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Normal saline versus lactated Ringer's solution for acute pancreatitis resuscitation, an open-label multicenter randomized controlled trial: the WATERLAND trial study protocol

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Abstract

Background: Some evidence suggests that fluid resuscitation with lactated Ringer's solution (LR) may have an anti-inflammatory effect on acute pancreatitis (AP) when compared to normal saline (NS), and may be associated with a decrease in severity, but existing single center randomized controlled trials showed conflicting results. The WATERLAND trial aims to investigate the efficacy and safety of fluid resuscitation using LR compared to NS in patients with AP.

Methods: The WATERLAND trial is an international multicenter, open-label, parallel-group, randomized, controlled, superiority trial. Patients will be randomly assigned in a 1:1 ratio to receive LR versus NS-based fluid resuscitation for at least 48 hours. The primary outcome will be moderately severe or severe AP, according to the revision of the Atlanta classification. The secondary objectives of the WATERLAND trial are to determine the effect of LR versus NS fluid resuscitation on several efficacy and safety outcomes in patients with AP.

A total sample of 720 patients, 360 in the LR group and 360 in the NS group, will achieve 90% power to detect a difference between the group proportions of 10%, assuming that the frequency of moderately severe or severe AP in the LR group will be 17%. A loss to follow-up of 10% of patients is expected, so the total sample size will be 396 patients in each treatment arm (792 patients overall). The test statistic used is the two-sided Z test with pooled variance set at a 0.05 significance level.

Discussion: The WATERLAND study aims to improve the early management of AP. Fluid resuscitation is an inexpensive treatment available in any hospital center worldwide. If a better evolution of pancreatitis is demonstrated in one of the treatment arms, it would have important repercussions in the management of this frequent disease.

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Keywords

Acute pancreatitis. Fluid resuscitation. Lactated Ringer solution. Normal saline. Randomized controlled trial.

Administrative information

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Role of sponsor {5c}	This is a researcher-driven study. The sponsor participated in the study design and will participate in the collection, management, analysis, and interpretation of data, writing of the report, and the decision to submit the report for publication. The sponsor declares

	no conflicts of interest. Funders are not directly involved in the study. Private companies with economic interest in fluid resuscitation will not be involved in this study.

{}: SPIRIT checklist item numbers. (2)

Introduction

Background and rationale (SPIRIT checklist item number {6a}) (2)

Acute pancreatitis (AP) is the third leading cause of hospital admission and readmission for digestive diseases (nearly 300,000 diagnoses in the United States in 2018). (3) Furthermore, its incidence is increasing, and the median total cost per hospitalization in 2018 amounted to $22,817. (3) AP is an acute inflammatory disease with variable severity. According to the revision of the Atlanta classification (RAC), severe AP is defined by the development of persistent organ failure (lasting more than 48 hours), moderately severe by the presence of local complications, exacerbation of comorbidity, or transient organ failure (lasting ≤ 48 hours), and mild AP by the absence of organ failure, exacerbation of comorbidity and local complications. (4) Mild cases have minimal local and systemic inflammation with an uncomplicated clinical course and often a prompt recovery. Local complications, e.g. acute peripancreatic fluid collections, pancreatic or peripancreatic fat necrosis (4), occur in one-third of patients and are associated with a longer hospital stay, greater morbidity, and increased hospital costs. (5, 6) Of greatest concern are patients who develop uncontrolled systemic inflammatory response syndrome (SIRS) that can lead to organ failure, which is associated with significant mortality (5).

The control of inflammation in the initial phase of AP may alter the clinical course of the disease by reducing the development of local and systemic complications and thus decreasing patient suffering, mortality, and costs. Unfortunately, no treatment has

consistently been shown to decrease the incidence of moderately severe or severe AP. (7-9) The current early management of AP consists of supportive treatment in which fluid resuscitation has played a central role in the last two decades. (10) Research in fluid resuscitation has focused on the volume of fluids (aggressive or moderate) (11-14) and the type of fluid. The recently published WATERFALL study demonstrated that early aggressive fluid resuscitation was associated with three times more episodes of fluid overload than moderate hydration and does not appear to reduce the severity of AP compared to moderate hydration. (15)

Regarding the type of fluid which is best for AP, published results are conflicting. The two major types of fluids used in medicine are crystalloids and colloids. Crystalloids have an osmotic pressure equivalent to plasma and contain water-soluble electrolytes such as sodium. (16) Colloids, which have a higher oncotic pressure, were designed to allow the supplied water to remain more effectively and durably in the intravascular compartment than crystalloids. However, published trials do not suggest that they improve clinical results in intensive care patients (16-18) which has dampened enthusiasm for their widespread use. The two crystalloids most frequently used in clinical practice include normal saline (NS) and lactated Ringer's solution (LR). NS contains water and 0.9% sodium chloride (154 mEq/L of sodium and chlorine). With a chlorine content higher than plasma, large-volume infusions of NS may result in hyperchloremic acidosis. (16) LR contains less sodium and chloride (130 and 109 mEq/L respectively) and contains 28 mEq/L of lactate, in addition to calcium and potassium. LR is a balanced crystalloid due to its more neutral effect on acid-base physiology. (16) In

vitro studies suggest that the lactate present in the LR may have an anti-inflammatory effect. (19)

In 2011, Wu *et al*. published an open-label clinical trial that included 40 patients with double randomization to A) LR or NS, and B) to a goal-directed volume protocol (titration to blood urea nitrogen levels) or standard management. No differences were detected in goal-directed versus standard management, but patients treated with LR had a lower incidence of SIRS and lower C-reactive protein (CRP) blood levels 24 hours after recruitment. (20) In 2018, our group published a triple-blind randomized clinical trial with 40 patients from a single center. We described that LR was associated with lower CRP levels at 48 and 72 hours. (19) In a 2018 open-label randomized clinical trial by Choosakul *et al.*, 47 patients received LR or NS, demonstrating a lower proportion of patients with SIRS at 24 hours but not thereafter. (21) We conducted a larger double-blind randomized clinical trial with 121 patients with predicted mild AP. In this study, LR was associated with a similar degree of inflammation as NS but with a shorter hospital stay and lower intensive care unit (ICU) admission. (22) A recent single-center randomized clinical trial with 51 patients (Karki *et al*.) also described less inflammation with LR. (23) There have been several meta-analyses of these studies, including our review, which incorporated unpublished data by contacting trial authors (248 patients from 4 trials were included). (24) In these studies, patients who received LR were less likely to suffer moderately severe or severe pancreatitis (odds ratio [OR] 0.49, 95% confidence interval [CI] 0.25-0.97), there were no differences in inflammation (SIRS) or organ failure, but they were less likely to be admitted to the ICU (OR 0.33, 95% CI 0.13-0.81) or to develop local complications (OR 0.42, 95% CI 0.20-0.88). It has been

described in other different clinical scenarios than AP that NS is associated with renal failure. (25) Clinical trials in other diseases have shown conflicting results. In a double-blind clinical trial at 4 hospital centers of critically ill patients, no benefit was shown for balanced fluids (Plasma-Lyte 148, which does not contain lactate) compared to NS. (26) In another single-center open-label clinical trial of critically ill patients comparing Plasma-Lyte A or LR versus NS, it was shown that NS was associated with a greater probability of renal failure. (25) Very recently, a double-blind study was published of critically ill patients from 53 ICUs that did not observe advantages of Plasma-Lyte 148 compared to NS. (27)

Reporting guidelines

This protocol follows the recommendations of SPIRIT 2013 Statement: Definition of Standard Protocol Elements for Clinical Trials. (2) Numbers in curly brackets, e.g. {5a} are SPIRIT element identifiers.

Objectives {7}

The null hypothesis is that there is no difference in the incidence of moderately severe or severe disease in patients with AP receiving fluid resuscitation based on LR compared to NS. The alternative hypothesis is that fluid resuscitation based on LR is associated with a lower incidence of moderately severe or severe AP.

The primary objective of the WATERLAND trial is to investigate the effect of fluid resuscitation based on LR versus NS on the severity of AP (frequency of moderately severe or severe disease).

The secondary objectives of the WATERLAND trial are to determine the effect of LR versus NS fluid resuscitation on several efficacy and safety outcomes in patients with AP.

Trial design {8}

The WATERLAND trial is an international multicenter, open-label, parallel-group, randomized, controlled, superiority trial promoted by the ERICA (int**ER**national league aga**I**nst biliary-pancreati**C** dise**A**ses) consortium. Patients will be randomly assigned in a 1:1 ratio to receive LR versus NS-based fluid resuscitation. WATERLAND trial is a low-risk interventional pharmacological clinical trial.

Methods

Participants, interventions, and outcomes

Study setting {9}

The WATERLAND study is open to international academic or non-academic level 2 and level 3 hospitals. Current participating centers can be found on the following link: https://clinicaltrials.gov/ct2/show/NCT05781243

Eligibility criteria {10}

Center eligibility: hospitals that care for patients admitted for AP that can offer continuous care, with the availability of blood tests, abdominal ultrasound, abdominal computed tomography (CT), magnetic resonance imaging, endoscopic retrograde cholangiopancreatography (ERCP), interventional radiology, and ICU.

Patient eligibility: Inclusion and exclusion criteria are provided in Table 1.

Informed consent {26a}

The local study collaborators will obtain informed consent from potential trial participants or authorized surrogates. Informed consent is provided in the protocol Appendix.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Biological samples will not be obtained.

Interventions

Explanation for the choice of comparators {6b}

As discussed in the background, some randomized clinical trials suggest that LR may be associated with less inflammation and better outcomes than NS. WATERLAND will compare LR—and NS-based fluid resuscitation in patients with AP.

Intervention description {11a}

The volume of fluids is based on the moderate treatment arm of the WATERFALL trial (1.5 mL/kg/hour preceded by bolus 10 mL/kg if the patient has hypovolemia). (15) The "participant timeline" shows more details; see below.

Criteria for discontinuing or modifying allocated interventions {11b}

LR and NS are fluids routinely used to treat AP and other diseases. The incidence of adverse effects in both is very low. In case of hyperkalemia or hypercalcemia, the

treating physician may discontinue the infusion of LR, which has a small amount of potassium and calcium, and the adverse effect will be recorded. NS can be associated with hyperchloremic acidosis if administered in massive amounts, so the treating physician may decide to suspend this fluid in case of this complication, as mentioned above. Patients may leave the study at any time after signing the informed consent.

Strategies to improve adherence to interventions {11c}

Adherence is assessed based on the percentage of subjects receiving ≥80% of the planned amount of fluids according to the study protocol in the first 48 hours. No measures are required to improve adherence to the interventions since it is an acute disease, and the study fluid is administered during the first days of hospitalization.

Relevant concomitant care permitted or prohibited during the trial {11d}

Potassium administration should be 40 mEq per day in both arms of treatment during fasting unless a higher or lower dose is clinically indicated. LR contains potassium at a concentration of 4 mEq/l and NS contains no potassium, which will be considered in the calculation of daily potassium administration. The attending physician will decide on feeding, treatment with analgesics, antibiotics, indications for ERCP, drainage, and all other treatment measures and administer as clinically appropriate.

Provisions for post-trial care {30}

Patients will be managed after the trial by the attending physician at his or her discretion.

Outcomes {12}

Most outcomes will be assessed between randomization and 30 days after randomization unless assessment at 24, 48, or 72 hours is specified; see Tables 2 and 4.

Efficacy outcomes

The primary outcome will be moderately severe or severe AP, defined according to the RAC. (4) Moderately severe AP is defined in the first four weeks after disease onset as the presence of local complications (acute peripancreatic fluid collections, acute necrotic collection, gastric outlet dysfunction, splenic or portal vein thrombosis, and colonic necrosis) or systemic complications (exacerbation of a preexisting coexisting condition, such as coronary artery disease or chronic lung disease, precipitated by AP) or transient organ failure (organ failure that resolves within 48 hours). Severe AP is defined as persistent (lasting more than 48 hours) organ failure. Organ failure is defined according to the modified Marshall score by the presence of any of the following criteria: A) kidney failure as a creatinine ≥1.9 mg/dL or >170 μmol/L, B) cardiovascular failure as a systolic blood pressure <90 mmHg despite fluid resuscitation, and C) respiratory failure as a PaO2/FIO2≤300. (4) Patients with moderately severe or severe AP have increased morbidity (more time to oral refeeding, greater need for invasive treatment, more frequency of ICU admission, higher hospital stay, and increased mortality risk). (5) Moderately severe or severe AP was the primary efficacy outcome used in the WATERFALL trial, which compared aggressive versus moderate fluid resuscitation in acute pancreatitis. (15) RAC definitions for local complications diagnosed within the first 4 weeks after disease onset (4) are as follows:

Acute peripancreatic fluid collections: peripancreatic fluid associated with interstitial edematous pancreatitis with no associated peripancreatic necrosis. This term applies only to areas of peripancreatic fluid seen within the first four weeks after the onset of interstitial edematous pancreatitis and without the features of a pseudocyst.

Acute necrotic collection: a collection containing variable amounts of both fluid and necrosis associated with necrotizing pancreatitis; the necrosis can involve the pancreatic parenchyma and/or the peripancreatic tissues. Heterogeneous and non-liquid density of varying degrees in different locations (some appear homogeneous early in their course). No definable wall encapsulating the collection. Location: intrapancreatic and/or extrapancreatic.

Gastric outlet dysfunction: Gastric outlet dysfunction typically presents with early satiety, weight loss, nausea, vomiting, and abdominal pain. (28) RAC provides no definition. In WATERLAND, it will be defined as a delay in gastric emptying that requires medical treatment (fasting, nasogastric or nasojejunal tube, prokinetics, etc.) or invasive treatment. Paralytic ileus should be ruled out.

Splenic or portal vein thrombosis: RAC provides no definition. In WATERLAND it will be defined as partial or complete thrombosis in the splenic or portal vein in imaging. Mesenteric vein thrombosis will also be recorded.

Colonic necrosis: RAC provides no definition. In WATERLAND it will be defined as colonic necrosis in imaging, endoscopy, or evidenced in surgical intervention.

Infection of pancreatic collections or necrosis: Extraluminal gas in the pancreatic and/or peripancreatic tissues on CT scan or when a sample from the collection/necrosis contains pus or it is positive for bacteria and/or fungi on Gram stain or culture (adapted from RAC).

Mild AP without imaging tests: If a patient has mild AP, with rapid resolution of pain, absence of SIRS 48 hours after admission, and discharge within the first five days of admission, it is assumed that the patient has no local complications even without imaging evidence.

Table 2 lists secondary outcome variables and their definitions. The PAN-PROMISE scale will be used to measure patient wellness. PAN-PROMISE is a Patient-Reported Outcome Measurement (PROM) that measures seven symptoms (range 0 to 10 for each symptom; overall range 0 to 70, with higher scores indicating higher symptom intensity). (6)

Safety outcomes

The safety outcomes will be a A) a composite variable involving any of the following: fluid overload, acute kidney injury, hyperkalemia, hypercalcemia, hyperchloremia, or acidosis; B) the individual components of the composite variable. The attending physician will manage these complications as clinically appropriate. Fluid overload is defined in Table 3. (15) Safety outcomes are defined in Table 4.

Severity of fluid overload is defined (15) as:

A. Mild: patients respond to medical treatment or decrease in volume infusion rate, and the PaO2/FIO2 never decreases <300

B. Moderate: patients respond to medical treatment or decrease in volume/infusion rate and have at least one measurement with PaO2/FIO2 <300

C. Severe: patients require invasive or non-invasive mechanical ventilation, and/or hemofiltration, or expire due to overload

NS has been associated with an increased risk of renal failure. (25) Acute kidney injury will be defined according to the KDIGO classification: increase in serum creatinine of

≥0.3 mg/dL within 48 hours or ≥50% within 7 days or urine output of <0.5 mL/kg/hour for >6 hours. (29) LR contains small quantities of potassium and calcium, so hyperkalemia and hypercalcemia are safety outcomes. As mentioned, the recommended daily potassium administration will be 40 mEq/day in both treatment arms during fasting unless a higher or lower dose is clinically indicated. NS has high chloride content, and this fluid has been associated with hyperchloremic acidosis, (30) so levels of chloride and pH will be measured.

Other variables

The volume of fluids administered in the first 48 hours after recruitment will be provided. This trial promotes the participation of patients from diverse backgrounds. Race will be recorded following the "Collection of Race and Ethnicity Data in Clinical Trials" 2016 recommendations of the FDA. (31) Sex will be recorded as sex assigned at birth (male/female).

Participant timeline {13}

The participant timeline is summarized in Figure 1.

STEP 1. AT RECRUITMENT: check for baseline hypovolemia criteria (Table 3) and SIRS (Table 2). Patients without hypovolemia will receive a continuous LR or NS intravenous infusion of 1.5 mL/kg/hour. Patients who meet hypovolemia criteria will first receive an LR or NS 10 mL/kg intravenous bolus (over 2 hours) of the study fluid, followed by an LR or NS infusion of 1.5 mL/kg/hour. Oral food is allowed if the patient is willing to start oral feeding. The baseline PAN-PROMISE scale will be assessed. (6)

STEP 2. FOLLOW-UP UNTIL THE 24-HOURS CHECKPOINT: in case of systolic blood pressure < 90 mmHg or urine output <0.5 mL/kg/hour, a 10 mL/kg intravenous bolus over 30 to 120 minutes will be administered, depending on the physician's assessment of the patient's condition. The bolus can be repeated if needed, as many times as necessary. In case of suspicion of fluid overload (Table 3), the attending physician can decrease or stop fluid resuscitation and administer treatment for fluid overload if needed. Tests to rule out other medical conditions (ischemic heart disease, lung embolism, etc...) will be performed according to the attending physician's assessment of the patient.

STEP 3. 24-HOURS CHECKPOINT. Anamnesis, blood test, and physical examination will be performed. Oral feeding will be considered in patients under null per mouth. All patients will maintain an infusion of 1.5 mL/kg/hour except those suspected of fluid overload (in that case, the physician will proceed as in step 2). PAN-PROMISE, hypovolemia, fluid overload, SIRS, and outcomes based on blood determinations (except for CRP) will be assessed.

STEP 4. FOLLOW-UP UNTIL THE 48 HOURS CHECKPOINT: the patient will be managed as in step 2.

STEP 5. 48 HOURS CHECKPOINT. Anamnesis, blood test, and physical examination will be performed. Fluid resuscitation will be stopped in those patients tolerating oral feeding for more than 8 hours, with normal or hypervolemia. In case of hypovolemia or patients without tolerance to oral food, proceed as in step 2 until normal volemia and

oral tolerance are reached. PAN-PROMISE, SIRS, hypovolemia, fluid overload, and outcomes based on blood determinations will be assessed.

STEP 6. FOLLOW-UP UNTIL DISCHARGE. The patient can be discharged at the 48 hours checkpoint in case of mild pancreatitis and tolerance to oral diet or later, according to the patient status determined by the attending physician. Fluid overload will be assessed also at 72h. CT scan for the diagnosis of local complications should be performed on day three or later in case of SIRS at 48 hours, increased CRP at 48 hours (more than 15 mg/dL or more than 150 mg/L), or when clinically indicated according to the attending physician.

STEP 7. FOLLOW-UP UP TO 30 DAYS AFTER RANDOMIZATION. Many outcome variables are assessed 30 days after randomization (Table 2). When this period has elapsed, an assessment will be performed to determine whether the patient has been readmitted; this can be done by phone call.

Sample size {14}

The WATERFALL trial had a frequency of moderately severe or severe AP in the moderate fluid resuscitation arm of treatment (based on LR) of 17%. (15) In a recent systematic review, patients who received LR-based fluid resuscitation were less likely to develop moderately severe or severe pancreatitis than patients receiving NS, with an OR of 0.49, 95 % CI 0.25-0.97. (24) The differences in the incidence of moderately severe or severe pancreatitis in the four included randomized controlled trials between LR and NS ranged from 10 to 14%, favoring LR. (24) For this reason, we expect an incidence of

moderately severe or severe AP in the NS arm of 27%. Patients will be assigned in a 1:1 ratio. A total sample of 720 patients, 360 in the LR group and 360 in the NS group, will achieve 90% power to detect a difference between the group proportions of 10% (the smaller difference observed in the four RTCs (24)), assuming that the frequency of moderately severe or severe AP in LR group will be 17%. The frequency in the NS group is assumed to be 17% under the null hypothesis and 27% under the alternative hypothesis. A loss to follow-up of 10% of patients is expected, so the sample size will be 396 patients in each treatment arm (792 patients in total). The test statistic used is the two-sided Z test with pooled variance set at a 0.05 significance level.

Recruitment {15}

WATERLAND is an international multicenter study. Current participating centers can be found on the following link: https://clinicaltrials.gov/ct2/show/NCT05781243

The study has been shared through these communication channels:

1. Previous ERICA consortium collaborators (5, 6, 14, 15)
2. National and international gastroenterology, surgery, and pancreatology associations (see acknowledgements)
3. ERICA consortium website (ericaresearch.com)
4. ERICA consortium and the researchers 'personal social networks
5. Meetings and symposiums

Assignment of interventions: allocation

Sequence generation {16a}

Sequence assignment will be performed using computer-generated random numbers. Random assignments will be stratified by center, presence or absence of baseline SIRS, and presence or absence of baseline hypovolemia. The randomization process will be performed using the block.random function of the "psych" library of R. Only the study coordinator (AVR) and the Dr. Balmis General University Hospital's Department of Clinical Pharmacology will have access to the sequence.

Concealment mechanism {16b}

Randomization will be integrated into the web-based electronic case report form (REDCap). (32).

Implementation {16c}

The allocation sequence will be generated by the Department of Clinical Pharmacology of the Dr. Balmis General University Hospital and entered in REDCap by the study coordinator. REDCap will randomize every new patient the study collaborators enter in their centers.

Assignment of interventions: Blinding

Who will be blinded {17a}

Only data analysts will be blinded. For this purpose, they will be administered a database in which the arm of treatment (NS or LR) will be replaced by randomly assigned labels A and B.

Procedure for unblinding if needed {17b}

The design is open label so unblinding will not occur.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Before recruitment begins, collaborators will receive training on the study through a teleconference with the study coordinator. Video tutorials on the study will be available in the electronic case report form (REDCap). The electronic case report form, the randomization process, the importance of avoiding missing data, and the importance of accurate data entry will be explained and highlighted. The web-based electronic case report is based on the REDCap platform, (32, 33) provided by the Spanish Association of Gastroenterology (AEG). The promotors have extensive experience in this platform.

Plans to promote participant retention and complete follow-up {18b}

The WATERLAND trial only covers from randomization to 30 days thereafter, so complete follow-up will be easily achievable. Centers that do not adequately follow patients may be dropped from the study. The study coordinator, AVR, will oversee patient and center monitoring.

Data management {19}

The forms have been designed to explain every variable to promote data quality. Quantitative variables will include alarms for extreme values. To minimize errors and ensure timely monitoring, filling out the web form directly online will be required. Logical alarms will be set when two or more variables are contradictory, e.g., classifying

AP as severe in a patient without persistent organ failure. Local collaborators caring for patients with AP will enter the study data into the electronic case report form.

Confidentiality {27}

The data will be stored in the REDCap node of the AEG, a secure database. Each center has a "Data Access Group" that ensures that only patient records from their center can be accessed. Patient data are entered after the informed consent of the patient or their legal guardian has been obtained, which will have been previously approved by the ethics committees of the participating centers after checking compliance with current legislation (in terms of data protection in Europe: Organic Law 3/2018 of December 5, Regulation 2016/679 of the European Parliament and of the Council of April 27, 2016), which includes information to the patient on the processing of their data, with the right to access, rectification, cancellation, and opposition.

The data are anonymized. Participants will be allocated using an individual trial identification number; information that can identify the patient is not included in the database. The Steering Committee, coordination committee, and data analysts will have access to the final dataset. The ownership of the data belongs to the ERICA consortium; collaborating centers are offered the possibility of access to the global database if they wish to carry out an ancillary study (post hoc studies with different objectives to the original) to WATERLAND trial based on its database, after submitting a report containing an introduction, hypothesis, objectives, methodology, and expected impact. The decision will be made unanimously by the trial Steering Committee. After the central Institutional Review Board approval, the database exported to a statistical package (SPSS or Stata) will be shared in a password-protected zip file that will be sent to the

collaborator by another means of communication from which he/she receives the file. The provision of specific anonymized data to other researchers for meta-analysis will be encouraged. To this end, data will be provided (see {31a}) without providing granular details that could compromise patient privacy.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Biological samples will not be collected.

Statistical methods

The Statistical Analysis Plan version 1, June 30, 2024, available in the protocol Appendix, specifies detailed statistical methods.

Statistical methods for primary and secondary outcomes {20a}

Normality will be assessed using the Lilliefors-corrected Kolmogorov-Smirnov test. The number and percentage of primary and secondary categorical outcomes will be reported for each treatment group. Continuous data will be reported by mean and standard deviation if data are normal and median and interquartile range if data are skewed. To calculate the p-value for the primary outcome and secondary safety outcomes, the Cochran–Mantel–Haenszel method will be utilized, with adjustments made for randomization stratification factors including center, baseline SIRS presence, and baseline hypovolemia presence. In addition, this procedure will yield adjusted relative risks and their corresponding 95% confidence intervals for all outcomes, also accounting for any variables that display imbalances among randomized groups. For

continuous variables, adjusted relative risks will be calculated using multiple regression models adjusted for randomization stratification factors and any variable that display imbalances among randomized groups to analyze the effect of the continuous variable itself. Additionally, the Cochran-Mantel-Haenszel method will be applied to compare high values (above the median) to low values (at or below the median), providing a comprehensive analysis of the data. Briefly (see more details in the Statistical Analysis Plan version 1, June 30, 2024, available in the protocol Appendix), the intention-to-treat population will include all randomized patients, following the intention-to-treat principle. The safety (per-protocol) population will include all randomized patients, according to the fluid that was actually received. Patients receiving no fluid will not be included in the safety population. Efficacy outcomes will be tested in the intention-to-treat population, and safety outcomes will be tested in the safety population.

The analysis will be conducted using SPSS version 29 or higher (IBM), SAS software version 9.4 or higher (SAS Institute), and R software version 4.4.1 or higher. Statistical analysis will be performed by PM, PZ, and EMNM.

Interim analyses {21b}

Given the low expected incidence of adverse events in both arms of treatment, no interim analysis has been predefined. There will be two a priori stopping rules: clear evidence of harm in one trial group over the other (safety) as adjudicated by the Data and Safety Monitoring Committee and a slow recruitment rate determined by the Steering Committee.

Methods for additional analyses {20b}

The following pre-specified subgroup analyses will be performed on the primary and secondary outcomes:

- Baseline presence and absence of SIRS
- Baseline presence and absence of hypovolemia
- Sex

There is no provision for correction for multiplicity for subgroup analysis, so results will be reported as point estimates with two-sided 95% CI.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Adherence is assessed based on the percentage of subjects receiving ≥80% of the planned volume of fluids according to the study protocol in the first 48 hours after randomization. The attending physician will assess it. Details about intention-to-treat and per-protocol populations are available in the paragraph "Statistical methods for primary and secondary outcomes" above. More details are specified in the Statistical Analysis Plan version 1, June 30, 2024, available in the protocol Appendix.

Our goal is to reduce or eliminate missing data during recruitment through concerted efforts. If, despite these efforts, missing data occur, we will assess the amount and pattern of missing data. The purpose of this assessment is to analyze the amount of missing data on the primary variables and other variables and determine the nature of the missingness (missing completely random, missing at random, or missing at non-random). We will use the Multivariate Imputation by Chained Equations (MICE) using creating multiple datasets for ten times and results will be combined using Rubin's Rules. (34) MICE is useful when the pattern of missing data is random (MAR) or when the

proportion of missing data exceeds 5% and does not follow a Missing Not at Random (MNAR) pattern. (35) In cases where missing data follow a Missing Not at Random (MNAR) pattern, we will employ sensitivity analyses to examine the impact of different assumptions about the missing data mechanism on our results. Additionally, we will consider Bayesian imputation methods to address the potential bias introduced by MNAR data.

Plans to give access to the full protocol, participant level-data and statistical code {31c}
Members of the ERICA consortium that recruited patients in the WATERLAND trial may claim access to the final dataset to perform ancillary studies; as discussed above, the Steering Committee will study these proposals. The datasets analyzed during the current study will be published in an open-access repository. Statistical codes are available from the corresponding author on reasonable request, as is the full protocol.

Oversight and monitoring

Composition of the coordinating center and trial Steering Committee {5d}

The study will be coordinated by the Gastroenterology and the Clinical Pharmacology Departments of the Dr. Balmis General University Hospital, Alicante, Spain. The Coordination Committee will include the principal investigator and promotor (EdM, gastroenterologist), the study coordinator (AVR), and a clinical pharmacologist (PZ). This committee will provide daily support to the study collaborators. The Coordination Committee will meet every month or in situations requiring important decisions.

The trial Steering Committee comprises a group of international pancreatologists (LG, AGGP, AC, YHB, GC, JLB), an acute pancreatitis patient advocate (CLV) and an expert in

statistics (PM). They will meet (via teleconference) every three months or in situations requiring important decisions. The Steering Committee had the following tasks: A) to supervise the overall progress of the trial, B) to review and consider the Data and Safety Monitoring Committee (DSMC) reports and recommendations, C) to discuss and decide post hoc analyses after the study is complete, D) to participate in writing the final publication.

Composition of the Data and Safety Monitoring Committee, its role, and reporting structure {21a}

The DSMC will comprise a nephrologist, an intensivist, and a clinical pharmacologist. It will evaluate all reported adverse events. Safety reports will be issued as reported and analyzed by the Steering Committee.

Adverse event reporting and harms {22}

The WATERLAND trial investigates two fluids used routinely for over 100 years; adverse events and harms are expected to be very low. The DSMC oversees the detection of possible adverse events and harms and proposes to the Steering Committee how to proceed. The local collaborators can report safety problems to the study coordinator, who would contact the DSMC.

Frequency and plans for auditing trial conduct {23}

AVR, the study coordinator, will oversee the study audits. Participant enrolment, eligibility, allocation to study groups, adherence to trial interventions, reporting of harms, and completeness, accuracy, and timeliness of data collection will be monitored.

Given the international nature of the study, the audits will be carried out through the analysis of the electronic case report form (REDCap) and telematic contact with the collaborating researchers. An initial audit of the participating centers (completeness, accuracy, and timeliness of data collection) will be performed after the complete data entry of the first three patients, then every ten patients. Also, the funding institutions (particularly Instituto de Salud Carlos III, the main funding source) can decide to perform external audits.

Plans for communicating important protocol amendments to relevant parties {25}

The Steering Committee can decide to make protocol amendments. In that case, the study coordinator will inform the Institutional Review Boards, change the study registries, and inform the study collaborators. All amendments will be registered, and the changes and their dates will be explained in the final publication supplementary material. All changes from this protocol will be identified as post hoc analyses in the final publication.

Dissemination plans {31a}

The results of the WATERLAND trial will be presented at international meetings and published in a peer-reviewed scientific journal. The article will be published with an open-access license if the scientific journal has that possibility. The results will be shared through the social networks of the ERICA consortium (Twitter: @ERICAconsortium) and its website (www.ericaresearch.com). The authors will write a lay summary to share with all participants. With the help of the patient advocate, informative material will be produced for the general public, and a press release will be issued. The data will be

available in a public open data repository. The register records will be updated for EudraCT and ClincalTrials.gov

Authorship criteria:

1 to 15 patients. Two investigators from the center will be acknowledged as collaborators in the supplementary appendix of the final publication.

16 to 30 patients. One investigator will be included as a co-author of the study, and two other investigators from the center will be acknowledged as collaborators in the supplementary appendix of the final publication.

31 to 50 patients. Two investigators will be included as co-authors of the study, and 1 investigator from the center will be acknowledged as a collaborator in the supplementary appendix of the final publication.

51 or more. Three investigators will be included as co-authors of the study.

Discussion

The WATERLAND trial is an international multicenter, open-label, parallel-group, randomized, controlled, superiority trial aiming to compare the efficacy and safety of moderate fluid resuscitation based on LR versus NS in AP. The study has been designed to recruit both patients with predicted mild and predicted severe AP, thus, with different ranges of severity of disease, but patients that have baseline criteria for moderately severe or severe disease will be excluded, as this is the main efficacy outcome, and the hypothesis of the study is that fluid therapy may improve the course of the disease, preventing the development of complications. The study will be open-label, as the logistics for an international double-blinded randomized controlled trial on fluid

resuscitation are challenging. The efficacy outcome is moderately severe or severe disease, a compound variable that includes local complications, organ failure, and exacerbation of previous comorbidity. (4) Patients with those complications have more morbidity and risk of mortality. (5) Both arms of treatment are safe, but concerns about hyperchloremic acidosis have been raised in patients receiving high doses of NS. (30) LR and NS administration will be based on the results of the WATERFALL trial, which demonstrated that 1.5 mL/kg/hour (preceded by a bolus of 10 mL/kg only in patients with hypovolemia) is safer that a more aggressive strategy (20 mL/kg bolus in all patients, followed by 3 mL/kg/hour). (15) LR and NS are fluids used in AP daily for more than 100 years, so this is a low interventional pharmacological randomized controlled trial. Low interventional clinical trials, according to the European Union Clinical Trials Regulation No 536/2014 should fulfill the following requirements: A) the investigational medicinal products, excluding placebos, are authorized; B) according to the protocol of the clinical trial, the investigational medicinal products are used in accordance with the terms of the marketing authorization or the use of the investigational medicinal products is evidence-based and supported by published scientific evidence on the safety and efficacy of those investigational medicinal products in any of the Member States concerned, and C) the additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical practice in any Member State concerned. Most countries do not require insurance for patients included in low interventional trials, which helps the WATERLAND trial to be performed in an international scenario; If a center or country requires insurance, an attempt will be made to cover it through the grants that support this project.

Finally, the ERICA consortium has experience in international multicenter studies (5-7) and studies on fluid resuscitation. (14, 15, 19)

Trial status

Protocol version 4, September 18, 2023. Recruitment started in June 2023. Recruitment is expected to be completed in December 2024.

Abbreviations

AEG: Spanish association of gastroenterology.

AP: acute pancreatitis.

CI: confidence intervals.

CRP: c-reactive protein.

CT: computed tomography.

DSMC: data safety and monitoring committee.

ERCP: endoscopic retrograde cholangiopancreatography.

ERICA: international league against biliary-pancreatic diseases.

ICU: intensive care unit.

ISCIII: Instituto de Salud Carlos III.

LR: lactated Ringer's solution.

NS: normal saline.

OR: odds ratio.

RAC: revision of the Atlanta classification.

SIRS: systemic inflammatory response syndrome.

Declarations

Acknowledgments

The researchers acknowledge the Spanish Association of Gastroenterology (AEG) for providing the REDCap electronic case report form, and AEG, the European Pancreatic Club (EPC), and the Spanish Association of Pancreatology (AESPANC) for their endorsement and support in sharing the trial through their communication channels to help recruiting centers. The researchers would like to thank the Scientific Committee of the United European Gastroenterology (UEG) for the UEG Research Prize 2024 to Enrique de-Madaria and the research grant that it entails, and the scientific committee of AEG for the Gonzalo Miño grant. Finally, thanks to the Instituto de Salud Carlos III (ISCIII) for making this study possible through a public competitive grant; the ISCIII has supported all our randomized controlled trials with these grants.

Authors' contributions {31b}

EdM is the principal investigator; he conceived the study, applied for grants, and led the proposal and protocol development. PM, PZ and EMNM were the lead trial methodologists. The other authors contributed to the study design and the proposal's development. All authors read and approved the final manuscript for this protocol.

Funding {4}

The WATERLAND trial is funded by a grant (grant number PI22/00980) from ISCIII, Madrid, Spain, the national and international reference in biomedical research and public health in Spain. It is the Public Research Organization of the Government of Spain,

responsible for funding and executing national biomedical research. It depends on the Ministry of Science, Innovation, and Universities.

Other grants:

- UEG Research Prize, United European Gastroenterology, grant number: UEG Research Prize 2024, Vienna, Austria.
- Beca Gonzalo Miño, Spanish Association of Gastroenterology (Asociación Española de Gastroenterología, AEG), grant number: Gonzalo Miño Grant 2024, Madrid, Spain.
- Beca Intramural, Alicante Institute of Health and Biomedical Research (ISABIAL), grant number: ayudas intramurales de ISABIAL modalidad C 2023, Alicante, Spain.

None of the funders were or will be involved in the study design, collection, analysis, data interpretation, or manuscript writing.

Availability of data and material {29}

The Steering Committee, coordination committee, and statistical advisors will have access to the final dataset. Members of the ERICA consortium that recruited patients in the WATERLAND trial may claim access to the final dataset to perform ancillary studies; the Steering Committee will study these proposals. Any data required to support the protocol can be supplied on request.

Ethics approval and consent to participate {24}

Dr. Balmis General University Hospital's research ethics committee (Comité de Investigación con Medicamentos, CEiM) will be considered as the central institutional

review board. All participating centers must have permission from their local institutional review boards to participate in WATERLAND.

Consent for publication {32}

Not applicable - no identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form available from the corresponding author on request.

Competing interests {28}

This is a researcher-driven study. There is little if any, commercial interest in the results of the WATERLAND trial. No industry involvement will be allowed in this study, and the study committee members and collaborators will not be able to participate in this trial in case of conflicts of interest. A declaration of conflicts of interest will be mandatory to be part of the WATERLAND trial.

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Appendix

Statistical Analysis Plan, Version 1, June 30, 2024

Normal saline versus lactated Ringer's solution for acute pancreatitis resuscitation, an open-label multicenter randomized controlled trial: the WATERLAND trial

Contributors

Trial Design:

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This plan follows the Guidelines for the Content of Statistical Analysis Plans (SAP) in Clinical Trials by Gamble et al, JAMA 2017. (1) The numbers in parentheses preceded by an R (e.g., R1a: recommendation 1a) are the recommendations provided in the table entitled "SAP Guidance Document: Recommended Items to Address in a Clinical Trial SAP".

Section 1: Administrative Information

Title (R1a)	Normal saline versus lactated Ringer's solution for acute pancreatitis resuscitation, an open-label multicenter randomized controlled trial: the WATERLAND trial
Trial registration (R1b)	EudraCT number 2023-000010-18, date of registration January 4, 2023. ClinicalTrials.gov Identifier: NCT05781243, first posted March 23, 2023
SAP version (R2)	June 2024, version 1
Protocol version (R3)	Version 4, September 18, 2023. Version 1 was approved by the central ethics committee (Comité Ético de Investigación con Medicamentos, Hospital General Universitario Dr. Balmis, Alicante, Spain, project code 2023-031) on March 9, 2023. Version 4 was approved on September 29, 2023.
SAP revisions (R4a, R4b, R4c)	This is the first version of the SAP.
Roles and responsability (R5)	Ankit Chhoda, [1] Pedro Zapater, [2 3 4] Patrick Maisonneuve, [5] Eva M. Navarrete-Muñoz [6 7] and Enrique de-Madaria. [8 9] [1] Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA. [2] Clinical Pharmacology Department, Dr. Balmis General University Hospital, ISABIAL, Alicante, Spain. [3] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain. [4] IDiBE, Miguel Hernandez University, Elche, Spain. [5] Division of Epidemiology and Biostatistics, IEO European Institute of Oncology IRCCS, Milan, Italy. [6] Grupo de Investigación en Terapia Ocupacional (InTeO), Department of Surgery and Pathology, Miguel Hernandez University-ISABIAL, Alicante, Spain. [7] Joint research unit UMH-Fisabio (STATSALUT), Alicante, Spain. [8] Gastroenterology Department, Dr. Balmis General University Hospital, ISABIAL, Alicante, Spain. [9] Department of Clinical Medicine, Miguel Hernandez University, Elche, Spain. AC and EdM wrote the first draft of the SAP. PZ, EMNM, and PM critically reviewed and contributed to this SAP.
Signatures of	Persons writing the SAP (R6a): Ankit Chhoda and Enrique de-Madaria
	Senior statistician responsible (R6b): Patrick Maisonneuve
	Chief investigator/clinical lead (R6c): Enrique de-Madaria

Section 2: Introduction

Background and Rationale (R7)

Acute pancreatitis (AP) is the third leading cause of hospital admission and readmission for digestive diseases, with increasing incidence and high costs. (2) AP is characterized by varying severity, with mild cases having minimal inflammation and favorable outcomes, while moderately severe and severe cases involve morbidity, and increased risk of mortality. (3-5) The control of inflammation in the initial phase of AP may alter the clinical course of the disease by reducing the development of local and systemic complications and thus, overall morbidity, mortality, and costs. However, no treatment has consistently been shown to decrease the incidence of moderately severe or severe AP. (6-8) Fluid resuscitation has been attributed a central role in AP management over the last two decades. (9) Research in fluid resuscitation has been focused on the volume and the type of fluids. (10-13) The recently published WATERFALL study demonstrated superior safety of moderate hydration over aggressive fluid resuscitation since the latter was associated with three times higher fluid overload and similar severity of AP. (14)

Regarding the type of fluid best for AP, published results are conflicting. The two major types of fluids are crystalloids and colloids. (15) Colloids, although suspected to remain more effectively and durably in the intravascular compartment than crystalloids, were shown not to improve clinical results in intensive care patients. (15-17). Among the crystalloids, normal saline (NS) contains water, and 0.9% sodium chloride (154 mEq/L of sodium and chlorine), and lactated Ringer's solution (LR) contains less sodium and chloride (130 and 109 mEq/L, respectively), 28 mEq/L of lactate, in addition to calcium and potassium. With a chlorine content higher than plasma, large-volume infusions of

NS may result in hyperchloremic acidosis, whereas LR is a balanced crystalloid due to its more neutral effect on acid-base physiology, and in-vitro studies suggest an anti-inflammatory effect of lactate. (15, 18)

Prior clinical trials have shown conflicting results regarding using LR versus NS in AP management. Some studies have reported a lower incidence of systemic inflammatory response syndrome (SIRS) or reduced c-reactive protein blood levels with LR, (18-20) while others found no significant difference in inflammation. (21, 22) In the study by Lee et al, (22) LR was associated with a shorter hospital stay and lower intensive care unit (ICU) admission. A meta-analysis of original studies, including unpublished data suggested that LR may be associated with a lower likelihood of developing severe AP (odds ratio[OR] 0.49, 95% confidence interval [CI] 0.25-0.97), reduced odds of intensive care unit (ICU) admission (OR 0.33, 95% CI 0.13-0.81), and fewer local complications (OR 0.42, 95% CI 0.20-0.88) but no differences in inflammation (SIRS) or organ failure. (23) In conclusion, the optimal fluid choice for AP management remains uncertain; LR has shown some potential benefits, including reduced inflammation and improved clinical outcomes; however, more research is needed to establish definitive recommendations regarding fluid resuscitation in AP.

Objectives (R8)

Research hypothesis:

The null hypothesis is that there is no difference in the incidence of moderately severe or severe disease in patients with AP receiving fluid resuscitation based on LR compared

to NS. The alternative hypothesis is that fluid resuscitation based on LR is associated with a lower incidence of moderate severe or severe AP.

Study objectives:

The primary objective of the WATERLAND trial is to investigate the effect of fluid resuscitation based on LR versus NS on the severity of AP (frequency of moderately severe or severe disease).

The secondary objectives of the WATERLAND trial are to determine the effect of LR versus NS fluid resuscitation on several efficacy and safety outcomes in patients with AP (see "outcomes" below).

Section 3: Trial Methods

Trial Design (R9)

The WATERLAND trial is an international multicenter, randomized, open-label, parallel-group, controlled, superiority trial promoted by the ERICA (int**ER**national league aga**I**nst biliary-pancreati**C** dise**A**ses) consortium. The patients will be randomly assigned to receive either LR or NS-based fluid resuscitation in a 1:1 ratio.

Randomization (R10)

Random assignment will be performed centrally, using a computer-based central randomization system integrated in a Web-based electronic case-report form (REDCap) to guarantee adequate allocation concealment. Random assignments will be stratified by center, presence or absence of baseline SIRS, and presence or absence of baseline hypovolemia. Permuted 12-patients blocks will be used.

Sample Size (R11)

The WATERFALL trial had a frequency of moderately severe or severe AP in the moderate fluid resuscitation arm of treatment (based on LR) of 17%. (14) In a recent systematic review, patients who received LR-based fluid resuscitation were less likely to develop moderately severe or severe pancreatitis than patients receiving NS, with an OR of 0.49, 95 % CI 0.25-0.97. (23) The differences in the incidence of moderately severe or severe pancreatitis in the four included randomized controlled trials between LR and NS ranged from 10 to 14% favoring LR. (23) For this reason, we expect an incidence of moderately severe or severe AP in the NS arm of 27%. Patients will be assigned in a 1:1 ratio. A total sample of 720 patients, 360 in the LR group and 360 in the NS group, will achieve 90% power to detect a difference between the group proportions of 10% (smaller difference observed in the 4 RTCs (23)), assuming that the frequency of moderately severe or severe AP in LR group will be 17%. The frequency in the NS group is assumed to be 17% under the null hypothesis and 27% under the alternative hypothesis. A loss to follow-up of 10% of patients is expected, so the sample size will be 396 patients in each treatment arm (792 patients in total). The test statistic used is the two-sided Z test with pooled variance set at a 0.05 significance level.

Framework (R12)

The WATERLAND trial is a superiority trial.

Statistical Interim Analyses and Subgrouping Guidance.

Information on interim analyses specifying what interim analyses will be carried out and listing of time points (R13a)

Given that NS and LR are both commonly used and effective IV fluids for resuscitation and generally safe and well tolerated, no interim analysis has been predefined. The Data and Safety Monitoring Committee will evaluate all reported adverse events. Safety reports will be issued as reported and analyzed by the Steering Committee.

Any planned adjustment of the significance level due to interim analysis (R13b)

Not applicable.

Details of guidelines for stopping the trial early (R13c)

This is a low-intervention study, with fluids that have been shown to be safe. There will be two a priori stopping rules: clear evidence of harm in one trial group over the other (safety) as adjudicated by the Data and Safety Monitoring Committee and a slow recruitment rate determined by the Steering Committee.

Timing for Final Analysis (R14)

The final analysis is planned at the end of the study. All outcomes will be analyzed collectively.

Timing of Outcome Assessments (R15)

The timing of outcome assessments is provided in Table 2 and 4 of the protocol.

Section 4: Statistical Principles

Confidence Intervals and P Values

Level of statistical significance (R16)

All statistical tests for will be two-sided using a 5% significance level.

Description and rationale for any adjustment for multiplicity and, if so, detailing how the type 1 error is to be controlled (R17)

The WATERLAND trial aims to test the primary outcome and safety secondary outcomes for superiority (a two-sided P value of less than 0.05 will be considered to indicate statistical significance for the primary efficacy outcome and safety outcomes). There is no provision for correction for multiplicity for secondary efficacy outcomes, so their results will be reported as point estimates with 95% confidence intervals.

Confidence intervals to be reported (R18)

Primary and secondary outcomes will be reported as relative risk, adjusted for randomization stratification factors (center, presence or absence of SIRS at recruitment, and presence or absence of hypovolemia at recruitment) with 95% CI. Additionally, adjustments may also include variables that exhibit imbalances across randomized groups.

Adherence and Protocol Deviations

Definition of adherence to the intervention and how this is assessed, including extent of exposure (R19a)

Adherence is defined as receiving ≥80% of the planned volume of fluids according to the study protocol in the first 48 hours after randomization. Non-adherence refers to discontinuing study-allocated fluid (<80% of the total amount of fluids planned for the first 48 hours after randomization) as per the patient's decision and will be assessed by the attending physician. The protocol allows to decrease or stop fluid resuscitation in case of fluid overload. In such cases, it is not considered non-adherence; this is part of the goal-directed protocol to avoid dangerous fluid overload.

Description of how adherence to the intervention will be presented (R19b)

The number and % of participants per arm of treatment receiving ≥80% of the prescribed fluids will be presented in a table in the final manuscript, together with the number and % of participants with fluid overload.

Definition of protocol deviations for the trial (R19c)

The following are pre-defined protocol violations:

A) Deviations type A: protocol deviations that are not considered to affect the study outcomes as those patients will not be included in the final analysis, thus not affecting the scientific value of the trial. Includes A1: patients who are randomized by mistake (e.g., patients meeting exclusion criteria), A2: computer error that prevents randomization of the patient; A3: patients who, once randomized, refuse to participate in the study before the study fluid is started, and A4: patients who

were randomized, received the study fluid, but then refuse to participate in the study and revoke the permission for their data to be analyzed. Those patients will not be analyzed, as A1 are patients who do not meet the criteria to enter the study; in A2, randomization was not possible; A3 patients are not willing to participate before receiving the study fluid, and A4 revoked their consent for the data to be included in the analysis.

B) Deviations type B: protocol deviations that may affect the study outcomes as those patients will be included in the final analysis. Thus, they could affect the trial's scientific value. Includes B1: patients with inaccurate collection and/or documentation of data in RedCAP database, B2: patients receiving <80% planned volume of fluids according to the study protocol in the first 48h after randomization due to mistakes or patient decisions, and B3: patients receiving the wrong fluid, LR instead of NS or vice versa. In case of missing data, the multiple imputation technique will be performed, see below.

Description of which protocol deviations will be summarized (R19d)

Deviations type A will be summarized (number of cases) in the study flow chart. Type B deviations (number of cases per arm of treatment) will be summarized in the final manuscript.

Analysis populations (R20)

The **intention-to-treat population** will include all randomized patients without type A deviation from the study protocol, following the intention-to-treat principle (see R19c).

The **safety (per-protocol) population** will include all randomized patients without type A deviations from study protocol, according to the fluid that was actually received. Patients receiving no fluid will not be included in the safety population.

Efficacy outcomes will be tested in the intention-to-treat population, and safety outcomes will be tested in the safety population.

See also, "Any planned sensitivity analyses for each outcome where applicable (R27e)"

Section 5: Trial Population

Screening Data (R21)

The following summaries will be presented for all screened patients: Enrolment: the number of patients screened, the number of patients recruited, the number of screened patients not recruited, and the reason for non-recruitment.

Eligibility (R22)

The trial inclusion and exclusion criteria are specified in protocol Table 1. The number of ineligible patients randomized, if any, will be reported, with reasons for ineligibility.

Recruitment (R23)

A CONSORT flow diagram (Statistical Analysis Plan Figure 1) (24) will be used to summarize the number of patients who were eligible, consented, randomized, receiving study-allocated intravenous fluids, withdrawing, or lost to follow-up.

Withdrawal/Follow-Up

Level of withdrawal (R24a)

Participants may A) withdraw from the intervention but continue with follow-up; B) withdraw from follow-up but allow data collected to date to be used; C) withdraw from follow-up and withdraw consent for data collected to date to be used; or D) be lost to contact/follow-up.

Timing of withdrawal/lost to follow up data (R24b)

A time-to-event Kaplan Meier Curve will incorporate the timing of withdrawal or loss of follow-up.

Reasons and details of how withdrawal/lost to follow-up data will be presented (R24c)

The numbers (with reasons) of losses to follow-up (drop-outs and withdrawals) throughout the trial will be summarized in the CONSORT flow diagram.

Baseline Patient Characteristics

List of baseline characteristics to be summarized (R25a)

Patients will be described for age, sex assigned at birth, first episode of pancreatitis, gallstone cause of pancreatitis, body-mass index, Charlson comorbidity index, BISAP score, HAPS score, PAN-PROMISE score, urea, hematocrit, creatinine, SIRS, hypovolemia, potassium, calcium, pH, and chlorine, both overall and separately for the two randomized groups. The representativeness of the study participants will be discussed in another table in the supplementary material containing information about

the disease studied, special considerations about sex and gender, age, race, geography, other considerations, and the overall representativeness of this trial. (25)

Details of how baseline characteristics will be descriptively summarized (R25b)

Categorical data will be summarized by numbers and percentages. Continuous data will be summarized by mean and SD if data are normal and median and interquartile range if data are skewed. Tests of statistical significance will not be undertaken for baseline characteristics; instead, the clinical importance of any imbalance will be noted.

Section 6: Analysis

Outcome Definitions

Specification of outcomes and timings (R26a), specific measurement and units (R26b), and any calculation or transformation used to derive the outcome (R26c)

The specifications on outcomes and timings, specific measurements and units, and calculations used to derive outcomes are explained in the study protocol (main text: "Outcomes," and Tables 2, 3, and 4). To calculate the change from the baseline PAN-PROMISE scale at 24 and 48 hours, we will subtract the baseline value from the values at those time points. In addition, we will analyze the data at 24 and 48 hours, considering both baseline-adjusted and non-adjusted values.

Analysis methods

What analysis method will be used, and how the treatment effects will be presented (R27a)

Normality will be assessed using the Lilliefors-corrected Kolmogorov-Smirnov test. The number and percentage of primary and secondary categorical outcomes will be reported for each treatment group. Continuous data will be reported by mean and SD if data are normal and median and interquartile range if data are skewed.

To calculate the p-value for the primary outcome and secondary safety outcomes, the Cochran–Mantel–Haenszel method will be utilized, with adjustments made for randomization stratification factors including center, baseline SIRS presence, and baseline hypovolemia presence. In addition, this procedure will yield adjusted relative risks and their corresponding 95% confidence intervals for all outcomes, also accounting

for any variables that display imbalances among randomized groups. For continuous variables, adjusted relative risks will be calculated using multiple regression models adjusted for randomization stratification factors and any variable that display imbalances among randomized groups to analyze the effect of the continuous variable itself. Additionally, the Cochran-Mantel-Haenszel method will be applied to compare high values (above the median) to low values (at or below the median), providing a comprehensive analysis of the data

Any adjustment for covariates (R27b)

As explained in R27a, p-values and relative risk will be adjusted for randomization stratification factors (center, baseline presence or absence of SIRS, and baseline presence or absence of hypovolemia). Additionally, adjustments may also include variables that exhibit imbalances across randomized groups.

Methods used for assumptions to be checked for statistical methods (R27c)

The Lilliefors-corrected Kolmogorov-Smirnov test will be used to assess the normality of continuous data. We will also assess the assumptions of the regression models used in the data analysis

Details of alternative methods to be used if distributional assumptions do not hold (R27d)

If continuous variables do not adhere to normal distribution assumptions, alternative methods such as variable transformations, robust regression techniques, or bootstrap methods will be considered. Similarly, if regression models do not meet assumptions, non-parametric models or robust models will be explored to ensure robustness of the findings.

Any planned sensitivity analyses for each outcome where applicable (R27e)

The intention-to-treat population will be used to test efficacy outcomes, while safety outcomes will be tested in the safety population with complete cases (see R20). For sensitivity analyses, we will perform per-protocol analyses to ensure the robustness of the primary efficacy outcomes and the safety outcomes in the intention-to-treat population, and we will include multiple imputation for missing data to account for potential biases and provide a more comprehensive assessment of the treatment effects.

Any planned subgroup analyses for each outcome including how subgroups are defined (R27f)

The following pre-specified subgroup analyses will be performed on the primary and secondary outcomes:

- Baseline presence and absence of SIRS
- Baseline presence and absence of hypovolemia
- Sex

There is no provision for correction for multiplicity for subgroup analysis, so results will be reported as point estimates with two-sided 95% CI.

Missing data (R28)

Our goal is to reduce or eliminate missing data during recruitment through concerted efforts. If, despite these efforts, missing data occur, we will assess the amount and pattern of missing data. The purpose of this assessment is to analyze the amount of missing data on the primary variables and other variables and determine the nature of

the missingness (missing completely random, missing at random, or missing at non-random). We will use the Multivariate Imputation by Chained Equations (MICE) using creating multiple datasets for ten times and results will be combined using Rubin's Rules. (26) MICE is useful when the pattern of missing data is random (MAR) or when the proportion of missing data exceeds 5% and does not follow a Missing Not at Random (MNAR) pattern. In cases where missing data follow a Missing Not at Random (MNAR) pattern, we will employ sensitivity analyses to examine the impact of different assumptions about the missing data mechanism on our results. Additionally, we will consider Bayesian imputation methods to address the potential bias introduced by MNAR data.

Additional analyses (R29)

No additional analyses have been planned.

Harms (R30)

Safety outcomes (Study Protocol Table 4) have been addressed above in this section.

Statistical software (R31)

The analysis will be conducted using SPSS version 29 or higher (IBM), SAS software version 9.4 or higher (SAS Institute), and R software version 4.4.1 or higher.

References

32a References to be provided for nonstandard statistical methods (R32a)

No non-standard statistical method is planned.

32b Reference to Data Management Plan (R32b)

The study protocol includes a reference to this Statistical Analysis Plan (see “Data management and confidentiality”): “Data analysis is specified in the Statistical Analysis Plan version 1, June 30, 2024, available in the protocol Appendix.”

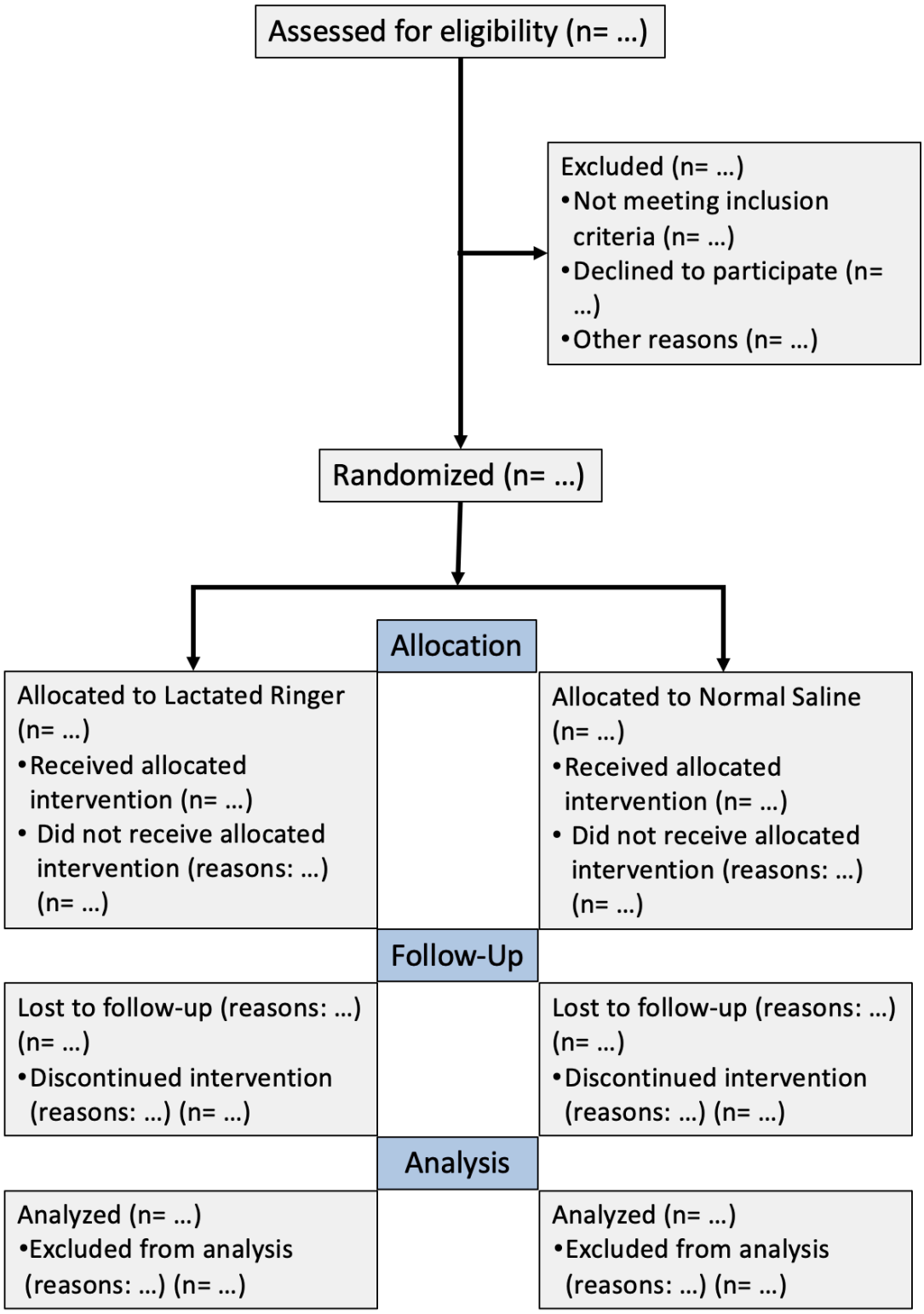
32c Reference to the Trial Master File and Statistical Master File (R32c)

Access to the Trial Master File is restricted to the clinical trial coordinator, the Dr. Balmis General University Hospital's Clinical Pharmacology Department, and collaborators in charge of statistical analysis.

32d Reference to other standard operating procedures or documents to be adhered to (R32d)

None provided.

Statistical Analysis Plan, Figure 1. Flow diagram, based on CONSORT 2010 recommendations. (24)



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PATIENT INFORMATION SHEET

Project Name: Normal saline versus lactated Ringer's solution for acute pancreatitis resuscitation, an open-label multicenter randomized controlled trial: the WATERLAND trial

Principal Investigator: PI OF THE CENTER

Department: DEPARTMENT

Center: CENTER

We are writing to request your consent to participate in a research project. This project has been approved by the CENTER'S ETHICS COMMITTEE. The project will be conducted in accordance with the standards of Good Clinical Practice and international ethical principles applicable to medical research in humans (Declaration of Helsinki and its latest revision).

In order for you to decide whether you wish to participate in this project, it is important that you understand why this research is necessary, what your participation will involve, how your information will be used, and its potential benefits, risks, and discomforts. Detailed information about the project can be found in this document. Please take the time to read the information provided below carefully and we will answer any questions you may have. Once you have understood the project you will be asked to sign the informed consent form if you wish to participate.

If you decide to participate in this study, you should know that you do so voluntarily and that you may withdraw from the study at any time. In the event that you decide to discontinue your participation, this will not entail any type of penalty or loss or detriment to your rights and medical care.

The project will be carried out in the CENTER'S HEALTH AREA.

WHY IS THIS PROJECT BEING CARRIED OUT?

Acute pancreatitis is a common disease; it is the third leading cause of hospital admission due to digestive disease. It is manifested by pain in the upper abdomen and requires hospital admission. Most patients (2/3 of cases) have a mild course of disease, with discomfort that subsides quickly, and a short hospital stay. However, in 1/3 of patients there are local or distant complications of the pancreas that make the recovery slower, with more discomfort, longer hospital stay, with greater need for invasive treatments and greater risk to life, although fortunately mortality from pancreatitis is very low, less than 3%.

Medical researchers have been searching for decades for treatments that, when administered early in the course of acute pancreatitis, can improve its prognosis, allowing more patients to have mild pancreatitis by preventing the onset of complications. No such treatment has been found, but there are clinical trials suggesting that the administration of certain types of fluids may have an anti-inflammatory effect in this early phase of acute pancreatitis. There are two types of fluids that are administered intravenously in patients with acute pancreatitis during the first few days of admission: normal saline and lactated Ringer's solution. These intravenous fluids consisting of water and salts are administered to ensure hydration and sodium intake in patients with acute illnesses, especially those involving nil by mouth since the patient is unable to drink. Some studies have described that lactated Ringer's solution has an anti-inflammatory effect in acute pancreatitis and could improve the evolution of this

disease, but other studies do not show that it is better than saline, which is the most frequently used.

In the WATERLAND study we want to check whether patients receiving lactated Ringer's solution or normal saline have a better evolution, or whether there are no significant differences between the two types of fluids.

WHAT IS THE AIM OF THE PROJECT?

Our objective is to test the effect of lactated Ringer's solution or normal saline on the severity of pancreatitis.

HOW WILL THE STUDY BE PERFORMED?

After being informed of the objectives, procedure, potential benefits, and risks of this study, we will proceed to decide randomly, thanks to a computer program, whether you will receive lactated Ringer's solution or normal saline. You will then receive this type of fluid for the first few days of admission for acute pancreatitis (at least for the first 48 hours).

It is of utmost importance that you understand that these two types of fluids are routinely and daily used in the management of acute pancreatitis, so you are going to receive one of these two fluids anyway, the only thing that changes is that instead of being decided by the attending physician, it will be drawn by a computer program, but you will be exposed to the same risks and benefits as both are a recommended treatment in the guidelines for the management of acute pancreatitis.

Your participation in the study is simply limited to the duration of hospital admission. This study will recruit patients over two years, although it could be extended if it has not

achieved the total number of patients we have calculated are needed to see if there is a treatment that is better. As soon as you start eating after that period and it is not clinically indicated, we will proceed to suspend the treatment of the fluid under study.

To participate you only have to sign this document.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

As we have mentioned, whether you participate in this study, you will receive one of the two types of fluids that we are studying, the only difference is that it is done in a controlled manner in order to be able to compare the two groups of patients. Your help in this study will allow us to know if one of the two types of fluids has a beneficial effect on the evolution of acute pancreatitis, or on the contrary both are the same and it is not worthwhile to continue asking this question in new studies. You will not receive financial compensation for your participation in the study. This study is sponsored by physicians and researchers who have no financial interest in these types of fluids, which are very cheap and of little or no interest to the pharmaceutical industry. The researchers participating in this study do not receive financial compensation for collaborating in the recruitment and follow-up of patients, they participate, just as you do, out of altruism, so that science advances and we know more about the treatment of acute pancreatitis.

WHAT ARE THE RISKS OF PARTICIPATING IN THE STUDY?

This study does not involve exposure to risks that you will not suffer anyway, since regardless of whether or not you participate in it, you will be given one of the two fluids. It has been speculated that saline may slightly more frequently produce renal failure, and blood acidity, and that lactated Ringer's solution may give increases in blood calcium

and potassium. Despite this, these are fluids that are used daily on any hospital floor, and we have a great deal of experience in the handling of both fluids, and the problems they cause are anecdotal.

WHAT DATA WILL BE COLLECTED?

We will collect your age, sex, weight, and other variables present at the beginning of the study. Throughout the admission we will note a series of variables that give an idea of the evolution of the disease, and we will compare these variables between the group of patients who received lactated Ringer's solution and those who received saline. For example, we will see if one of the two groups of patients has a greater severity of disease. We will measure other variables such as the presence of local complications in and around the pancreas, the presence of organ problems at a distance from the pancreas, inflammation measured by different ways, time taken to eat, analytical parameters and similar variables.

HOW WILL MY PERSONAL DATA BE TREATED AND HOW WILL CONFIDENTIALITY BE PRESERVED?

The collection, processing and use of the data required by this study will be in accordance with the provisions of Organic Law 3/2018, of December 5, 2018, on the Protection of Personal Data and guarantee of digital rights, and with the provisions of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on Data Protection (GDPR).

Access to your personal information will be restricted to the study doctor/collaborators, health authorities and the advisory bodies of the Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), when required, to check the data and procedures of

the study, but always maintaining the confidentiality of the same in accordance with current legislation. The Researcher, when processing and treating your data will take appropriate measures to protect them and prevent access to them by unauthorized third parties.

You may exercise your rights of access (to request information about your information stored in the database), of opposition (to refuse to provide the data), of cancellation (to request that the data be destroyed) and rectification (if over time any data is modified, or an error is detected). You may revoke your consent to the processing of your personal data by contacting the researcher.

In addition to these rights, and in accordance with the GDPR, you can also limit the processing of data that are incorrect, request a copy or that the data you have provided for the study be transferred to a third party (portability). To exercise your rights, please contact the principal investigator of the study. We remind you that the data cannot be deleted even if you no longer participate in the study to ensure the validity of the research and to comply, if applicable, with legal obligations and drug authorization requirements. You also have the right to contact the Data Protection Agency if you are not satisfied.

Both the Center and the Sponsor are respectively responsible for the processing of your data and undertake to comply with the data protection regulations in force. The data collected for the study will be identified by a code, so that no information that can identify you is included, and only your study doctor/collaborators will be able to relate this data to you and your medical history. Therefore, your identity will not be disclosed to any other person except to health authorities, when required or in cases of medical emergency. The Research Ethics Committees, the representatives of the Health

Authority for inspection and the personnel authorized by the Sponsor, will only have access to check the personal data, the procedures of the clinical study and the compliance with the rules of good clinical practice (always maintaining the confidentiality of the information).

The Investigator and the Sponsor are obliged to keep the data collected for the study for at least 25 years after its completion. Thereafter, your personal information will only be retained by the Center for your health care and by the Sponsor for other scientific research purposes if you have given your consent to do so, and if permitted by applicable law and ethical requirements.

If we transfer your encrypted data outside the EU to our group entities, service providers or scientific researchers collaborating with us, the participant's data will be protected by safeguards such as contracts or other mechanisms by data protection authorities. If the participant wants to know more about this, he/she can contact the CENTER'S PRINCIPAL RESEARCHER.

WHO CAN I CONTACT IF I HAVE ANY QUESTIONS?

If you need more information about the study, you can contact the CENTER'S PRINCIPAL INVESTIGATOR, Telephone: TELEPHONE