

Clinical Study Protocol

Title

Preoperative Antibiotic vs No antibiotics in Early Laparoscopic Cholecystectomy In Mild-Moderate Acute Calculous Cholecystitis: an international RCT

Short Title

PANEL ChIMMACC

Study Type:	Prospective, international, multicentre, open-label, randomized controlled, parallel-group, non-inferiority clinical trial with investigational medicinal product/intervention.
Study Registration:	Clinicaltrial.gov (to be registered)
Study Identifier:	EU CT number 2026-528153-79-00
Sponsor, Sponsor-Investigator or Principal Investigator:	Sponsor: Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi, 19, Pavia, Italy. Sponsor-Investigator: Luca Ansaloni. Coordinating centre: Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi, 19, Pavia, Italy. SC Chirurgia Generale 1 - Dipartimento di Emergenza-Urgenza l.ansaloni@smatteo.pv.it
Investigational Product/intervention:	Preoperative antibiotic strategy. Control arm: amoxicillin/clavulanic acid 2000 mg/200 mg IV every 8 hours from randomization until early laparoscopic cholecystectomy. Experimental arm: omission of preoperative antibiotic therapy before early laparoscopic cholecystectomy. Rescue antibiotic therapy will be allowed whenever clinically indicated.
Protocol Version and Date:	Version 01 dated 20/04/2026



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PROTOCOL SIGNATURE FORM

Study number	EU CT number 2026-528153-79-00
Study Title	Preoperative Antibiotic vs No antibiotics in Early Laparoscopic Cholecystectomy In Mild-Moderate Acute Calculous Cholecystitis: an international RCT

The Sponsor-Investigator and trial statistician have approved the protocol version 01 dated 20/04/2026 and confirm hereby to conduct the study according to the protocol, the current version of the World Medical Association Declaration of Helsinki [1], ICH E6(R3) guidelines [2] and the local legally applicable requirements.

Sponsor-Investigator

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Place/Date

Signature

Study Statistician

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Affiliation: Biostatistics, Epidemiology & Research Design (BERD) Office

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Dipartimento Ricerca Clinica e Sperimentale

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Study Coordinator

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Direzione Scientifica- Clinical Trial Center

Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Place/Date

Signature

DRAFT



Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki [1], ICH E6(R3) guidelines, [2] and the local legally applicable requirements.

Site _____

Principal investigator _____

Place/Date

Signature

DRAFT



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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	Sponsor: Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi, 19, Pavia, Italy. Sponsor-Investigator: Luca Ansaloni. Coordinating centre: Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. SC Chirurgia Generale 1 - Dipartimento di Emergenza-Urgenza l.ansaloni@smatteo.pv.it
Study Title:	Preoperative Antibiotic vs No antibiotics in Early Laparoscopic Cholecystectomy In Mild-Moderate Acute Calculous Cholecystitis: an international RCT
Short Title / Study ID:	PANEL ChIMMACC
Protocol Version and Date:	Version 01, 20/04/2026
EU CT number	2026-528153-79-00
Trial registration:	Clinicaltrial.gov (to be registered)
Clinical Phase:	Phase 4 clinical trial based on clinical equipoise, evaluating omission of preoperative antibiotics versus standard amoxicillin/clavulanic acid therapy before early laparoscopic cholecystectomy.
Background and Rationale:	Acute calculous cholecystitis is a frequent cause of emergency surgical admission, and early laparoscopic cholecystectomy represents the standard treatment in selected patients. The routine use of preoperative antibiotics in mild-moderate acute calculous cholecystitis remains debated, with available randomized evidence and guidelines not providing a definitive answer. Reducing unnecessary antibiotic exposure is a key strategy to promote antimicrobial stewardship and reduce antimicrobial resistance. The PANEL ChIMMACC trial aims to determine whether early laparoscopic cholecystectomy without preoperative antibiotics is non-inferior to the standard strategy including preoperative antibiotics in selected low-risk patients with mild-moderate acute calculous cholecystitis.

Objective(s):	Primary objective: to assess whether omission of preoperative antibiotic therapy is non-inferior to preoperative antibiotic therapy in terms of 30-day infectious postoperative morbidity in selected patients undergoing early laparoscopic cholecystectomy for mild-moderate acute calculous cholecystitis. Secondary objectives: to compare postoperative infectious and non-infectious complications, surgical site infections, bacteriobilia and bacteremia, inflammatory markers, length of hospital stay, readmission, need for postoperative radiological or surgical interventions, rescue antibiotic therapy, and safety outcomes between the two treatment arms.
Outcome(s):	Primary outcome: patient-level composite incidence of postoperative infectious morbidity within 30 days after surgery, including SSI and other predefined postoperative infectious complications. Primary estimand/estimator: between-group absolute risk difference in 30-day infectious postoperative morbidity between the no-antibiotic arm and the antibiotic arm, assessed in a non-inferiority framework. Main secondary outcomes: bacteriobilia, bacteremia, C-reactive protein, procalcitonin, leukocyte count, non-infectious postoperative complications according to the Clavien-Dindo classification, length of hospital stay, readmission, need for radiological or surgical treatment after discharge, use of rescue antibiotics, and adverse events.
Study design:	☐ single center study; or ☒ multicenter study; • ☒ coordinated by the proposer ☐ single arm clinical trial or ☒ randomised controlled clinical trial (RCT) ○ blindness: ☒ no; ☐ single; ☐ double; ☐ triple ○ design: ☒ parallel groups; ☐ crossover; ☐ factorial; ☐ other _____

Inclusion / Exclusion criteria:	Inclusion criteria: • Adult patients aged ≥ 18 years. • Informed Consent as documented by signature before randomization. • Diagnosis of acute calculous cholecystitis according to Tokyo Guidelines 2018 diagnostic criteria. • Mild-to-moderate acute calculous cholecystitis, defined as TG18 grade I or selected TG18 grade II disease. For the purposes of this protocol, selected grade II disease ("grade IIa") is defined as grade II acute calculous cholecystitis with preserved gallbladder wall integrity and inflammatory changes confined to the gallbladder; • Candidate for early laparoscopic cholecystectomy, to be performed as soon as possible and within 72 hours from hospital admission. • Low surgical risk for early laparoscopic cholecystectomy, defined as CHOLE-POSSUM Physiological Score < 25. • Low risk of common bile duct stones, defined as Israeli Score 0–1. Exclusion criteria: • Age < 18 years. • Known or suspected pregnancy, including a positive pregnancy test when performed. Pregnancy status will be assessed before randomization according to local clinical practice and medical judgement; testing, which may include urine or serum hCG, will be performed when pregnancy cannot be reasonably excluded, when the participant is uncertain, or when required by local procedures. • CHOLE-POSSUM Physiological Score ≥ 25. • Israeli Score ≥ 2. • TG18 grade IIb disease with preoperative evidence of gallbladder perforation, biliary peritonitis or pericholecystic abscess • Known hypersensitivity or allergy to amoxicillin/clavulanic acid, penicillins or other beta-lactam antibiotics. • Previous amoxicillin/clavulanic acid-associated jaundice or hepatic dysfunction. • Severe renal impairment, defined as creatinine clearance < 30 mL/min, when the protocol-defined amoxicillin/clavulanic acid 2000
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mg/200 mg regimen cannot be safely administered.

- Poorly controlled or decompensated diabetes mellitus, defined as HbA1c $\geq 8.0\%$ (≥ 64 mmol/mol) documented within the previous 3 months or available during the eligibility assessment, or clinically relevant acute metabolic decompensation.
- Ongoing systemic immunosuppressive therapy, including chemotherapy, biological immunomodulatory therapy, post-transplant immunosuppression or other systemic immunosuppressive drugs.
- Ongoing chronic systemic corticosteroid therapy.
- Any other clinically relevant immunosuppressed condition which, in the investigator's judgement, may significantly increase the risk of postoperative infectious complications or make participation in the trial inappropriate.
- Concurrent operation on other organs.
- Suspected or confirmed malignancy.
- History of previous upper abdominal surgery if considered relevant by the investigator.
- Suspicion of hollow organ injury.
- Any systemic antibiotic administered for the current episode of acute calculous cholecystitis before randomization. Antibiotic treatment received for a previous, clinically resolved episode of acute cholecystitis will not constitute an exclusion criterion, provided that no antibiotic therapy is ongoing at screening.

Measurements and procedures:	Patients presenting to the Emergency Department of participating centres with suspected acute calculous cholecystitis will be assessed by the emergency surgical team and screened for potential eligibility. After written informed consent, eligibility will be confirmed and baseline demographic, clinical, laboratory and imaging data will be collected. CHOLE-POSSUM Physiological Score, Israeli Score and TG18 severity grade will be assessed before randomization. Eligible participants will be randomized 1:1 to either omission of preoperative antibiotic therapy or standard preoperative antibiotic therapy before early laparoscopic cholecystectomy. Early laparoscopic cholecystectomy should be performed as soon as possible and within 72 hours from hospital admission. Intraoperative findings, operative details, conversion, bail-out procedures, postoperative complications, rescue antibiotic therapy, laboratory parameters, length of stay, readmissions and 30-day outcomes will be prospectively recorded in the electronic case report form using REDCap. Postoperative follow-up will include a POD7 ± 2 clinical assessment, which may be performed either as an outpatient visit or by telephone contact according to local practice and participant availability. POD7 laboratory tests are not mandatory study-specific procedures and will be recorded only if performed according to local clinical practice, centre procedures or clinical indication. A mandatory POD30 ± 2 outpatient follow-up visit will be scheduled for standardized assessment of the surgical wounds and ascertainment of the primary, secondary and safety outcomes occurring within 30 days after surgery.
Study Product / Intervention:	Experimental intervention, No-antibiotic arm: no planned preoperative antibiotic therapy before early laparoscopic cholecystectomy. Antibiotic therapy may be administered as rescue therapy if clinically indicated, for example in case of clinical deterioration, intraoperative findings requiring antibiotics, or postoperative infectious complications. Any rescue antibiotic administration will be recorded in REDCap.

Control Intervention:	Control intervention, Antibiotic arm: standard preoperative antibiotic therapy with amoxicillin/clavulanic acid 2000 mg/200 mg IV every 8 hours, diluted in 100 mL of 0.9% sodium chloride solution and administered over 30–40 minutes, starting from randomization until early laparoscopic cholecystectomy.
Number of Participants with Rationale:	The planned sample size is 2,596 patients, 1,298 per group. To account for an expected drop-out rate of approximately 10%, the study may enrol up to 2,884 patients, 1,442 per group. The sample size is based on a non-inferiority hypothesis comparing the incidence of infectious postoperative morbidity in the no-antibiotic arm versus the antibiotic arm, with a one-sided alpha of 2.5% and 80% power.
Study Duration:	Recruitment is planned over approximately 24 months. Each participant will be followed for 30 days after surgery. The expected duration from first-patient-in to last-patient-last-visit is approximately 25 months, excluding database cleaning, analysis and reporting.
Study Schedule:	First Participant In: January 2027. Last Participant Out: February 2029.
Investigator(s):	Sponsor-Investigator: Prof. Luca Ansaloni, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. Local Principal Investigators: one qualified investigator will be appointed at each participating centre.
Study Centre(s):	See the list of centres (attached).
Statistical Considerations:	The primary analysis will test the non-inferiority of no preoperative antibiotics compared with preoperative antibiotics for 30-day infectious postoperative morbidity. The main effect measure will be the absolute difference between groups. Non-inferiority will be concluded if the upper bound of the confidence interval for the difference in infectious postoperative morbidity does not exceed the pre-specified 2% non-inferiority margin. The primary analysis will be performed on the per-protocol population. A sensitivity analysis will be performed on the intention-to-treat population. Secondary outcomes will be analysed using appropriate descriptive and inferential statistics according to variable type and distribution.

Sample Size	The sample size calculation assumes infectious postoperative morbidity rates based on previous RCT evidence in comparable patients with mild-moderate acute calculous cholecystitis. With a one-sided alpha of 2.5%, 80% power and a pre-specified 2% non-inferiority margin, 2,596 patients are required. Allowing for approximately 10% drop-out, up to 2,884 patients may be enrolled.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the World Medical Association Declaration of Helsinki [1], ICH E6(R3) guidelines [2] as well as all national legal and regulatory requirements.

GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
BUN	Blood urea nitrogen
CA	Competent Authority (e.g. AIFA)
CEC	Competent Ethics Committee
CRF	Case Report Form
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
ECG	Electrocardiogram
GCP	Good Clinical Practice
IB	Investigator's Brochure
H0	Null hypothesis
H1	Alternative hypothesis
HbA1c	Glycated haemoglobin
hCG	Human Chorionic Gonadotropin
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ITT	Intention to treat
IVDR	In Vitro Diagnostic Medical Device Regulation
MD	Medical Device
MDR	Medical Device Regulation
PCT	Procalcitonin
PI	Principal Investigator
SDV	Source Data Verification



SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

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STUDY SCHEDULE

See Appendix A: “Table of study procedures and assessments”.

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1. STUDY ADMINISTRATIVE STRUCTURE

1.1 *Sponsor, Sponsor-Investigator*

The Sponsor of the study is Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi 19, 27100 Pavia, Italy.

The Sponsor-Investigator is Prof. Luca Ansaloni, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. Prof. Luca Ansaloni is responsible for the overall scientific, clinical and regulatory conduct of the trial and for the submission of the study protocol to the competent Ethics Committee and regulatory authorities, as applicable.

The coordinating centre is SC Chirurgia Generale 1 - Fondazione IRCCS Policlinico San Matteo, Dipartimento di Emergenza-Urgenza, Pavia, Italy.

The Sponsor and Sponsor-Investigator are responsible for the design of the study, coordination of the participating centres, regulatory and ethics submissions, insurance coverage where applicable, oversight of trial conduct, data collection procedures, data management, statistical analysis, interpretation of results, preparation of the final report, and dissemination of study findings.

1.2 *Principal Investigator(s)*

The Principal Investigator of the study is:

Prof. Luca Ansaloni
Fondazione IRCCS Policlinico San Matteo
Dipartimento di Emergenza-Urgenza / SC Chirurgia Generale 1
Viale Camillo Golgi 19, 27100 Pavia, Italy
E-mail: l.ansaloni@smatteo.pv.it

The Co-Principal Investigator is:

Prof. Lorenzo Cobianchi
Fondazione IRCCS Policlinico San Matteo
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Dr. Enrico Cicuttin

Dr. Tommaso Dominioni

Dr. Simone Frassini

Dr. Marcello Maestri

Dr. Johanness Rademacher

Dr. Giuseppe Soffiantini

Dr. Antonio Stabile

Dr. Matteo Tomasoni

Dr. Jacopo Viganò

In this multicentre study, each participating centre will appoint one qualified Local Principal Investigator. The Local Principal Investigator will be responsible for all trial-site related medical decisions, participant safety, informed consent procedures, compliance with the protocol and ICH E6(R3) Good Clinical Practice [2], supervision of delegated study personnel, and accuracy and completeness of data entered in the eCRF.

The complete and updated list of participating centres, Local Principal Investigators, contact details and delegated study personnel will be maintained in the Trial Master File and/or in a dedicated study site list, delegation log or appendix, and will be updated as new centres are activated.

1.3 Statistician ("*Biostatistician*")

The trial biostatistician is:

Catherine Klersy, MD MSc Epid

c.klersy@smatteo.pv.it - int. 3557

Biostatistics, Epidemiology & Research Design (BERD) Office

SC Gestione, supporto e monitoraggio della ricerca clinica e sperimentale

Dipartimento Ricerca Clinica e Sperimentale

Direzione Scientifica

Fondazione IRCCS Policlinico San Matteo, Pavia

The biostatistician will be responsible for the statistical aspects of the study, including contribution to the statistical design, sample size calculation, randomization procedures, preparation of the statistical analysis plan, statistical analyses, and support in the interpretation and reporting of study results.

1.4 Laboratory

No central study laboratory is planned for this trial.

Routine laboratory tests and microbiological analyses, including blood tests, bile cultures, blood cultures and other microbiological samples when collected, will be performed by the local certified laboratories of each participating centre according to standard clinical practice and local procedures.

1.5 Monitoring institution

Monitoring will be performed by the Sponsor-Investigator.

The Principal Investigator is responsible for ensuring the quality of the data entered into the CRF through remote monitoring.

1.6 Data Safety Monitoring Committee

No independent Data Safety Monitoring Committee (DSMC) is planned for this trial.

A DSMC is not considered necessary because the study is an open-label Phase IV trial conducted in a selected low-risk population of adult patients with mild-to-moderate acute calculous cholecystitis undergoing

early laparoscopic cholecystectomy. The control arm uses an authorized antibiotic regimen according to standard clinical practice, while the experimental intervention consists of omission of preoperative antibiotic therapy in carefully selected patients.

1.7 Any other relevant Committee, Person, Organisation, Institution

The study will receive scientific and network support from national and international scientific societies and professional organizations in the fields of emergency surgery, general surgery and surgical infections.

1.8 Data Management Team

Dr. Valeria Musella

SC Gestione, supporto e monitoraggio della ricerca clinica e sperimentale
Dipartimento Ricerca Clinica e Sperimentale

Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi 19, 27100 Pavia, Italy

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2. ETHICAL AND REGULATORY ASPECTS

2.1 Study registration

The trial will be registered with an authorized registry, according to ICMJE (International Committee of Medical Journal Editors) Guidelines, prior to the start of recruitment. [3]. The registration number will be recorded in this protocol once available.

2.2 Competent Ethics Committee (CEC)

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents will be submitted to a properly constituted Competent Ethics Committee (CEC) and to the competent authority (CA). The decision of the CEC and the CA concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

The Competent Ethics Committee for the Sponsor Institution, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, is Comitato Etico Territoriale Lombardia 6 (CET Lombardia 6).

Before the site can enroll patients into the trial, the PI or designee will apply to the hospital for permission to conduct the study and be granted written permission. The study may start at each participating centre only after all required Ethics Committee approvals, regulatory authorisations, contracts and site activation procedures have been completed. Any conditions, recommendations or additional requirements issued by the Ethics Committee or competent authority will be implemented as applicable.

No changes to the approved protocol or other approved study documents will be implemented without prior approval from the Sponsor and, where legally required, from the Competent Ethics Committee and/or competent authority. The only exception will be changes necessary to eliminate an apparent immediate hazard to study participants; in such cases, the change will be implemented without delay and reported to the Sponsor, Competent Ethics Committee and competent authority as soon as possible, according to applicable requirements.

The Sponsor-Investigator and responsible investigators will ensure timely reporting to the Competent Ethics Committee and competent authorities, as applicable, of all relevant changes in the research activity, substantial amendments, unanticipated problems involving risks to participants, serious safety concerns, premature interruption or premature termination of the study, regular study end and final study report.

Premature study end or temporary interruption of the study will be reported to the Competent Ethics Committee within 15 days, according to applicable requirements. The regular end of the study will be reported to the Competent Ethics Committee within 90 days.

Protocol amendments will be submitted and managed as described in Section 2.9 of this protocol and according to applicable regulatory requirements.

2.3 Competent Authorities (CA)

Where required by applicable legislation, the Sponsor will obtain approval from the competent authority before initiation of the clinical trial. For Italian centres, the competent authority is the Italian Medicines Agency (AIFA), according to the applicable regulatory framework for clinical trials on medicinal products. For international participating centres, the Sponsor, in collaboration with the responsible local investigator, will ensure that approval or authorisation from the relevant national competent authority is obtained before site activation, where required by local regulations.

The study may start at each participating centre only after all required competent authority approvals have been obtained.

2.4 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, the ethical principles enunciated in the current version of the World Medical Association Declaration of Helsinki [1], ICH E6(R3) Good Clinical Practice guidelines [2], the European Regulation 536/2014 for clinical studies and all applicable national and international regulatory requirements.

The Competent Ethics Committee and regulatory authorities will receive safety reports, interim reports, notification of study interruption or termination, and notification of regular study end, as required by applicable regulations and local requirements.

2.5 Declaration of interest

No conflicts of interest to declare.

The study is investigator-initiated and is not promoted or sponsored by any pharmaceutical company. Any potential conflict of interest arising during the course of the study will be promptly declared, documented and managed according to Sponsor procedures and applicable regulatory requirements.

2.6 Patient Information and Informed Consent

Written informed consent will be obtained before randomization and before any study-specific procedure is performed.

When a patient presents to the Emergency Department with suspected acute calculous cholecystitis and the on-call surgeon identifies the patient as potentially eligible for the trial, the patient will be considered screened. At this stage, the investigator or an appropriately delegated member of the study team will provide the patient with oral and written information about the study.

The patient will be informed about the nature of the study, its purpose, the study procedures, the expected duration of participation, the treatment strategies under investigation, the potential risks and benefits, and any discomfort that participation may entail. The patient will also be informed that participation is voluntary and that he/she may refuse to participate or withdraw consent at any time, without any effect on subsequent medical care or treatment.

All patients will receive the approved Patient Information Sheet and Informed Consent Form. Adequate time will be given to read the document, ask questions and decide whether to participate. Because the study is conducted in an emergency surgical setting, the time available for decision-making may depend on the patient's clinical condition and urgency of treatment; however, consent will always be obtained before randomization and without delaying necessary clinical care.

The participant will be informed that relevant medical records and study data may be reviewed by authorised individuals other than the treating physician, including study monitors, Sponsor representatives, Ethics Committee members and competent authorities, in accordance with applicable confidentiality and data protection requirements.

Patient's understanding of the information will be assessed. If the patient agrees to participate, the informed consent form will be signed and dated. The authorized person obtaining the informed consent must also sign the ICF. Written informed consent must be documented before any study-specific screening procedure. A copy of the signed consent form will be given to the participant, and the original will be retained as part of the study records.

Patients who decline informed consent will not be enrolled in the study and will receive standard clinical care. Consented patients who are subsequently found not to meet the inclusion criteria or to meet one or more exclusion criteria will be considered screening failures, will not proceed to randomization, and will be recorded in the screening log.

No financial compensation for participation in the study is planned.

2.7 Participant privacy and confidentiality

For participating centres located in the European Union or European Economic Area, personal data will be processed in accordance with Regulation (EU) 2016/679 (General Data Protection Regulation; GDPR) [4] and applicable national data-protection legislation. Participating centres outside the European Union or European Economic Area will comply with applicable local data-protection requirements. Participant privacy and confidentiality will be protected throughout the study. No directly identifiable participant information will be disclosed in scientific publications, congress presentations or other public communications.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilizing subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (e.g. AIFA), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.8 Early termination of the study

The Sponsor-Investigator may prematurely terminate or temporarily suspend the study, in whole or at individual participating centres, if justified by clinical, ethical, regulatory, safety or operational reasons.

Reasons for premature termination or temporary suspension may include, but are not limited to:

- ethical concerns;
- insufficient participant recruitment;
- concerns regarding participant safety;
- new evidence suggesting that continuation of the study may expose participants to unacceptable risk;
- alterations in accepted clinical practice that make continuation of the trial no longer appropriate;
- early evidence of benefit or harm of the experimental intervention;
- major protocol non-compliance or inability to ensure adequate study conduct;
- decision or request by the Competent Ethics Committee, competent authority or Sponsor.

In case of premature study termination or temporary suspension, the Sponsor-Investigator will promptly inform the participating centres, the Competent Ethics Committee and competent authorities, as applicable, according to regulatory requirements and predefined reporting timelines. Participants already enrolled will be followed and managed according to their clinical condition and standard medical care.

2.9 Protocol amendments

Substantial amendments will be submitted to the Competent Ethics Committee and competent authority, where applicable, and will be only implemented after approval has been obtained, unless immediate action is required to protect the rights, safety or well-being of study participants.

Under emergency circumstances, deviations from the protocol or urgent safety measures necessary to protect participants may be implemented without prior approval from the Sponsor-Investigator, Competent Ethics Committee and competent authority. Such deviations or measures will be documented and reported to the Sponsor-Investigator, Competent Ethics Committee and competent authority as soon as possible, according to applicable requirements.

Non-substantial amendments will be managed according to applicable regulations and Sponsor procedures. Where required, they will be communicated to the competent authority as soon as possible and to the Competent Ethics Committee within the Annual Safety Report or according to local requirements.

3. BACKGROUND AND RATIONALE

3.1 *Background and Rationale*

Clinical burden of acute calculous cholecystitis

Gallstone disease is common worldwide and generates a substantial burden of acute and elective care. The prevalence of cholelithiasis in the general population is estimated at approximately 10–15%; 20–40% of affected individuals may develop gallstone-related complications during their lifetime, and acute calculous cholecystitis is a frequent first complication [5–7]. Acute calculous cholecystitis is therefore among the most common conditions requiring emergency surgical assessment, hospital admission and urgent operative treatment. Approximately 200,000 people are diagnosed with acute cholecystitis each year in the United States, and gallstone-related cystic duct obstruction accounts for approximately 90–95% of cases [8]. In a separate analysis of 2018 US hospital data, the combined category of cholelithiasis and cholecystitis accounted for approximately 316,000 principal hospital discharge diagnoses [9]. Although estimates vary according to population, case definition and healthcare system, they consistently indicate a high-volume clinical problem requiring emergency-department evaluation, imaging, inpatient treatment, operating-room capacity and postoperative follow-up.

The impact of acute calculous cholecystitis is not limited to admission frequency. Disease progression may lead to gangrene, empyema, perforation, pericholecystic or hepatic abscess, biliary peritonitis, sepsis and organ dysfunction. Treatment may be associated with postoperative morbidity, conversion to open surgery, prolonged or repeat hospitalization, and the use of radiological or additional surgical procedures. Acute calculous cholecystitis is associated with clinically relevant perioperative morbidity and may require substantial hospital resources [9,10]. The frequency of the condition and the resources required for timely treatment make even modest differences in routine management potentially important for patients and healthcare systems.

Early laparoscopic cholecystectomy as definitive treatment and source control

The current guidelines describe laparoscopic cholecystectomy as the definitive treatment for acute calculous cholecystitis. Removal of the obstructed, inflamed gallbladder treats the underlying gallstone disease and provides surgical source control by removing the principal focus of inflammation and potential infection [5–7]. Delaying definitive treatment exposes patients to continuing symptoms, possible clinical deterioration and recurrent biliary events, while also increasing total use of hospital resources. A network meta-analysis of randomized trials found that earlier cholecystectomy was associated with shorter total hospital stay and, for some early timing strategies, lower conversion and postoperative complication rates than delayed surgery; its overall conclusion was that surgery should be performed as soon as possible [11]. PANEL ChIMMACC considers early laparoscopic cholecystectomy to be performed as soon as possible and within 72 hours from hospital admission. This prompt source-control strategy is integral to the clinical question. The trial is restricted to selected low-risk adults with mild-to-moderate acute calculous

cholecystitis because this is the population in which equipoise regarding routine preoperative antibiotic therapy is most credible. Acute calculous cholecystitis spans a wide spectrum, and the balance between the potential benefits and burdens of antibiotics cannot be assumed to be constant across uncomplicated localized inflammation, severe local complications, sepsis and organ failure. The protocol's strict eligibility criteria identify patients in whom early definitive source control is planned and exclude patients whose disease severity, physiological risk or associated clinical condition makes omission of antibiotics inappropriate. Any conclusions will therefore apply to the protocol-defined population and should not be extrapolated to patients outside it.

Controversial role of preoperative antibiotics

The central clinical distinction is between therapeutic antibiotic treatment and routine preoperative antibiotic administration in selected patients proceeding promptly to source control. In severe, septic, complicated or otherwise high-risk presentations, antimicrobials are administered therapeutically to treat established or strongly suspected infection and to accompany urgent source-control measures. General intra-abdominal infection guidance emphasizes early antimicrobial treatment in sepsis or septic shock, assessment of individual risk and timely source control [12]. Biliary-infection guidance similarly differentiates treatment according to disease severity and clinical context [13]. These indications are outside the question tested by PANEL ChIMMACC.

By contrast, in a stable, low-risk patient with mild-to-moderate acute calculous cholecystitis who will undergo early laparoscopic cholecystectomy, the incremental value of routinely administered antibiotics before surgery remains uncertain. In this setting, antibiotics may be regarded partly as treatment of a potentially infected gallbladder and partly as perioperative prophylaxis against contamination and surgical-site infection. The relative contribution of each function is difficult to determine, particularly when source control is obtained shortly after admission. PANEL ChIMMACC does not challenge the use of antibiotics when clinically indicated and does not propose withholding treatment from patients with severe, septic, complicated or deteriorating disease. The control arm receives the protocol-defined standard preoperative amoxicillin/clavulanic acid therapy, whereas routine planned preoperative antibiotics are omitted in the experimental arm. Rescue antibiotics are permitted whenever clinically indicated before, during or after surgery; their use and the indication will be recorded in accordance with the protocol.

Guidelines and unresolved recommendations

Current recommendations reflect the uncertainty rather than resolving it for the precise PANEL ChIMMACC population. The 2022 Surgical Infection Society guideline recommends perioperative antibiotics for patients undergoing laparoscopic cholecystectomy for acute calculous cholecystitis, while recommending against postoperative antibiotics after laparoscopic cholecystectomy for mild or moderate disease [14]. The Tokyo Guidelines 2018 recommend antimicrobial therapy for grade I and II disease before and at the time of surgery, but not routine continuation once effective source control has been achieved; longer treatment is reserved for severe or complicated cases and is guided by the clinical course and adequacy of source

control [13]. Broader intra-abdominal infection guidance likewise makes antimicrobial timing and duration dependent on severity, risk and source control [12].

Evidence on postoperative therapy supports separating the preoperative and postoperative questions. In a multicentre randomized non-inferiority trial of 414 patients with grade I or II acute calculous cholecystitis, all participants received amoxicillin/clavulanic acid before and at surgery and were then assigned to no postoperative treatment or a further five-day course. Postoperative infection occurred in 17% and 15%, respectively, in the imputed intention-to-treat analysis and in 13% in both groups in the per-protocol analysis; no postoperative treatment was non-inferior within the study's 11% margin [15]. This trial supports avoidance of routine postoperative continuation after effective source control in the population studied, but it cannot establish whether the preoperative component is necessary. Thus, guidance that supports preoperative or perioperative exposure can coexist with guidance against postoperative treatment. Neither position definitively answers whether planned preoperative therapy may be omitted in carefully selected low-risk patients who undergo early source control.

Biological rationale: bacteriobilia and bile colonization

The historical rationale for antibiotic use includes the possibility of intraoperative contamination by infected bile. Under physiological conditions the biliary tree is generally considered sterile, whereas gallstone-related obstruction and inflammation may favour bacterial colonization [16–18]. In published evidence, bile cultures are positive in approximately 35–60% of patients with acute calculous cholecystitis [17,18]. Commonly isolated organisms include Gram-negative bacteria, particularly *Escherichia coli* and *Klebsiella* species, as well as *Enterococcus* species [13,17,18]. Clinically relevant resistance may occur within these groups, which supports attention to local epidemiology and susceptibility patterns when therapeutic antibiotics are required [13].

Bacteriobilia is therefore biologically plausible as a contributor to postoperative infection, but its presence does not by itself establish that routine preoperative antibiotics improve patient-important outcomes. Associations between positive bile cultures and postoperative complications are inconsistent, and bacteriobilia may also be a marker of disease severity, obstruction, prior instrumentation or patient risk [16–18]. The biological rationale supports careful investigation and microbiological assessment; it does not substitute for comparative randomized evidence in the population of interest.

Available randomized trials and meta-analysis

The randomized evidence directly evaluating routine preoperative antibiotics in acute calculous cholecystitis is limited to a small number of trials with different regimens, eligibility criteria and analytical approaches. Jaafar et al. conducted a single-centre, double-blind, placebo-controlled trial in patients with grade I or II disease. Among 90 patients included in the principal analysis, postoperative infectious complications occurred in 8 of 42 patients assigned to piperacillin/tazobactam and 14 of 48 assigned to placebo ($P=0.193$). The study did not identify a statistically significant reduction, but the small sample, protocol violations and exclusions limited precision [19].

Park et al. performed a double-blind, placebo-controlled randomized trial in patients with mild-to-moderate disease undergoing emergency laparoscopic cholecystectomy, generally within 24 hours after randomization. The antibiotic group received cefazolin and the comparator group received saline placebo. In the imputed per-protocol analysis, infectious complications occurred in 10 of 116 patients (8.6%) in the antibiotic group and 9 of 118 patients (7.6%) in the placebo/no-antibiotic group, corresponding to an absolute between-group difference of approximately 1 percentage point in the investigators' framework (95% confidence interval, -8.1% to 6.1%; $P=0.815$) [20]. These event rates are central to the statistical assumptions in the PANEL ChIMMACC protocol. Park et al. concluded non-inferiority using a 10% margin. The small observed difference is consistent with limited incremental benefit from routine preoperative antibiotics in that trial, but the width of the confidence interval and the margin used do not provide a definitive answer for the more rigorous non-inferiority question addressed by PANEL ChIMMACC.

The PEANUTS II multicentre randomized non-inferiority trial compared a single pre-incision dose of cefazolin with no prophylaxis in adults with mild-to-moderate acute calculous cholecystitis. In the intention-to-treat analysis, postoperative infectious complications occurred in 16 of 226 patients (7.1%) in the prophylaxis group and 29 of 231 patients (12.6%) in the no-prophylaxis group, an absolute difference of 5.5 percentage points (95% confidence interval, -0.4 to 11.3). Because the confidence interval crossed the prespecified 10% non-inferiority margin, non-inferiority of omission was not established. Surgical-site infection was more frequent in the no-prophylaxis group (12.1% versus 5.3%; $P=0.010$) [21]. This result is an important counterpoint to the other trials and prevents the evidence from being interpreted as uniformly supportive of omission.

A systematic review and meta-analysis pooled these three randomized trials, including 781 patients: 384 assigned to single-dose preoperative antibiotics and 397 to placebo or no antibiotics. The pooled risk ratio for postoperative infectious complications was 0.69 (95% confidence interval, 0.46 to 1.03; $P=0.07$), with reported statistical heterogeneity of $I^2=0\%$ [22]. The analysis did not demonstrate a statistically clear additional benefit from antibiotics, although its point estimate favoured prophylaxis and its confidence interval did not exclude a clinically relevant benefit. Moreover, low measured statistical heterogeneity does not eliminate important clinical and methodological differences in antibiotic selection and timing, duration of exposure, blinding, eligibility, outcome definitions, management of protocol deviations and non-inferiority assumptions. A UK-perspective cost-effectiveness analysis based on PEANUTS II found prophylaxis marginally less costly and slightly more effective than no prophylaxis, but characterized the result as modest and sensitive to model assumptions, warranting caution in generalization [23]. Economic modelling therefore does not resolve the clinical uncertainty.

Taken together, the available evidence suggests no clear additional benefit of routine preoperative or perioperative antibiotics in all patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy, but certainty remains limited. The number of trials is small; individual studies are modest in size; event rates are relatively low; treatments and methods differ; and the findings are not fully concordant. Failure to demonstrate superiority of antibiotics is not equivalent to establishing non-

inferiority of omission, and prior non-inferiority margins of 10–11% are wider than the degree of precision sought by the current protocol.

Residual evidence gap and need for PANEL ChIMMACC

The residual question is whether routine preoperative antibiotic therapy can be omitted without an unacceptable increase in 30-day postoperative infectious morbidity in the carefully selected low-risk population defined by PANEL ChIMMACC. Existing trials do not answer this question with sufficient precision or consistency. PANEL ChIMMACC addresses the gap through strict eligibility criteria, a large international multicentre design, centralized randomization, protocol-defined early source control, a predefined 30-day postoperative infectious morbidity endpoint, and a per-protocol primary analysis aligned with the non-inferiority objective. Prospective documentation of rescue antibiotics, protocol deviations and relevant clinical events is intended to support an interpretable estimate of the treatment difference.

A definitive, adequately powered non-inferiority trial is required to determine whether omission excludes a clinically unacceptable increase in infection, rather than merely failing to show a statistically significant difference.

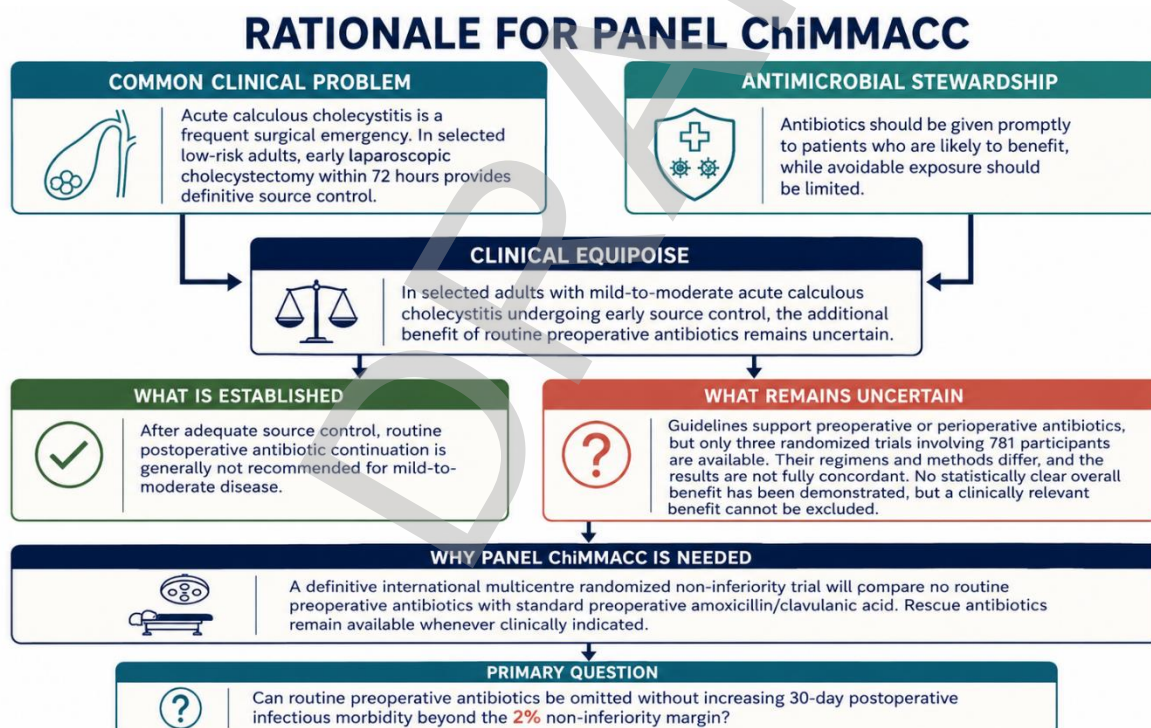


Figure 1: Rationale for PANEL ChIMMACC

Expected impact on antimicrobial stewardship and clinical practice

Observational, ecological and modelling studies available in the literature associate antimicrobial consumption with resistance patterns, while burden analyses demonstrate the clinical importance of antimicrobial resistance [24–28]. Within that context, antimicrobial stewardship seeks appropriate antimicrobial use, not simply lower consumption: antibiotics should be given promptly to patients likely to

benefit, using an appropriate agent, dose, route and duration, while avoidable exposure should be limited [29–32]. Appropriate use also seeks to limit avoidable drug toxicity and secondary complications, including *Clostridioides difficile* infection, without compromising effective treatment [29–32]. Stewardship programmes apply coordinated, evidence-based interventions to improve prescribing, and implementation studies have reported reductions in use and improvements in selected clinical or resistance indicators, although effects depend on setting and study design [33–35].

If non-inferiority is demonstrated, PANEL ChIMMACC may support omission of unnecessary routine preoperative antibiotic exposure and more rational prescribing in the narrowly defined population studied. It may also help emergency surgeons distinguish patients requiring therapeutic antibiotics from those in whom prompt source control alone is sufficient. The trial will not directly demonstrate a reduction in antimicrobial resistance at population level, and its findings will not apply to patients with severe, septic, complicated or high-risk disease or to those without timely source control. Its principal potential contribution is reliable evidence to guide responsible antibiotic prescribing in a common emergency surgical condition.

3.2 Investigational Product (treatment, device, intervention) and Indication

The present study does not investigate a new medicinal product, medical device or in vitro diagnostic device. The investigational intervention is the omission of preoperative antibiotic therapy in selected patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy.

The control intervention consists of standard preoperative antibiotic therapy with amoxicillin/clavulanic acid, an authorised beta-lactam/beta-lactamase inhibitor combination.

The indication under investigation is the use, or omission, of preoperative antibiotic therapy in adult patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy.

No deviation from commercially available authorised amoxicillin/clavulanic acid formulations is planned. The Summary of Product Characteristics of the locally available product will be used as reference for preparation, administration, contraindications, warnings and safety information.

3.3 Preclinical Evidence

This study does not investigate a new medicinal product, a new formulation, or a new route of administration. The study evaluates a clinical strategy, namely omission of preoperative antibiotic therapy versus standard preoperative amoxicillin/clavulanic acid therapy in selected patients undergoing early laparoscopic cholecystectomy. Amoxicillin/clavulanic acid is an authorised medicinal product with established pharmacological and safety information described in the relevant Summary of Product Characteristics. Therefore, no additional preclinical evidence is required for the purposes of this trial.

3.4 Clinical Evidence to Date

Not applicable.

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Not applicable.

3.6 Explanation for choice of comparator (or placebo)

The comparator chosen for this study is standard preoperative antibiotic therapy with amoxicillin/clavulanic acid, which represents a commonly used antibiotic strategy in patients with acute calculous cholecystitis undergoing early laparoscopic cholecystectomy.

The choice of this comparator is justified by the current clinical equipoise regarding the need for routine preoperative antibiotic therapy in selected patients with mild-to-moderate acute calculous cholecystitis. Although preoperative antibiotics are commonly used and recommended in several clinical settings, available evidence does not provide a definitive answer on whether they are necessary in low-risk patients undergoing early source control by laparoscopic cholecystectomy.

The experimental strategy consists of omitting preoperative antibiotic therapy. This is considered ethically acceptable because patients will be carefully selected, early laparoscopic cholecystectomy will provide definitive source control, and rescue antibiotic therapy will be allowed at any time if clinically indicated.

3.7 Risks / Benefits

The main potential risk for participants randomized to the no-antibiotic arm is the occurrence or worsening of infectious complications due to omission of preoperative antibiotic therapy. This risk will be mitigated by careful patient selection, exclusion of high-risk patients, early laparoscopic cholecystectomy as definitive source control, close clinical monitoring, and the possibility to start rescue antibiotic therapy at any time if clinically indicated.

Participants randomized to the antibiotic arm may be exposed to known risks of amoxicillin/clavulanic acid, including hypersensitivity reactions, gastrointestinal adverse events, antibiotic-associated diarrhoea, *Clostridioides difficile* infection, renal impairment, and infusion-related reactions. These risks will be mitigated by excluding patients with known contraindications or severe renal impairment and by monitoring adverse events according to the protocol.

No direct individual benefit can be guaranteed. Participants may benefit from close standardized follow-up and early detection of postoperative complications. The expected benefit of the study is to generate high-

quality evidence on the appropriate use of preoperative antibiotics in mild-to-moderate acute calculous cholecystitis, potentially reducing unnecessary antibiotic exposure and contributing to antimicrobial stewardship and reduction of antimicrobial resistance.

Post-trial care and treatment of any complication will be provided according to standard clinical practice at each participating centre.

3.8 Justification of choice of study population

The study population consists of adult patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy. This population was selected because early laparoscopic cholecystectomy represents definitive source control, and clinical equipoise remains regarding the need for routine preoperative antibiotic.

Eligible patients will be adults aged ≥ 18 years with TG18 grade I or grade IIa acute calculous cholecystitis, low surgical risk defined by CHOLE-POSSUM Physiological Score < 25 , and low risk of common bile duct stones defined by Israeli Score 0–1. This selection aims to exclude patients with severe disease, high surgical risk, suspected common bile duct stones or other conditions in which antibiotic therapy or additional procedures potentially delaying surgery would be clearly indicated and could confound the study question.

Vulnerable participants, including minors, pregnant women and patients unable to provide informed consent, will not be included. Written informed consent will be obtained before randomization. If a patient is clinically unstable, unable to consent, or requires urgent management that does not allow appropriate study procedures, the patient will not be enrolled and will receive standard clinical care.

The choice of this study population is therefore justified by both scientific and safety considerations: it allows evaluation of the effect of omitting preoperative antibiotics in the subgroup of patients most likely to benefit from an antibiotic-sparing strategy, while minimizing avoidable risks.

4. STUDY OBJECTIVES

4.1 Overall Objective

The overall objective of the PANEL ChIMMACC trial is to evaluate whether omission of preoperative antibiotic therapy is non-inferior to standard preoperative amoxicillin/clavulanic acid therapy in selected adult patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy.

The study aims to generate high-quality evidence on the appropriate use of preoperative antibiotics in this setting, contributing to antimicrobial stewardship and reduction of unnecessary antibiotic exposure.

4.2 Primary Objective

The primary objective is to determine whether omission of preoperative antibiotic therapy is non-inferior to standard preoperative antibiotic therapy in terms of 30-day postoperative infectious morbidity after early laparoscopic cholecystectomy. The non-inferiority margin was set at 2%.

4.3 Secondary Objectives

Secondary objectives are to compare the two treatment strategies in terms of individual infectious complications, surgical site infection, microbiological outcomes, postoperative inflammatory markers, non-infectious postoperative complications, length of hospital stay, readmission, need for postoperative radiological or surgical intervention, use of rescue antibiotics, and 30-day mortality.

4.4 Safety Objectives

The safety objectives are to assess the safety of omitting preoperative antibiotic therapy.

Safety assessment will include adverse events, serious adverse events, clinical deterioration requiring rescue antibiotic therapy or urgent treatment, sepsis or septic shock, postoperative complications, and suspected adverse reactions to amoxicillin/clavulanic acid.

5. STUDY OUTCOMES

5.1 Primary Outcome

The primary outcome will be the incidence of postoperative infectious morbidity within 30 days after early laparoscopic cholecystectomy.

Postoperative infectious morbidity will be defined as the occurrence of at least one predefined postoperative infectious complication after the index surgical procedure and within 30 days after surgery. The outcome will be assessed as a binary patient-level variable: yes/no. Patients experiencing more than one infectious complication will be counted once for the primary composite outcome, while each individual infectious event will be recorded separately.

The primary composite outcome will include:

- surgical site infection (SSI), including superficial incisional SSI, deep incisional SSI and organ/space SSI;
- intra-abdominal infection or abscess;
- bacteremia;
- pneumonia;
- urinary tract infection;
- sepsis or septic shock;
- other clinically relevant postoperative infections requiring antimicrobial therapy and/or radiological or surgical treatment.

Postoperative infectious complications will be defined according to the applicable CDC/NHSN criteria [36,37]. Potential blood-culture contaminants will be adjudicated according to CDC/NHSN criteria and established microbiological guidance [37,38]. Sepsis and septic shock will be defined according to Sepsis-3 [39]. Surgical site infections will be classified as superficial incisional, deep incisional or organ/space SSI. For the purpose of SSI assessment, laparoscopic trocar sites, including the umbilical trocar site, will be considered surgical incisions. When more than one tissue level is involved, the SSI type will reflect the deepest tissue level fulfilling the criteria during the surveillance period.

The primary outcome was selected because the clinical question of the trial is whether omission of preoperative antibiotic therapy is non-inferior to standard preoperative antibiotic therapy in preventing clinically relevant postoperative infectious complications in selected patients with mild-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy. A composite infectious morbidity endpoint is clinically appropriate because the potential effect of preoperative antibiotic therapy is not limited to surgical site infection, but may also involve other postoperative infectious complications.

Intraoperative findings already present at the time of surgery, such as purulent bile, gangrenous cholecystitis, gallbladder perforation or local inflammatory findings, will be recorded separately and will not by themselves define the primary postoperative outcome unless a postoperative infectious complication fulfilling the predefined criteria occurs within the 30-day follow-up period.

Rescue antibiotic therapy will be recorded. However, rescue antibiotic administration alone, in the absence

of a postoperative infectious complication fulfilling the predefined criteria, will not by itself define the primary outcome.

5.1.1 Primary estimand

The primary estimand is the between-group difference in the risk of 30-day postoperative infectious morbidity in selected patients with mild-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy, comparing omission of preoperative antibiotic therapy with standard preoperative antibiotic therapy.

The treatment conditions are:

- No-antibiotic arm: no preoperative antibiotic therapy before early laparoscopic cholecystectomy;
- Antibiotic arm: preoperative antibiotic therapy with amoxicillin/clavulanic acid 2000 mg/200 mg IV every 8 hours from randomization until surgery.

The target population for the primary estimand will be the per-protocol population, defined as randomized patients fulfilling eligibility criteria, undergoing early laparoscopic cholecystectomy within the predefined time window, and without major protocol deviations affecting the allocated preoperative treatment strategy.

The variable of interest is the occurrence of postoperative infectious morbidity within 30 days after surgery, assessed as a binary patient-level outcome. Postoperative infectious morbidity is defined as the occurrence of at least one predefined postoperative infectious complication, including surgical site infection, intra-abdominal infection or abscess, bacteremia, pneumonia, urinary tract infection, sepsis or septic shock, or other clinically relevant postoperative infection requiring antimicrobial therapy and/or radiological or surgical treatment.

The population-level summary measure will be the absolute risk difference between No-antibiotic arm and Antibiotic arm in the incidence of 30-day postoperative infectious morbidity.

Intercurrent events will be handled as follows:

- rescue antibiotic therapy due to suspected or confirmed postoperative infection will be recorded and, when the predefined criteria for postoperative infectious morbidity are met, will contribute to the primary outcome;
- rescue antibiotic therapy alone, in the absence of predefined infectious morbidity criteria, will be recorded but will not by itself define the primary outcome;
- major deviations from the allocated treatment strategy, including non-administration of the assigned intervention or inappropriate administration of preoperative antibiotics in No-antibiotic arm, will be considered for exclusion from the per-protocol population;

- bail-out procedures, conversion to laparotomy, severe intraoperative findings not compatible with the target study population, or failure to undergo surgery within 72 hours from hospital admission will be recorded and handled according to the predefined statistical analysis plan;

- withdrawal of informed consent and loss to follow-up will be handled as described in the statistical analysis plan.

The primary clinical question addressed by this estimand is whether, among patients managed according to the protocol, omission of preoperative antibiotic therapy is non-inferior to standard preoperative antibiotic therapy with respect to 30-day postoperative infectious morbidity. The non-inferiority margin was set at 2%.

5.1.2 Primary estimator

The primary estimator will be the absolute risk difference in the incidence of 30-day postoperative infectious morbidity between No-antibiotic arm and Antibiotic arm, calculated as:

Risk difference = Risk in No-antibiotic arm – Risk in Antibiotic arm

The primary analysis will be performed on the per-protocol population. The incidence of the primary composite outcome will be calculated in each treatment group as the proportion of patients experiencing at least one predefined postoperative infectious complication within 30 days after surgery.

Non-inferiority of the no-antibiotic strategy will be concluded if the upper bound of the confidence interval for the absolute risk difference does not exceed the 2% non-inferiority margin.

A sensitivity analysis will be performed on the intention-to-treat population, including all randomized patients according to their assigned treatment group. Additional sensitivity or adjusted analyses may be performed as described in the statistical analysis plan.

5.2 Secondary Outcomes

Secondary outcomes will include the individual components of the primary composite outcome and additional clinically relevant postoperative, microbiological, inflammatory and healthcare-resource outcomes.

The following secondary outcomes will be assessed within 30 days after early laparoscopic cholecystectomy, unless otherwise specified:

- overall surgical site infection (SSI);
- superficial incisional SSI;
- deep incisional SSI;
- organ/space SSI, including intra-abdominal infection or abscess;
- pneumonia;
- urinary tract infection;
- sepsis or septic shock;
- other clinically relevant postoperative infections requiring antimicrobial therapy and/or radiological or surgical treatment;

- rescue antibiotic therapy, including indication, timing, antibiotic agent and duration;
- bile and blood cultures: strongly recommended but not mandatory;
- positive intra-abdominal fluid culture, when collected;
- positive umbilical wound culture, when collected;
- microbiological profile of positive cultures, including identification of isolated organisms and antimicrobial resistance when available;
- postoperative inflammatory markers, including white blood cell count, C-reactive protein, procalcitonin, total and direct bilirubin, and creatinine, according to the predefined study timepoints;
- laboratory parameters at ED admission, including haemoglobin, white blood cell count, total and direct bilirubin, creatinine, urea or BUN, sodium, potassium, C-reactive protein and procalcitonin;
- laboratory parameters at POD1, including haemoglobin, white blood cell count, total and direct bilirubin and C-reactive protein; procalcitonin will be recorded if performed according to local clinical practice or clinical indication;
- Postoperative complications, including infectious and non-infectious complications, graded according to the Clavien–Dindo classification at POD30 [40];
- conversion to laparotomy;
- bail-out procedures;
- length of hospital stay;
- readmission within 30 days after surgery;
- need for postoperative radiological intervention;
- need for postoperative surgical reintervention;
- mortality within 30 days after surgery.

Each secondary outcome will be recorded separately in the electronic case report form. For infectious outcomes, the date of event, diagnostic criteria, microbiological confirmation when available, treatment received and clinical consequences will be recorded.

Surgical site infections will be classified according to CDC/NHSN criteria as superficial incisional, deep incisional or organ/space SSI. If more than one SSI tissue level is involved, the deepest tissue level fulfilling the criteria during the surveillance period will be used for classification.

The rationale for these secondary outcomes is to describe the clinical, microbiological and healthcare-resource impact of omitting preoperative antibiotic therapy, to identify which components of infectious morbidity contribute to the primary composite outcome, and to assess whether the no-antibiotic strategy affects postoperative recovery, rescue antibiotic use, readmission or need for further intervention.

5.3 Other Outcomes of Interest

Other outcomes of interest will include exploratory, feasibility and process-related outcomes aimed at describing trial implementation, protocol adherence and the potential impact of the study on antimicrobial stewardship.

The following outcomes will be assessed:

- number of screened patients per centre;
- number and proportion of eligible patients;
- number and proportion of randomized patients;
- screening failure rate and reasons for screening failure;
- recruitment rate per centre;
- time from emergency department admission to surgical evaluation;
- time from randomization to surgery;
- proportion of patients undergoing early laparoscopic cholecystectomy within 72 hours from hospital admission;
- number of preoperative amoxicillin/clavulanic acid doses administered in Antibiotic arm;
- time from last amoxicillin/clavulanic acid dose to surgical incision, when applicable;
- operating surgeon role or experience category, as recorded in the eCRF;
- protocol deviations and major protocol deviations;
- proportion of patients included in the per-protocol population;
- reasons for exclusion from the per-protocol population;
- use of non-study antibiotics before surgery;
- total antibiotic exposure during the index admission;
- antibiotic exposure within 30 days after surgery;
- feasibility of 30-day follow-up completion;
- completeness of REDCap/eCRF data entry;
- availability and completeness of microbiological data, when collected.

5.4 Safety Outcomes

Safety outcomes will be assessed from randomization until 30 days after early laparoscopic cholecystectomy.

The rationale for these safety outcomes is to monitor both potential harms related to omission of preoperative antibiotic therapy, such as clinical deterioration or infectious complications, and potential

harms related to antibiotic exposure, such as allergic reactions, antibiotic-associated diarrhoea, *Clostridioides difficile* infection and other drug-related adverse events. The following safety outcomes will be specifically recorded:

- serious adverse events occurring from randomization to 30 days after surgery;
- all-cause mortality within 30 days after surgery;
- clinical deterioration requiring urgent treatment, rescue antibiotic therapy, intensive care admission or urgent surgery;
- sepsis or septic shock;
- postoperative infectious complications requiring radiological drainage, surgical reintervention or readmission;
- non-infectious postoperative complications, classified according to the Clavien-Dindo classification;
- conversion to laparotomy and bail-out procedures;
- suspected adverse reactions to amoxicillin/clavulanic acid, including hypersensitivity reactions, anaphylaxis, rash or severe cutaneous reactions, antibiotic-associated diarrhoea, suspected or confirmed *Clostridioides difficile* infection, hepatic dysfunction, renal impairment, thrombophlebitis at the infusion site, nausea or vomiting;
- laboratory abnormalities of clinical relevance, including worsening renal function, hepatic dysfunction, marked inflammatory response or other clinically significant abnormalities according to the judgement of the treating physician.

Rescue antibiotic therapy will always be allowed when clinically indicated. The need for rescue antibiotic therapy will be recorded as a safety-related event and analysed according to the predefined statistical analysis plan.

6. STUDY DESIGN

6.1 General study design and justification of design

PANEL ChIMMACC is a prospective, international, multicentre, randomized, parallel-group, open-label, non-inferiority clinical trial.

The study will compare two preoperative management strategies in selected adult patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy. Participants will be randomized in a 1:1 ratio to either standard preoperative antibiotic therapy or omission of preoperative antibiotic therapy.

The control arm, Antibiotic arm, will receive amoxicillin/clavulanic acid 2000 mg/200 mg IV every 8 hours from randomization until surgery. The experimental arm, No-antibiotic arm, will not receive preoperative antibiotic therapy. Rescue antibiotic therapy will always be allowed in both groups whenever clinically indicated.

The study is designed to test whether omission of preoperative antibiotic therapy is non-inferior to standard preoperative antibiotic treatment in terms of 30-day postoperative infectious morbidity. This design was chosen because the role of preoperative antibiotics in selected low-risk patients with mild-to-moderate acute calculous cholecystitis undergoing timely surgical source control remains uncertain, and available randomized evidence is limited and not definitive.

The planned maximum sample size is **2,884 participants**, including allowance for drop-out or major protocol deviations. Participants will be recruited from Italian and international centres, with scientific and network support from the World Society of Emergency Surgery (WSES) and the Global Alliance for Infections in Surgery (GAIS). Details of sample size assumptions and statistical analysis are provided in the statistical section of this protocol.

Randomization will be performed centrally through REDCap, with allocation concealment until assignment. The study is open-label because the allocated strategy consists of administration or omission of preoperative antibiotics and placebo administration is not planned. Treating clinicians and participants will therefore be aware of the allocated strategy.

Eligible participants will be adults with mild-to-moderate acute calculous cholecystitis, low surgical risk according to CHOLE-POSSUM Physiological Score, and low risk of common bile duct stones according to the Israeli Score, as defined in the eligibility section of this protocol.

Early laparoscopic cholecystectomy should be performed as soon as possible and within 72 hours from hospital admission. Surgical procedures will be standardized according to protocol principles and local standard practice. Laparoscopic cholecystectomy will generally be performed using a three- or four-trocar technique by surgeons experienced in emergency laparoscopic cholecystectomy. Operative details,

intraoperative findings, conversion to laparotomy, bail-out procedures, drain placement and intraoperative complications will be recorded.

Bile cultures and blood cultures are strongly recommended but not mandatory. Additional microbiological samples may be collected when clinically indicated according to local practice. The gallbladder surgical specimen will be managed and analysed according to routine pathology procedures and is not collected for study-specific research purposes.

No routine postoperative antibiotics will be prescribed in either group unless clinically indicated. Postoperative treatment, analgesia and discharge will follow local standard clinical practice.

Postoperative follow-up will include a POD7 ± 2 clinical assessment, which may be performed either as an outpatient visit or by telephone contact according to local practice and participant availability. POD7 laboratory tests are not mandatory study-specific procedures and will be recorded only if performed according to local clinical practice, centre procedures or clinical indication.

A mandatory POD30 ± 2 outpatient follow-up visit will be scheduled for standardized assessment of the surgical wounds and ascertainment of the primary, secondary and safety outcomes occurring within 30 days after surgery.

The expected duration of participation for each participant will extend from screening and randomization until completion of the 30-day postoperative follow-up. The formal primary endpoint assessment will be completed at the POD30 follow-up visit.

Recruitment is planned over approximately 24 months. Each participant will be followed for 30 days after surgery. The expected duration from first-patient-in to last-patient-last-visit is approximately 25 months, excluding database cleaning, analysis and reporting.

6.2 Methods of minimising bias

Several measures will be implemented to minimise bias and ensure methodological robustness.

Selection bias will be minimised through predefined inclusion and exclusion criteria, prospective screening, use of a screening log, and centralised web-based randomisation after written informed consent and confirmation of eligibility.

Allocation bias will be minimised through central randomisation using the REDCap platform hosted at the Coordinating Center. The allocation sequence will be generated by an independent statistician and will not be accessible to investigators before randomisation.

Performance bias cannot be completely avoided because the study is open-label and placebo administration is not planned. However, perioperative management, surgical principles, rescue antibiotic criteria, outcome definitions and follow-up procedures will be predefined in the protocol and eCRF.

Detection bias will be minimised by using predefined outcome definitions, including CDC/NHSN criteria for surgical site infection, standardised eCRF fields, and investigator training.

Data quality and reporting bias will be minimised through prospective data collection, central data checks, monitoring according to the monitoring plan, audit trail in REDCap, query resolution procedures and predefined statistical analyses.

6.2.1 Randomisation

The unit of randomisation will be the individual participant.

After written informed consent has been obtained and eligibility has been confirmed, participants will be randomised in a 1:1 ratio to one of the two study arms:

Antibiotic arm: preoperative antibiotic therapy with amoxicillin/clavulanic acid;

No-antibiotic arm: omission of preoperative antibiotic therapy.

Randomisation will be performed centrally through the REDCap platform hosted at the Coordinating Center, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. The allocation sequence will be generated by an independent statistician using Stata software, release 19.5 or later (StataCorp, College Station, TX, USA), with random block randomisation and variable block sizes.

Allocation concealment will be ensured by the central web-based randomisation system. The allocated treatment arm will be disclosed only after the participant has been consented, eligibility has been confirmed, and randomisation has been completed.

Screening failures and patients who decline informed consent will not proceed to randomisation and will be recorded in the screening log.

6.2.2 Blinding procedures

The study will be open-label so blinding procedures are not provided. Treating ward physicians, anaesthesiologists and surgeons will not be blinded for practical and safety reasons, including the need to manage clinical deterioration, intraoperative findings and rescue antibiotic therapy.

6.2.3 Other methods of minimising bias

Additional measures to minimize bias will include the use of predefined inclusion and exclusion criteria, based on TG18 severity grade, CHOLE-POSSUM Physiological Score and Israeli Score; standardized perioperative procedures; prospective recording of intraoperative findings and postoperative outcomes; and uniform follow-up up to 30 days after surgery.

All participating centres will use the same electronic case report form in REDCap. Data fields will be predefined and will include mandatory variables for eligibility, randomization, allocated treatment, surgical procedure, protocol interruption, rescue antibiotic therapy, postoperative infectious complications, non-infectious complications, readmission and 30-day follow-up. Data quality checks and central data validation will be performed by the coordinating centre according to the data management plan.

The primary analysis will be performed on the per-protocol population. A sensitivity analysis will be performed on the intention-to-treat population. Protocol interruptions, rescue antibiotic administration, bail-out procedures and conversion to open surgery will be prospectively recorded and handled according to the prespecified statistical analysis plan.

6.3 Unblinding Procedures (Code break)

Not applicable.

DRAFT

7. STUDY POPULATION

7.1 Eligibility criteria

INCLUSION CRITERIA

Participants must meet all of the following criteria to be eligible for randomization:

- Adult patients aged ≥ 18 years.
- Informed Consent as documented by signature before randomization.
- Diagnosis of acute calculous cholecystitis according to Tokyo Guidelines 2018 diagnostic criteria [41], which classifies ACC into 3 grades according to disease severity.
- Mild-to-moderate acute calculous cholecystitis, defined as TG18 grade I or selected TG18 grade II disease [41]. For the purposes of this protocol, selected grade II disease ("grade IIa") is defined as grade II acute calculous cholecystitis with preserved gallbladder wall integrity and inflammatory changes confined to the gallbladder [20]
- Candidate for early laparoscopic cholecystectomy, to be performed as soon as possible and within 72 hours from hospital admission.
- Low surgical risk for early laparoscopic cholecystectomy, defined as CHOLE-POSSUM Physiological Score < 25 [42].
- Low risk of common bile duct stones, defined as Israeli Score 0–1 [43].

EXCLUSION CRITERIA

- Age < 18 years.
- Known or suspected pregnancy, including a positive pregnancy test when performed. Pregnancy status will be assessed before randomization according to local clinical practice and medical judgement; testing, which may include urine or serum hCG, will be performed when pregnancy cannot be reasonably excluded, when the participant is uncertain, or when required by local procedures.
- CHOLE-POSSUM Physiological Score ≥ 25 .
- Israeli Score ≥ 2 .
- TG18 grade IIb disease with preoperative evidence of gallbladder perforation, biliary peritonitis or pericholecystic abscess [20]
- Known hypersensitivity or allergy to amoxicillin/clavulanic acid, penicillins or other beta-lactam antibiotics.
- Previous amoxicillin/clavulanic acid-associated jaundice or hepatic dysfunction.

- Severe renal impairment, defined as creatinine clearance <30 mL/min, when the protocol-defined amoxicillin/clavulanic acid 2000 mg/200 mg regimen cannot be safely administered.
- Poorly controlled or decompensated diabetes mellitus, defined as HbA1c $\geq 8.0\%$ (≥ 64 mmol/mol) documented within the previous 3 months or available during the eligibility assessment, or clinically relevant acute metabolic decompensation.
- Ongoing systemic immunosuppressive therapy, including chemotherapy, biological immunomodulatory therapy, post-transplant immunosuppression or other systemic immunosuppressive drugs.
- Ongoing chronic systemic corticosteroid therapy.
- Any other clinically relevant immunosuppressed condition which, in the investigator's judgement, may significantly increase the risk of postoperative infectious complications or make participation in the trial inappropriate.
- Concurrent operation on other organs.
- Suspected or confirmed malignancy.
- History of previous upper abdominal surgery if considered relevant by the investigator.
- Suspicion of hollow organ injury.
- Any systemic antibiotic administered for the current episode of acute calculous cholecystitis before randomization. Antibiotic treatment received for a previous, clinically resolved episode of acute cholecystitis will not constitute an exclusion criterion, provided that no antibiotic therapy is ongoing at screening.

7.2 Recruitment and screening

Participants will be recruited among adult patients presenting to the Emergency Department of participating centres with suspected acute calculous cholecystitis and indication for early laparoscopic cholecystectomy. Screening will be performed by the local investigator or by delegated members of the study team, in collaboration with the treating surgical team. Eligibility will be assessed according to the predefined inclusion and exclusion criteria, including diagnosis and severity grading of ACC according to TG18, CHOLE-POSSUM Physiological Score, Israeli Score, clinical examination, laboratory tests and imaging findings routinely performed as part of standard care. An ECG will be performed and/or recorded according to local clinical practice, local preoperative procedures and medical judgement, and the ECG category required for the CHOLE-POSSUM Physiological Score will be recorded in the eCRF.

Written informed consent will be obtained before randomization and before any study-specific procedure. A screening log will be maintained at each participating centre to record all screened patients, including screening failures and reasons for non-enrolment.

7.3 Assignment to study groups

After confirmation of eligibility criteria and after written informed consent has been obtained, participants will be randomized in a 1:1 ratio to the preoperative antibiotic group (Antibiotic arm) or to the no-antibiotic group (No-antibiotic arm).

Randomization will be performed by the local investigator or by an authorized delegated member of the study team through the web-based REDCap platform. The allocation sequence will be generated by an independent statistician using random block randomization with blocks of varying size. The statistician will not be involved in patient recruitment, treatment allocation or outcome assessment.

Treatment allocation will not be concealed to participants or treating clinicians, as the study is open-label and no placebo administration is planned.

7.4 Criteria for withdrawal / discontinuation of participants

Participants may withdraw consent for study participation at any time, without any effect on subsequent medical care or treatment. In case of withdrawal of consent, no further study-specific procedures will be performed. Data already collected up to the time of withdrawal may be retained and analysed according to the informed consent form and applicable regulations, unless otherwise requested by the participant where legally applicable.

Discontinuation of the allocated study strategy or protocol interruption may occur when the participant's clinical condition requires deviation from the assigned preoperative management strategy or from the planned study pathway. This may include, but is not limited to, rescue antibiotic therapy, clinical deterioration, urgent clinical treatment, inability to undergo early laparoscopic cholecystectomy within the protocol-defined time window, conversion to laparotomy, bail-out procedures, or intraoperative findings suggesting disease outside the target mild-to-moderate population.

These events will not automatically imply withdrawal from the study. Whenever consent is not withdrawn, participants will continue to receive appropriate clinical care and will remain followed until 30 days after surgery for assessment of primary, secondary and safety outcomes.

Protocol interruptions, major protocol deviations and reasons for exclusion from the per-protocol population will be prospectively recorded in the eCRF and handled according to the predefined Statistical Analysis Plan. The intention-to-treat population will include randomized participants according to their assigned treatment group, as defined in the statistical section of this protocol.

Rescue antibiotic therapy will always be allowed when clinically indicated. The use of rescue antibiotics will be recorded, including indication, timing, molecule, route and duration. Rescue antibiotic therapy will not automatically define the primary outcome unless predefined criteria for postoperative infectious morbidity are met.

8. STUDY INTERVENTION

8.1 *Identity of Investigational Products*

8.1.1 Experimental Intervention

The experimental intervention will consist of omission of preoperative antibiotic therapy before early laparoscopic cholecystectomy. Participants randomized to the experimental arm (No-antibiotic arm) will not receive active preoperative antibiotic treatment.

Rescue antibiotic therapy may be administered if clinically indicated, as described in the protocol.

8.1.2 Control Intervention

The control intervention will consist of standard preoperative antibiotic therapy before early laparoscopic cholecystectomy.

Participants randomized to the control arm (Antibiotic arm) will receive intravenous amoxicillin/clavulanic acid. The investigational medicinal product will be amoxicillin/clavulanic acid 2000 mg/200 mg powder for solution for infusion, or an equivalent commercially available authorized formulation providing 2000 mg amoxicillin and 200 mg clavulanic acid per administration.

The study drug will be supplied through the local hospital pharmacy according to local procedures. No deviation from the commercially available product is planned.

Amoxicillin/clavulanic acid will be administered intravenously every 8 hours from randomization until early laparoscopic cholecystectomy. Each dose will be diluted in 100 mL of 0.9% sodium chloride solution and administered by intravenous infusion over 30–40 minutes.

The selected regimen is consistent with the high-dose intravenous regimen reported for amoxicillin/clavulanic acid and with the Summary of Product Characteristics, which reports 2000 mg/200 mg as the formulation for intravenous infusion, administration over 30–40 minutes, and compatibility with dilution in 100 mL of normal saline solution.

8.1.3 Packaging, Labelling and Supply (re-supply)

Amoxicillin/clavulanic acid will be commercially available and supplied by the local hospital pharmacy of each participating centre, according to local procedures. No deviation from the commercially available products is planned.

Amoxicillin/clavulanic acid will be supplied as powder for solution for infusion, in vials containing 2000 mg amoxicillin and 200 mg clavulanic acid, or an equivalent authorized formulation providing the same dose.

Study infusions will be prepared by authorized unblinded clinical staff.

Participating centres will be responsible for ensuring adequate local availability of amoxicillin/clavulanic acid throughout the recruitment period. Re-supply will be managed according to

local pharmacy procedures.

8.1.4 Storage Conditions

Amoxicillin/clavulanic acid will be stored at each participating centre according to the Summary of Product Characteristics and local hospital pharmacy procedures.

Unopened amoxicillin/clavulanic acid vials will be stored in the original container and not above 25°C, unless otherwise specified by the locally available product information.

After reconstitution and dilution, amoxicillin/clavulanic acid infusion should preferably be administered immediately. In any case, the prepared infusion must be administered within the stability time specified in the Summary of Product Characteristics and local procedures. Prepared study infusions will not be stored for later use.

Any unused medicinal product or waste material will be discarded according to local hospital procedures and applicable regulations.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

Participants randomized to the experimental arm (No-antibiotic arm) will not receive active preoperative antibiotic therapy before early laparoscopic cholecystectomy. No pharmacologically active investigational product will be administered in the experimental arm.

Rescue antibiotic therapy may be started at any time if clinically indicated, as described in the protocol.

8.2.2 Control Intervention

Participants randomized to the control arm (Antibiotic arm) will receive standard preoperative antibiotic therapy before early laparoscopic cholecystectomy.

The control intervention will consist of amoxicillin/clavulanic acid 2000 mg/200 mg IV every 8 hours, from randomization until surgery. Each dose will be diluted in 100 mL of 0.9% sodium chloride solution and administered by intravenous infusion over 30–40 minutes.

The study drug will be administered by authorized clinical staff according to local hospital procedures. The first dose should be administered as soon as possible after randomization. Additional doses will be administered every 8 hours up until surgery, as expected from standard clinical practice.

The rationale for the selected regimen is to provide standard active preoperative antibiotic treatment during the interval between diagnosis/randomization and early laparoscopic cholecystectomy. The total exposure to antibiotic therapy will depend on the time elapsed between randomization and surgery.

From the start of the operation, the assigned preoperative antibiotic treatment will be stopped. No

postoperative antibiotic therapy will be routinely administered after effective source control, unless clinically indicated as rescue therapy.

8.3 Dose / Device modifications

No dose administration is planned for the experimental intervention.

In the control arm, amoxicillin/clavulanic acid will be administered according to the clinical standard regimen. Dose adjustment is not planned within the trial protocol; patients in whom the clinical standard regimen cannot be safely administered, including severe renal impairment, will be excluded before randomization.

The allocated intervention may be discontinued in case of hypersensitivity reaction, serious adverse event, clinical deterioration, need for rescue antibiotic therapy, intraoperative findings requiring antibiotic treatment, participant request, or according to the judgement of the treating physician. Any discontinuation or modification of the allocated intervention will be recorded in REDCap.

8.4 Compliance with study intervention

Study interventions will be administered during hospitalization by authorized clinical staff; therefore, adherence is expected to be directly controlled.

Non-compliance will include failure to administer the allocated intervention, administration of the wrong intervention, relevant delay or omission of scheduled doses, unauthorized preoperative antibiotic administration in No-antibiotic arm, or administration of rescue antibiotics outside predefined clinical indications.

All cases of non-compliance will be documented and assessed for their impact on the intention-to-treat and per-protocol analyses.

8.5 Data Collection and Follow-up for withdrawn participants

Participants may withdraw consent at any time without consequences for their subsequent medical care.

In case of withdrawal of consent, no further study-specific data will be collected unless the participant agrees to continued follow-up. Data already collected up to the time of withdrawal may be retained and analysed according to the informed consent form and applicable regulations.

Whenever possible, participants who discontinue the allocated intervention but do not withdraw consent will continue follow-up until 30 days after surgery. Data on protocol interruption, rescue antibiotic therapy, bail-out procedures, conversion, postoperative complications, readmission and mortality will be recorded in REDCap.

8.6 Trial specific preventive measures

Before randomization, all participants will be screened for contraindications to the assigned study strategy and to amoxicillin/clavulanic acid, where applicable.

Specific preventive measures before randomization will include assessment of:

- known hypersensitivity or allergy to amoxicillin/clavulanic acid, penicillins or other beta-lactam antibiotics;
- previous amoxicillin/clavulanic acid-associated jaundice or hepatic dysfunction;
- severe renal impairment, defined as creatinine clearance <30 mL/min, when the protocol-defined amoxicillin/clavulanic acid 2000 mg/200 mg regimen cannot be safely administered;
- pregnancy status. For participants for whom pregnancy is clinically possible, pregnancy status will be assessed before randomization according to local clinical practice and medical judgement. If pregnancy can be reasonably excluded on the basis of clinical history and the participant's statement, no study-mandated pregnancy test is required. If pregnancy cannot be reasonably excluded, the participant is uncertain, or testing is required by local procedures, pregnancy testing will be performed according to local practice and may include urine or serum hCG. Known or suspected pregnancy, including a positive pregnancy test when performed, is an exclusion criterion. No study-specific procedure or randomization will occur until pregnancy has been reasonably excluded;
- poorly controlled or decompensated diabetes mellitus. HbA1c will be checked if no result is available from the previous 3 months. Patients with HbA1c $\geq 8.0\%$ (≥ 64 mmol/mol) or clinically relevant acute metabolic decompensation will be excluded;
- ongoing systemic immunosuppressive therapy, including chemotherapy, biological immunomodulatory therapy, post-transplant immunosuppression or other systemic immunosuppressive drugs;
- ongoing chronic systemic corticosteroid therapy;
- any other clinically relevant immunosuppressed condition which, in the investigator's judgement, may significantly increase the risk of postoperative infectious complications or make participation in the trial inappropriate.

Rescue antibiotic therapy will be allowed at any time if clinically indicated, including in case of clinical deterioration, suspected or confirmed infection, persistent fever, hemodynamic instability, worsening inflammatory parameters, peritonitis, bacteremia, intraoperative findings requiring antibiotics, or according to the judgement of the treating physician.

The need for rescue antibiotic therapy will be recorded in REDCap, including indication, date and time of initiation, antibiotic molecule, route of administration, duration and clinical reason. Rescue antibiotic therapy will not automatically define the primary outcome unless predefined criteria for postoperative

infectious morbidity are met. Its impact on protocol adherence and analysis populations will be assessed according to the predefined statistical analysis plan.

No study procedure or allocated treatment strategy should delay clinically necessary treatment.

8.7 Concomitant Interventions (treatments)

There are no restrictions on medicine or treatment in this study. All standard perioperative treatments required for the management of acute calculous cholecystitis and early laparoscopic cholecystectomy are permitted, including analgesics, antiemetics, intravenous fluids, thromboprophylaxis, anaesthetic drugs and other supportive treatments according to local clinical practice.

Postoperative antibiotics will not be routinely administered after effective source control, unless clinically indicated as rescue therapy.

Any relevant concomitant treatment, including non-study antibiotics, rescue antibiotics, analgesics and other treatments potentially affecting outcomes, will be recorded in REDCap.

8.8 Study Drug / Medical Device Accountability

Amoxicillin/clavulanic acid accountability will be managed according to local hospital pharmacy and ward procedures.

8.9 Return or Destruction of Study Drug / Medical Device

No return of study drug to the Sponsor is planned.

Unused amoxicillin/clavulanic acid, empty vials, infusion bags and other related materials will be discarded or destroyed according to local hospital pharmacy procedures and applicable regulations.

9. STUDY ASSESSMENTS

9.1 *Study flow chart(s) / table of study procedures and assessments*

See Appendix A: “Table of study procedures and assessments” and Appendix B: “Study Flowchart”.

9.2 *Assessments of outcomes*

Unless otherwise specified, all endpoint data will be prospectively collected in the eCRF from source medical records, operative reports, laboratory reports, microbiology results, imaging reports, discharge summaries, outpatient visit records and follow-up assessments.

9.2.1 **Assessment of primary outcome**

The primary outcome will be assessed in accordance with the definitions and classification criteria specified in the Study Outcomes section. It will be treated as a binary patient-level composite endpoint. Each qualifying infectious event will be recorded separately in the eCRF, including the event type, date of onset, supporting clinical, laboratory, microbiological and imaging evidence, treatment received and clinical consequences. Participants experiencing more than one qualifying infectious event will be counted only once in the primary composite outcome.

Primary outcome surveillance will be performed throughout the index hospital stay, at discharge, at the POD7 assessment, at the POD30 follow-up visit and whenever clinically indicated.

Primary outcome surveillance will be performed throughout the index hospital stay, at discharge, at the POD7 ± 2 assessment, at the POD30 ± 2 outpatient follow-up visit and whenever clinically indicated. The formal primary endpoint determination will be completed at the mandatory POD30 ± 2 outpatient follow-up visit, which will include direct assessment of the surgical wounds and review of all qualifying events occurring from completion of the index surgical procedure through POD30 inclusive.

The POD30 ± 2 outpatient visit is planned to ensure standardized ascertainment of 30-day postoperative infectious morbidity, including surgical site infection. This timepoint is consistent with CDC/NHSN surgical site infection surveillance definitions for gallbladder surgery/cholecystectomy, which use a 30-day postoperative surveillance period. [36, 37]

9.2.2 **Assessment of secondary outcomes**

Secondary outcomes will be assessed prospectively using source medical records, laboratory reports, microbiology reports, operative reports, imaging reports, discharge summaries, outpatient visit records and REDCap/eCRF entries.

Secondary infectious outcomes will include the individual components of the primary composite outcome, including surgical site infection and its subtypes, intra-abdominal infection or abscess, bacteremia, pneumonia, urinary tract infection, sepsis or septic shock, and any other clinically relevant postoperative infection. Surgical site infections will be assessed according to predefined CDC/NHSN

criteria and classified as superficial incisional, deep incisional or organ/space infection.

Microbiological outcomes will include bile culture results, blood culture results and other microbiological findings when available. Bile cultures and blood cultures are strongly recommended but not mandatory. Their collection, timing, positivity, isolated microorganisms and available antimicrobial susceptibility results will be recorded in the eCRF when applicable.

For secondary analyses, laboratory parameters obtained at ED as part of standard clinical assessment will include haemoglobin, white blood cell count, total and direct bilirubin, creatinine, urea or BUN, sodium, potassium, C-reactive protein and procalcitonin. At POD1, laboratory parameters obtained as part of routine postoperative care will include haemoglobin, white blood cell count, total and direct bilirubin and C-reactive protein. Procalcitonin at POD1 will be recorded if performed according to local clinical practice or clinical indication. At POD7 ± 2 days, results from the same laboratory panel will be collected on an optional, as-available basis when the tests are performed according to local clinical practice, centre procedures or clinical indication. Procalcitonin (PCT) at POD7, if collected, will be recorded when available. POD7 laboratory data will be analysed descriptively as exploratory outcomes. The absence of POD7 laboratory tests will not constitute a protocol deviation and will not affect eligibility, randomization, primary endpoint assessment, follow-up completion or per-protocol status. Additional laboratory tests may be performed at any time according to clinical indication and local practice but will not constitute protocol-mandated outcome assessments unless otherwise specified. Additional laboratory tests may be performed at any time according to clinical indication and local practice.

HbA1c will be assessed during baseline/eligibility evaluation if no HbA1c result is available from the previous 3 months. HbA1c will be used to identify poorly controlled or decompensated diabetes mellitus according to the eligibility criteria.

Secondary non-infectious outcomes will include overall postoperative complications, non-infectious complications, conversion to laparotomy, bail-out procedures, bile leak, postoperative ERCP, reoperation, radiological intervention, length of hospital stay, readmission within 30 days and 30-day mortality.

Postoperative complications will be classified according to the Clavien-Dindo classification. Clavien-Dindo classification will be assessed at POD7 and POD30.

Length of hospital stay will be calculated from the date of hospital admission to the date of discharge. Readmissions, radiological interventions, surgical reinterventions and mortality will be recorded if occurring within 30 days after surgery.

The POD7 assessment should preferably be performed on postoperative day 7, with an acceptable window of ± 2 days. The POD30 outpatient follow-up visit should preferably be performed on postoperative day 30, with an acceptable window of ± 2 days. Events occurring within 30 days after surgery will be included in the endpoint assessment regardless of the exact date of the follow-up visit.

9.2.3 Assessment of other outcomes of interest

Other outcomes of interest described in Section 5.3 will be assessed prospectively using the screening log, REDCap/eCRF, source medical records, medication administration records, operative reports, discharge summaries and follow-up documentation.

Screening and recruitment outcomes, including the number of screened patients, eligible patients, consented patients, screening failures, reasons for screening failure, randomized patients and recruitment rate per centre, will be recorded in the screening log and/or eCRF. Patients who decline informed consent will be recorded as consent refusals and will not proceed to randomization. Consented patients who do not meet inclusion criteria or meet one or more exclusion criteria will be recorded as screening failures and will not be randomized.

Process-related time intervals will be calculated from documented date and time fields in the source records and eCRF. These will include, as applicable, time from Emergency Department admission to surgical evaluation, time from hospital admission to surgery, and the proportion of patients undergoing early laparoscopic cholecystectomy within 72 hours from hospital admission.

Protocol adherence outcomes will include protocol deviations, major protocol deviations, eligibility violations, use of non-study antibiotics before surgery, rescue antibiotic therapy, and reasons for exclusion from the per-protocol population. These data will be collected from source documentation, medication administration records, clinical notes and eCRF entries. In Antibiotic arm, the number and timing of preoperative amoxicillin/clavulanate doses administered before surgery will be recorded when applicable.

Antibiotic exposure will be assessed during the index admission and within 30 days after surgery. The indication, molecule, start and stop date, route of administration and duration of any antibiotic therapy will be recorded when available.

Follow-up feasibility will be assessed by recording completion of POD7 assessment and POD30 outpatient follow-up visit, including missed visits and reasons for missing data when available. The POD7 clinical assessment may be completed without laboratory testing. Absence of optional POD7 laboratory data will not render follow-up incomplete, constitute a protocol deviation or generate a missing-data query. Completeness of REDCap/eCRF data entry will be assessed through database checks, missing data queries and monitoring procedures.

Availability and completeness of microbiological data will be assessed only when microbiological samples are collected. Bile cultures and blood cultures are strongly recommended but not mandatory. No additional biological samples, biobanking procedures, sample storage or shipment to external laboratories are planned for these exploratory outcomes.

9.2.4 Assessment of safety outcomes

Safety outcomes will be assessed from randomization until 30 days after early laparoscopic cholecystectomy. Safety data will be collected from clinical records, medication administration records,

laboratory results, microbiology results, imaging reports, operative reports, discharge summaries, outpatient visit records and participant follow-up assessments.

Safety assessment will include adverse events, serious adverse events, clinical deterioration, rescue antibiotic therapy, ICU admission, urgent treatment, postoperative infectious and non-infectious complications, reinterventions, readmissions and mortality. All safety outcomes will be recorded in the eCRF according to the definitions and reporting procedures described in Section 10.

9.2.4.1 Adverse events

Adverse events will be assessed throughout hospitalization, at discharge, at the POD7 assessment, at the POD30 outpatient follow-up visit and whenever clinically indicated.

At each clinical assessment and follow-up visit, the investigator or delegated study team member will review the participant's clinical course and actively ask about new symptoms, complications, hospital readmissions, additional treatments, antibiotic therapy and any other relevant medical event. Spontaneously reported adverse events will also be collected.

For each adverse event, the following information will be recorded in the eCRF when available: date and time of onset, duration or resolution date, description of the event, severity, seriousness, outcome, action taken, need for treatment or hospitalization, and suspected relationship with the study intervention or treatment strategy, when applicable.

Particular attention will be paid to suspected adverse reactions to amoxicillin/clavulanic acid in the control arm, including hypersensitivity reactions, anaphylaxis, rash or severe cutaneous reactions, antibiotic-associated diarrhoea, suspected or confirmed *Clostridioides difficile* infection, hepatic dysfunction, renal impairment, thrombophlebitis at the infusion site, nausea and vomiting.

Events potentially related to omission of preoperative antibiotic therapy, such as clinical deterioration, rescue antibiotic therapy, sepsis or septic shock, postoperative infectious complications, radiological drainage, surgical reintervention or readmission, will also be specifically recorded.

Rescue antibiotic therapy will always be allowed when clinically indicated. The indication, molecule, route, start and stop date, duration and clinical reason for rescue antibiotic therapy will be recorded in the eCRF.

9.2.4.2 Laboratory parameters

Laboratory parameters relevant to safety will be obtained from tests performed as part of standard clinical care. At Emergency Department admission, the laboratory parameters of interest will include haemoglobin, white blood cell count, total and direct bilirubin, creatinine, urea or BUN, sodium, potassium, C-reactive protein and procalcitonin. At POD1, they will include haemoglobin, white blood cell count, total and direct bilirubin and C-reactive protein; procalcitonin will be recorded if performed according to local clinical practice or clinical indication.

POD7 laboratory tests are not mandatory study-specific procedures. At POD7 $\pm$ 2 days, haemoglobin, white blood cell count, total and direct bilirubin and C-reactive protein will be recorded only when tests

are performed according to local clinical practice, centre procedures or clinical indication; procalcitonin will be recorded if performed. Their absence will not constitute a protocol deviation or affect eligibility, follow-up completion, primary endpoint assessment or per-protocol status.

No additional study-specific laboratory panel is mandated for safety purposes. Additional laboratory tests may be performed at any time according to clinical indication and local practice.

HbA1c will be assessed during baseline/eligibility evaluation if no HbA1c result is available from the previous 3 months. HbA1c $\geq 8.0\%$ (≥ 64 mmol/mol), or clinically relevant acute metabolic decompensation, will be considered an exclusion criterion as defined in Section 7.1.

Pregnancy status will be assessed before randomization according to local clinical practice and medical judgement. Pregnancy testing, which may include urine or serum hCG, will be performed only when pregnancy cannot be reasonably excluded, when the participant is uncertain, or when required by local procedures.

Laboratory tests will be analysed by the local certified laboratory of each participating centre. No central laboratory analysis, biobanking, long-term sample storage or shipment of biological samples to external laboratories is planned.

Abnormal laboratory values will be recorded as adverse events only when considered clinically significant by the treating physician or investigator, for example when they require treatment, additional diagnostic procedures, modification of clinical management, prolongation of hospitalization, readmission, or are associated with clinical deterioration.

9.2.4.3 Vital signs

Vital signs will be assessed according to standard clinical practice during Emergency Department evaluation, preoperative management, postoperative hospitalization, discharge assessment and whenever clinically indicated.

Systolic blood pressure, heart rate and Glasgow Coma Scale (GCS) will be recorded at baseline, before randomization, because they are required for eligibility/scoring and baseline physiological assessment.

Other vital signs, including body temperature, blood pressure, heart rate, respiratory rate and peripheral oxygen saturation, will be assessed according to local clinical practice and good clinical care during preoperative and postoperative monitoring and whenever clinically indicated. Apart from the specified baseline parameters, the protocol does not mandate additional or fixed-schedule vital-sign measurements at POD1, discharge, POD7 or POD30. Additional assessments, including ECG or further monitoring, will be performed according to clinical indication, the treating physician's judgement and local practice. A baseline ECG will be performed and/or recorded as part of the Emergency Department/baseline or preoperative clinical assessment according to local clinical practice, local preoperative procedures and medical judgement. The ECG category required for the CHOLE-POSSUM Physiological Score will be recorded in the eCRF. Additional ECGs or further monitoring may be performed whenever clinically indicated according to the treating physician's judgement and

local practice.

9.2.5 Assessments in participants who prematurely stop the study

Participants who prematurely discontinue the allocated study strategy or experience protocol interruption will remain under clinical care and, whenever consent is not withdrawn, will continue to be followed for safety and outcome assessment until 30 days after surgery.

Premature discontinuation of the allocated strategy may occur in case of rescue antibiotic therapy, clinical deterioration, conversion to laparotomy, bail-out procedure, intraoperative findings requiring deviation from the protocol, major protocol deviation, withdrawal of consent, or other clinical or operational reasons.

Whenever possible, participants who prematurely stop study participation will undergo a final safety assessment, including review of adverse events, serious adverse events, postoperative complications, rescue antibiotic therapy, concomitant treatments, vital signs, clinically indicated laboratory parameters, readmissions, reinterventions and survival status.

If a participant withdraws consent for further study participation, no further study-specific procedures will be performed. Data already collected up to the time of withdrawal may be retained and analysed unless the participant requests otherwise, in accordance with applicable regulations and the informed consent form. No follow-up information will be collected unless permitted by the participant's consent and applicable data protection requirements.

All premature discontinuations, withdrawals of consent, protocol interruptions and reasons for discontinuation will be documented in the eCRF.

9.3 Procedures at each visit

All visits required for the purposes of this study will occur during routine clinical care rather than as separate study-specific visits.

Screening / Emergency Department assessment

When a patient presents to the Emergency Department with suspected acute calculous cholecystitis and the on-call surgeon is called, the surgeon will assess whether the patient is potentially eligible for the trial. If the patient is considered potentially eligible, the patient will be considered screened and will be recorded in the screening log.

During this phase, routine clinical assessment will be performed according to standard care, including medical history, physical examination, vital signs, diagnostic imaging, laboratory tests and ECG assessment according to local clinical practice, local preoperative procedures and medical judgement. Systolic blood pressure, heart rate and Glasgow Coma Scale will be recorded at baseline before randomization because they are required for eligibility/scoring and baseline physiological assessment. A baseline ECG will be performed and/or recorded, and the ECG category required for the CHOLE-POSSUM Physiological Score will be recorded in the eCRF. Diagnostic imaging will include ultrasound

and/or CT scan, as clinically indicated, and will be used to confirm the diagnosis, assess local disease severity and record common bile duct diameter <7 mm yes/no.

At Emergency Department admission/baseline, laboratory parameters collected as part of standard clinical assessment will include haemoglobin, white blood cell count, total and direct bilirubin, creatinine, urea or BUN, sodium, potassium, C-reactive protein and procalcitonin. HbA1c will be assessed only if no result is available from the previous 3 months or when needed to assess the diabetes-related exclusion criterion. HbA1c will be assessed if no HbA1c result is available from the previous 3 months. Pregnancy status will be assessed before randomization according to local clinical practice and medical judgement. If pregnancy can be reasonably excluded on the basis of clinical history and the participant's statement, no study-mandated pregnancy test is required. If pregnancy cannot be reasonably excluded, the participant is uncertain, or testing is required by local procedures, pregnancy testing will be performed according to local practice and may include urine or serum hCG.

Consent and eligibility confirmation

The investigator or delegated study team member will provide oral and written information about the study and obtain written informed consent before randomization and before any study-specific procedure is performed.

After consent, eligibility will be confirmed according to the predefined inclusion and exclusion criteria. This will include confirmation of acute calculous cholecystitis according to TG18 criteria, assessment of CHOLE-POSSUM Physiological Score, Israeli Score, disease severity, HbA1c when applicable, confirmation, where pregnancy is clinically possible, that pregnancy has been reasonably excluded before randomization according to local clinical practice and medical judgement; assessment of contraindications to amoxicillin/clavulanic acid; and review of all other exclusion criteria.

Consented patients who do not meet inclusion criteria or meet one or more exclusion criteria will be considered screening failures, will not be randomized and will be recorded in the screening log with the reason for screening failure.

Randomization / recruitment

Only consented patients who meet all inclusion criteria and have no exclusion criteria will be randomized through the centralized REDCap system.

The allocated treatment strategy will be applied only after randomization.

Preoperative phase after randomization

Participants assigned to Antibiotic arm will receive amoxicillin/clavulanic acid according to the protocol until surgery. The timing and number of administered preoperative antibiotic doses will be recorded.

Participants assigned to No-antibiotic arm will not receive preoperative antibiotic therapy, unless rescue antibiotic therapy becomes clinically indicated.

During the preoperative phase, the participant will be clinically monitored according to standard care. Vital signs, clinical deterioration, rescue antibiotic therapy, concomitant medications and any adverse events will be recorded when applicable.

Blood cultures are strongly recommended but not mandatory and should preferably be obtained before antibiotic administration when clinically and logistically feasible. Their collection, timing and results will be recorded in the eCRF.

Surgery / intraoperative assessment

Early laparoscopic cholecystectomy should be performed as soon as possible and within 72 hours from hospital admission.

Intraoperative data will be recorded, including date and time of surgery, surgical approach, operative duration, intraoperative findings, gallbladder condition, bile spillage, conversion to laparotomy, bail-out procedures, drain placement, operating surgeon role or experience category, intraoperative complications and any deviation from the planned procedure.

Bile culture is strongly recommended but not mandatory. If collected, timing, positivity, isolated microorganisms and available antimicrobial susceptibility results will be recorded in the eCRF.

Postoperative day 1

On POD1, clinical assessment and vital signs will be performed according to standard care. The planned POD1 laboratory panel includes haemoglobin, white blood cell count, total and direct bilirubin and C-reactive protein. Procalcitonin will be recorded if performed according to local clinical practice or clinical indication.

Postoperative complications, adverse events, rescue antibiotic therapy, microbiological results, concomitant treatments and clinical deterioration will be recorded when applicable.

Discharge

At discharge, the investigator or delegated study team member will record length of hospital stay, discharge date, clinical status, postoperative complications, adverse events, antibiotic exposure, rescue antibiotic therapy, readmission plans if any, and relevant laboratory or microbiological results available at that time.

Participants will receive instructions for postoperative follow-up. A POD7 ± 2 clinical assessment will be scheduled and may be performed either as an outpatient visit or by telephone contact. POD7 laboratory tests will be recorded only if performed according to local clinical practice, centre procedures or clinical indication. A mandatory POD30 ± 2 outpatient follow-up visit will be scheduled for direct wound assessment and ascertainment of 30-day outcomes.

POD7 assessment

The POD7 assessment should preferably be performed on postoperative day 7, with an acceptable window of ± 2 days. The assessment may be conducted either as an outpatient visit or by telephone contact, according to local practice, participant availability and clinical judgement.

At this assessment, clinical status, wound condition, signs or symptoms of infection, adverse events, rescue antibiotic therapy, readmissions, reinterventions and postoperative complications will be assessed and recorded.

POD7 laboratory tests are not mandatory study-specific procedures. Laboratory results at POD7 ± 2

days will be recorded only when tests are performed according to local clinical practice, centre procedures or clinical indication. The absence of POD7 laboratory tests will not constitute a protocol deviation and will not affect follow-up completion, primary endpoint assessment or per-protocol status. Clavien-Dindo classification will be assessed at POD7.

POD30 outpatient follow-up visit

The POD30 follow-up is planned as an outpatient visit and should preferably be performed on postoperative day 30, with an acceptable window of ± 2 days.

At this visit, the investigator or delegated study team member will assess the primary outcome and all relevant secondary and safety outcomes occurring within 30 days after surgery. This will include surgical site infection, other infectious complications, non-infectious complications, adverse events, serious adverse events, readmissions, radiological interventions, surgical reinterventions, rescue antibiotic therapy and mortality.

Wound status will be assessed and surgical site infection will be classified according to the predefined criteria. Clavien-Dindo classification will be assessed at POD30.

Whenever clinically indicated

Whenever clinically indicated, additional clinical assessments, laboratory tests, imaging, microbiological samples or treatments may be performed at any time according to the participant's clinical condition and local standard practice.

Participants who prematurely discontinue the allocated treatment strategy or experience protocol interruption will continue to receive appropriate clinical care. Whenever consent is not withdrawn, they will continue to be followed for safety and outcome assessment until 30 days after surgery, as described in Section 9.2.5.

10. SAFETY

The safety of participants will be monitored throughout the study. Safety data will be collected, documented, assessed, reported and managed according to the protocol, Good Clinical Practice, applicable pharmacovigilance requirements, Sponsor procedures and local regulatory requirements. For the purposes of this study, the safety reporting period starts at the time of signature of the informed consent form and ends at completion of the POD30 follow-up visit, corresponding to the last protocol-specific follow-up assessment. If the Investigator becomes aware after the end of the study of a serious adverse reaction or relevant safety information considered related to the study drug or study intervention, this information must be reported to the Sponsor-Investigator as soon as possible.

All adverse events and serious adverse events will be documented in the source documents and recorded in the eCRF, according to the procedures described in this section. Serious adverse events will also be reported using the study-specific SAE Form.

In the event of a serious adverse event (SAE), the Investigator will send a safety report form immediately (no later than 24 hours) after becoming aware of the SAE to: Sponsor's Pharmacovigilance Team by e-mail farmacovigilanza@smatteo.pv.it. In addition, the Investigator will send a safety report form immediately (no later than 24 hours) to the above contact after becoming aware of any adverse events of special interest (AESI) or pregnancies by e-mail farmacovigilanza@smatteo.pv.it.

Full contact details and SAE reporting procedures will be provided to each participating centre before site activation.

This is an open-label Phase IV trial evaluating a preoperative antibiotic strategy in selected patients with mild-to-moderate acute calculous cholecystitis undergoing early laparoscopic cholecystectomy. The control intervention consists of an authorised medicinal product, amoxicillin/clavulanic acid, used according to standard clinical practice. The experimental intervention consists of omission of preoperative antibiotic therapy in carefully selected patients, with rescue antibiotic therapy allowed whenever clinically indicated.

10.1 Drug studies

Safety assessment, causality and expectedness for adverse drug reactions will specifically refer to the medicinal product used as part of the protocol-defined control intervention, amoxicillin/clavulanic acid.

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

Adverse Event

An Adverse Event is any untoward medical occurrence in a participant or clinical study participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event may therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Medical conditions, symptoms or abnormal findings already present before informed consent or at baseline will not be considered adverse events unless they worsen in severity, frequency or clinical relevance during the study. Worsening of pre-existing conditions after informed consent will be recorded as an adverse event when clinically relevant.

Serious Adverse Event

A Serious Adverse Event is any untoward medical occurrence that, at any dose or at any time during the study period:

- results in death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability or incapacity;
- results in a congenital anomaly or birth defect;
- is otherwise considered an important medical event that may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed above.

The term “life-threatening” refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that might hypothetically have caused death if it had been more severe.

Planned hospitalization, hospital admission or prolongation of hospitalization that is part of the planned management of acute calculous cholecystitis, early laparoscopic cholecystectomy or routine postoperative care will not, by itself, be considered a serious adverse event. However, prolongation of hospitalization due to an adverse medical occurrence, postoperative complication, infection, clinical deterioration or drug-related reaction will be assessed as a potential SAE.

Adverse Drug Reaction

An Adverse Drug Reaction is any adverse event for which there is at least a reasonable possibility of a causal relationship with amoxicillin/clavulanic acid.

A Serious Adverse Drug Reaction is an adverse drug reaction that meets one or more seriousness criteria.

Unexpected Adverse Drug Reaction

An unexpected adverse drug reaction is an adverse reaction whose nature, severity or outcome is not consistent with the current applicable product information for amoxicillin/clavulanic acid, including the Summary of Product Characteristics.

Suspected Unexpected Serious Adverse Reaction

A Suspected Unexpected Serious Adverse Reaction is an adverse event that is serious, suspected to be related to the study drug or study intervention, and unexpected according to the applicable reference safety information.

The Sponsor-Investigator will evaluate each reported SAE in terms of seriousness, causality and expectedness. If an event is assessed as serious, related to the study drug or study intervention, and unexpected, it will be classified as a SUSAR and reported according to applicable regulatory requirements.

As the study is open-label, no unblinding procedure is required for SUSAR assessment.

Adverse Event of Special Interest

An Adverse Event of Special Interest (AESI) is an event of scientific and medical concern specific to the investigational product, study intervention, or study population that requires enhanced monitoring, prompt communication, and/or additional data collection, regardless of its seriousness, severity, or assessed relationship to the study intervention.

Assessment of severity

The Investigator will assess the severity of each adverse event using the following categories:

- Mild: transient or mild discomfort; no or minimal medical intervention required; no limitation of usual activities.
- Moderate: sufficient discomfort to interfere with usual activities; medical evaluation or treatment may be required.
- Severe: considerable interference with usual activities; inability to perform normal daily activities; significant symptoms requiring intensive therapeutic intervention or medical management. Severity is distinct from seriousness. An event assessed as severe is not necessarily serious, and a serious event may be mild, moderate, or severe in intensity.

Postoperative complications will also be classified according to the Clavien-Dindo classification at POD7 and POD30, as specified elsewhere in the protocol.

Assessment of causality

The Investigator will assess the relationship of each adverse event to:

- amoxicillin/clavulanic acid, when applicable;
- omission of preoperative antibiotic therapy, when applicable;
- rescue antibiotic therapy, when applicable;
- surgical procedure;
- underlying acute calculous cholecystitis;
- concomitant diseases or concomitant treatments;
- other causes.

Causality will be classified as follows:

- Not related: a causal relationship can be ruled out; the event is clearly explained by another cause.
- Unlikely related: a causal relationship is unlikely; alternative explanations are more plausible.
- Possibly related: a temporal relationship exists and a causal relationship cannot be excluded; alternative explanations may also be present.
- Probably related: a reasonable temporal relationship exists, alternative explanations are unlikely, and the event is clinically compatible with the known or expected effects of the investigational product or study intervention.
- Definitely related: a clear causal relationship is established based on temporal association, absence of alternative explanations, known pharmacology of the study intervention.

The Sponsor-Investigator may perform an independent causality assessment. If the Sponsor-Investigator disagrees with the Investigator's causality assessment, both assessments will be documented according to applicable requirements.

Assessment of expectedness

Expectedness of adverse drug reactions related to amoxicillin/clavulanic acid will be assessed using the current Summary of Product Characteristics as reference safety information.

Expectedness of events related to the clinical condition, surgical procedure or study strategy will be assessed in relation to the known and anticipated risks of acute calculous cholecystitis, early laparoscopic cholecystectomy, omission of preoperative antibiotics, rescue antibiotic therapy and perioperative care, as described in this protocol.

Events that are anticipated in the clinical context of acute calculous cholecystitis and early laparoscopic cholecystectomy will still be recorded and reported as AE or SAE when applicable. Their anticipated nature does not exclude SAE reporting if seriousness criteria are met.

Expected adverse reactions to amoxicillin/clavulanic acid

The following adverse reactions are considered expected adverse drug reactions to amoxicillin/clavulanic acid when consistent with the current Summary of Product Characteristics:

- gastrointestinal disorders, including diarrhoea, nausea, vomiting, abdominal discomfort and antibiotic-associated diarrhoea;
- suspected or confirmed *Clostridioides difficile* infection or antibiotic-associated colitis;
- hypersensitivity reactions, including rash, pruritus, urticaria, angioedema, bronchospasm and anaphylaxis;
- severe cutaneous adverse reactions, when consistent with the reference safety information;
- hepatic abnormalities, including increased liver enzymes, cholestatic jaundice, hepatitis or clinically relevant hepatic dysfunction;
- renal abnormalities, including clinically relevant renal impairment or interstitial nephritis when suspected;
- haematological abnormalities, including leukopenia, neutropenia, thrombocytopenia, haemolytic anaemia or coagulation abnormalities when clinically relevant;
- neurological events such as convulsions, particularly in predisposed patients or in the presence of renal impairment;
- infusion-site reactions, including pain, inflammation or thrombophlebitis at the infusion site;
- other adverse reactions listed in the applicable product information.

Expected clinical and surgical complications of acute calculous cholecystitis and early laparoscopic cholecystectomy

The following events are recognised as anticipated clinical or surgical complications in the context of acute calculous cholecystitis, early laparoscopic cholecystectomy and postoperative care. They will be collected as clinical outcomes and/or adverse events as applicable:

- surgical site infection, including superficial incisional, deep incisional and organ/space infection;
- intra-abdominal infection, abscess or infected collection;
- bacteremia;
- pneumonia;
- urinary tract infection;
- sepsis or septic shock;
- bile leak;
- postoperative ERCP;
- radiological drainage or other radiological intervention;
- reoperation or surgical reintervention;

- readmission within 30 days;
- conversion to laparotomy;
- bail-out procedures;
- bleeding or postoperative haemorrhage;
- pancreatitis;
- ileus or delayed recovery of bowel function;
- thromboembolic events;
- cardiovascular or respiratory complications;
- intensive care unit admission;
- death.

These events will not automatically be considered adverse drug reactions. Causality will be assessed according to the Investigator's judgement, considering the underlying disease, surgery, allocated treatment strategy, antibiotic exposure and other clinical factors.

Adverse Events / AESI

The following events will be considered safety events of special interest and will be specifically monitored and recorded. They may or may not meet seriousness criteria:

- severe hypersensitivity reaction or anaphylaxis;
- suspected or confirmed *Clostridioides difficile* infection;
- clinically significant hepatic dysfunction;
- clinically significant renal deterioration;
- severe clinical deterioration requiring urgent treatment, rescue antibiotic therapy, ICU admission or urgent surgery;
- sepsis or septic shock;
- postoperative infectious complications requiring radiological drainage, surgical reintervention, ICU admission or readmission;
- death from any cause within 30 days after surgery.

Safety events of special interest will be recorded in the eCRF and reported as SAE when seriousness criteria are met.

Rescue antibiotic therapy

Rescue antibiotic therapy will always be allowed when clinically indicated. The need for rescue antibiotic therapy will be recorded, including indication, date and time of initiation, molecule, route of

administration, duration and clinical reason.

Rescue antibiotic therapy alone will not automatically define the primary outcome unless predefined criteria for postoperative infectious morbidity are met. It will be considered in the assessment of protocol adherence, safety outcomes and analysis populations according to the Statistical Analysis Plan.

Pregnancy

Known or suspected pregnancy, including a positive pregnancy test when performed, is an exclusion criterion. Pregnancy status will be assessed before randomization according to local clinical practice and medical judgement. Pregnancy testing, which may include urine or serum hCG, will be performed only when pregnancy cannot be reasonably excluded, when the participant is uncertain, or when required by local procedures.

If pregnancy is discovered after randomization, the participant will discontinue the allocated study strategy, will receive standard clinical care, and the pregnancy will be reported to the Sponsor-Investigator according to the procedures described in Section 10.1.2. Pregnancy itself will not be considered an adverse event or serious adverse event, but any complication affecting the participant, pregnancy, fetus or newborn will be recorded and reported as AE or SAE when applicable.

Documentation of adverse events

For each adverse event, the following information will be recorded in the source documents and eCRF when available:

- participant study identification number;
- event diagnosis or description;
- date and time of onset;
- date and time of resolution or status at last follow-up;
- severity;
- seriousness;
- seriousness criterion, if applicable;
- causality assessment;
- expectedness, when applicable;
- action taken;
- treatment required;
- outcome;
- relationship with study drug, study intervention, surgery, underlying disease or other causes;
- whether the event is an event of special interest;
- whether the event contributed to a study outcome.

All adverse events will be followed until resolution, stabilization, explanation, loss to follow-up,

withdrawal of consent, or end of the study, as applicable. Serious adverse events will be followed until resolution or stabilization, even if this occurs after the POD30 follow-up visit.

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

General reporting principles

All adverse events will be recorded in the source documents and in the eCRF. Serious adverse events must also be reported using the study-specific SAE Form.

The Investigator must immediately (no later than 24 hours after becoming aware of the event) inform the Sponsor's Pharmacovigilance team by sending the SAE form to farmacovigilanza@smatteo.pv.it

Reporting of Serious Adverse Events

All SAEs must be reported to the Sponsor's Pharmacovigilance team within 24 hours of awareness, using the study-specific SAE Form.

The initial SAE report should include, at a minimum, the following information:

- study title or study code;
- participating centre;
- coded participant identification number;
- reporter name and contact details;
- date of awareness of the event;
- event diagnosis or medical description;
- seriousness criterion;
- date and time of onset;
- current outcome or status;
- action taken;
- relevant clinical information available at the time of reporting;
- suspected relationship with amoxicillin/clavulanic acid, omission of preoperative antibiotics, rescue antibiotic therapy, surgical procedure, underlying disease or other causes;
- Investigator's causality assessment.

The initial SAE report should not be delayed because some information is missing. Additional information must be provided in follow-up SAE reports as soon as it becomes available.

The Medical Advisor will review each SAE and assess seriousness, causality, expectedness and reporting requirements. The Medical Advisor may request additional clinical information from the site, and the Local Investigator must provide follow-up information in a timely manner.

Events related to the planned hospitalization for acute calculous cholecystitis and early laparoscopic cholecystectomy will not be considered SAE solely because hospitalization occurred. However, any

adverse medical occurrence leading to prolongation of hospitalization, readmission, ICU admission, urgent intervention, significant disability, life-threatening condition or death will be assessed as a potential SAE.

Expected postoperative complications, infectious complications or clinical events related to the underlying disease or surgical procedure must still be reported as SAE when seriousness criteria are met. Their expected nature in the clinical context does not remove the obligation for SAE reporting to the Sponsor-Investigator.

Onward reporting by the Sponsor-Investigator

The SAEs unexpected and related require expedited reporting to Competent Authorities, according to applicable laws and local requirements. It will be performed by Sponsor's Pharmacovigilance team.

Expected serious events related to acute calculous cholecystitis, early laparoscopic cholecystectomy, postoperative complications or progression of the underlying disease will be documented and followed. They will not normally be classified as SUSARs unless they are considered related to the study drug or study intervention and unexpected according to the applicable reference safety information.

Reporting of SUSARs

The Sponsor-Investigator will evaluate all reported SAEs for seriousness, causality and expectedness. If an SAE is assessed as related to the study drug or study intervention and unexpected according to the applicable reference safety information, it will be classified as a Suspected Unexpected Serious Adverse Reaction.

Fatal or life-threatening SUSARs will be reported by the Sponsor-Investigator according to applicable regulatory requirements as soon as possible and no later than 7 calendar days after the Sponsor-Investigator first becomes aware of the event. Relevant follow-up information will be submitted as soon as possible, and where applicable within a further 8 calendar days.

All other SUSARs will be reported by the Sponsor-Investigator according to applicable regulatory requirements as soon as possible and no later than 15 calendar days after the Sponsor-Investigator first becomes aware of the event.

In this open-label study, no unblinding procedure is required for SUSAR assessment.

The Sponsor-Investigator will inform participating investigators of SUSARs and relevant safety information as required by applicable regulations and Sponsor procedures.

Reporting of safety signals

Any suspected new risk, relevant new aspect of a known risk, unexpected pattern of adverse events, or safety concern that may affect participant safety or the risk-benefit assessment of the trial must be reported by the Local Principal Investigator to the Sponsor's Pharmacovigilance team within 24 hours of awareness.

The Sponsor-Investigator will evaluate all safety signals and will determine whether urgent safety measures, protocol amendments, temporary suspension, additional investigator communication, or reporting to Ethics Committees and Competent Authorities are required.

Urgent safety measures

If an urgent measure is required to protect participants from an immediate hazard, the Investigator may implement the necessary action without prior approval from the Sponsor, Ethics Committee or Competent Authority.

The urgent safety measure must be documented and reported to the Sponsor-Investigator as soon as possible. The Sponsor-Investigator will ensure further notification to the Ethics Committee and Competent Authorities according to applicable requirements.

Reporting and handling of pregnancy

Known or suspected pregnancy, including a positive pregnancy test when performed before randomization, is an exclusion criterion.

If pregnancy is discovered after randomization, the participant will discontinue the allocated study strategy and will receive standard clinical care. The pregnancy must be reported to the Sponsor's Pharmacovigilance team within 24 hours of awareness using the appropriate pregnancy reporting form or SAE Form, according to Sponsor procedures.

Pregnancy itself will not be considered an AE or SAE. However, any complication affecting the participant, pregnancy, fetus or newborn, including spontaneous abortion, congenital anomaly, birth defect, serious maternal complication or other clinically significant abnormal outcome, must be recorded and reported as AE or SAE when applicable.

The course and outcome of pregnancy will be followed, where possible and permitted by consent and applicable regulations.

Periodic safety reporting

The Sponsor-Investigator will prepare and submit periodic safety reports according to applicable regulatory requirements. For this clinical trial, annual safety reporting will include cumulative safety information from all participating centres, including centres outside Italy where applicable.

Participating investigators will provide the Sponsor-Investigator with all necessary safety information for periodic reporting. The Sponsor-Investigator will submit annual safety reports to the Competent Authorities (in CTIS) according to applicable requirements and will provide relevant safety updates to participating investigators.

Reporting in international centres

For participating centres outside Italy, the Local Principal Investigator is responsible for complying with

local safety reporting requirements in addition to the reporting obligations to the Sponsor-Investigator described in this protocol.

Any country-specific reporting requirements, timelines or local forms will be defined before site activation and documented in the site-specific regulatory file, study manual or local agreement.

Reporting to the Marketing Authorisation Holder

Amoxicillin/clavulanic acid is an authorised medicinal product. If reporting to the Marketing Authorisation Holder is required by applicable regulations or local pharmacovigilance procedures, this will be performed by the Sponsor's Pharmacovigilance team according to Sponsor procedures and applicable requirements.

10.1.3 Follow up of (Serious) Adverse Events

All adverse events will be followed until resolution, stabilization, explanation, loss to follow-up, withdrawal of consent, or completion of the study follow-up period, as applicable.

All serious adverse events will be followed until resolution, stabilization, return to baseline condition, death, loss to follow-up, withdrawal of consent, or until the Sponsor-Investigator determines that no further clinically meaningful follow-up information can be obtained.

If an SAE is ongoing at the POD30 follow-up visit, the participant will continue to be followed for that SAE until resolution or stabilization, even if this occurs after completion of the scheduled study follow-up period, unless consent for further follow-up is withdrawn or further follow-up is not possible.

Follow-up information must be documented in the source documents and reported to the Sponsor-Investigator using follow-up SAE Forms or other Sponsor-approved reporting procedures.

Follow-up reports should include, when available:

- updated diagnosis or event description;
- clinical course;
- relevant laboratory, imaging, microbiology or operative findings;
- treatment administered;
- changes in severity or seriousness;
- outcome;
- updated causality assessment if applicable;
- discharge summary, readmission records, operative reports or death certificate when relevant and available;
- any additional information requested by the Sponsor-Investigator.

Clinically significant abnormal laboratory values or vital signs will be followed until resolution, stabilization or clinical explanation. Abnormal laboratory values will be recorded as adverse events only

when considered clinically significant by the treating physician or investigator, for example when they require treatment, additional diagnostic procedures, modification of clinical management, prolongation of hospitalization, readmission, or are associated with clinical deterioration.

For participants who prematurely discontinue the allocated study strategy or experience protocol interruption, safety follow-up will continue until 30 days after surgery whenever consent is not withdrawn. These participants will remain included in safety data collection and will be followed for adverse events, serious adverse events, rescue antibiotic therapy, postoperative complications, readmissions, reinterventions and mortality.

For participants lost to follow-up, reasonable attempts will be made to obtain safety information, including review of hospital records, outpatient records, telephone contact, contact with the treating physician or other methods permitted by local regulations and the informed consent form. All attempts to obtain follow-up information will be documented.

If a participant withdraws consent for further study participation, no further study-specific procedures will be performed. No safety follow-up information will be collected unless permitted by the consent and applicable data protection requirements. Data already collected up to the time of withdrawal may be retained and analysed according to the informed consent form and applicable regulations.

10.2 Medical Device studies

Not applicable.

10.3 Radiation

Not applicable.

11. STATISTICAL METHODS

All analyses will be performed using the Stata software (release 19.5 or later, StataCorp, College Station, TX, USA). A 95% confidence level will be used. Descriptive statistics will be computed separately for each arm. Counts and percentages will be reported for categorical variables and mean and standard deviation or median and quartiles (if skewed) for continuous variables. Normalizing transformations may be applied for the purpose of the analysis.

11.1 Hypothesis

The null hypothesis is that the experimental arm is inferior to the control arm in terms of incidence of postoperative infectious morbidity within 30 days after early laparoscopic cholecystectomy.

The alternative hypothesis is that the experimental arm is non inferior to the control arm.

11.2 Determination of Sample Size

The sample size is computed based on the non-inferiority hypothesis that the incidence of infectious postoperative morbidity (IPM) in the no-antibiotic arm is not superior to the incidence of IPM in the antibiotic arm. Data on IPM incidence are based on the study by Park et al [20] which enrolled ACC patients with presenting characteristics similar to those in the present study (PANEL ChIMMACC). In this study the obtained IPM in the antibiotic group and in the no-antibiotic group were respectively 8.6% and 7.6%. These figures are consistent in size with most of the literature on the topic [19, 22].

Given these low IPM rates and the need for a clear evidence to allow for a non-inferiority claim (given the sparse and in part contradictory results from the literature), we consider a conservative non inferiority (NI) margin of 2%: hence an IPM rate in the no-antibiotic group which is less than 2% (absolute) higher than the IPM rate in the antibiotic group is considered non inferior.

The null (H_0) and alternative (H_1) hypotheses are as follows:

H_0 : $PN-PA \geq NI$

H_1 : $PN-PA < NI$

where (PN) and (PA) denote the incidence of IPM in the no-antibiotic arm and antibiotic arm, respectively.

We compute the sample size based on these hypotheses, a one-sided alpha of 2.5% and a power of 80%, using the following command in Stata:

artbin, pr(0.086 0.076) margin(0.020) alpha(0.025) power(0.8) unf onesided wald force

that yields a sample size of **2596 patients (1298 per group)**. To account for a drop-out rate of 10%, we may enroll up to **2884 patients (1442 per group)**.

11.3 Statistical criteria of termination of trial

There are no statistical criteria for the termination of the trial.

11.4 Planned Analyses

11.4.1 Datasets to be analysed, analysis populations

Given the non-inferiority design, the primary analysis will be per-protocol (patients as randomized, in whom the primary endpoint is recorded and no major deviations are observed). A sensitivity analysis will use the intention to treat population (patients as randomized, in whom the primary endpoint is recorded). Intercurrent events will be handled as described in §5.1.1

11.4.2 Primary Endpoint Analysis

Analysis of the primary endpoint.

The difference in rate of IPM between the two arms (no-antibiotic arm-antibiotic arm) will be computed together with its 95% confidence interval (CI), using a generalized linear model extended for the binomial family. If the upper limit of the 95%CI is below 2%, non-inferiority will be claimed. If the non-inferiority is attained, the superiority will be assessed. Superiority of the no-antibiotic arm will be concluded if the upper limit of the 95% confidence interval for the risk difference is below 0. Huber White robust standard errors will be computed to account for intra-center correlation of measures. The primary analysis will be performed in the per-protocol population. The secondary analysis will be performed in the intention to treat population.

11.4.3 Secondary Endpoint Analyses

#	Endpoint at 30 days POD	Analysis
2.1	overall surgical site infection (SSI);	The same model as for the primary endpoint will be fitted
2.2	superficial incisional SSI;	The same model as for the primary endpoint will be fitted
2.3	deep incisional SSI;	The same model as for the primary endpoint will be fitted
2.4	organ/space SSI, including intra-abdominal infection or abscess;	The same model as for the primary endpoint will be fitted
2.5	pneumonia;	The same model as for the primary endpoint will be fitted
2.6	urinary tract infection;	The same model as for the primary endpoint will be fitted

2.7	sepsis or septic shock;	The same model as for the primary endpoint will be fitted
2.8	other clinically relevant postoperative infections requiring antimicrobial therapy and/or radiological or surgical treatment;	The same model as for the primary endpoint will be fitted
2.9	rescue antibiotic therapy, including indication, timing, antibiotic agent and duration;	The same model as for the primary endpoint will be fitted
2.10	bile and blood cultures: strongly recommended but not mandatory;	The same model as for the primary endpoint will be fitted
2.11	positive intra-abdominal fluid culture, when collected;	The same model as for the primary endpoint will be fitted
2.12	positive umbilical wound culture, when collected;	The same model as for the primary endpoint will be fitted
2.13	microbiological profile of positive cultures, including identification of isolated organisms and antimicrobial resistance when available;	Description of the microbiological profile by treatment arm
2.14	postoperative inflammatory markers, including white blood cell count, C-reactive protein, procalcitonin, total and direct bilirubin, and creatinine, according to the predefined study timepoints;	A generalized linear model will be fitted. Huber White robust standard errors will be computed to account for intra-center correlation of measures. The difference between groups of the continuous variable and 95%CI will be derived from the model. Data may be log-transformed, given the expected skewed distribution. In this case, the effect estimate, and 95%CI will be back-transformed and expressed as a ratio on the original scale
2.15	laboratory parameters at ED admission and POD1;	The same model as for EP2.14 will be fitted
2.16	non-infectious postoperative complications, classified according to the Clavien-Dindo classification, specifically assessed at POD30;	Descriptive statistics by group and category will be computed at POD30. Groups will be compared with the test for trend at the time point. The

		Bonferroni correction will be applied.
2.17	conversion to laparotomy;	The same model as for the primary endpoint will be fitted
2.18	bail-out procedures;	The same model as for the primary endpoint will be fitted
2.19	length of hospital stay;	The same model as for EP2.14 will be fitted
2.20	readmission within 30 days after surgery;	The same model as for the primary endpoint will be fitted
2.21	need for postoperative radiological intervention;	The same model as for the primary endpoint will be fitted
2.22	need for postoperative surgical reintervention;	The same model as for the primary endpoint will be fitted
2.23	mortality within 30 days after surgery.	The same model as for the primary endpoint will be fitted

11.4.4 Interim analyses

No formal interim analyses are planned for this study.

Safety will be monitored throughout the study by the investigators and may indicate the need for study termination.

11.4.5 Safety analysis

Adverse events and serious adverse events will be described by treatment arm and by patient and treatment arm

11.4.6 Deviation(s) from the original statistical plan

Any deviation(s) from the original statistical plan will be reported and justified in the final Statistical Analysis Plan (SAP).

11.5 Handling of missing data and drop-outs

No missing data imputation will be performed. Given the short duration of the study, we do not expect missing data to occur.

12. QUALITY ASSURANCE AND DATA CONTROL

The Sponsor is responsible for implementing and maintaining appropriate quality assurance and quality control procedures for the study. The Principal Investigator at each participating centre is responsible for ensuring that all delegated study personnel are adequately trained on the protocol, Good Clinical Practice and study procedures.

12.1 Data handling and record keeping / archiving

Study data will be collected, handled and archived according to the protocol, Sponsor procedures, Good Clinical Practice and applicable regulatory requirements.

Records and documents (e.g., signed Informed Consent Forms, copies of eCRFs, regulatory documents) will be retained at each study center, by the investigator per local regulations or institutional policies. The Investigator should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. No records may be destroyed during the retention period without the written approval of the sponsor.

12.1.1 Case Report Forms

Study data will be recorded with an electronic Case Report Form (eCRF) based on the REDCap® platform (REDCap 15.5.36 - © 2026 Vanderbilt University).

Participants will be identified in the eCRF only by a coded study identification number. Direct identifiers will not be entered into the central study database. Only authorised and trained study personnel will be allowed to enter or modify data. All entries and modifications will be traceable through the system audit trail.

Each local Principal Investigator is responsible for keeping in a separate document patients identification data and the corresponding patients code.

12.1.2 Specification of source documents

Source documents will include all original records relevant to the study, including medical records, informed consent forms, screening logs, eligibility documentation, randomization records, medication administration records, operative reports, laboratory and microbiology reports, imaging reports, discharge summaries, adverse event and serious adverse event documentation, and follow-up records.

12.1.3 Record keeping / archiving

Essential study documents, source documents and study data will be archived according to applicable legal and regulatory requirements. The Investigator will retain copies of all the essential documents at site for 25 years after study termination or premature termination of the clinical trial (as defined by ICH-

GCP). The Investigator will inform the Sponsor of the storage location of these essential documents. If the Investigator wishes to remove them to another location, he/she should consult with Sponsor about this change.

12.2 Data management

12.2.1 Data Management System

The data will be collected in a database built using the REDCap platform (REDCap 15.5.36-© 2026 Vanderbilt University), hosted on a server of the Information Systems at the coordinating Center and administered by the Clinical Trial Center. Databases are backed-up on a regular basis, as prescribed by the local security rules. The access to the database is web-based. A double authentication process is applied: Each authorised user will access the database using a dedicated username and password and a double authentication via OTP. Every participating Center will be only able to see and edit its own data. Every access to REDCap is monitored by the system, as are any changes to the data. Patients will be pseudo-anonymised and no identifying data will be included. It will be the Local Principal Investigator's responsibility to maintain a separate file containing the subject's personal data (and file number) together with the code assigned by REDCap. The study will be approved by the hospital Institutional Review Boards of the different national leading institutions and will be registered at clinicaltrials.gov.

12.2.2 Data security, access and back-up

Patients will be pseudo-anonymized and no personal identifiers will be collected. They will be only identified by a unique identification (ID) number in the CRFs and in the other study documents. Personal data recorded on all documents will be regarded as confidential, and to preserve each patient's pseudoanonymity, only their patient study number will be recorded on the CRFs. The Investigator site must maintain the patient's pseudoanonymity in all communications and reports related to the research. It will be the responsibility of the PI to maintain a mapping linking code and patient's ID separately, to allow for follow-up.

REDCap supports a secure web-based application for developing fully functional case report forms (CRFs). In particular, through REDCap we will implement:

- full user authentication (log-on/password/authentication code) to restrict users to study functions;
- real-time data validation, integrity checks for ensuring data quality;
- de-identification options to be applied to data exports to remove fields that contain notes and other information that could identify patient;
- centralized, secure storage of research data with back-ups.

Access to REDCap will be restricted to authorised users through individual credentials and double authentication. Each participating centre will be able to access and manage only its own data.

12.2.3 Analysis and archiving

Data will be imported into Stata by the study statistician before database lock in order to identify outliers and missing information for the local PIs to correct. After database lock, a final data import will be performed for the analyses described above. They will be stored on the study statistician computer. Further import may be performed in case of post-hoc analyses authorized by the PI. The electronic database will be archived on the REDCap platform and maintained for 2 years from the end of the study.

12.2.4 Electronic and central data validation

For central data validation, data completeness will be monitored in REDCap; in addition, data will be imported into Stata by the study statistician before database lock, in order to identify outliers and missing information as described in 12.2.3.

12.3 Monitoring

Monitoring will be performed by the Sponsor-Investigator.

The Sponsor-Investigator is responsible for ensuring the quality of the data entered into the CRF through remote monitoring.

12.4 Audits and Inspections

The study may be subject to audits or inspections by the Sponsor, competent authorities, Ethics Committees or other authorised institutions. Investigators will allow access to study documentation and source data, while ensuring participant confidentiality.

All parties involved in audits or inspections must maintain strict confidentiality of participant data.

12.5 Confidentiality, Data Protection

Participant confidentiality and personal data will be protected in accordance with Regulation (EU) 2016/679 (GDPR) [4], applicable national data-protection legislation and, for centres outside the European Union or European Economic Area, applicable local data-protection requirements. Direct access to source documents will be permitted only to authorised monitoring, auditing, inspection and regulatory purposes.

Access to the protocol, dataset, statistical code and study documents during and after the study will be controlled by the Sponsor according to the protocol, publication policy, data sharing policy and applicable regulations.

12.6 Storage of biological material and related health data

No study-specific biobanking, long-term storage of biological samples or shipment of biological samples to external laboratories is planned.

Blood, bile or other microbiological samples, when collected, will be processed by local certified laboratories according to standard clinical practice and local procedures. Samples will be stored or destroyed according to local standard procedures.

The gallbladder surgical specimen removed during laparoscopic cholecystectomy will be managed and analysed according to routine clinical practice and local pathology procedures. Collection and pathological examination of the gallbladder specimen are part of standard surgical care and are not specifically required by the study protocol for research purposes.

13. PUBLICATION AND DISSEMINATION POLICY

The results of the PANEL ChIMMACC trial will be disseminated through publication in peer-reviewed scientific journals, presentation at national and international scientific meetings, and reporting in public clinical trial registries, according to applicable regulatory requirements.

The Sponsor-Investigator and the study coordination group will have ultimate responsibility for the interpretation of study results, preparation of the final report, submission of manuscripts and dissemination of study findings. The decision to submit the results for publication will not be influenced by commercial interests. The study is investigator-initiated and no pharmaceutical company will have any role in manuscript preparation, interpretation of results or decision to publish.

The main trial results will be submitted for publication regardless of whether the findings are positive, negative or inconclusive. Results will also be reported in the trial registry according to applicable requirements. When appropriate, trial findings may be communicated to healthcare professionals, participating centres and the scientific community through congress presentations, educational activities and study-related communications.

Authorship will be defined according to scientific contribution to the study and in accordance with ICMJE recommendations, where applicable.

The final authorship list, order of authors and composition of the collaborative group will be defined by the study coordination group before manuscript submission, taking into account recruitment contribution, scientific contribution, data quality, compliance with study procedures and applicable journal requirements.

No professional medical writers are currently planned. If professional writing or editorial support is used, this will be transparently acknowledged in any resulting publication, including the source of support.

The full protocol and statistical analysis plan may be made available through publication, trial registry records or upon reasonable request, according to Sponsor procedures. De-identified participant-level data and statistical code may be shared after publication upon reasonable scientific request, subject to Sponsor approval, applicable ethics and data protection requirements, and completion of an appropriate data sharing agreement.

No public disclosure will include directly identifiable participant information. Dissemination of results will comply with applicable confidentiality, privacy and data protection requirements.

14. FUNDING AND SUPPORT

14.1 Funding

The PANEL ChIMMACC trial is funded by the Italian Ministry of Health within the framework of the Ricerca Finalizzata programme.

Funding source: Italian Ministry of Health, Bando Ricerca Finalizzata 2024.

Project code: RF-2024-12378141.

Project type: Ordinary project / Progetto ordinario di Ricerca Finalizzata.

Project duration: 36 months.

The applicant institution and project leader centre is Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

The study is investigator-initiated and is not funded by any pharmaceutical company. The funder will have no role in individual patient management, treatment allocation, data collection, data analysis, interpretation of results, manuscript preparation or decision to publish, unless otherwise required by applicable reporting obligations related to the funded project.

14.2 Other Support

Not applicable.

15. INSURANCE

Insurance coverage will be provided by the Sponsor for all participants enrolled in the study, including participants enrolled at international participating centres.

The insurance policy will cover study-related risks and injuries according to applicable national and international legal and regulatory requirements. For participating centres outside Italy, insurance coverage and any additional country-specific requirements will be arranged before site activation, in accordance with local regulations.

A copy of the insurance certificate and any relevant insurance documentation will be filed in the Trial Master File and, where applicable, in each Investigator Site File.

No participant may be enrolled at a participating centre before confirmation that appropriate insurance coverage is in place for that centre.

DRAFT

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17. APPENDICES

Appendix A: “Table of study procedures and assessments”

Appendix B: “Study Flowcharts”

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Appendix A: “Table of study procedures and assessments”

Procedure / assessment	ED screening (suspected ACC)	Consent + eligibility confirmation	Randomization / recruitment	Pre-op after randomization	Surgery / intra-op	POD1	Discharge	POD7	POD30 outpatient visit	Whenever clinically indicated
Enrolment, consent and allocation										
Patient presents to ED with suspected acute calculous cholecystitis	X									
On-call surgeon assessment: potentially eligible patient identified as screened	X									
Screening log entry, including screening failures and reasons	X									
Study information and written informed consent		X								
Final inclusion/exclusion criteria check		X								If needed
Randomization through centralized REDCap system			X							
Patient considered recruited/randomized			X							
Baseline clinical, laboratory and imaging assessment										
Demographics, medical history and relevant comorbidities	X									
Physical examination / baseline clinical assessment	X									If needed
Baseline physiological parameters for eligibility/scoring: systolic blood pressure, heart rate and GCS**	X									
ECG / electrocardiogram for baseline preoperative assessment and CHOLE-POSSUM category†	X									
TG18 diagnosis and severity grading	X									
CHOLE-POSSUM Physiological Score components and total score		X								
Israeli Score components and total score		X								
Pregnancy status assessment/testing in women of childbearing potential*‡	X									If needed
Diagnostic imaging: ultrasound and/or CT scan; CBD diameter <7 mm yes/no	X			If needed						If needed
ED/baseline laboratory panel*	X									
Study intervention and preoperative management										
Antibiotic arm: amoxicillin/clavulanate according to protocol			X	X						
No-antibiotic arm: omission of preoperative antibiotic therapy			X	X						
Rescue antibiotic therapy: indication, start/stop date, molecule and duration				Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	If needed



Procedure / assessment	ED screening (suspected ACC)	Consent + eligibility confirmation	Randomization / recruitment	Pre-op after randomization	Surgery / intra-op	POD1	Discharge	POD7	POD30 outpatient visit	Whenever clinically indicated
Concomitant medications and relevant clinical management				Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	If needed
Surgery and microbiology										
Early laparoscopic cholecystectomy within 72 hours from hospital admission					X					
Operative details: timing, duration, approach, intraoperative findings and operating surgeon role or experience category					X					
Conversion to laparotomy or bail-out procedure					X					
Bile culture					Rec.					
Blood cultures				If needed						If needed
Other microbiological samples/results					If needed	If needed	If needed	If needed	If needed	If needed
Postoperative assessments and outcomes										
Routine postoperative vital signs according to local clinical practice†						Ongoing	Ongoing			If needed
POD1 laboratory panel‡						X				
POD7 ±2 clinical assessment: outpatient visit or telephone contact								X		
POD7 ±2 laboratory tests§, if performed								If performed		
Primary outcome: 30-day postoperative infectious morbidity (formal ascertainment at POD30)						Ongoing	Ongoing	Ongoing	Mandatory	If needed
SSI assessment according to predefined criteria						Ongoing	Ongoing	Ongoing	Mandatory	If needed
Other infectious complications: pneumonia, UTI, bacteremia, sepsis/septic shock and other infections						Ongoing	Ongoing	Ongoing	X	If needed
Non-infectious postoperative complications						Ongoing	Ongoing	Ongoing	X	If needed
Clavien-Dindo classification								X	X	If needed
Length of hospital stay							X			
Readmission, radiological intervention and surgical reintervention								Ongoing	X	If needed
POD30 ±2 outpatient follow-up visit: mandatory									Mandatory	
POD30 ±2 surgical wound assessment and 30-day outcome ascertainment									Mandatory	
Safety, follow-up and data management										
Adverse events and serious adverse events				Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	If needed
Clinical deterioration / urgent treatment / ICU admission				Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	If needed
Withdrawal of consent or premature discontinuation				Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	If needed



Procedure / assessment	ED screening (suspected ACC)	Consent + eligibility confirmation	Randomization / recruitment	Pre-op after randomization	Surgery / intra-op	POD1	Discharge	POD7	POD30 outpatient visit	Whenever clinically indicated
Additional laboratory tests, ECGs, imaging, microbiological samples or clinical assessments										If needed
eCRF / REDCap data entry and source data completion	X	X	X	X	X	X	X	X	X	If needed

Notes

* ED/baseline laboratory panel: haemoglobin, white blood cell count, total and direct bilirubin, creatinine, urea or BUN, sodium, potassium, C-reactive protein and procalcitonin. These tests are collected as part of standard Emergency Department/baseline clinical assessment according to local clinical practice.

¶ ECG will be performed and/or recorded as part of Emergency Department, baseline or preoperative clinical assessment according to local clinical practice. The ECG category required for the CHOLE-POSSUM Physiological Score will be recorded in the eCRF.

** Systolic blood pressure, heart rate and Glasgow Coma Scale are recorded at baseline because they are required for protocol-defined eligibility/scoring and baseline physiological assessment.

*II Pregnancy status will be assessed according to local clinical practice and medical judgement. Pregnancy testing, including urine or serum hCG, will be performed when pregnancy cannot be reasonably excluded or when required by local procedures. Known or suspected pregnancy, including a positive pregnancy test when performed, is an exclusion criterion.

† Routine postoperative vital signs may include body temperature, heart rate, blood pressure, respiratory rate and peripheral oxygen saturation, and are assessed according to local clinical practice and good postoperative care.

‡ POD1 laboratory panel: haemoglobin, white blood cell count, total and direct bilirubin, C-reactive protein, and procalcitonin if performed according to local practice or clinical indication. These results are recorded from routine postoperative care.

POD7 clinical assessment. The POD7 ± 2 assessment may be performed as an outpatient visit or by telephone contact and includes clinical status, wound-related symptoms/signs, signs or symptoms of infection, adverse events, rescue antibiotic therapy, readmissions, reinterventions and postoperative complications.

§ POD7 laboratory tests are not mandatory study-specific procedures. When performed according to local clinical practice, centre procedures or clinical indication, the laboratory parameters of interest are haemoglobin, white blood cell count, total and direct bilirubin, C-reactive protein and procalcitonin if performed. Absence of POD7 laboratory tests will not constitute a protocol deviation and will not affect follow-up completion, primary endpoint assessment or per-protocol status.

POD30 follow-up. The POD30 ± 2 outpatient visit is mandatory and is planned for direct wound assessment and standardized ascertainment of 30-day postoperative infectious morbidity, including surgical site infection, as well as relevant secondary and safety outcomes. The formal primary endpoint determination will be completed at POD30; qualifying events may be collected throughout hospitalization, at discharge, at POD7, at POD30 and whenever clinically indicated. POD7 laboratory availability is not required for primary endpoint assessment.

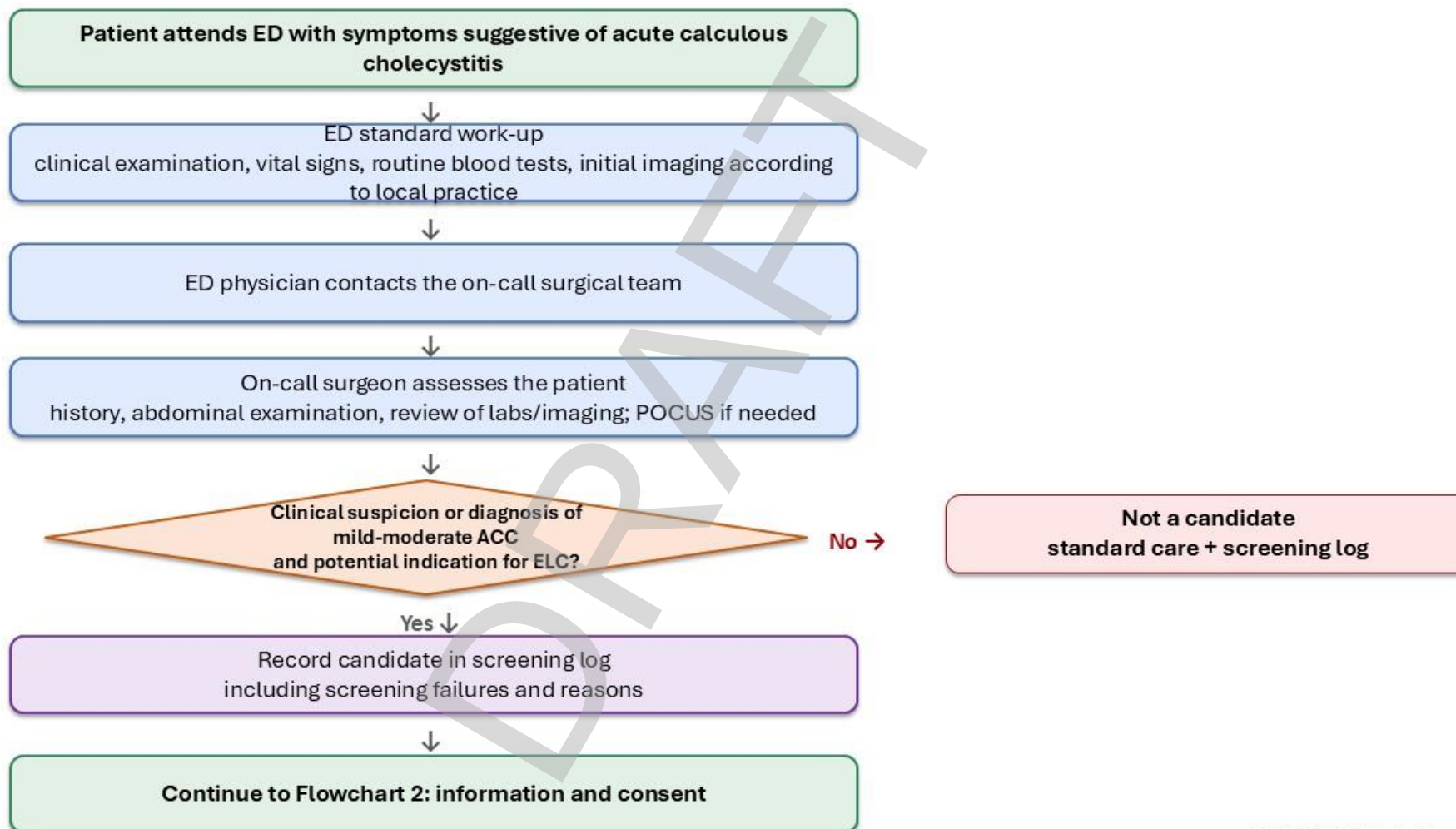
Clavien-Dindo. Clavien-Dindo classification will be assessed at POD7 and POD30. At POD7, classification will be recorded when sufficient clinical information is available, including when the assessment is performed by telephone.

Additional assessments. Additional laboratory tests, ECGs, imaging, microbiological samples or clinical assessments may be performed at any time according to clinical indication and local standard practice.

Marking key. X = scheduled assessment or data collection; Ongoing = continuous surveillance or data collection; Rec. = recommended; If needed = according to clinical indication; If performed = recorded only when performed; Mandatory = required protocol assessment.

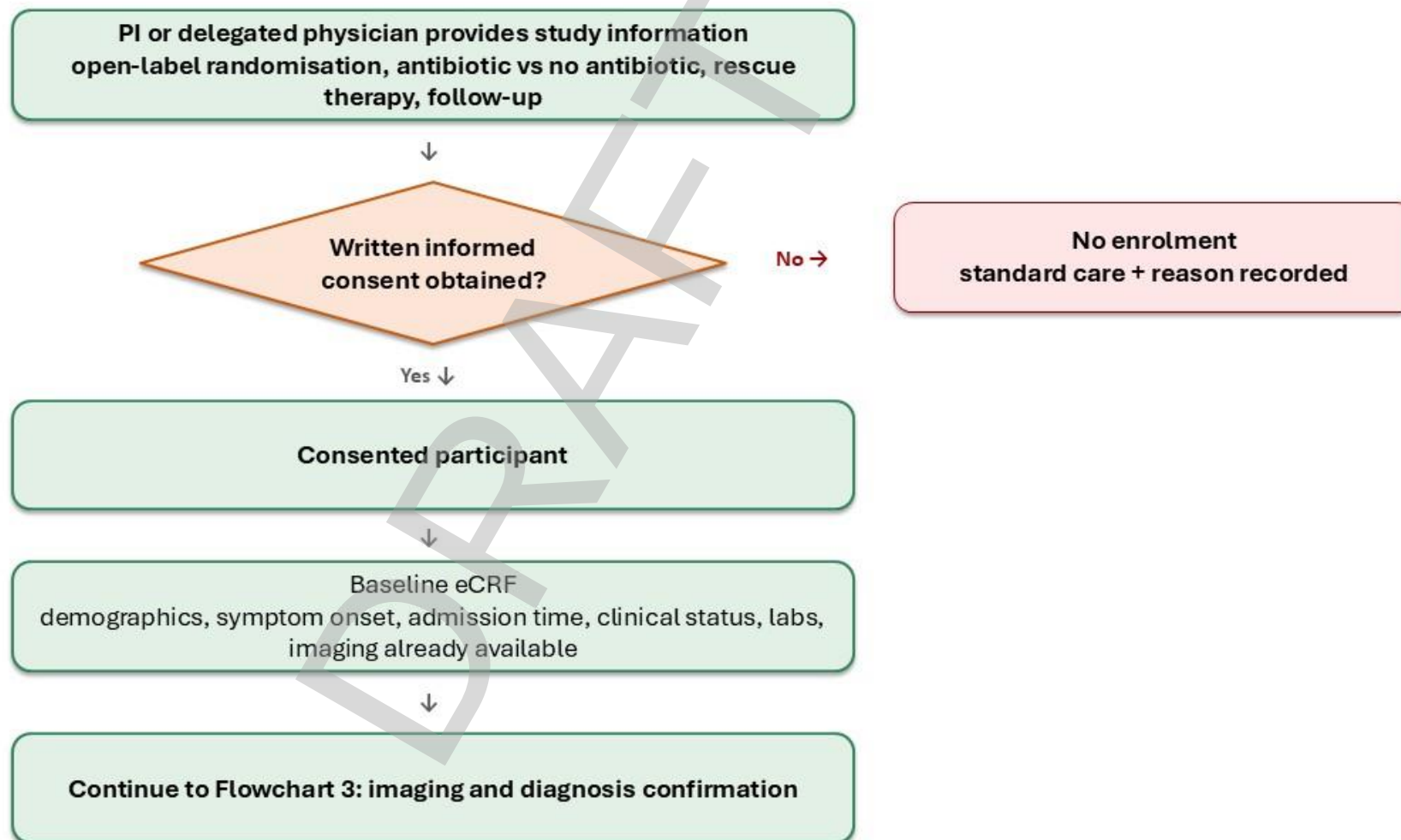
Appendix B: “Study Flowcharts”

Study Flowchart 1 - ED pre-screening and surgical assessment

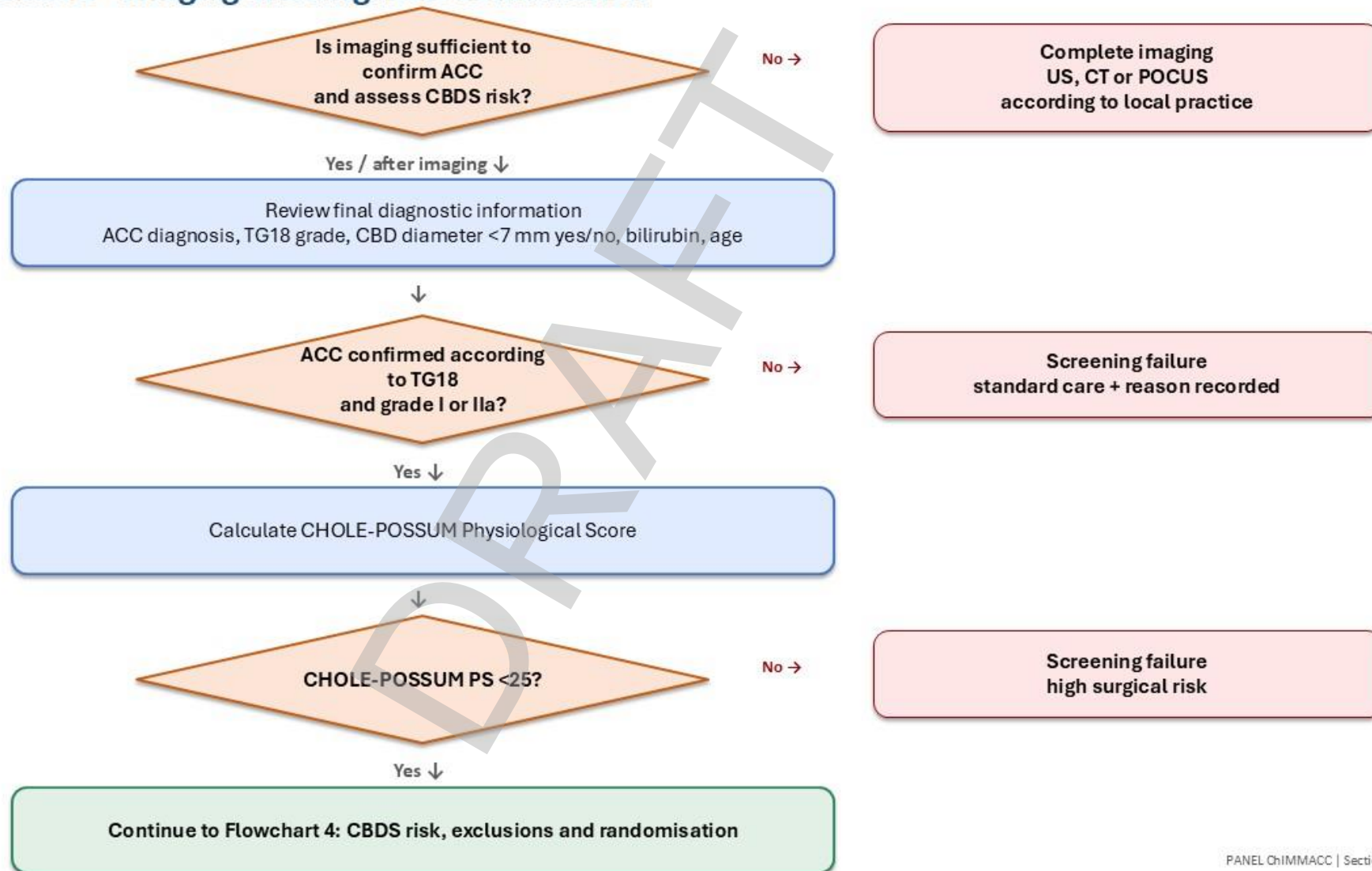


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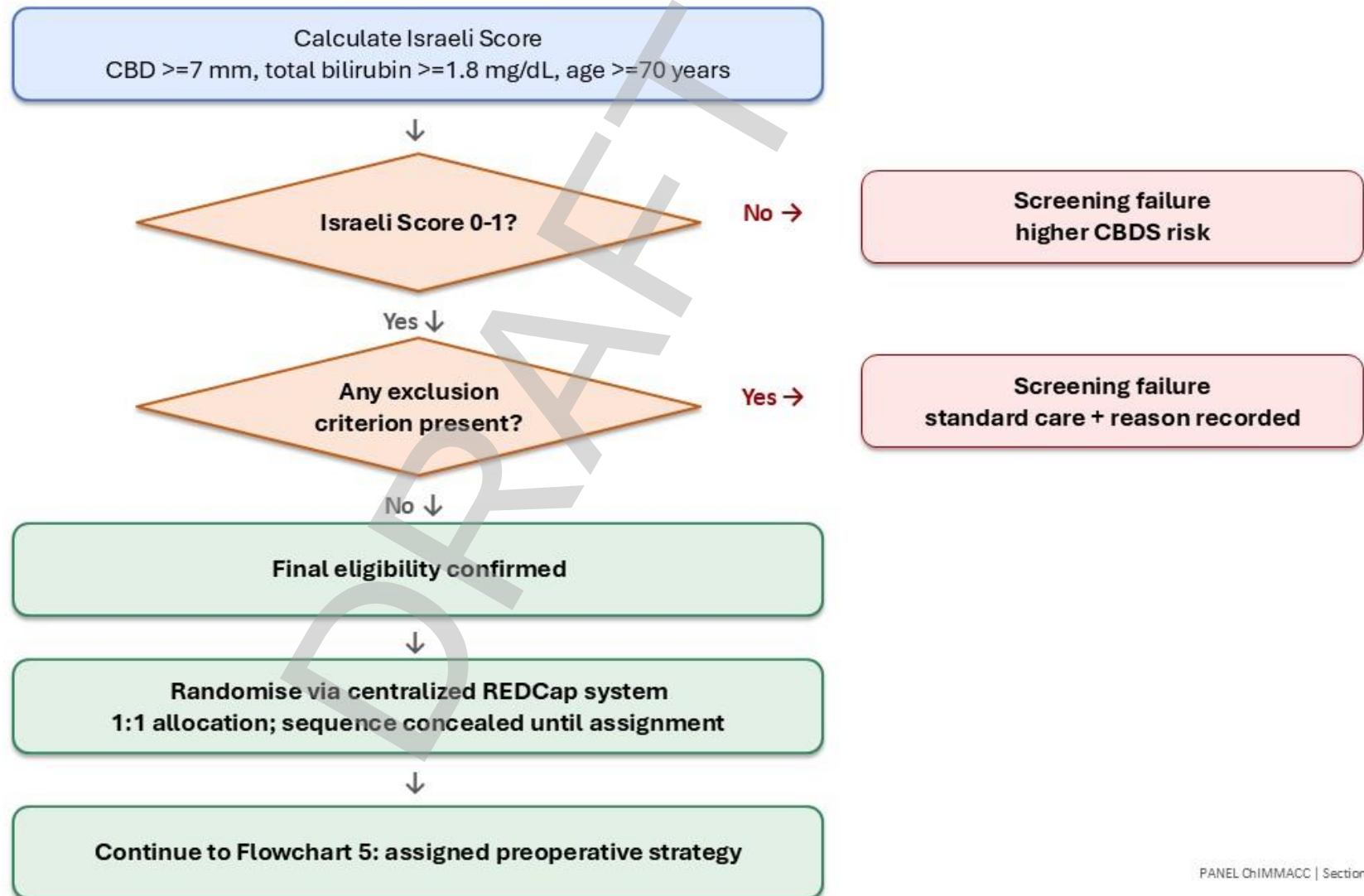
Study Flowchart 2 - Information, consent and baseline enrolment



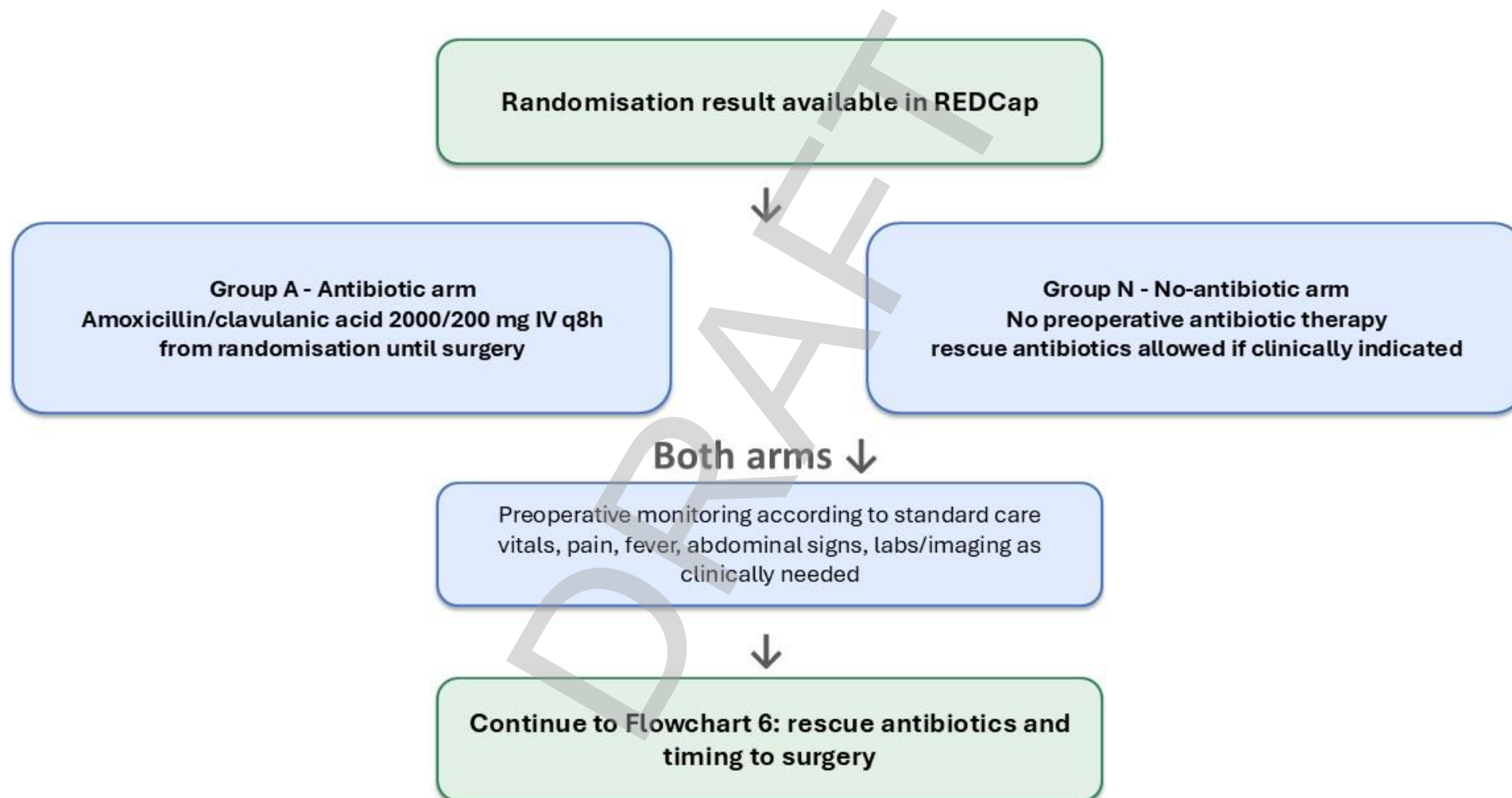
Study Flowchart 3 - Imaging and diagnosis confirmation



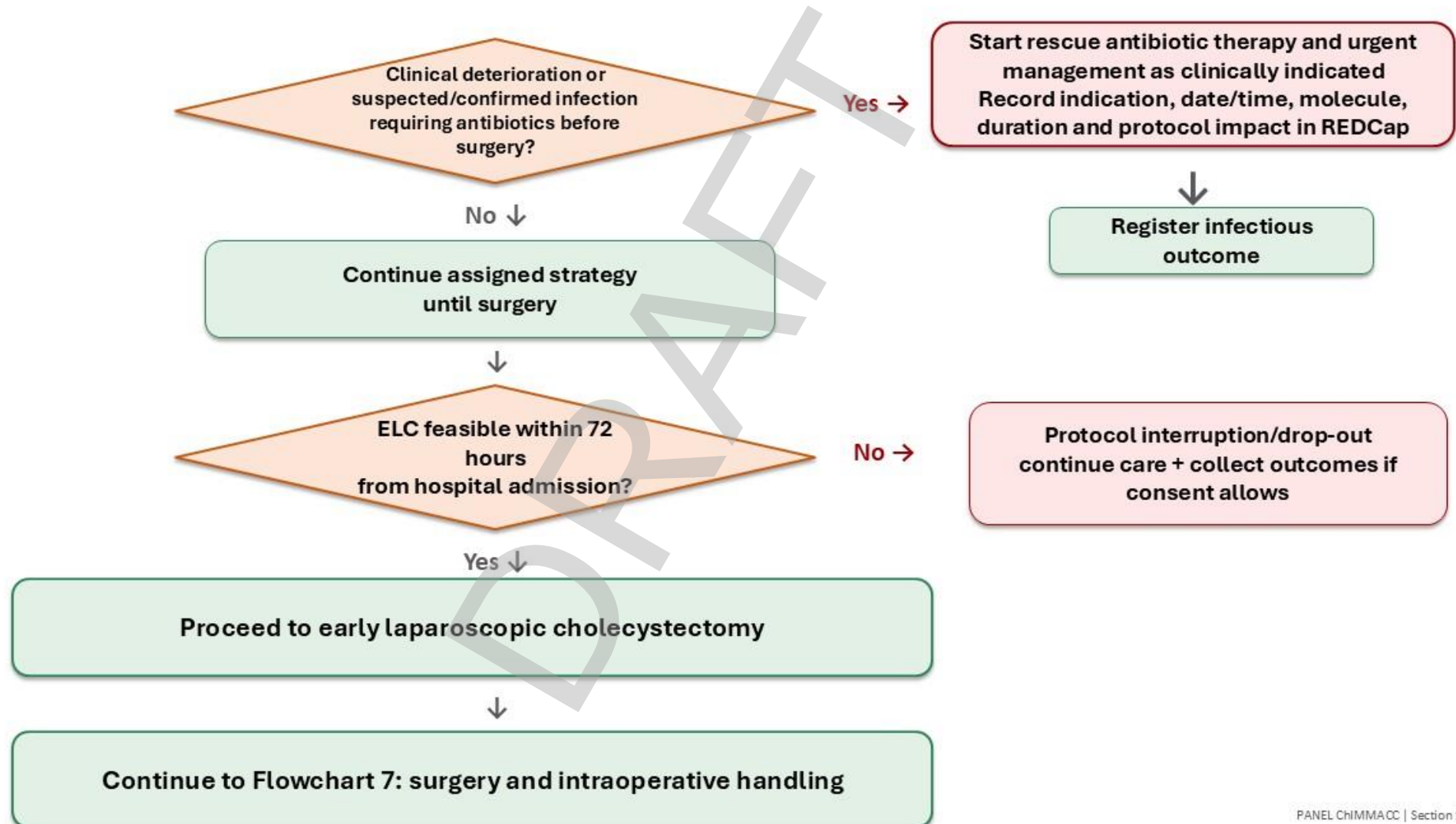
Study Flowchart 4 - CBDS risk, exclusions and randomisation



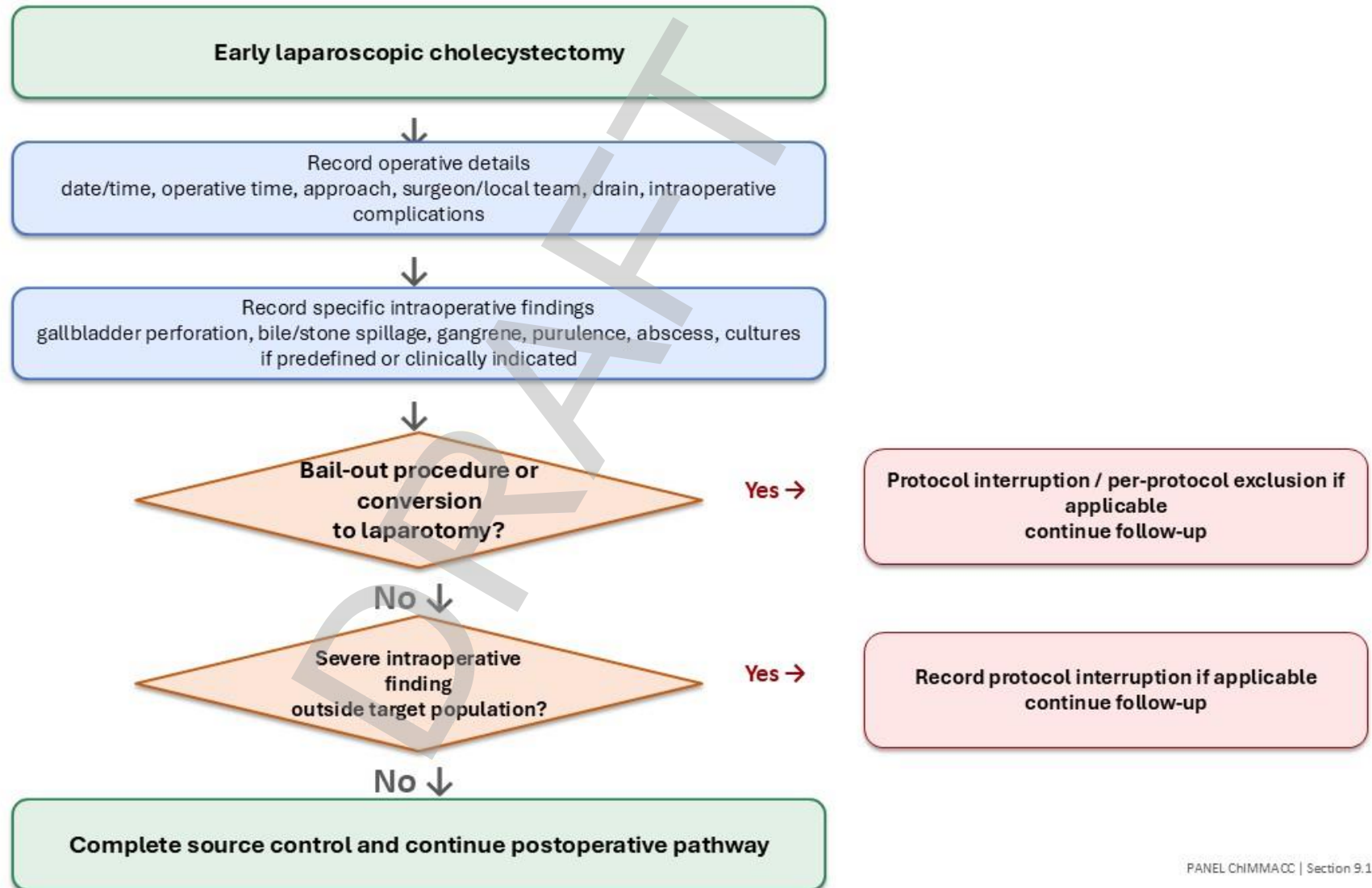
Study Flowchart 5 - Assigned preoperative strategy



Study Flowchart 6 - Rescue therapy and 72-hour surgery window

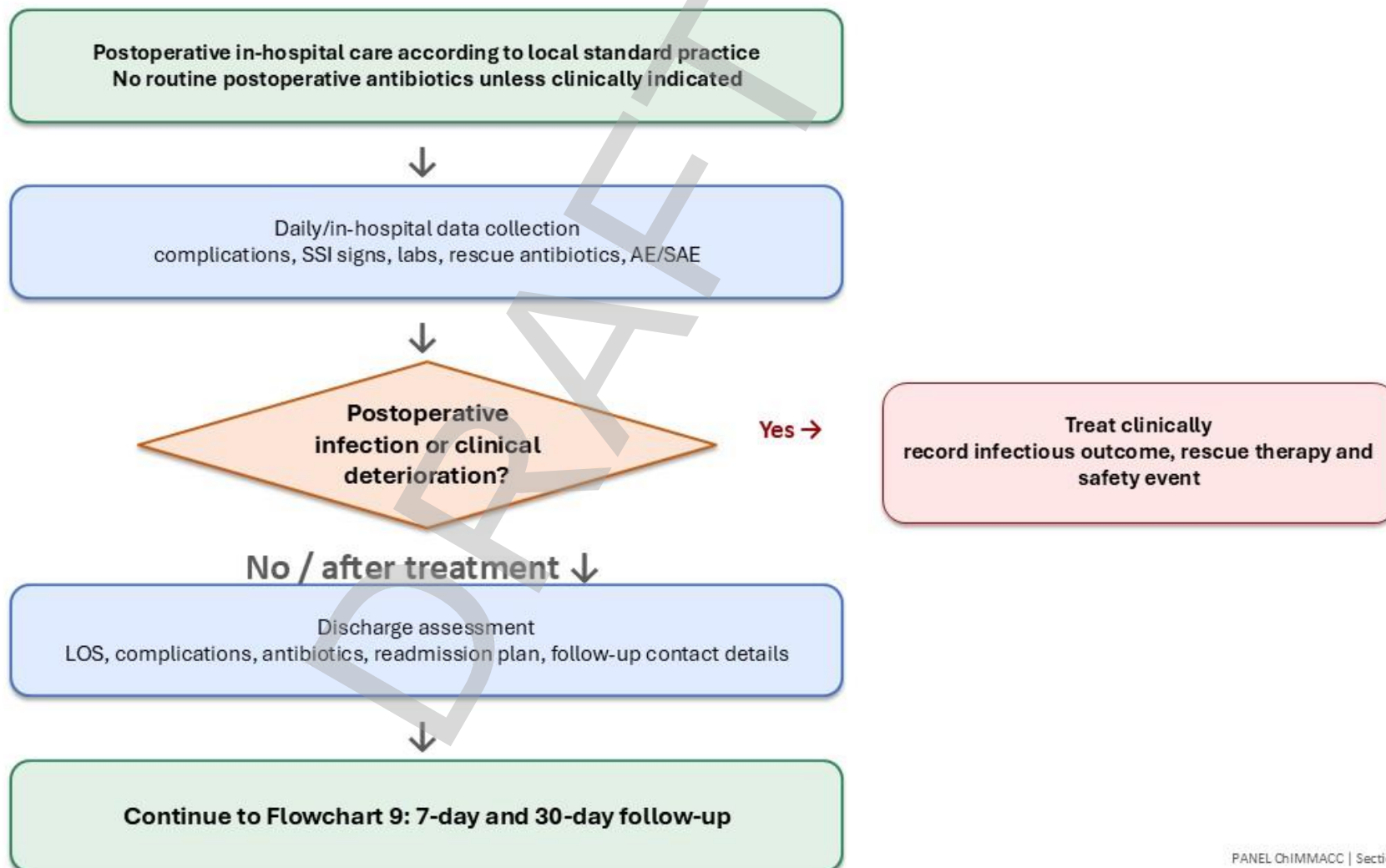


Study Flowchart 7 - Surgery and intraoperative handling



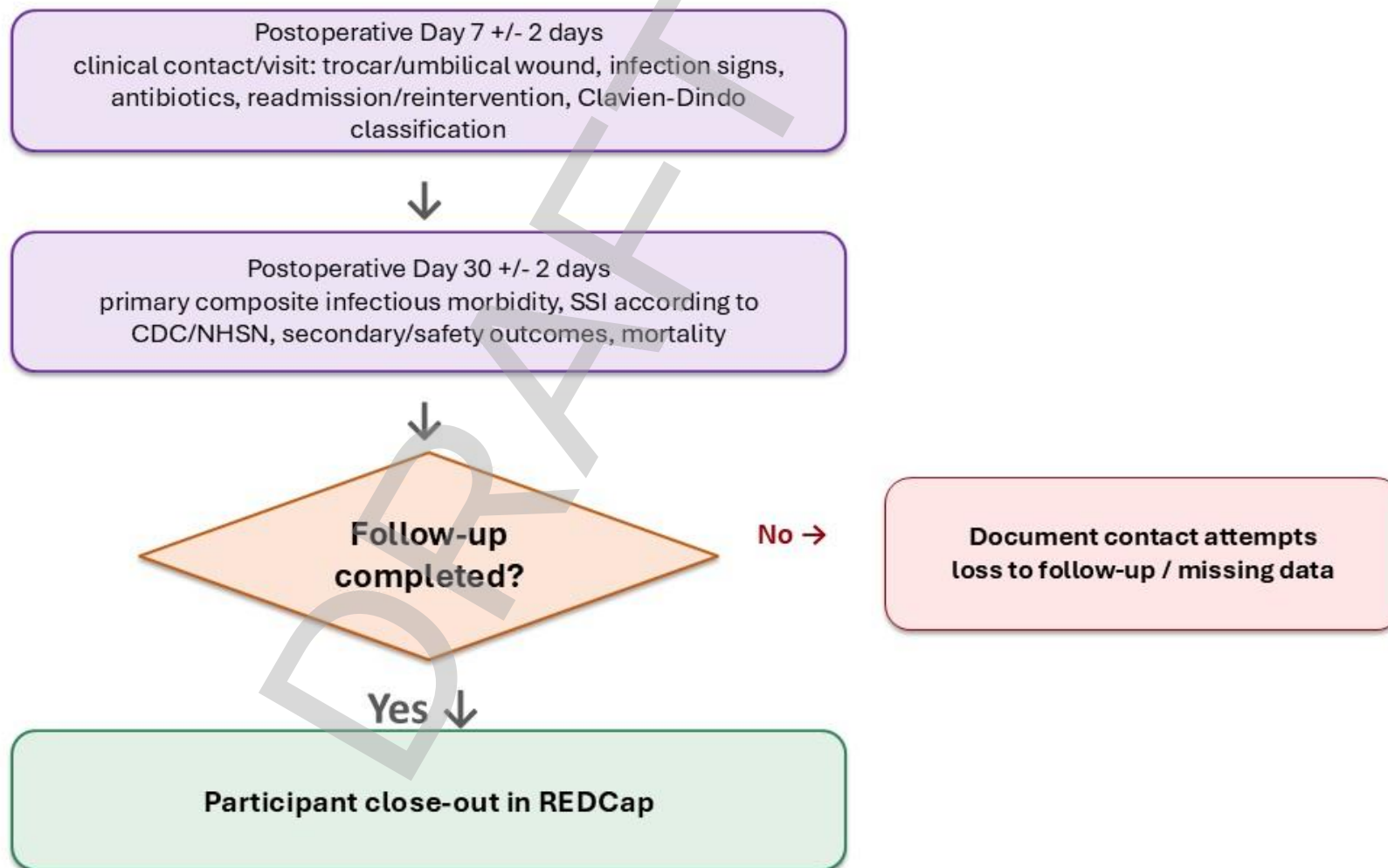
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Study Flowchart 8 - In-hospital postoperative surveillance and discharge



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Study Flowchart 9 - 7-day and 30-day follow-up





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