Synopsis Final Clinical Study Report for Study PHYDELIO
(ICH Topic E 3 Structure and Content of Clinical Study Reports)

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2) Name of finished product	1.Anticholium® 0.02 mg/kg BW as bolus and 0.01 mg/kg BW per hour intravenously (i.v.) for 24 hours, ATC code: V03AB19 MA number: 6073341.00.00 2. NaCl isotonic physiological solution for infusion 0.9% NaCl isotonic physiological solution as bolus and intravenously (i.v.) for 24 hours ATC code: B05BB01 Fresenius Kabi 50ml/100ml/200ml MA number: 6096595.00.00 B.Braun 50ml/100ml MA number: 6726174.00.00 3. NaCl isotonic physiological solution for injection Berlin Chemie 50ml MA number: 1299.95.99
3) Name of active substance	Ad 1. Anticholium® 2 mg solution for injection 1 ampoule of 5 ml contains: 2.0 mg physostigmine salicylate. Excipient with known effect: sodium pyrosulfite 2.5 mg / 5 ml (corresponding to 1.68 mg SO_2) Ad 2. 0,9% NaCl isotonic physiological solution for infusion contains: Sodium chloride 9,0 g Ad 3. 0,9% NaCl isotonic physiological solution for injection contains: Sodium chloride 9,0 g
5) Title of study (according to EudraCT)	Perioperative physostigmine prophylaxis for liver resection patients at risk for delirium and postoperative cognitive dysfunction Perioperative Gabe von Physostigmin bei Leberteilresktion zur Prophylaxe von Delir und postoperativem kognitivem Defizit Code number: PHYDELIO ISRCTN18978802

EudraCT-Number of the protocol: 2008-007237-47

BfArM approval:
Date of approval: 17.12.2008 (Protocol V1.1)
Number: 4034796

1. Amendment Protocol V1.2: 25.05.2011
2. Amendment Protocol V1.3: 21.03.2012
3. Amendment Protocol V1.4: 01.01.2013
4. Amendment Protocol V1.5: 11.12.2014
5. Amendment Protocol V1.6: 20.01.2016
6. Amendment Protocol V1.7: 12.05.2016
7. Amendment New SMPC Anticholium 0872015: 01.09.2016
8. Amendment Protocol V1.8: 15.08.2017

Ethical approval:
Date of approval: 15.01.2009 (Protocol V1.1)

1. Amendment Protocol V1.2: 13.05.2011
2. Amendment Protocol V1.3: 29.03.2012
3. Amendment Protocol V1.4: 07.01.2013
4. Amendment Protocol V1.5: 30.12.2014
5. Amendment Protocol V1.6: 15.02.2016
6. Amendment Protocol V1.7: 17.06.2016
7. Amendment Protocol V1.8: 15.08.2017

Date	**Amendment**
25.05.2011	Amendment 01 changes of the protocol V1.1 to V1: 2 Primary reason for amendment changes concern a specification of the inclusion and exclusion criteria. In addition, the goal-directed hemodynamic protocol should be extended by a hemodynamic factor based on previous publications in order to increase the safety of the study. Safety documentation changes have been made to increase data quality.
29.03.2012	Amendment 02: Primary reason for amendment changes concern a specification of the inclusion and exclusion criteria and the extension of the recruitment period. The secondary endpoint polysomnographic evaluation of sleep quality has been added.
07.01.2013	Amendment 03: Primary reason for amendment was to include the secondary endpoint hemodynamic and heart frequency and further laboratory markers (kidney and hematology). Notification of a the other representative of the principle investigator.
30.12.2014	Amendment 04: Primary reason for the amendment was the notification of the result of the blinded interim analysis (recalculation of case number) after 200 included patients in october 2014: an unblinded interim analysis should follow if 284 patients are recruited = final case number) and the extension of the recruitment period (8 years). Collection of additional blood samples (immunological parameters, expression of clock genes and light level and light frequencies, cortisol and nagalase (reference samples as well) for possible correlation to early delirium and additional measurement of heart rate variability and transthoracic echocardiography.
15.02.2016	Amendment 05: Primary reason for the amendment was the notification of a planned second blinded interim analysis (if 246 patients are recruited) for the recalculation of case number and a planned stop of recruitment; the time of recruitment was set to 7 years and 11 months.

	The secondary endpoints were reduced: no analysis of blood samples (expression of clock genes and no cortisol of hair samples), no measurement of light level and light frequencies and sleep architecture.
17.03.2016	**Recruitment stop**: After inclusion of 281, of which 35 patients were declared drop-outs a blinded interim analysis was conducted. In the course of this clinical trial, no suspected unexspected serious drug reactions occurred during the reporting period (11.08.2009- 17.03.2016). The risk-benefit ratio remained unchanged positive during this clinical trial. The follow up examinations (3 months and one year) of the study patients were not interrupted.
17.06.2016	Amendment 06: primary reason for the amendment was an additional POCD control group (45 patients); 25 new ASA II/III patients have to be included with informed consent forms in the cklinical trial, so planned number of subjects to be included = 271. Further 20 patients are matched from the observational study (EA1/296/12 „ Cognitive Outcome after two-stage Liver-Operation – a Pilotstudy)", in which 20 ASA II and III POCD control patients have been included since 27.07.2015 with analogous inclusion and exclusion criateria (ClinicalTrials.govIdentifier:NCT01809782).
01.09.2016	Amendment 07 BfArM: primary reason for amendment was the new SMPC Anticholium 08/2015
15.08.2017	Amendment 07 Ethics commission/Amendment 08 BfArM: Primary reason was the notification that the evaluation report of 271 included patients will be submitted in September 2017 and the and last patient last visit of the POCD control group patient is scheduled for August 10th 2017; application of another representative of the principle investigator. Nomination of an additional laboratory "Sciomics GmbH" for analyzes of reference samples.

6) Investigators

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7) study centers	Monocenter, 1 active center: Department of Anesthesiology and Operative Intensive Care Medicine Campus Charité Mitte and Campus Virchow - Klinikum Charité - University Medicine Berlin Campus Virchow - Klinikum Augustenburger Platz 1 13353 Berlin Germany
8) Publication of the trial (Reference)	Publication is planned in a peer reviewed journal. Planned publication title: Postoperative Delirium and Long-Term Cognitive Dysfunction Are Not Reduced By Physostigmine – a double-blinded randomized controlled trial ISRCTN18978802
9) Study period	First patient, first visit: 11.08.2009 Last patient, last visit: 10.08.2017 (last POCD control subject) Recruitment stop: 17.03.2016 At the 17.03.2016 the recruitment of the trial was stopped (281 study patients were already randomized and included, of these were 246 study patients per protocol and 15 per intention to treat and 20 Drop-outs)), because of the second blinded interim analysis.

	Study ending: 19.10.2017 The cancellation of the study took place in October 2017 The study was designed as an internal pilot study with recalculation of sample size. Supposing a POCD-rate of 30%, a rate of delirium of 25% and a reduction of both by 50%, a draft sample size calculation resulted in 200 patients per group. Since this data was not sufficiently backed up by greater studies, the study was started with 100 patients each and performed blinded interim analyses in 2014 and 2017, respectively. Therefore, after 200 patients (100 per group) and after 246 patients (123 per group), a blinded interim evaluation was performed to re-estimate the number of cases. Result of second interim analysis (statistical report 19.10.2017): In the second interim analysis with 246 patients (123 per group), the resulting new case number was already sufficient for the evaluation of the primary target parameter (Delir / POCD) based on a acceptable power of at least 75%. Neither a higher serious adverse event (SAE) rate nor a higher adverse event (AE) rate was clearly recognizable in the Anticholium® arm based on statistical evidence. No Suspected Unexspected Serious Adverse Drug Reactions (SUSAR) were reported. The risk-benefit ratio of Anticholium® in this clinical trial is favourable.
10) Phase of development	Therapeutic use (Phase IV)
11) Objectives	Differences of treatment (Anticholium® versus placebo) in patients undergoing liver resection regarding their mental outcome (delirium and post-operative cognitive deficit). The aim of this study is to find out whether giving patients the drug Physostigmine (Anticholium®) reduces the incidence of delirium and POCD. Primary outcome parameters 1.Delirum according to Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV), measured pre-operatively and up to hospital discharge 2.POCD (post-operative cognitive deficit) according to Cambridge Neurophysiological Test Automated Battery (CANTAB), measured preoperatively, on the 7th, 90th and 365th post-operative day. For the POCD evaluation, a POCD-control group of 25 additionally recruited patients with systemic disease and 20 patients with systemic disease from another non-interventional study (EA1/296/12 Code: Cognitive Outcome after two-stage Liver-Operation)) (ClinicalTrials.gov Identifier: NCT01809782) are analyzed. Secondary outcome parameters 1. Diagnostics of delirium: 1.1. Confusion Assessment Method (CAM)/Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) 1.2. Intensive Care Delirium Screening Checklist (ICDSC) 1.3. Delirium Detection Scale (DDS) 1.4. Delirium Rating Scale (DRS) 1.5. Nursing Delirium Screening Scale (NuDESC) 2. Evaluation of intensive care unit performance: 2.1. Simplifies Acute Physiology Score (SAPS II) 2.2. Acute Physiological and Chronic Health Evaluation (Apache II) 2.3. Sequental Organ Failure Assessment (SOFA) 2.4. Therapeutic Interventions Scoring System (TISS)

	2.5. Richmonds Agitation Sedations Scale (RASS) 2.6. Glasgow Coma Scale (GCS) 2.7. Risk Injury Failure Loss End Stage Kidney Disease (RIFLE) 3. Length of post-operative hospital stay, measured by Post-anaesthesia Discharge Scoring Stay (PADSS) 4. Length of post-operative intensive care unit stay according to the criteria of internal standard operating procedures (SOP) 5. Pain: 5.1. Numeric Rating Scale (NRS) 5.2. Verbal Rating Scale (VRS) 5.3. Visual Analogue Scale (VAS) 5.4. Behavioural Pain Scale (BPS) The secondary outcome parameter "Pain" will be measured pre-operatively and up to hospital discharge 6. The rate of post-operative organ dysfunctions and complications 7. Incidence of systemic inflammatory response syndrome (SIRS) and infection, measured by CDC and American Thoracic Society (ATS) criteria and via laboratory parameters of immunology 8. Quality of life questionnaires (questionnaires): 8.1 Quality of life questionnaires 36-item short form health survey (SF-36), EuroQoL instrument (EQ-5D), 8.2. Barthel Index: Activities of Daily Living/Instrumental Activity of Daily Living (ADL/IADL) and Instrumentelle Aktivität im täglichen Leben (IATL) 8.3. Geriatric Depression Scale (GDS), Cornell Depression Scale (CDS), Hospital Anxiety and Depression Scale deutsche Version (HADS-D) 9. Mortality, postoperative survival after 90 days, after 6 months and after one year 10. Immune parameters 11. Parameters of Hematology (Sysmex Europe GmbH) 12. Parameters of renal function 13. Cortisol level in all study patients from amendment 04, 14. Venous return in all study patients from amendment 04 15. Heart rate variability in all study patients from amendment 04 16. Calcification propensity in all study patients from amendment 04 17. Transthoracacic echocardiography in all study patients from amendment 04 18. Frequency of Delirium, Duration of Delirium and Delirium-free days: 18.1. Confusion Assessment Method (CAM)/Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) 18.2. Intensive Care Delirium Screening Checklist (ICDSC) 18.3. Delirium Detection Scale (DDS) 18.4. Delirium Rating Scale (DRS) 18.5 Nursing Delirium Screening Scale (NuDESC) 18.6 Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) 19. Frequency of subsyndromal Delirium, subsyndromal Duration of Delirium and subsyndromal Delirium-free days: 19.1 Intensive Care Delirium Screening Checklist (ICDSC) 19.2 Delirium Detection Scale (DDS) 19.3 Nursing Delirium Screening Scale (NuDESC)
12) Methodology	A prospective, randomised, controlled, double-blinded, two-armed single centre pilot study trial with sample size recalculation

	Study group: Patients of both genders, aged greater than or equal to 18 years with planned elective liver resection with or without additional elective surgery in the same session were screened during their visit in the preoperative anesthesia clinic and evaluated regarding regarding their inclusion criteria. Treatment /placebo assignment was performed due to randomization. A unique identifier was assigned to each patient included. The allocation of the patients to the therapy groups took place in the ratio 1: 1 taking into account the following stratifications: Stratum1: • Patients with ASA I and II • Patients with ASA III and IV Stratum 2: • Planned trisectomy • Planned hemihepatectomy Baseline period: Baseline assessment including baseline charactersitics and sociodemgraphic data and further investigations regarding the planned operation were performed after the informed consent in the preoperative anesthesia clinic. Preoperative liver function was evaluated using liver enzymes, INR, albumin levels and the LiMAx test serving as a surrogate of prospective liver function capacity. During the screening process, special attention was paid to rule out preoperative dementia. Cognitive testing included the Modified Mini Mental State Examination (MMSE), CANTAB test battery along with delirium screening prior to surgery and questions on sleep quality. Depression was identified using the Cornell Depression Scale (CDS) and the Hospital Anxiety and Depression Scale. (HADS-D, German version). If patients were 65 years or older, we additionally applied the Geriatric Depression Scale. Information on comorbidities was collected and classified according to ASA (American Society of Anesthesiologists) classification and Charlson´s Comorbidity Index (CKI). Additionally, patients were asked to report on their health-related quality of life measured by standardized questionnaires including the 12-Item Short Form Health Survey (SF-12), the EQ-5D and Activities of Daily Living (ADL) and Instrumental ADL (IADL). Treatments to be compared: Patients were randomized either to receive physostigmine (Anticholium®, Köhler comp, Germany) or placebo starting after induction of anesthesia for 24 hours. Procedures until postoperative day 30: All patients received guideline oriented anaesthesiological and surgical treatment according to our standard operating procedures. Intraoperative data including neuro-monitoring for guidance of anesthesia and ICU admission scores were documented.

	For 7 postoperative days the overall complication rate, which included infections, cardiopulmonary insufficiency, delirium, bleeding and other complications, was recorded. All infections were diagnosed according to criteria recommended by the Centers for Disease Control and Prevention. From day 1 to day 7 after surgery, phlebotomy was performed every morning, Labor Berlin – Charité Vivantes GmbH carried out the analysis of all samples. The results of routine blood samples were also recorded. Non-routine samples were stored, processed, and analyzed in the research laboratory of the Department of Anesthesiology and Operative Intensive Care Medicine CCM/CVK, Charité – Universitätsmedizin Berlin. IL-8 from whole blood was determined preoperatively, 1 and 7 days after surgery by the Inst. of Immunology. Circulating IL-8, which can be measured in plasma, higher amounts of IL-8 in the blood is cell-associated and binds to the Duffy antigen on the surface of erythrocytes. With the method of total IL-8 determination after erythrocyte lysis, considerably higher values are regularly found, which can be easily assessed in the course of a disease. Total IL-8 content was determined after lysing 100 µl EDTA whole blood with 100 µl 1% Triton-X100 in RPMI medium. IL-8 in the resulting blood lysate was measured with the IMMULITE® IL-8 immunoassay (DPC Biermann). The detection limit of the assay is 5 ng/L. The length of intensive care unit (ICU) and hospital stays was documented. Follow up visits: The follow up was until1 year after drug application for all arms. The cognitive tests and quality of life measurements, questions on sleep quality/ADL/IADL were repeated on the 7th, 90th and 365th days after surgery. **POCD Control Group** The trial also included 45 POCD-control group participants. The received cognitive tests and quality of life measurements at baseline, 7days, 90 and 365 days later. Data were blinded until the analysis had been completed, protocol violations were documented.
Number of patients (planned and analyzed)	Study group <u>Planned number of patients:</u> Planned number of patients was 400 (200patients in each group: physostigmine (Anticholium®), placebo group (0,9% NaCl). <u>Sample size calculation</u> The study was designed as an internal pilot study with recalculation of sample size. Supposing a POCD-rate of 30%, a rate of delirium of 25% and a reduction of both by 50%, a draft sample size calculation resulted in 200 patients per group. Two group Fisher’s test of equal proportions (odds ratio = 1) (equal n's)

	Delir	POCD
Test significance level, □	0.025	0.025
1 or 2 sided test?	2	2
Group 1 proportion, □1	0.250	0.300
Group 2 proportion, □ 2	0.125	0.150
Power (%)	81	90
n per group	**200**	**200**

Since this data was not sufficiently backed up by greater studies, we started the study with 100 patients each and performed blinded interim analyses in 2014 and 2017, respectively. Therefore, after 200 patients (100 per group) and after 246 patients (123 per group), a blinded interim evaluation was performed to re-estimate the number of cases. In the second interim analysis with 246 patients (123 per group), the resulting new case number was already sufficient for the evaluation of the primary target parameter (Delir / POCD) based on a acceptable power of at least 75%.

Analyzed number of patients:
1255 study patients were assessed for eligibility between August 2009 and March 2016. Of these patients 216 refused to participate, 745 fulfilled exclusion criteria and 13 patients were not included due to other reasons (Figure 1). In total 281 patients were allocated to participate in this study.

Drop-Out:
20 Patients were declared as Drop-Outs:
From the 281 included patients, 20 cases were excluded from analysis due to withdrawal of patient´s consent, cancellation of surgery or occurrence of exclusion criteria after inclusion. They received no study medication.

Per- protocol analysis:
This analysis was restricted to the participants who fulfil the protocol in the terms of the eligibility, interventions, and outcome assessment: From 261 patients 245 patients could be analyzed per protocol.

Intention to treat analysis
In the following 15 patients protocol deviations occurred (analysis intention to treat):

6 protocol violations in the Verum group:

No.	Pseudonym	Major protocol violation
1.	PH11120	Baseline – POCD-measurement is not complete
2.	PH12005	Missing 4 POCD measurements
3.	PH11113	Study medication < 21 hours
4.	PH12041	Study medication < 21 hours
5.	PH21036	Study medication < 21 hours
6.	PH21041	Study medication < 21 hours

9 protocol violations in the Placebo group:

No.	Pseudonym	Major protocol violation
1.	PH11006	Missing 4 POCD measurements

	2.	PH11010	Missing 4 POCD measurements
	3.	PH11088	Missing second, third and fourth POCD measurement
	4.	PH11098	Missing second, third and fourth POCD measurement
	5.	PH11138	Missing third and fourth POCD measurement
	6.	PH12002	Missing 4 POCD measurements
	7	PH12034	Missing second, third and fourth POCD measurement
	8	PH11130	Study medication < 21 hours
	9.	PH12049	Study medication < 21 hours

261 patients were analyzed according to intention to treat.

POCD control group

Planned number of POCD control group patients:
Planned number of POCD control patients was 1/5 - 1/6 from the full sample.

Analyzed number of POCD control group patients:
25 POCD control group patients were assessed for eligibility between 26. July 2016 and 10. August 2018. Of these patients no patient refused to participate.
20 patients with systemic disease from another non-interventional study between July 2015 and June 2017 (EA1/296/12 Code: Cognitive Outcome after two-stage Liver-Operation)) (ClinicalTrials.gov Identifier: NCT01809782) were analyzed.
In total 45 POCD control group patients were allocated to undergo POCD evaluation in this study.

14) Diagnosis and main criteria for inclusion

Study group (Physostigmine and Placebo group):
Inclusion criteria

1.Written informed consent
2.Age greater than or equal to 18 years
3.Scheduled for liver resection with or without additional surgery in the same session
4.No participation in another clinical trial during the trial and one month before inclusion
5.Negative pregnancy testing (beta-human chorionic gonadotrophin [B-HCG])

Study group (Physostigmine and Placebo group):
Exclusion criteria

1.Aged less than 18 years
2.Pregnancy or lactation
3.Lacking willingness to save and hand out pseudonymised data within the clinical study
4. Accommodation in an institution due to an official or judicial order
5. Advanced disease of the oesophagus of nasopharyngeal cavity
6. Illiteracy
7. Unability of German language use
8. Visual and acustical impairment
9. Score on the mini mental state examination (MMSE) at screening of 23 or less

	10. American Society of Anaesthesiologists (ASA) Classification greater than IV 11. Wedge resection 12. Ascertained psychiatric disease 13. Intake of psychotropic drugs (including sleeping pills and Benzodiazepine) 14. Acquired immune deficiency syndrome (AIDS) (Centers for Disease Control and Prevention [CDC] - classification "C") 15. Neoadjuvant Chemo- or radiotherapy within the last 28 days 16. Rheumatoid diseases 17. Colitis ulcerosa 18. Vagotomy 19. Symptomatic bradycardia 20. Known prolongation of QTc - interval greater than 456 ms 21. Regular intake of amiodarone or cholinesters 22. Vagus nerve stimulation in epilepsy 23. Bronchial asthma 24. Allergies and sensibility to physostigmine salicylate 25. Operations in the area of the oesophagus or nasopharynx within the last two months 26. Gangrene 27. Dystrophia myotonica 28. Intoxications by irreversibly acting cholinesterase inhibitor, e.g. organophosphate 29. Closed craniocerebral trauma with medical intervention within one year before inclusion of this study 30. Parkinsons disease 31. Positive history of a depolarisation block after application of a depolarising muscle relaxant or rather after basal narcosis with a depolariser 32. Coronary heart disease Canadian Society of Anaesthesiologists criteria (CSC) stadium IV or the presentation of a coronary heart disease that needs intervention 33. Symptomatic obstructions in gastrointestines and efferent urinary tract 34. Symptomatic cardiac arrythmia 35. Staff of Charite University hospital Berlin, Virchow Klinikum 36. Allergies to any ingredient of the electrode fixing material (only for participants of sleep stage assessment) POCD control group: Inclusion criteria 1.Written informed consent 2.Age greater than or equal to 18 years 3.Patients with systemic disease 4.American Society of Anesthesiologists (ASA) Classification score II or III 5.Not any surgery performed in the last half year before inclusion 6.Not scheduled for any elective surgery within the upcoming year POCD-control group: Exclusion criteria 1.Mini-Mental-State-Examination at baseline

	assessment ≤ 23 Points 2. Neuropsychiatric morbidity that limits the conduction of the neurocognitive testing 3. Visual defect or hearing impairment 4. Regular intake of psychotropic drugs (including sleeping medication and benzodiazepines)
15) Test product, dose and mode of administration, batch number	Perfusor of Physostigmine (Anticholium®) in a dose of 0.02 mg/kg BW as bolus and 0.01 mg/kg BW per hour intravenously (i.v.) for 24 hours; Manufacturer: Dr. Franz Köhler Chemie GmbH Batch numbers: 1422551, 1219251,1033451, 082661.
16) Duration of treatment	The maximum duration of the study protocol (administration of Anticholium) is 24 hours after induction of anesthesia. The study patients were followed up for30 postoperative days in the hospital and until 90 and 365 days in follow up visits.
17) Reference therapy, dose and mode of administration, batch number	.9% NaCl isotonic physiological solution as bolus and for 24 hours after induction of anesthesia. Manufacturer: Fresenius Kabi Batch numbers: 50 ml: 14DH21,14DD26, 14DC22, 14DB28, 14CL36,14CF26, 14EK25, 14CF39, 14EE42, 14ED41, 14DK23, 14EC35, 14EC31, 14EB33, 14DK38, 14EA25, 14CK38, 14DH38, 14DK22, 14DH21, 14DI38, 14DE29 ,14CD33, 14CE20,134101, 134103, 14GD24, 14GF28, 14GB27, 14FK34, 14FM21, 14FK21 14FF28, 14FH37, 14FI58, 14FI34, 14FH21, 14FG28, 14FF30, 14FF27, 14FF30, 14FF22, 14FF23, 14FE32, 14FD26, 14FL29 14FC29, 14FB29, 14EM25, 14FA21, 14EM37. 100ml: 19EG13WB. 250ml: 15EF046B1, 15FD034B1. Manufacturer: B.Braun: Batch numbers: 50ml:152438091, 151148091, 126050, 151218091, 145118091, 143918093, 143618091, 143518091, 143118091, 143618091, 143518091, 143118091, 142718091, 142118091, 142758091, 142318092, 141538091, 141118091, 141328091, 135018091, 140858091, 140618091, 140358091, 134718092, 134218091, 133258091, 133918091, 133758092, 133568092, 132478091, 121938091, 113348091, 114218091, 1057A191. 100ml: 1540040, 154878092, 154548091, 154158091, 153118091, 153148091, 154078091, 153838091, 152918091, 111248091, 152618091, 152548091, 152118092, 151738091, 1540006, 1540013, 1540011, 15B2582004, 1450011, 1440110, 1540004, 1540001, 1440103, 1440098, 1440080, 1440083, 1440068, 1440071, 1440061, 1440011, 134101, 134103 Manufacturer: Berlin-Chemie Batch numbers: 50ml: 1065A191, 111248091

18) Criteria for evaluation: Efficacy and Safety	**Evaluation of Efficacy** Primary outcome parameters 1.Delirum according to Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV), measured pre-operatively and up to hospital discharge was the primary efficacy variable. 2.POCD (post-operative cognitive deficit) was the second primary efficacy variable. POCD according to Cambridge Neurophysiological Test Automated Battery (CANTAB), measured preoperatively, on the 7th, 90th and 365th post-operative day. For the POCD evaluation, a POCD-control group of 25 additionally recruited patients with systemic disease and 20 patients with systemic disease from another non-interventional study (EA1/296/12 Code: Cognitive Outcome after two-stage Liver-Operation)) (ClinicalTrials.gov Identifier: NCT01809782) are analyzed. Secondary outcome parameters 1. Diagnostics of delirium: 1.1. Confusion Assessment Method (CAM)/Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) 1.2. Intensive Care Delirium Screening Checklist (ICDSC) 1.3. Delirium Detection Scale (DDS) 1.4. Delirium Rating Scale (DRS) 1.5. Nursing Delirium Screening Scale (NuDESC) 2. Evaluation of intensive care unit performance: 2.1. Simplifies Acute Physiology Score (SAPS II) 2.2. Acute Physiological and Chronic Health Evaluation (Apache II) 2.3. Sequental Organ Failure Assessment (SOFA) 2.4. Therapeutic Interventions Scoring System (TISS) 2.5. Richmonds Agitation Sedations Scale (RASS) 2.6. Glasgow Coma Scale (GCS) 2.7. Risk Injury Failure Loss End Stage Kidney Disease (RIFLE) 3. Length of post-operative hospital stay, measured by Post-anaesthesia Discharge Scoring Stay (PADSS) 4. Length of post-operative intensive care unit stay according to the criteria of internal standard operating procedures (SOP) 5. Pain: 5.1. Numeric Rating Scale (NRS) 5.2. Verbal Rating Scale (VRS) 5.3. Visual Analogue Scale (VAS) 5.4. Behavioural Pain Scale (BPS) The secondary outcome parameter "Pain" will be measured pre-operatively and up to hospital discharge 6. The rate of post-operative organ dysfunctions and complications 7. Incidence of systemic inflammatory response syndrome (SIRS) and infection, measured by CDC and American Thoracic Society (ATS) criteria and via laboratory parameters of immunology 8. Quality of life questionnaires (questionnaires): 8.1 Quality of life questionnaires 36-item short form health survey (SF-36), EuroQoL instrument (EQ-5D), 8.2. Barthel Index: Activities of Daily Living/Instrumental Activity of Daily Living (ADL/IADL) 8.3. Geriatric Depression Scale (GDS), Cornell Depression Scale (CDS), Hospital Anxiety and Depression Scale deutsche Version (HADS-D) 9. Mortality, postoperative survival after 90 days, after 6 months and after one year 10. Immune parameters

	11. Parameters of Hematology (Sysmex Europe GmbH) 12. Parameters of renal function 13. Cortisol level in all study patients from amendment 04, 14. Venous return in all study patients from amendment 04 15. Heart rate variability in all study patients from amendment 04 16. Calcification propensity in all study patients from amendment 04 17. Transthoracacic echocardiography in all study patients from amendment 04 18. Frequency of Delirium, Duration of Delirium and Delirium-free day 19. Frequency of subsyndromal Delirium, subsyndromal Duration of Delirium and subsyndromal Delirium-free days **<u>Evaluation of Safety</u>** Adverse events
19) Statistical methods	The study was designed as an internal pilot study with recalculation of sample size. Supposing a POCD-rate of 30%, a rate of delirium of 25% and a reduction of both by 50%, a draft sample size calculation resulted in 200 patients per group. Since this data was not sufficiently backed up by greater studies, we started the study with 100 patients each and performed blinded interim analyses in 2014 and 2017, respectively. Therefore, after 200 patients (100 per group) and after 246 patients (123 per group), a blinded interim evaluation was performed to re-estimate the number of cases. In the second interim analysis with 246 patients (123 per group), the resulting new case number was already sufficient for the evaluation of the primary target parameter (Delir / POCD) based on a acceptable power of at least 75%. After the second interim analysis the data were unblinded accordingly. Results are expressed as median (25% - 75% quartiles), or frequencies (%), respectively. The primary endpoints (POD and POCD) as frequencies were tested by the exact Chi-Square-test; the same test was applied for other qualitative data. After checking the distributions for normality, differences between the regarded groups in terms of interesting continuous clinical parameters were tested by using non-parametric exact Mann-Whitney tests for independent groups. Associations between POCD and interesting clinical factors were not only univariately proved, but also multivariabely using the logistic regression analysis with a two-step reduction process: 1st univariate logistic regressions for each factor and selection of significant variables, 2nd stepwise multivariable backward selection. Odds ratios (OR) with 95%-confidence intervals (CI) and the corresponding p-values were calculated for each risk factor. Changes in interesting clinical outcomes with respect to time were analyzed using nonparametric analysis of longitudinal data in a two-factorial design (1st (independent) factor: groups, 2nd (dependent) factor: repetitions in time) , package ‘nparLD’, in R software (R Core Team 2017) including an interaction between time and treatment arm as indicator for group differences. Relative effects (treatment effect of the regarded group, relative to all groups, therefore to a “mean“ treatment effect) were determined. After global testing, post-hoc analyses were carried out to detect specific differences (of clinical interest) between certain time points (Wilcoxon tests) as well as with respect to the 1st factor (groups) for fixed times (Mann-Whitney tests). Mortality rates in both treatment groups were estimated according to Kaplan-Meier analysis and reported with 95% confidence intervals. Survival curves until 90 days, 180 days and 12 months were compared between treatment groups using the Breslow test (Generalized Wilcoxon). A two-tailed p-value < 0.05 was considered statistically significant. All tests of secondary endpoints have to be

	understood in the area of exploratory data analysis. No adjustments for multiple testing have been made. All numerical calculations were performed with IBM© SPSS© Statistics, Version 25, © Copyright 1989, 2010 SPSS Inc. Calculations or R Software
20) Summary Conclusion Efficacy Results Safety Results Conclusion	ANALYZED DATA Following the data collection was carried out the investigators performed a detailed plausibility check of the data. Only after approval by the clinical monitor the data has been transferred to the database. Within the database, also a plausibility check under the 4-eye-principle by two investigators took place. After completion of the database, the randomization group was unblended and the whole database was transferred to the statistician Prof. Dr. Klaus-Dieter Wernecke. Of the 261 patients, 246 patients were treated and evaluated in accordance with the study protocol or underwent minor protocol violations. In the remaining 15 patients were major protocol violations before, so they were evaluated “intention-to-treat”. Serious protocol violations, which lead to the ITT analysis include: 1. Study medication < 21 hours 2. Missing 4 POCD measurements 3. Missing 3 POCD measurements 4. Missing 2 POCD measurements 5. Baseline POCD measurement is not completed **EFFICIACY RESULTS:** Tables and Figures are shown in the attachment of this synopsis. Efficacy: <u>Primary outcome parameters</u> Postoperative Delirium and Long-Term Cognitive Dysfunction are not reduced by physostigmine. The incidence of postoperative delirium (POD) measured by DSM-IV did not differ between physostigmine and placebo (20% [26 of 130] versus 15% [20 of 131]; p=0.334) in the first 7 days. The overall frequency of POD evaluated by DSM-IV was 26 patients (20%) in the physostigmine group compared to 20 patients (15%) in the placebo group (p=0.334) in the first 7 days. There was no difference in POCD rates between treatment groups (Table 9). <u>Secondary outcome parameters</u> For the secondary efficacy endpoints, there was, except in TISS-28 and mortality, no difference between the p-values. 1. Diagnostics of delirium: Cumulative delirium incidence (CDI) Considering all validated POD scores applied (Delirium CDI, see methods), POD was seen in 65 patients (50%) in the physostigmine and 52 patients (40%) in the placebo group (p=0.106) in the first 7 days. Incidence of Subsyndromal Delirium (SSD): Overall incidence of SSD was 70 (54%) in the physostigmine group and 77 (59%) in the placebo group (p=0.455). Delirium Rating Scale (DRS) was not evaluated. 2. Evaluation of intensive care unit performance:

	SOFA-score Morbidity in ICU according to maximum SOFA-score and according to Clavien classification was comparable in both interventional groups (Table 5). TISS-28 Scoring by TISS-28 showed comparable values on admission (Table 5), however, was decreased in physostigmine patients during ICU stay (p=0.016, Table 8). SAPS II and Apache II Morbidity according SAPS II and Apache II was comparable in both interventional groups (Table 5). RASS Level of sedation assessed by RASS was similar between groups, oversedation (RASS < -2) was observed in 16 patients both in the physostigmine group (12%) and in the placebo group (12%) (p>0.999). GCS and ROFLE criteria are not evaluated yet. 3. Length of post-operative hospital stay, measured by Post-anaesthesia Discharge Scoring Stay (PADSS) Hospital length of stay (LOS) did not significantly differ between groups (Table 5). Discharge criteria according to PADSS did not differ significantly between interventional groups at day 7 (p=0.228) (Table 5). 4. Length of post-operative intensive care unit stay ICU and hospital length of stay (LOS) did not significantly differ between groups (Table 5). 5. Pain: In total, 105 (81%) in the physostigmine group and 109 (83%) in the placebo group reported postoperative pain (p=0.632) (Table 5). 6. The rate of post-operative organ dysfunctions and complications Morbidity according to Clavien classification was comparable in both interventional groups (Table 5). 7. Incidence of systemic inflammatory response syndrome (SIRS) and infection, measured by CDC and American Thoracic Society (ATS) criteria and via laboratory parameters of immunology – Incidence of systemic inflammatory response syndrome (SIRS) and infection are not evaluated yet. Incidence of SIRS: Physostigmine 58 (7.3%) vs. Placebo 41 (5.1%), Incidence if infection: Physostigmine 11 (1.4%) vs. Placebo 18 (2.3%) 8. Quality of life questionnaires (questionnaires): Health related quality of life Health related quality of life was assessed preoperatively, at discharge, 90 and 365 days after surgery using standardized questionnaires (EQ-5D and the short form 12 survey to assess self-reported physical and mental health; SF-12). Reported quality of life was comparable in interventional groups at all time points. Activities of daily living and instrumental activities of daily living Patients were evaluated on both activities of daily living and instrumental activities of daily living preoperatively, at discharge, 90 and 365 days after surgery. No difference was seen between treatment groups at any time point. Depression

	All patients were evaluated for depression at discharge, and at 90 and 365 days after surgery. Screening tools included the Hospital Anxiety and Depression Scale – German version (HADS-D) as well as the Geriatric Depression Scale (GDS) for patients 65 years or over. Occurrence of depression was comparable between interventional groups at any time point. 9. Mortality, postoperative survival after 90 days, after 6 months and after one year A reduced mortality rate was seen in the physostigmine compared to the placebo group after 3 and 6 months [2% (95%CI 0 - 4) versus 11% (95%CI 6 - 16); p=0.002 and 7% (95%CI 3 - 12) versus 16% (95%CI 10 - 23) p=0.012, respectively] while this was less pronounced one year after surgery [20% (95%CI 13 - 27) versus 26% (95%CI 18 - 33); p=0.093]. 10. Immune parameters No statistically significant difference in IL-8 level change over time was seen between interventional groups (p=0.297) (Table 7) For CRP and PCT no significant change over time was seen neither between interventional groups nor for POD and POCD (data not shown). 11. Parameters of Hematology (Sysmex Europe GmbH)- not evaluated yet 12. Parameters of renal function- not evaluated yet 13. Cortisol level in all study patients from amendment 04 - not evaluated yet 14. Venous return in all study patients from amendment 04- not evaluated yet 15. Heart rate variability in all study patients from amendment 04- not evaluated yet 16. Calcification propensity in all study patients from amendment 04- not evaluated yet 17. Transthoracacic echocardiography in all study patients from amendment 04- not evaluated yet 18. Frequency of Delirium, Duration of Delirium and Delirium-free days 18.1 Frequency of Delirium was assessed according to DSM-4 and as CDI (cumulative delirium incidence). No difference was seen between treatment groups regarding frequencies of delirium (p>0.999 and p=0.171 respectively) (Table 4). 18.2 Duration of POD Duration of POD did not differ between treatment groups (Table 4). Delirium days did not differ between treatment groups (Table 4). 18.3 Delirium-free day were not evaluated but Delirium days: Delirium days 1.5 (1.0 - 2.5) and 1 (0.5 - 1.5) in the physostigmine and the placebo group, did not differ between treatment groups (p=0.052). 19. Frequency of SSD, Duration of SSD 19. 1 Frequency of subsyndromal Delirium was assessed as number of episodes of subsyndromal delirium. The number of episodes of SSD developed in the intervention groups were similar (p=0.237) (Table 4).

	19.2 Duration of SSD was 1.0 (0.5 - 1.5) and 1.0 (0.5 - 1.5) in the physostigmine and the placebo group, respectively (p=0.361) (Table 4). <u>Screening parameters</u> Baseline and sociodemographic characteristics Baseline and sociodemographic characteristics of the 261 patients treated per protocol are shown in Table 1 and 2 (addendum). Basic patient characteristics, sociodemographic characteristics as well as preoperative cognitive, mental and functional status did not significantly differ between groups (Tables 1 and 2). Preoperative Cognitive Impairment Out of 261 patients, 129 and 128 patients had complete baseline cognitive assessment of the physostigmine and the placebo group, respectively. Four patients (1 in the physostigmine and 3 in the placebo group) had no cognitive baseline assessment. Preoperative cognitive impairment (PreCI) was present in 17 (13%) and 23 (18%) patients in the physostigmine and placebo group, respectively. Patients with PreCI had higher IL-8 levels preoperatively and 7 days postoperatively (p=0.007) (Table 7). Risk factors for POD and POCD Potential risk factors for POCD identified in univariate testing were included in a multinomial regression analysis. Along with these, we considered other factors as clinically relevant to cognitive performance, e.g. preoperative CRP levels and presence of alcohol use disorder according to the AUDIT questionnaire. However, an association between POD and POCD was shown. POD [OR 3.84 (95%CI 1.20-12.26)], severity of delirium [OR 0.99 (95%CI 0.98-1.00)], and low intraoperative glucose (OR 1.02 (95%CI 1.00-1.04)] were influential factors for POCD in multivariable logistic regression (Table 6). Patients who developed POD showed elevated IL-8 preoperatively and on day 7 after surgery compared to non-delirious individuals (p=0.016; Table 7). SAFETY RESULTS The safety analysis is based on 261 study patients (Anticholium® (n=130),NaCl 0,9% (n=131)). 20 study patients were declared as Drop-outs prior to unblinding. They received no study medication. Of these, 20 cases were excluded from analysis due to withdrawal of patient´s consent, cancellation of surgery or occurrence of exclusion criteria after inclusion. 4 in Verum group, 9 in the Placebo group (see Figure 1). A detailed Safety analysis was sent to the authority (Bundesinstitut für Arzneimittel und Medizinprodukte; BfArM)) and the Berlin State Ethics Committee (Department for Health and Social Affairs (LAGeSo)) in October 2018. Incidence of side effects by Investigational medicinal product (IMP) 4 Adverse drug reactions (ADRs) and 2 serious adverse drug reactions (SARs) were evaluated as being definitely causally related to the IMP Anticholium®. The serious adverse drug reaction “temporary AV block” of patient (PH11113) and the serious adverse drug reaction “hemodynamic instability” of patient

	(PH21032) were definitely assessed with causality to Anticholium®. The events were fully recovered. One early unblinding (Placebo arm) was necessary during the trial in patient PH 11130 because of adverse events “Bradycardia” (38 beats per minute) and adverse event “Hypotonia” (105:55) during the operation. The study medication was stopped after 3,5 hours. The events were fully recovered. 4 non-severe drug reactions “Bradycardia” in patients PH11096, PH21038, PH11129, and PH11140 were definitely assessed with causality to Anticholium®. The events were fully recovered. During this study no Suspected Unexspected Serious Drug Reactions were observed. The use of study medications (Anticholium®/Placebo) has to be considered as safe. The risk-benefit ratio did not change during this trial. Adverse events (AEs): 1654 AEs in 260 study patients were documented in this study. In 130 study patients of the verum group 840 AEs occurred; in 130 study patients in the Placebo group 814 AEs occurred. This difference is not significant. The intensity of the adverse events was mild (79,1%) and moderate (19%) and severe (1,9%). 1650 adverse events were not considered as related to the study drugs and no unblinding was necessary. 2 AEs were evaluated as possibly causally related to the study drug, which showed after unblinding (Placebo arm) that they were no adverse drug reactions: adverse event “Sinus Tachyarrhythmia” in patient PH21039 and adverse event Bradycardia in patient PH12061. The events were fully recovered Serious adverse events (SAEs): 241 SAEs in 126 study patients occurred in this study, 104 SAEs occurred in the Verum group, 137 SAEs occurred in the Placebo group. The intensity of the SAEs was mainly moderate (52,3%), mild (29,5%) and severe (18,3%). 239l serious adverse events were not considered as related to the study drugs and no prior unblinding was necessary. The serious adverse drug reaction “temporary AV block” of patient (PH11113) and the serious adverse drug reaction “hemodynamic instability” of patient (PH21032) were definitely assessed with causality to Anticholium®. The events were fully recovered. Death of study patients: There are a total of 65 deaths of the study group patients and 1 death of the POCD control group, and 9 deaths were recorded outside the observation period (> 12 months after the operation). There were 35 patients in the placebo group and 30 patients in the anticholium group died.The occurrence of deaths

	is not causally related to the administered investigational medicinal product within the scope of the study. There was no premature un blinding. DISCUSSION AND OVERALL CONCLUSION: The primary efficacy endpoints (postoperative Delirium and Long-Term Cognitive Dysfunction) are not reduced by Physostigmine. For the secondary efficacy endpoints, there was, except in TISS-28 and mortality, no difference between the p-values in the treatment arms. The mortality was significantly reduced by physostigmine up to 6 months postoperatively. This is of importance since the pre- and intraoperative data with respect to functional status and organ function in particular parameters of liver function such as LiMAx and albumin, were similar in both groups. The reason for the difference in mortality rates remains unclear. Postoperative delirium increased the POCD risk to 3.8-fold. In addition, patients with coincident POD and POCD were at significantly increased risk to die within one year. The mortality was significantly reduced by physostigmine up to 6 months postoperatively. This is of importance since the pre- and intraoperative data with respect to functional status and organ function in particular parameters of liver function such as LiMAx and albumin, were similar in both groups. In conclusion, physostigmine did not reduce the incidence of POD or POCD. This might be partly due to its short acting mechanism and loss of effect after discontinuation of the infusion.
Date of report: 16.10.2018, amended on 16.04.2019	

Attachment: Tables and Figures

Tables and Figures:

Table 1 Basic patient characteristics

	Physostigmine (n=130)	Placebo (n=131)	p-value between intervention groups	Controls (n=45)	p-value between intervention groups and controls
Age in years	61 (51 - 69)	60 (51 - 69)	0.817‡	60 (50 - 76)	0.712#
Patients ≥ 65 years, n (%)	53 (41%)	53 (41%)	0.990†	18 (40%)	>0.999†
Sex, female/male n (%)	59 (45%)/ 71 (55%)	50 (38%)/ 81 (62%)	0.260*	23 (51%)/ 22 (49%)	0.340†
Diagnosis, n (%)					
Hepatocellular Carcinoma	20 (15%)	19 (14%)			
Cholangiocarcinoma	45 (35%)	44 (34%)			
Extrahepatic Cholangiocarcinoma / gallbladder carcinoma	3 (2%) 40 (31%)	3 (2%) 42 (32%)			
Metastases	7 (6%)	11 (8%)			
Benign tumor	3 (2%)	1 (1%)			
Infection	4 (3%)	1 (1%)			
Tumor of unknown entity	3 (2%)	1 (1%)			
Living donor hepatectomy	5 (4%)	9 (7%)	0.702†		
Other primary malignancy					
ASA, n (%)					
ASA I	13 (10%)	8 (6%)		0 (0%)	
ASA II	91 (70%)	92 (70%)		42 (93%)	
ASA III	26 (20%)	31 (24%)	0.448†	3 (7%)	0.013†
MMSE	29 (29 - 30)	29 (29 - 30)	0.608‡	30 (29 - 30)	0.469#
BMI kg/m^2	25.0 (22.9 - 28.7)	25.0 (23.1 - 28.2)	0.727‡	N/A	N/A
Obesity, n (%)	23 (18%)	22 (17%)	0.871*	N/A	N/A
CKI	6 (2 6)	6 (2 6)	0.408‡	0 (0 - 1)	<0.001#
Cardiac failure					
- NYHA I	38 (29%)	31 (24%)		3 (7%)	
- NYHA II	7 (5%)	1 (1%)	0.040†	4 (9%)	0.004†
β-Blocker, n (%)	30 (23%)	30 (23%)	>0.999*	N/A	N/A
Chronic pain, n (%)	9 (7%)	6 (5%)	0.440*	N/A	N/A
MET score group, n (%)					
group 1 (1-3pts)	2 (2%)	2 (2%)			
group 2 (4-6pts)	70 (53%)	61 (46%)			
group 3 (7-10pts)	56 (43%)	64 (49%)			
group 4 (>10pts)	2 (2%)	4 (3%)	0.616†	N/A	N/A

Malnutrition NRS, n (%) No malnutrition At risk Malnutrition	 48 (37%) 19 (15%) 63 (48%)	 57 (44%) 20 (15%) 54 (41%)	 0.493†	 N/A	 N/A
Albumin (g/L)	38 (35 – 42)	38 (34 –ä 41)	0.237‡	N/A	N/A
LiMAx (µg/kg/h)	11 (10 - 16)	13 (10 - 15)	0.355‡		
ADL	100 (100 - 100)	100 (100 - 100)	0.689‡	100 (100 - 100)	0.537#
IADL	8 (8 - 8)	8 (8 - 8)	>0.999‡	8 (8 - 8)	0.515#
Smoker, n (%)	24 (19%)	28 (21%)	0.642*	7 (16%)	0.699†
AUDIT positive, n (%)	8 (6%)	8 (6%)	> 0.999 †	2 (4%)	>0.999†

ASA: American Society of Anesthesiologists, MMSE: Mini mental state examination, BMI: Body Mass Index, CKI: Charlsons Comorbidity Index, MET: Metabolic Equivalent of Task score, NRS: Nutritional Risk Screening 2002, ADL: Activities of Daily Living, IADL: Instrumental Activities of Daily Living, AUDIT: Alcohol use disorders Identification Test (males cut-off for positive 8 points, females cut-off for positive 5 points)
N/A: not assessed.
Data are shown as median with quartiles (25% - 75%) or as frequencies n (%). P-values are calculated using the Chi Square test†, Fisher´s exact test *, exact Wilcoxon-Mann-Whitney-U test ‡ and Kruskal-Wallis test#

Table 2 Sociodemographic characteristics

	Physostigmine (n=130)	Placebo (n=131)	p-value between intervention groups	Controls (n=45)	p-value between intervention groups and controls
Educational level, n (%)					
Elementary/middle school	74 (57%)	80 (61%)		24 (53%)	
High school and above	51 (39%)	49 (37%)		19 (42%)	
other/no degree	3 (2%)	2 (2%)		2 (4%)	
N/A	2 (2%)	0 (0%)	0.782†	0 (0%)	0.461†
Civil status, n (%)					
Married	90 (69%)	90 (69%)		28 (62%)	
Married, living apart	4 (3%)	4 (3%)		0 (0%)	
Single	10 (8%)	16 (12%)		7 (16%)	
Divorced	18 (14%)	15 (11%)		5 (11%)	
Widowed	7 (5%)	5 (4%)		5 (11%)	
N/A	1 (1%)	1 (1%)	0.753†	0 (0%)	0.482†
No of people in household, n (%)					
1	24 (18%)	22 (17%)			
2	76 (59%)	84 (64%)			
3	14 (11%)	12 (9%)			
4	7 (5%)	9 (7%)			
≥5	8 (6%)	4 (3%)			
N/A	1 (1%)	0 (0%)	0.703†	N/A	N/A
Living with a partner, n (%)	97 (75%)	103 (79%)	0.547*	N/A	N/A
Monthly income, n (%)					
> 1475 €	63 (49%)	83 (63%)		30 (67%)	
< 1475 €	29 (22%)	19 (15%)		12 (27%)	
N/A	38 (29%)	29 (22%)	0.056†	3 (7%)	0.009†

Data are shown as frequencies n (%). N/A = not assessed. P-values are calculated using the Chi Square test†, Fisher´s exact test *

Table 3 Intraoperative Data

	Physostigmine (n = 130)	Placebo (n = 131)	p-value
Surgery, n (%) Extended hepatectomy Right/left hepatectomy Two or less hepatic segments Laparotomy/Miscellaneous	 46 (36%) 52 (40%) 16 (12%) 16 (12%)	 46 (35%) 51 (39%) 15 (11%) 19 (15%)	 0.464†
Pringle´s maneuvre, n (%)	55 (42%)	62 (47%)	0.316*
Duration of surgery, min	252 (210 - 338)	265 (203 - 358)	0.860‡
BIS levels	37 (32 - 41)	38 (33 - 43)	0.0642‡
Max. rate of norepinephrine, µg/kg/min	0.15 (0.08 - 0.25)	0.12 (0.08 - 0.22)	0.089‡
Σ Administered volume of study fluid, mL	99.8 (99.2 - 100.4)	99.8 (99.3 - 100.3)	0.821‡
Σ Intraoperative crystalloids, mL	2100 (1400 - 3000)	2200 (1500 - 3000)	0.874‡
Blood loss, ml	575 (300 - 888)	600 (400 - 1000)	0.366‡
Σ Units of FFP, n	3 (0 - 5)	3 (0 - 6)	0.572‡
Σ Piritramide, mg	6 (0.0 - 7.5)	4.5 (0.0 - 7.5)	0.181‡
Epidural anesthesia, n (%)	28 (22%)	28 (21%)	>0.999*

BIS = Bispectral Index; max = maximal Σ = sum PBRS: packed red blood cells, FFP: fresh frozen plasma (each unit approximately 200 mL).
Data are shown as median with quartiles (25% - 75%) or as frequencies n (%). P-values are calculated using the Chi Square test†, Fisher´s exact test * and exact Wilcoxon-Mann-Whitney-U test‡

Table 4. Duration and frequency of delirium and subsyndromal delirium within the first seven days after surgery

Delirious Patients – DSM IV	**Physostigmine (n=26)**	**Placebo (n=20)**	**p-value**
DSM 4-TR Incidence of delirium	**26/130 (20%)**	**20/131 (15%)**	**0.334**
Duration of delirium, d	0.5 (0.5 - 1.0)	0.5 (0.5 - 1.0)	0.410‡
Delirium frequency, 1, n (%) 2, n (%) 3, n (%) 4, n (%)	 23 (88%) 2 (8%) 0 (0%) 1 (4%)	 18 (90%) 2 (10%) 0 (0%) 0 (0%)	 > 0.999†
CDI Incidence of delirium	**65/130 (50%)**	**52/131 (40%)**	**0.106**
Delirium days, d	1.5 (1.0 - 2.5)	1 (0.5 - 1.5)	0.052‡
Delirium frequency, 1, n (%) 2, n (%) 3, n (%)	 53 (82%) 11 (16%) 1 (2%)	 48 (92%) 4 (8%) 0 (0%)	 0.171†
Incidence of SSD	**70/130 (54%)**	**77/131 (59%)**	**0.455**
Duration of SSD, d	1.0 (0.5 - 1.5)	1.0 (0.5 - 1.5)	0.361‡
Frequency of SSD, 1, n (%) 2, n (%) 3, n (%)	 57 (82%) 10 (14%) 3 (4%)	 54 (70%) 20 (26%) 3 (4%)	 0.237†

DSM = Diagnostic and Statistical Manual of Mental Disorders CDI = Cumulative Delirium Incidence SSD = Subsyndromal delirium

Data are shown as median with percentiles (25% - 75%). P-values are calculated using the Chi Square test†, Fisher´s exact test * and exact Wilcoxon-Mann-Whitney-U test‡

Table 5. Postoperative Course

	Physostigmine (n=130)	Placebo (n=131)	p-value
APACHE II score – PACU/ICU admission	11 (10 - 16)	13 (10 - 15)	0.579‡
SAPS II score – PACU/ICU admission	30 (21 - 39)	28 (20 - 37)	0.406‡
TISS-28 - PACU/ICU admission	25 (25 - 28)	25 (25 - 28)	0.794‡
SOFA score - maximum during ICU stay	7 (5 - 8)	7 (6 - 8)	0.459‡
Hospital Discharge Score at day 7	10 (9 - 10)	10 (9 - 10)	0.228‡
ICU LOS, h	23 (20 - 67)	22 (20 - 46)	0.497‡
Mechanical ventilation after surgery, n (%)	13 (10%)	8 (6%)	0.265*
Duration of mechanical ventilation after surgery, h	3 (0 - 6)	1.83 (0 - 3)	0.192‡
Oversedation (RASS < -2)	16 (12%)	16 (12%)	>0.999*
Pain (NRS ≥ 4 or BPS scores ≥6)	105 (81%)	109 (83%)	0.632*
Complications until day 7 after surgery Clavien grade 0, n (%) Clavien grade 1, n (%) Clavien grade 2, n (%) Clavien grade 3a, n (%) Clavien grade 3b, n (%) Clavien grade 4a, n (%) Clavien grade 4b, n (%) Clavien grade 5, n (%)	10 (8%) 44 (34%) 38 (29%) 22 (17%) 10 (8%) 5 (4%) 1 (1%) 0 (0%)	10 (8%) 38 (30%) 50 (38%) 17 (13%) 9 (7%) 2 (2%) 5 (4%) 0 (0%)	0.354†
LOS in hospital, d	12 (9 - 22)	13 (9 - 20)	0.699‡
In hospital mortality, n %	1 (1%)	8 (6%)	0.036†

APACHE: Acute Physiology and Chronic Health Evaluation within 24h after ICU admission, SAPS: Simplified Acute Physiology Score, Hospital Discharge Score according to PADS: Post-anesthetic discharge scoring system, ICU LOS: Intensive Care Unit Length of Stay, RASS: Richmond Agitation and Sedation Scale, NRS: Numeric Rating Scale, VRS: Visual Rating Scale, VAS: Visual Analogue Scale, BPS: Behavioral Pain Scale

Data are shown as median with quartiles (25% - 75%) or as frequencies n (%). P-values are calculated using the Chi Square test†, Fisher´s exact test * and exact Wilcoxon-Mann-Whitney-U test ‡

Table 6. Multivariable regression analysis, complete cases, outcome: POCD at any time point.

Risk factors	Odds Ratio (95% CI)	P-value
Delirium DSM-IV	3.840 (1.203 - 12.258)	0.023
Delirium severity	0.989 (0.976 - 1.002)	0.104
Low intraoperative glucose from ABG [mg/dL]	1.020 (1.002 - 1.039)	0.034

ABG= arterial blood gas analysis.
Final multivariable logistic regression analysis with POCD as outcome variable.
Nagelkerke R^2=0.101.

Table 7. IL-8 levels

Time Point	Preoperative	Day 1	Day 7	p-value*
Placebo (n=32)	195 (125 - 551)	712 (403 - 1282)	318 (187 - 597)	0.297
Physostigmine (n=32)	272 (101 - 454)	817 (412 - 1065)	228 (141 - 382)	
DSM-IV (Delir)				
neg (n=55)	210 (103 - 376)	760 (414 - 1102)	240 (159 - 416)	
pos (n=9)	520 (306 - 1327)	870 (392 - 1046)	390 (228 - 2642)	0.016
PreCI				
neg (n=54)	216 (112 - 450)	763 (413 - 1192)	240 (155 - 406)	
pos (n=10)	315 (146 - 832)	723 (398 - 934)	496 (249 - 1645)	0.007

Data are shown as median with quartiles (25% - 75%) * P-values refer to group differences in change over time assessed with nonparametric longitudinal data analysis
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders
PreCI: precognitive impairment
n.a. not available reference < 62pg/mL

Table 8. TISS 28 Therapeutic Interventions Scoring System

Time Point	6h post surgery	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Physostigmine	25 (25 - 28)	28 (25 - 30)	28 (24 - 31)	25 (23 - 31)	23 (20 - 31)	31 (20 - 35)	27 (25 - 32)	27 (26 - 29)
Placebo	25 (25 - 28)	28 (25 - 30)	28 (27 - 32)	28 (26 - 33)	32 (28 - 36)	35 (30 - 41)	38 (28 - 40)	36 (26 - 41)

Table 9. Frequency of pre-operative cognitive impairment (PreCI) at baseline, post-operative cognitive dysfunction (POCD) at discharge and follow-up after 3 months and 1 year.

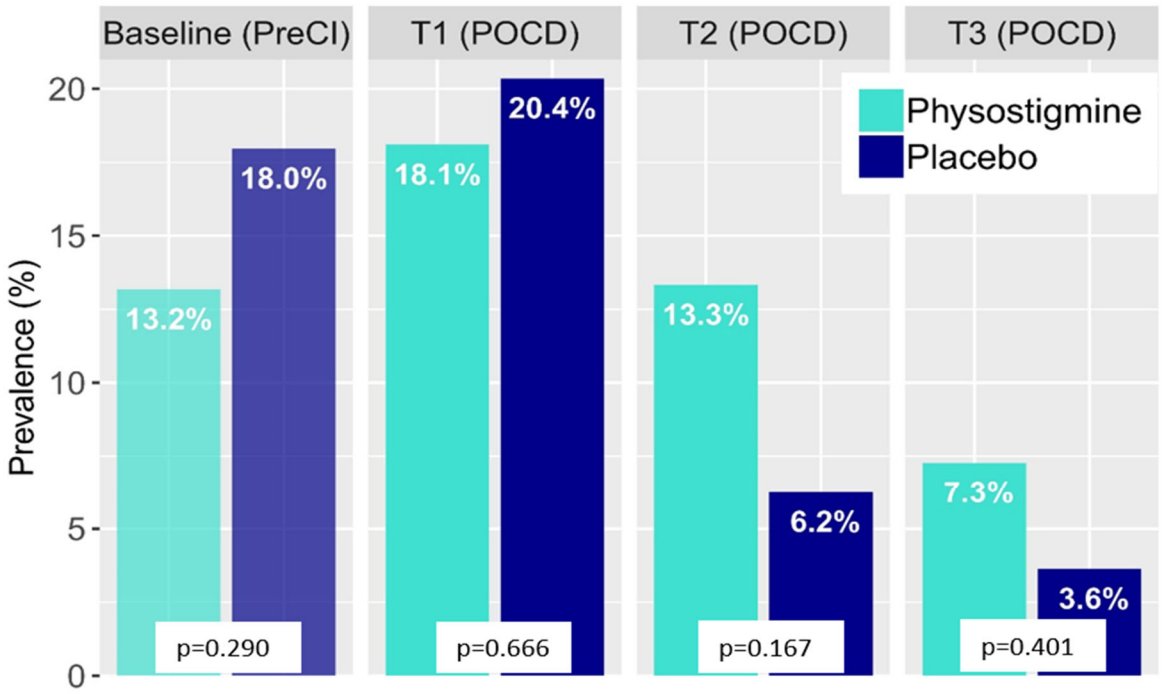
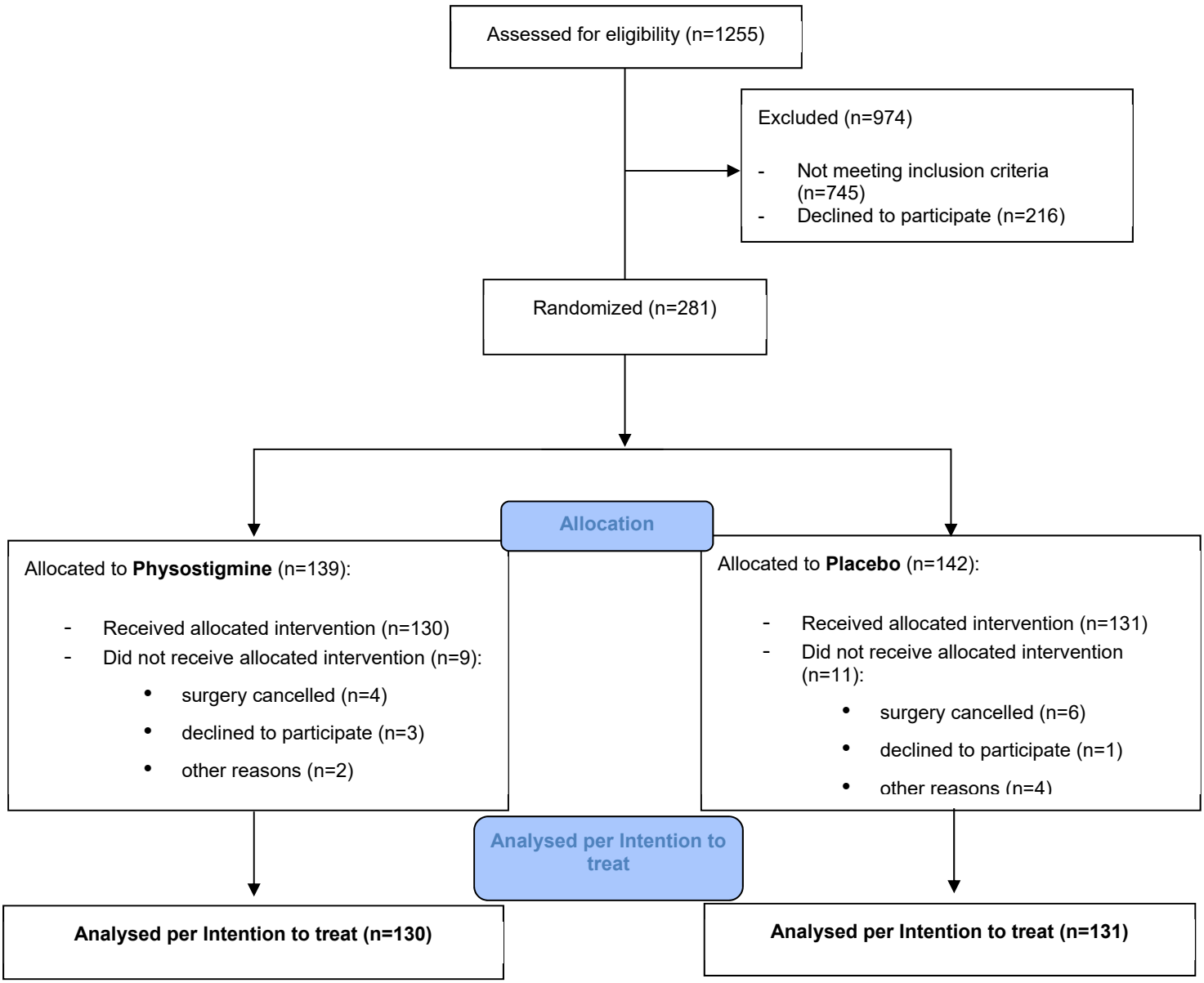


Figure 1


Assessed for eligibility (n=1255)
Excluded (n=974)
- Not meeting inclusion criteria (n=745)
- Declined to participate (n=216)
Randomized (n=281)
Allocation
Allocated to **Physostigmine** (n=139):
- Received allocated intervention (n=130)
- Did not receive allocated intervention (n=9):
• surgery cancelled (n=4)
• declined to participate (n=3)
• other reasons (n=2)
Allocated to **Placebo** (n=142):
- Received allocated intervention (n=131)
- Did not receive allocated intervention (n=11):
• surgery cancelled (n=6)
• declined to participate (n=1)
• other reasons (n=4)
Analysed per Intention to treat
Analysed per Intention to treat (n=130)
Analysed per Intention to treat (n=131)