufficient explanation and confirmation of the subject's understanding, obtain a consent form to participate in the experiment.

11. When recruiting vulnerable targets, protection measures

Do not recruit vulnerable targets.

12. Screening test items and methods

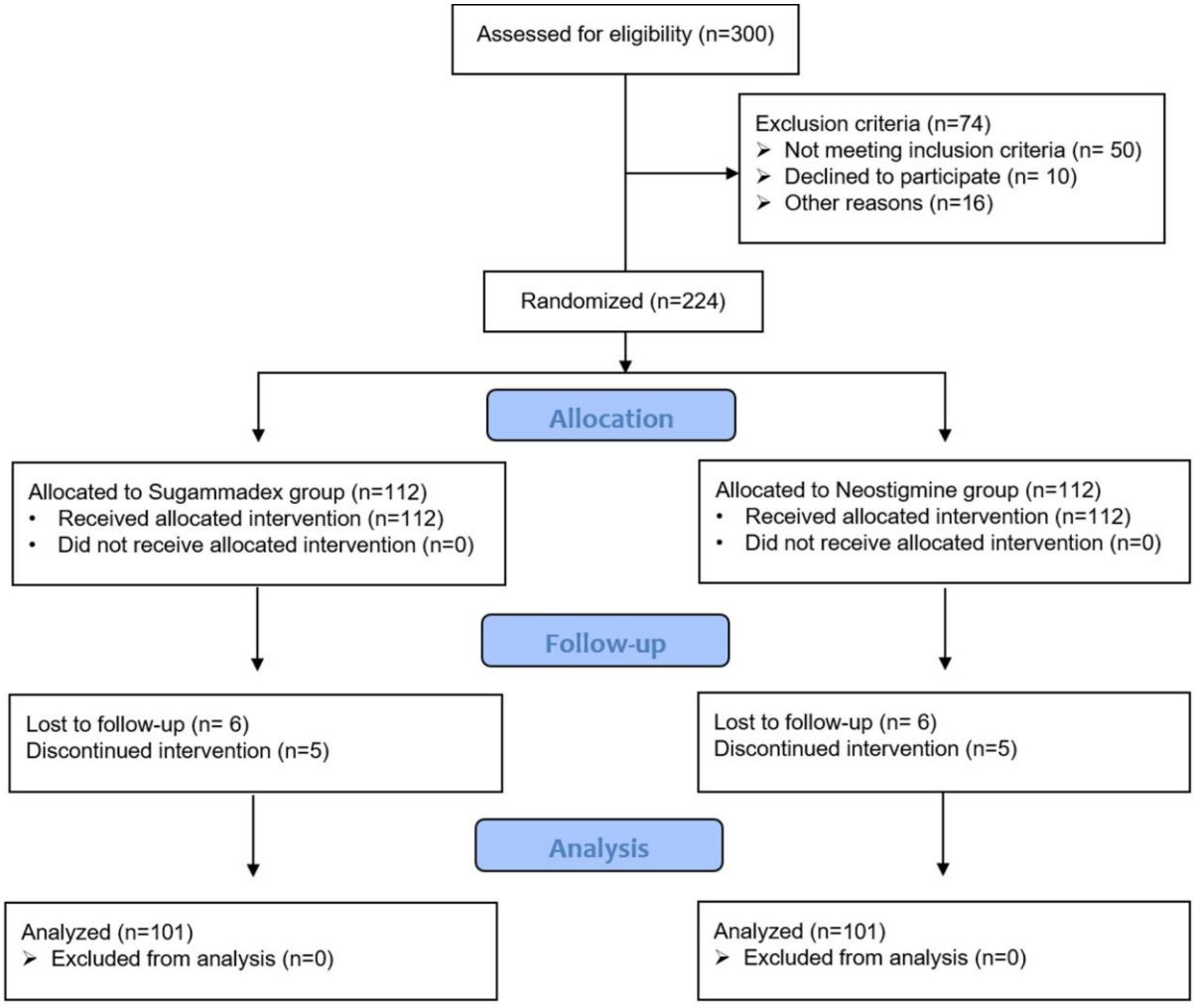
This study does not perform additional screening tests other than general anaesthesia and general screening for surgical preparation (including basic blood, urine tests and chest X-rays).

13. Study design and methods

1. This study is conducted in patients scheduled for surgery under general anesthesia in a

single-organ, randomized, parallel design, and double-blinded.

Study subjects who have completed screening tests and voluntarily agreed to participate in this study among subjects who meet the inclusion/exclusion criteria will be randomized 1:1 to the Sugammadex injection group or the neostigmine injection group (control). The assignment of the two groups is randomized by computer-generated randomization method by a table using www.randomization.com. The expected research flow chart is as follows.



2. After the patient enters the operating room, the patient is equipped with an electrocardiogram, oxygen saturation, esophageal thermometer, electroencephalography, non-invasive blood pressure monitoring device, and muscle relaxation monitoring equipment (TOF equipment). The method of anesthesia is according to the standard anesthesia method and is described in detail below.

3. Preanesthetic treatment: Midazolam 2 mg

4. Anesthesia inducer: Anterior venous anesthesia (propofol 2~1.5 mg/kg)

5. Neuromuscular blockers: Rocuronium 0.6 mg/kg IV in tracheal intubation , additional administration of rocuronium to keep TOF below 2 during surgery, 0.15 mg/kg.

6. Neuromuscular reversal: TOF results are checked at the end of surgery and neostigmine or sugammadex is administered according to the instructions. If TOF < 2, postpone administration until TOF 2 or higher spontaneously recovers. When recovering from TOF 2 or more, a prepared neuromuscular reversal agent is administered.
Neostigmine 50μg/kg + glycopyrollate 0.01mg/kg in the Neostigmine group
Sugammadex 2mg/kg from Sugammadex group
Neuromuscular reversal is received and prepared in advance by a separate medical staff (a separate anesthesiologist who does not participate in the patient's anaesthesia) using the drug and the corresponding prescription order is made separately at the time of leaving the patient recovery room. Since it is diluted with saline and prepared in a total of 5 cc, the patient and the patient's anesthesiologist will not know the drug until they leave the recovery room (double-blinded).
After checking the TOF ratio ≥ 0.9, the endotracheal tube is removed. 3If muscle relaxation recovery is delayed for more than 0 minutes, more than 2 mg / kg of sugammadex can be used regardless of the group, in which case the administration and dosage should be recorded separately.

7. Postoperative pain control
In the postanesthetic recovery room (PACU), 30 mg k etorolac is administered intravenously as a rescue analgesic as a first-line rescue medication, followed by an additional 50 μcg f entanyl (up to 2 doses).
In a general hospital room, tramadol 37.5 mg/a cetaminophen 325 mg is administered orally three times from the time of drinking water. If bowel function has not yet recovered or pain cannot be controlled with oral medications, nalbuphine 10 mg, pethidine 25 mg, and tramadol 100 mg are administered intravenously in that order. If the patient has adverse reactions to narcotic analgesics, 100 mg of ketoprofen or 1 g of propacetamol are administered.

8. Management of nausea and vomiting after surgery (PONV)
Ramosetron 0.3 mg is administered as a first-line antiemetic agent, and if vomiting / nausea persists, metoclopramide 10 mg and palonosetron 0.075 mg are administered.

9. Postoperative urinary retention (POUR) management
Regardless of whether the patient feels a feeling of urination, if the bladder volume exceeds 600 ml, it is considered a urinary retention POUR. A separate doctor who did not participate in intraoperative anesthesia measures and calculates bladder volume using ultrasound with a C onvex probe. Bladder volume is routinely assessed one hour after

arrival at the PACU, and if the patient feels urgency to urinate within one hour of arriving at the PACU, further evaluation of bladder volume is performed immediately. There are no additional costs for ultrasound examination.

The method of measuring the remaining amount of the bladder is as follows.

• After placing the ultrasonic probe on the upper part of the pubic bone, measure the widest width on the horizontal surface first, and measure the length on the longitudinal side. Finally, after measuring the depth from the front and rear sides, calculate the bladder volume with the formula (width x depth x length x coefficient (table below)).

Bladder Shape	Unknown	Triangular prism	Cylinder (Ellipsoid)	Cuboid	Spherical
Correction Coefficient	0.72 (most commonly used)	0.66	0.81	0.89	0.52

If urinary retention is identified, a Foley catheter is inserted into the patient. After placement of the Foley catheter, urine discharge is measured and bladder volume is reassessed. In principle, self-urination is not allowed, but in the case of autourination, the bladder volume is estimated by combining the amount of urination and the amount of residual urine.

10. End-point timeline

The occurrence of urinary retention after surgery is measured at 1 hour, 24 hours, and 48 hours, respectively, based on the arrival time in the postoperative recovery room.

The first fart time is measured from the time you arrive in the recovery room after surgery.

Two days after surgery, chest X-ray imaging is performed to check for changes in radiological aeration such as pneumonia infiltration, lobe, segmental and segmental atelectasis.

14. Standard treatment method for target disease

The anaesthesia method used in this review is the standard of care currently common in all patients undergoing general anaesthesia, and this review does not change the anaesthesia method or other medical interventions.

15. Effectiveness evaluation items and methods

Study endpoints

Primary endpoints

Whether urinary retention occurs 1 hour after surgery

Secondary endpoints

Surgery time and anesthesia time

Recovery time after anesthesia

Intraoperative bleeding, vital signs

Whether and how to administer additional sugammadex

Bladder volume assessed at 1 hour postoperative (summing of autologous urination volumes)

Recovery score and pain score assessed at 1 hour after surgery

First fart time

Whether urinary retention occurs at 24 or 48 hours after surgery

Presence of acute lesions on chest x-ray 2 days after surgery

Actual use of pain and nausea control medications

Other intraoperative events (such as arousal (open eyes), involuntary movements, adverse events and unexpected side effects)

Independent variables

Patient gender, age, weight, height

American Academy of Anesthesiology Physical Classification (ASA class)

Heart rate, blood pressure, oxygen saturation (SpO2), muscle relaxation monitoring score (TOF)

16. Statistical analysis method

Statistical analysis will be performed using SPSS software. (SPSS version 22.0, IBM Corporation, Armonk, NY, USA),

Continuous variables (variables expressed as mean ± standard deviations) will be compared using an independent t-test or Mann-Whitney U test, and categorical variables (including the presence of urinary retention and other adverse events) will be compared using a chi-squared test (or Fisher's exact test). Data are expressed as mean ± standard deviation, median (25; 75 median), or number of patients (%). If the p-value is less than 0.05, it is considered to have statistical significance.

17. Criteria for discontinuation and withdrawal from clinical studies

1) When the subject withdraws his/her consent to participate in the clinical trial

2) If the test cannot be continued due to a serious adverse reaction or sudden accident

3) If the test subject does not or is unable to comply with the visits and procedures specified in the plan.

4) If it is difficult to continue the test due to the manifestation of severe complications

5) If a test subject who does not meet the selection criteria participates in the test

6) If the test subject who meets the exclusion criteria participates in the test

In the event of dropout, the patient's records are kept confidential and are not used for study analysis. The patient is treated with known therapies even after opting out of the trial.

18. Evaluation criteria, evaluation methods, and reporting methods of safety including adverse events

Since this study is conducted with standard treatment methods, there is no risk from clinical trials except for general side effects caused by general anesthesia and surgery.

The test method used in the study is recorded for causality and any adverse events, and the severity, severity, duration, and causal relationship with the test method are subsequently assessed. Treatment and consequences for adverse reactions are also recorded. Adverse reactions are evaluated by abnormal screening or additional tests and examinations as necessary, in addition to the planned screening and findings of the test during the test period, and immediate action is taken. Frequency, expression rate, list of each, severity, and causal relationship with test methods are presented, If necessary, report in the form of a graph.

1) Severe adverse reactions are classified into mild, severe abnormalities, and severe according to the following definition. Mild is normal, transient, and does not interfere with daily activities. Moderation causes some discomfort or interferes with daily activities. Severe illness is inability to perform daily activities. 2) Causality Causation is classified as 'not thought to be relevant', 'likely to be related', 'highly likely', 'obviously related', or 'difficult to ascertain relevance'.

19. Measures to protect personal information and maintain confidentiality of research data

The subject's information completely removes the information that can identify the individual, and the document containing the inspector's record is stored in a locked place to prevent the leakage of information. Even after the end of the study, the subject's identity is accessible only to the participating researchers, and the subject's personal information is permanently destroyed three years after the end of the experiment. All researchers participating in this study are obliged to ensure the confidentiality of the research subjects.

20. Management, storage, and disposal measures for the collection of **human derivatives,** etc.

Not applicable.

21. Planning description of the statistical analysis of the study, including planning for interim analysis and the scope of early termination of the study if necessary

Not applicable.

22. Continuous safety monitoring plan and data safety monitoring plan

Since this study proceeds with the same setting as the actual clinical trial, except for the contents of blinding and group assignment, the additional risk that may arise from the study is minimal. Nevertheless, the principal investigator and the participating researcher entrusted by the principal investigator plan to monitor the safety of data and continuously monitor the safety of subjects during the experiment, under the supervision of the principal investigator.

(1) Monitor personnel

Only the principal investigator and participating researchers authorized by the principal investigator are eligible, and the qualifications of the researchers are limited to those registered at Korea University College of Medicine or the Industry-Academia Cooperation Group. The monitor must be able to prove his or her qualifications with resumes, etc., and perform his or her duties in accordance with the clinical trial protocol , standard work instructions, monitoring plan, etc. The principal investigator is responsible for the monitoring and supervision of monitoring and for the quality and reliability of the data collected on the trial.

(2) Continuous safety monitoring plan of the target person

In a clinical trial in patients undergoing general anesthesia and MAC anesthesia, researchers continuously monitor the patient's hemodynamic signs during the general anaesthesia and MAC sedation period. The duration of the double-blind examination is from the selection of the patient group to the primary endpoint 1 hour after surgery to the exit from the recovery room after confirming whether urinary retention occurs, and then up to 48 hours after surgery to check whether specific complications occur and determine whether the complications are relevant to the clinical trial. Details of the award are as follows: (relevant/irrelevant, start, end, last observation: recovered/recovering/not recovering/recovered but with sequelae/death/unknown)

(3) Data safety monitoring plan

The principal investigator will check whether it is conducted and recorded in accordance with the clinical study protocol, standard work guidelines, clinical study practice standards and related regulations during the entire clinical trial cycle, and interim monitoring will be conducted in December 2023, final monitoring in December after the end of the trial in 2024, and a total of two sessions. Conduct evidence document verification such as case records, supporting documents, and other clinical trial-related documents for accuracy, completeness, and mutual agreement, and if the monitoring-related work is to be coordinated, risk assessment shall be carried out and the application shall be carefully examined according to the results.

(4) Risk assessment

In adjusting and changing the type, scope, period, and scope of monitoring work, risk assessment is carried out and the results are documented. In this case, the procedure prescribed in the standard work manual shall be carried out including the following points.

1) Characteristics of clinical trial institutions identified through selection evaluation, etc.

2) Procedures that have a significant impact on the quality, reliability, and completeness of clinical trial results;

fulfilment

3) Types of clinical trial data collected.

4) Clinical trial evaluation variables

5) Matters related to clinical trial design and blindfold maintenance

6) Characteristics of investigational drugs

7) In addition, the protection and safety of test subjects, the quality and reliability of clinical trial data, etc.

What's related

If the risk assessment is not carried out appropriately, the monitoring method, period, scope, procedure, etc. shall not be arbitrarily reduced or changed, such as the reduction of visit monitoring or the task of checking supporting documents, etc. for the clinical trial conducting institution, the test subject, the collected items, etc., and the monitoring method, cycle, scope, procedure, etc. according to the risk assessment result shall be reduced and if matters that significantly affect the quality and reliability of the clinical trial results and the safety of the test subjects are identified, the method before the reduction, Monitoring should be carried out according to the periodic procedure and scope.

23. Research Implementation Plan (Schedule)

Detailed development details	Detailed Timeline									
	2023. 1.	2	3-6	7-9	10-12	2024. 1-3.	4-6	7-8	9-10	11-12.
Research Plan and Medical Research Review Committee Approval	■	■								
Recruitment and Conducting Research Procedures		■	■	■	■	■	■	■	■	
Data analysis and result reporting							■	■	■	■

24. References

1. Post-COVID conditions: Information for healthcare providers. Available at. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/postcovid-conditions. .html; July 14, 2021Accessed.

2. Dani, M., Dirksen, A., Taraborrelli, P., Torocastro, M., Panagopoulos, D., Sutton, R., & Lim, P. B. (2021). Autonomic dysfunction in 'long COVID': rationale, physiology and management strategies. Clinical medicine (London, England), 21(1), e63-e67.

3. Shouman K, Vanichkachorn G, Cheshire WP, Suarez MD, Shelly S, Lamotte GJ, Sandroni P, Benarroch EE, Berini SE, Cutsforth-Gregory JK, Coon EA, Mauermann ML, Low PA, Singer W. Autonomic dysfunction following COVID-19 infection: an early experience. Clin Auton Res. 2021 Jun;31(3):385-394. doi: 10.1007/s10286-021-00803-8. Epub 2021 Apr 16.

4. Bureau, B. L., Obeidat, A., Dhariwal, M. S., & Jha, P. (2020). Peripheral Neuropathy as a Complication of SARS-Cov-2. Cureus, 12(11), e11452.

5. Xue Y, Wang YY, Zhang K, Cong W, He B, Zeng FC. A Study of Complete Video-Assisted Thoracoscopic Surgery Lobectomy in Treatment of Elderly Patients with Non-Small Cell Lung Cancer: Curative Effect and Impact on Clinical Prognosis. Cell Biochem Biophys. 2015;73(2):399-404.

6. Blobner M, Eriksson LI, Scholz J, Motsch J, Della Rocca G, Prins ME. Reversal of rocuronium-induced neuromuscular blockade with sugammadex compared with neostigmine

during sevoflurane anesthesia: results of a randomized, controlled trial. Eur J Anaesthesiol. 2010;27(10):874-81.

7. Manzo Suzuki, Toshiichiro Inagi, Takehiko Kikutani, Takuya Mishima, Hiroyasu Bito. Negative Pressure Pulmonary Edema after Reversing Rocuronium-Induced Neuromuscular Blockade by Sugammadex. Anesthesiology Volume 2014, Article ID 135032, 3 pages.
8. Gopalaiah Venkatesh Kumar, Anita Pramod Nair, Hanuman Srinivasa Murthy, Koppa Ramegowda Jalaja. Residual Neuromuscular Blockade Affects Postoperative Pulmonary Function. Anesthesiology 2012; 117:1234-44.
9. Miller, R.D. Miller's Anesthesia, 9th ed.; Glopper, M.A., Cohen, N.H., Eriksson, L.I., Fleisher, L.A., Leslie, K., Wiener-Kronish, J.P., Eds.; Elsevier: Philadelphia, PA, USA, 2015; Volume 1, pp. 846-847.
10. Jiwon Han, Ah-Young Oh, Yong-Tae Jeon, Bon-Wook Koo, Bo Young Kim, Donghyun Kim, Insung Hwang. Quality of Recovery after Laparoscopic Cholecystectomy Following Neuromuscular Blockade Reversal with Neostigmine or Sugammadex: A Prospective, Randomized, Controlled Trial. J. Clin. Med. 2021, 10, 938. https://doi.org/10.3390/jcm10050938.