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Original Article

Glycopyrrolate versus atropine for preventing bradycardia induced by neostigmine injection after general anesthesia surgery: a randomized open, parallel-controlled multicenter clinical trial

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Abstract: Objective: With atropine as a positive control, randomized controlled clinical trials were conducted to verify the efficacy of glycopyrrolate injection in preventing bradycardia caused by neostigmine. Method: Patients undergoing elective general anesthesia and non-cardiac surgery were randomly divided into an experimental group (129 cases) and control group (127 cases) (ChiCTR2100046022, http://www.chictr.org.cn/showproj.aspx?proj=126075). At the end of the operation, the test group was given glycopyrrolate 6 ug/kg + neostigmine 0.04 mg/kg, and the control group was given atropine 0.016 mg/kg + neostigmine 0.04 mg/kg, bolus time 1 min, to antagonize muscle residual effects of relaxants. We compared the area under the time curve (AUC) of the difference between heart rate and baseline heart rate within 15 minutes of administration, the measured value of heart rate per minute, and the change in heart rate compared with baseline. We verified the safety of glycopyrrolate injection through laboratory tests, clinical symptoms, signs, and adverse events/serious adverse events. Results: The AUC of the experimental group's heart rate within 15 minutes after the administration was lower than the baseline heart rate change value, (P<005). The measured value of the heart rate at each time changed less than the control group; the experimental group's heart rate remained at the baseline level for longer than the control group (P<005). There was no significant difference in the incidence of adverse reactions between the two groups of patients (P>005). Conclusion: Glycopyrrolate and atropine are safe to prevent heart rate slowing induced by the non-depolarizing muscle relaxant antagonist neostigmine, and glycopyrrolate is more conducive to maintaining a stable heart rate in patients.

Keywords: Glycopyrrolate, atropine, bradycardia, neostigmine injection, general anesthesia surgery

Introduction

The residual paralysis of muscle relaxation during the recovery of general anesthesia is the main cause of complications after anesthesia [1]. At present, muscle relaxation antagonists are routinely used clinically to avoid or reduce the occurrence of residual muscle relaxation [2]. Commonly used muscle relaxation antagonists are cholinesterase inhibitors, such as neostigmine [2, 3]. However, neostigmine produces muscarinic reactions, such as slowing heart rate, increased airway secretions and other side effects [4]. In severe cases, slowing heart rate causes hemodynamic instability and endangers the life of the patient [5]. Therefore, it is necessary to use the anticholinergic drug atropine to antagonize the side effects of neostigmine. However, atropine has a fast onset of action and a short action time, which induce tachyarrhythmias early combined with neostigmine, and the heart rate may drop due to the

disappearance or weakening of the effect of atropine in the later period [6]. The central nervous system effects of atropine also limit its clinical use in large doses [7].

Glycopyrrolate (Robinol) is currently one of the most widely used anticholinergic drugs. Neostigmine is an anticholinesterase used to reverse neuromuscular blockade drugs (NMBD). Due to its cardiac muscarinic effect, glycopyrrolate or atropine are usually given in advance or simultaneously [8].

In 1959, Lunsford discovered that glycopyrrolate is an effective long-acting anticholinergic drug and has many potential clinical applications [9]. In 1972, a study reported the effects of various mixtures of glycopyrrolate and neostigmine, comparing it with atropine and neostigmine in the reversal of NMBD in 49 patients [10]. The study concluded that compared with the atropine group, when 0.2 mg glycopyrrolate and 1 mg neostigmine were used in combination, the incidence of arrhythmia and the range of heart rate changes were significantly reduced [10]. Subsequently, a study evaluated whether glycopyrrolate has a significant advantage over atropine in 85 adult patients. The conclusion was that glycopyrrolate shows the same protection as atropine for bradycardia caused by neostigmine, and significantly reduces the incidence of atropine-related tachycardia and other arrhythmias [11].

Wide use of glycopyrrolate has a stronger antisaliva secretion effect than atropine, without central anticholinergic activity, and little effect on cardiac conduction [8, 12]. This study intends to evaluate the effectiveness and safety of glycopyrrolate in preventing neostigmine-induced heart rate deceleration through a randomized, double-blind, double-simulated, parallel-controlled, multi-center clinical trial with atropine as the positive drug control. This study reveals that glycopyrrolate and atropine can safely and effectively prevent the heart rate slowing caused by neostigmine. In addition, glycopyrrolate can help patients maintain a stable heart rate, which is useful in clinical medication.

Materials and methods

Case selection

This study recruited 256 qualified subjects from 8-11 centers in China for safety and effectiveness evaluation and analysis. This study follows the Declaration of Helsinki, China's "GCP" and the ethical principles of clinical research. All participating units have been approved by the ethics committee, and all subjects participating in the trial have voluntarily participated and signed an informed consent. Researchers conducted experiments in strict accordance with the research protocol and GCP requirements to protect the legal rights and safety of subjects.

Inclusion criteria: adult males and females, aged 18-65 years old, patients undergoing elective general anesthesia surgery, expected operation time <4 hours, and neostigmine injection; ASA health status classification I~II; 18≤ body mass index (BMI) ≤25, all volunteered to participate in this trial and signed an informed consent form.

Exclusion criteria: patients allergic to anticholinergic drugs and their components, patients with respiratory, circulatory, and digestive diseases that affect heart rate, patients with abnormal liver and kidney function, patients who are using drugs that affect the cholinergic system, patients who have participated in drug clinical trials within 3 months. If a trial-related adverse event occurred during the study period, or a serious plan violation, or the patient withdrew informed consent, the trial was terminated. Written informed consent was obtained from every patient and the study was approved by the Ethics Committee of Beijing Chaoyang Hospital of Capital Medical University (Approval number: NUSTYYEC20200813).

This study was a block randomized, double-blind, positive drug parallel controlled, multicenter clinical study. SAS software was used to generate random numbers according to the requirements of the experimental design. Results were stratified by test center, and randomly divided into test drug group (test group) and control drug group (control group) at a ratio of 1:1. According to the purpose of this study and the principles of drug recommendations in the guidelines, atropine injection was selected as the positive control drug in this trial.

Test method

After the screening of qualified subjects after the last stitch of surgical suture and more than

30 minutes after the last muscle relaxant administration, glycopyrrolate injection or atropine injection and neostigmine injection were used in combination to antagonize Muscarinic adverse reactions andslow the heart rate.

Observation indicators

The analysis of the main efficacy index was to describe the area under the time curve (AUC) of the difference between the two groups' heart rate and the baseline heart rate within 15 minutes of the two groups of administration, and the non-inferiority unilateral test was performed, and the test level was 0.025. Taking the baseline heart rate as the covariate and considering the central factor, the covariance analysis model was used to compare the differences between groups and calculate the least square mean (LSMean) of the differences between the two groups and a 95% confidence interval. On the basis of this model, the interaction term between treatment and center was added to test the covariance analysis model of center effect. The analysis of the secondary efficacy index was used to describe the heart rate of the two groups at each time point within 15 minutes of administration and the difference from the baseline. The independent sample t test or Wilcoxon rank sum test was used to compare the differences between the two groups. The postoperative time points were compared with the preoperative baseline by paired t test or paired signed-rank test.

Safety analysis included adverse events, adverse reactions, serious adverse events, adverse events leading to shedding, laboratory and ECG examinations, and vital signs.

Statistical analysis

Data were analyzed by SAS 9.4 (Site 112021-65) software. The description of quantitative indicators calculated the mean, standard deviation, median, minimum, maximum, lower quartile (Q1), upper quartile (Q3), and classification indicators to describe the number of cases and percentages of each category. The features of the two groups were compared by appropriate methods according to the types of indicators. The group t test (homogeneity of variance, normal distribution) or Wilcoxon rank sum test was used for the comparison of quantitative data between groups, and the chi-squared test was used for categorical data. Fisher's exact test probability method (if the chi-square test was not applicable), was used, and the ranked data were compared by Wilcoxon rank sum test or CMH test. All statistical tests were two-sided. A *P* value less than or equal to 0.05 was considered statistically significant (Except for special instructions).

Results

A total of 260 cases were included in this experiment, and 18 cases were excluded from the experimental group. The phase-out rate was 7.09%. The control group excluded 28 cases. The elimination rate was 10.85%. The 4 dropped subjects did not take the test drug, and there was no random group information record in the database.

General information

127 subjects in the experimental group were (39.63±11.01) years old, weight (57.41±7.72) kg, height (161.42±7.77) cm. 129 subjects in the control group were (40.60±10.74) years old and weight (57.34±7.79) kg, height (160.93± 7.71) cm. There was no significant difference in age, height, weight, vital signs (body temperature, heart rate, respiration, blood pressure, and various system examinations), past medical history/concomitant diseases, or pregnancy of the two groups before the clinical trial medication (P>0.05). There was no significant difference in baseline heart rate between the two groups (P>0.05, **Table 1**).

Main efficacy indicators

The difference in AUC between the heart rate and the baseline heart rate within 15 minutes after the test group was given glycopyrrolate and neostigmine was (5.789±11.378) times. The difference in AUC between the heart rate and the baseline heart rate within 15 minutes after the control group was given atropine and neostigmine was (-27.620±11.359) times. The difference was significant (P<0.05, **Table 2**).

Secondary efficacy indicators

Measured value of heart rate per minute within 15 minutes after administration of the test drug: One minute after the end of the test drug administration, the heart rate of the two groups

Table 1. Comparison of baseline heart rate of the two groups of initially selected patients

Item	Index	FAS		PPS	
		Control group	Test group	Control group	Test group
1 min before medication	N (Missing)	129 (0)	127 (0)	115 (0)	118 (0)
(Times/min)	Mean (Sd)	61.06 (10.25)	61.24 (11.47)	61.24 (10.21)	60.80 (10.94)
	Median	60.00	59.00	60.00	59.00
	Q1, Q3	55.00, 66.00	55.00, 67.00	55.00, 66.00	55.00, 65.00
	Min, Max	40.00, 107.00	40.00, 107.00	40.00, 107.00	40.00, 107.00
	Statistics	-0.232	Wilcoxon rank sum test	0.650	Wilcoxon rank sum test
	P value	0.816		0.516	

Table 2. Corrected mean area under the time curve of heart rate changes from baseline within 15 minutes after medication

Item	FAS	
	Control group	Test group
Modified mean	-27.314	5.701
Standard error	11.290	11.318
Test statistic t	-2.525	-
P value	0.012	
Modified mean difference between two groups	33.014	
95% CI	7.2,58.773	

Covariance analysis was used to compare the corrected mean area under the time curve of heart rate changes from baseline within 15 minutes after medication. The 95% CI is the difference between the test group and the control group.

of patients began to increase, and the heart rate reached its highest value at 3 minutes after the end of administration, and then gradually slowed down. There was no significant difference in heart rate between the two groups immediately after the end of the administration and 1, 2, 4, and 5 minutes after the administration (P>0.05). After the administration, measuring at 3, 6, 7, 8, 9, 10, 11, and 12 minutes, there was a statistically significant difference in heart rate between the two groups at 13, 14, and 15 minutes (P<0.05).

Heart rate change within 15 minutes after administration of test drug: The heart rate change value is the difference between the measured heart rate per minute and the baseline heart rate after the test drug is administered.

There was no significant difference in heart rate change between the two groups of patients in the experimental group immediately after administration and 1, 2, 4, or 5 minutes after administration (P>0.05). The difference in heart rate change between the two groups of patients at 10, 11, 12, 13, 14, and 15 minutes was significant (P<0.05, **Figure 1**).

Safety results

During the study period, there was no significant difference in laboratory indexes, vital sign indexes, and ECG indexes between the two groups of patients before and after medication (P>0.05). Adverse events occurred in 176 patients (60.63%) in the test group and 144 patients (54.26%) in the control group. There was no significant difference between the two groups (P>0.05, **Table 3**). There were 2 serious adverse events in the experimental group and 1 serious adverse event in the control group (**Table 4**). Two serious adverse events in the experimental group were related to drugs (**Table 5**). During the study, there were 8 cases in the test group and 1 case in the control group. There was no statistical difference in the occurrence of adverse events related to the study drug in either system.

Discussion

Clinical application of neostigmine to reverse the effect of muscle relaxation requires the combined use of anticholinergic drugs to correct the cardiac side effects of neostigmine [13]. Compared with atropine, relevant foreign studies showed that glycopyrrolate had the advantages of no central function, less effect on the heart, and low incidence of tachyarrhythmia [8]. The "Expert Consensus on the Rational Use of Muscle Relaxants" issued by the Chinese Medical Association Anesthesiology Branch in

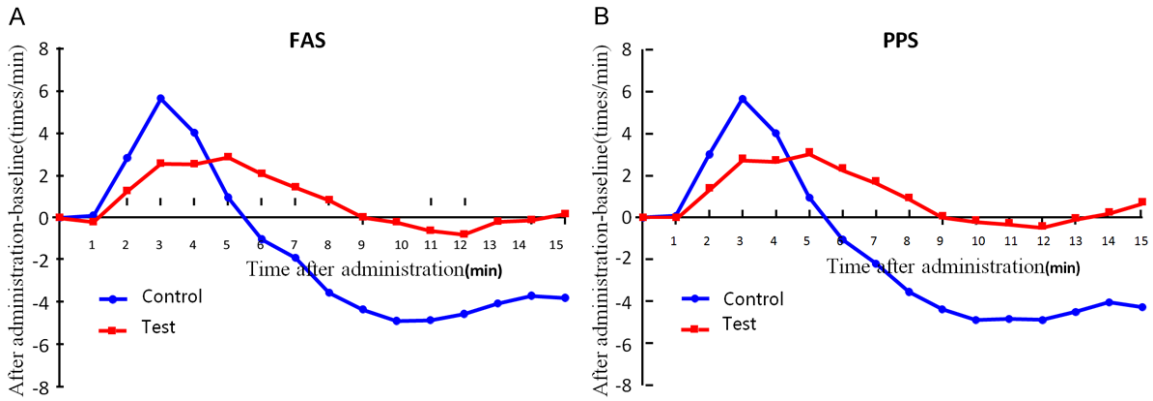

Figure 1. Time curve of the change in resting heart rate per minute (beats/min) from baseline after medication. (A) Using the full analysis set (FAS) population and (B) using the per-protocol (PPS) population set.

Table 3. Occurrence of adverse events and serious adverse events during the study

Item	Control group			Test group			*P* value
	Cases	Number	Percentage	Cases	Number	Percentage	
Adverse events	144	70	54.26%	176	77	60.63%	0.315
Adverse events related to study drug	16	12	9.30%	34	24	18.90%	0.031
Serious adverse events	0	0	0.00%	0	0	0.00%	-
Serious adverse events related to study drug	0	0	0.00%	0	0	0.00%	-
Adverse events leading to shedding	0	0	0.00%	0	0	0.00%	-

Table 4. Distribution of severity of adverse events during the study

Item	Control group			Test group			*P* value
	Cases	Number	Percentage	Cases	Number	Percentage	
Mild adverse events	72	40	31.01%	92	50	39.37%	0.191
Moderate adverse events	71	44	34.11%	81	43	33.86%	1.000
Severe adverse event	1	1	0.78%	3	2	1.57%	0.621

Table 5. Distribution of the severity of adverse events related to the study drug during the study

Item	Control group			Test group			*P* value
	Cases	Number	Percentage	Cases	Number	Percentage	
Mild adverse events	7	6	4.65%	15	12	9.45%	0.150
Moderate adverse events	9	9	6.98%	17	14	11.02%	0.282
Severe adverse event	0	0	0.00%	2	1	0.79%	0.496

2017 clearly stated: "Glycopyrrolate is the first choice for neostigmine [14]".

Research on the application of glycopyrrolate during anesthesia began in the 1970s. The original purpose was to verify that glycopyrrolate reduced gastric volume and gastric acid secretion to prevent reflux aspiration syndrome. While drawing conclusions, the study found that compared with atropine, glycopyrrolate had less interference with heart rate and circulation during the combined use of neostigmine [15-17]. Subsequently, the focus of the series of studies shifted to the evaluation of glycopyrrolate in antagonizing neostigmine's heart rate side effects and heart rate variability compared with atropine. Previous studies believed that glycopyrrolate doses lower than 5 μg/kg would not antagonize the bradycardia effect of neostigmine, and higher than 10 μg/kg would have

no benefit [18]. However, research on the efficacy and safety after application in China is lacking. The dose of glycopyrrolate used in this clinical trial was 6 μg/kg.

This was a randomized, open, positive drug parallel controlled, multi-center clinical study. The target population of this study was adult males and females, aged 18 to 65 years old, patients undergoing elective general anesthesia surgery, the expected operation time was less than 4 hours, and neostigmine injection; ASA health status classification I~II; 18≤ Body mass index (BMI) ≤25. Qualified subjects were given glycopyrrolate injection or atropine injection in combination with neostigmine injection after the last stitch of the skin was sutured and more than 30 minutes after the last muscle relaxant administration. This should antagonize muscarinic side effects, namelybradycardia.

Glycopyrrolate injection blocked the muscarinic effect caused by cholinesterase inhibitors, prevented bradycardia, and achieved a more stable heart rate change after surgery [18-20]. According to the actual needs of clinical practice, the stability of the heart rate within a period of time after surgery is more clinically meaningful than the stability of the heart rate at a time point. Therefore, according to the reference, time was the horizontal axis and the change in heart rate compared with baseline was the vertical axis. The area under the curve was the main indicator of effectiveness. According to literature reports, with a single intravenous injection of glycopyrrolate at 6 μg/kg, the time to reach peak pharmacodynamics is about 9 minutes. Therefore, this study evaluated the change in heart rate within 15 minutes after the medication compared to baseline. The main efficacy indicator of this study was the AUC of the difference between heart rate and baseline heart rate within 15 minutes after administration. The results showed that the AUC of the difference between heart rate and baseline heart rate in the test group was smaller than that of the control group. This suggests that the degree of change in the center rate of patients in the experimental group was significantly smaller than that in the control group.

In terms of data management and analysis, the research data were collected by eCRF (Oracle OCRDC5.1 version system). Data management was overseen by the CRO Xingdetong Medical Data Management Department. It was necessary to ensure the authenticity, completeness and integrity of clinical trial data. The data management process must comply with ICH GCP, FDA 21 CFR Part 11 and other specifications to ensure the traceability of clinical trial data. The analysis was carried out in two aspects: the full analysis set (FAS) population and the per-protocol (PP) population. The full analysis population was based on the ITT principle: all the cases that have been randomized and used at least one drug formed the FAS population of this study. The missing data of the main efficacy indicators in the FAS set were supplemented by the previous last observation data carry-over (LOCF) method; the population that meets the protocol, also called the Per Protocol Set (PPS, Per Protocol Set), referred to the cases in which the trial drug treatment is completed according to the protocol regulations, with no important protocol deviation, and all the evaluation content was completed.

The results of this study showed that 1 minute after the end of administration, the heart rates of the two groups of patients began to increase and reached the highest at 3 minutes, showing the effect of anticholinergic drugs. Subsequently, the heart rate gradually slowed down for 3-15 minutes, showing the effect induced by neostigmine. The heart rate changed in the two groups of patients, except immediately after the end of the administration and 3 minutes after the administration. Compared with the other time points, the heart rate changes of the control group were significantly greater than in the test group. The series of results in this study suggest that the anticholinergic effect was first displayed during the combined use of anticholinergic drugs and neostigmine, and the heart rate reached the highest value at 3 minutes after administration. Later, it showed a heart rate slowing effect caused by neostigmine, but the degree of heart rate change in the experimental group was significantly smaller than that of the control group ($P<0.05$). Both atropine and glycopyrrolate showed the effect of antagonizing neostigmine to slow down the heart rate, but compared with atropine, glycopyrrolate induced smaller heart rate variability.

There were 2 serious adverse reactions in the test group before and after the medication, and 1 adverse reaction in the control group. There was no significant difference in safety indicators. This suggests that under the premise of

equivalent antagonism to neostigmine, there was no significant difference between the two drugs in terms of safety.

The results of this study show that when neostigmine is used in combination with glycopyrrolate or atropine, the heart rate-increasing effect of anticholinergic drugs appears first. Glycopyrrolate and atropine both antagonized neostigmine's heart rate slowing, but glycopyrrolate was more conducive to maintaining the stability of heart rate.

Disclosure of conflict of interest

None.

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References

[1] Nemes R, Fülesdi B, Pongrácz A, Asztalos L, Szabó-Maák Z, Lengyel S and Tassonyi E. Impact of reversal strategies on the incidence of postoperative residual paralysis after rocuronium relaxation without neuromuscular monitoring: a partially randomised placebo controlled trial. Eur J Anaesthesiol 2017; 34: 609-616.

[2] Hunter JM. Reversal of residual neuromuscular block: complications associated with perioperative management of muscle relaxation. Br J Anaesth 2017; 119 Suppl 1: i53-i62.

[3] Zoremba N, Schälte G, Bruells C and Pühringer FK. Update on muscle relaxation: what comes after succinylcholine, rocuronium and sugammadex? Anaesthesist 2017; 66: 353-359.

[4] Luo J, Chen S, Min S and Peng L. Reevaluation and update on efficacy and safety of neostigmine for reversal of neuromuscular blockade. Ther Clin Risk Manag 2018; 14: 2397-2406.

[5] Honing G, Martini CH, Bom A, van Velzen M, Niesters M, Aarts L, Dahan A and Boon M. Safety of sugammadex for reversal of neuromuscular block. Expert Opin Drug Saf 2019; 18: 883-891.

[6] Atropine. LiverTox: clinical and research information on drug-induced liver injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.

[7] Abdelaal Ahmed Mahmoud A, Mansour AZ, Yassin HM, Hussein HA, Kamal AM, Elayashy M, Elemady MF, Elkady HW, Mahmoud HE, Cusack B, Hosny H and Abdelhaq M. Addition of neostigmine and atropine to conventional management of postdural puncture headache: a randomized controlled trial. Anesth Analg 2018; 127: 1434-1439.

[8] Howard J, Wigley J, Rosen G and D'Mello J. Glycopyrrolate: it's time to review. J Clin Anesth 2017; 36: 51-53.

[9] Lunsford CD, Ward JW, Pallotta AJ, Tusing TW, Rose EK and Murphey RS. Derivatives of 3-pyrrolidinols-I. The chemistry, pharmacology and toxicology of some N-substituted-3-benzhydryloxypyrrolidines. J Med Pharm Chem 1959; 1: 73-94.

[10] Klingenmaier C, Bullard R, Thompson D and Watson R. Reversal of neuromuscular blockade with a mixture of neostigmine and glycopyrrolate. Anesth Analg 1972; 51: 468-472.

[11] Ramamurthy S, Shaker MH and Winnie AP. Glycopyrrolate as a substitute for atropine in neostigmine reversal of muscle relaxant drugs. Can Anaesth Soc J 1972; 19: 399-411.

[12] Kuan AHY and Tey HL. Topical glycopyrrolate followed by microneedling: a novel treatment option for eccrine hidrocystomas. J Dermatolog Treat 2020; [Epub ahead of print].

[13] Hristovska AM, Duch P, Allingstrup M and Afshari A. Efficacy and safety of sugammadex versus neostigmine in reversing neuromuscular blockade in adults. Cochrane Database Syst Rev 2017; 8: CD012763.

[14] Leybaert L, Lampe PD, Dhein S, Kwak BR, Ferdinandy P, Beyer EC, Laird DW, Naus CC, Green CR and Schulz R. Connexins in cardiovascular and neurovascular health and disease: pharmacological implications. Pharmacol Rev 2017; 69: 396-478.

[15] Donati F, Bevan JC and Bevan DR. Neuromuscular blocking drugs in anaesthesia. Can Anaesth Soc J 1984; 31: 324-336.

[16] Mirakhur RK and Dundee JW. Glycopyrrolate. Gen Pharmacol 1981; 12: 423-427.

[17] Kanto J and Katevuo K. The effect of drugs with different mechanisms of action on the contraction of the human gallbladder. Int J Clin Pharmacol Ther Toxicol 1981; 19: 303-309.

[18] Nkemngu NJ and Tochie JN. Atrio-ventricular block following neostigmine-glycopyrrolate reversal in non-heart transplant patients: case report. Anesth Prog 2018; 65: 187-191.

[19] Teng IC, Chang YJ, Lin YT, Chu CC, Chen JY and Wu ZF. Sugammadex induced bradycardia and hypotension: a case report and literature review. Medicine (Baltimore) 2021; 30; 100: e26796.

[20] Brett K and Farrah K. Sugammadex for the reversal of rocuronium-induced neuromuscular blockade in surgical patients: a review of clinical effectiveness. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2019.