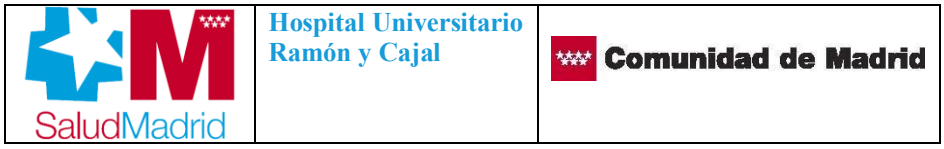

Study Title: Efficacy of oral and intravenous sodium chloride administration in the prophylaxis of post-contrast acute kidney injury in outpatients.

Short Title: PNIC-Na

Study phase: 2

Ethics Ref: 124-13

Project ID: EC11-132; 2012/0019.

EudraCT Number: 2013-001229-15

NCT03476460

Date and Version No.: 02-Aug-2013 V2.0 (ENG)

Principal Investigator: Luis Manzano Espinosa, Internal Medicine Dept., Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain.

Sponsor: The Foundation for Biomedical Research of the Hospital Universitario Ramón y Cajal (FIBioHRC)

Funder: The Spanish Health Ministry through the Fondo de Investigaciones Sanitarias (FIS) of the Instituto de Salud Carlos III, Madrid.

Contents

1. INVESTIGATOR COMMITMENT STATEMENT

I assume the responsibility for the proper conduct of the study at this site.

I agree to conduct the study in accordance with this protocol Version 2, August 2, 2013.

I will not make any changes to the protocol without the prior consent of the sponsor and the written authorization of the Clinical Research Ethics Committee (CEIC), except as necessary to counteract any imminent danger to patients or as it relates to the administrative aspects of the study (and when permitted by current legal regulations to this effect).

I am aware of and will comply with Good Clinical Practice (GCP) and other relevant legal requirements.

Signed: MD PhD Luis Manzano Espinosa. Principal investigator of the study.

Date: 2nd August of 2013.

2. SUMMARY

Type of request

Phase II clinical trial of oral sodium chloride in a new indication.

Sponsor

The Foundation for Biomedical Research of the Hospital Universitario Ramón y Cajal (FIBioHRC)

Funder

The Spanish Health Ministry through the Fondo de Investigaciones Sanitarias (FIS) of the Instituto de Salud Carlos III, Madrid.

Clinical trial title

Efficacy of oral and intravenous sodium chloride administration in the prophylaxis of post-contrast acute kidney injury in outpatients (PNIC-Na).

Protocol code

EudraCT: 2013-001229-15

Principal Investigator

Luis Manzano Espinosa.

Internal Medicine Department, Hospital Universitario Ramón y Cajal.

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Sites where the trial is planned to be carried out

Hospital Universitario Ramón y Cajal

Clinical Research Ethics Committee that evaluates the trial

Clinical Research Ethics Committee from Hospital Universitario Ramón y Cajal

Monitoring

Unit of Clinical Research and Clinical Trials (UICEC) of Hospital Universitario Ramón y Cajal, ISCIII National Network (SCReN). Tel/Fax +34 91 336 8825. e-mail: marisa.monitoracaiber@gmail.com

Experimental and control group

Experimental group: 500 mg capsules of sodium chloride (NaCl), supplied by the Pharmacy Department of the Hospital Universitario Ramón y Cajal. The total dose administered will be 100 mg/kg of body weight distributed in 60 hours, 48 hours before and 12 hours after the procedure. Appendix C describes the specific regimen according to weight.

Control group: 0.9% NaCl solution, supplied by the Pharmacy Department of the Hospital Universitario Ramón y Cajal. Dose: 3 ml/kg in the hour prior to the procedure and 2 ml/kg/hour in the following 4 hours.

Clinical trial phase

Phase II.

Main objective

To compare the incidence of post-contrast acute kidney injury (PC-AKI) in high-risk ambulatory patients after oral versus intravenous administration of sodium chloride prophylaxis.

Design

Phase 2, monocenter, randomized, open-label, controlled clinical trial for non-inferiority.

Disease or condition studied

Post-contrast acute kidney injury.

Main outcome

The main efficacy criteria for PC-AKI is defined as an increase in serum creatinine > 0.3 mg/dL or a reduction >25% in estimated glomerular filtration rate (MDRD-4), within 48 hours from baseline.

Study population

Patients at high risk of developing PC-AKI. The total number of patients will be 266, 133 for each treatment group.

Study duration

Three years.

Schedule and expected completion date

After approval by the Ethics Committee and other regulatory bodies:

Patient inclusion: 30 months.

Duration of treatment and follow-up: 72 hours.

Overall estimated completion of the study 36 months after approval.

3. KEY CONTACTS AND GENERAL INFORMATION

Sponsor's Contact Person

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Clinical Laboratories

Heart Failure and Vascular Risk Unit. Internal Medicine Department. Hospital Universitario Ramón y Cajal.

Nephrology Department. Hospital Universitario Ramón y Cajal.

Radiology Department. Hospital Universitario Ramón y Cajal.

Biochemistry Department. Hospital Universitario Ramón y Cajal.

4. RATIONALE AND OBJECTIVES

4.1. Background and current status

Post-contrast acute kidney injury (PC-AKI) is defined by an increase in plasma creatinine greater than 0.3 mg/dL, or greater than 25-50% of the baseline value, 24-48 hours after administration in the absence of other causes (surgery, arteriothrombotic disease, nephrotoxic, hemodynamically compromised or hypovolemia).[1] Despite it is usually transitory, it is a relevant complication. This is the third most frequent cause of acute renal failure in hospitalized patients, with an incidence of around 12%.[2] It may require permanent dialysis and is associated with an increased risk of other non-renal complications. It also increases hospitalization time and mortality.[3-5] Up to 30% of subjects who suffer PC-AKI will remain with some degree of renal failure.[6]

The existence of previous renal failure, diabetes, heart failure and the type and volume of contrast used have been recognized as risk factors.[4] The incidence of PC-AKI in general population is low (0.6-2.3%), increases in elderly and diabetic patients (5-29%), and is very striking in the presence of renal failure (incidence 15-55%).[6,7] Predictive scales have been developed for early identification of those at risk in whom preventive measures are especially indicated.[8] The European Society of Urogenital Radiology established a Contrast Safety Committee in 1996, which recommends the measurement of renal function prior to the test, and after it within a maximum of 7 days, as well as preventing measures in patients considered to be at risk.[9] Regarding the assessment of renal function, it is advisable to estimate the glomerular filtration rate using formula such as the Modification of Diet in Renal Disease (MDRD).[10] However, the MDRD formula may underestimate glomerular filtration rate in healthy subjects and overestimate it in diabetic patients with normal plasma creatinine.[11] Cystatin C is a low molecular weight protein freely filtered and metabolized after its reabsorption in the proximal tubule and its values show less variability than those of creatinin.[12]

Different in vitro and animal studies suggest that PC-AKI is a consequence of the existence of both direct and indirect tubular damage triggered by ischemia secondary to reactive oxygen species that induce local vasoconstriction.[13] On the other hand, the presence of contrast in the renal tubular lumen constitutes an osmotic force that induces natriuresis and stimulates tubule-glomerular feedback with reduction of renal perfusion.[14] These evidences constitute the pathophysiological basis for the benefit of hydration in patients submitted to contrast administration.

Numerous studies on PC-AKI prophylaxis have been performed but there is still not enough evidence to establish a clear recommendation. In addition to the use of hypo or isomolar contrast agents, experts advise the administration of intravenous isotonic solutions, with a wide variability between the type of solution and its administration schedule. Current recommendations are intravenous volume expansion, which is inapplicable in most outpatients undergoing radiological procedures.

Most of the preventive guidelines evaluated have used intravenous saline or sodium bicarbonate solutions.[15] It has been postulated that sodium bicarbonate offers greater protection than sodium chloride probably because of its urinary alkalinizing effect.[16,17] However, clinical trials and several recent meta-analyses have not demonstrated a greater efficacy of sodium bicarbonate vs sodium chloride (NaCl) in the prevention of PC-AKI.[18-20] A clinical trial is currently underway to conclusively clarify the efficacy of bicarbonate versus sodium chloride infusion (ClinicalTrials.gov NCT00930436).

N-acetyl-cysteine has been used in PC-AKI prophylaxis with heterogeneous results. Recently, a clinical trial with a large number of patients has shown no benefit associated with the use of N-acetyl-cysteine, so its administration should probably be abandoned.[21]

Regarding oral volume expansion, studies are scarce, with non-comparable designs, limited statistical power and heterogeneous results.[22-24] The findings of a recent study with NaCl suggest that oral administration is non-inferior to intravenous administration in the prevention of PC-AKI in high-risk subjects.[23] Nevertheless, due to the small number of patients in each group no conclusions can be drawn.

An important aspect of PC-AKI is the early detection, before the loss of renal function occurs.[25] In this sense, creatinine is not a good marker, since its elevation occurs when there is an established renal failure. Sensitive biomarkers of renal damage would allow not only to an early detection of renal injury, but also the identification of those patients at risk of suffering it.[25-27] Among the new candidate predictors of early kidney injury is the measurement in urine of neutrophil gelatinase-associated lipocalin (NGAL), an enzyme released after renal tissue injury, which is one of the most accurate, sensitive and specific methods.[28,29] Other state-of-the-art urinary markers related to the early diagnosis of renal damage are renal damage molecule 1[30], N-acetyl glucosaminidase[31], and other molecules patented by Spanish researchers: t-gelsolin (a proteolytic fragment of gelsolin) and ganglioside M2 activating protein (GM2AP).[32,33]

On the basis of the above, and taking into account the prevalence and clinical relevance of this complication, the possibility of introducing an effective oral regimen for the prevention of PC-AKI would have a considerable impact, both in terms of patient benefit and health care cost. Otherwise, the patient would have to be hospitalized. We consider the project to be sufficiently justified due to the high incidence of PC-AKI. The selected population group presents a high risk, so the expected benefit of this study will be especially relevant in this population. On the other hand, the incorporation of biomarkers of early diagnostic and/or prognostic utility would be of great help in identifying those patients at greater risk of suffering a severe nephropathy.

4.2. Benefits and risks for humans

The intended benefit is the demonstration that oral NaCl is non-inferior to an intravenous administration. This would allow the widespread application of a preventive treatment for contrast nephropathy, easy to administer to all patients. Likewise, the study could help us to identify patients at risk of PC-AK analyzing the molecules mentioned.

The administration of saline compounds can increase blood volume and increase congestive symptoms such as edema. However, at the doses used and taking into account the criteria required for participation, patients are not expected to suffer any complication from the study. In any case, oral treatment, which is the subject of the study, should not produce more volume overload at the proposed doses than conventional intravenous treatment.

4.3. Justification of the route of administration, dose, regimen and treatment period

The proposed intravenous regimen of 11 mL/kg body weight of 0.9% NaCl, over the course of 6 hours is within the accepted recommendations. The oral regimen is intended to provide volume support with NaCl for a reasonable period of 60 hours.

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4.5. Description of the study groups

Experimental group

Sodium chloride capsules of 500 mg, supplied by the Pharmacy Department of the hospital. The total dose administered will be 100 mg/kg distributed in 60 hours, 48 hours before and 12 hours after the procedure. Water (250 mL) will be administered with each oral dose, ensuring a minimum daily water intake of 750 mL.

Control group

Sodium chloride solution 0.9% will be supplied by the Pharmacy Department of the hospital at a dose of 3 mL/kg in the hour before to the procedure and 2 mL/kg/hour in the 4 hours after.

4.6. Description of the study population

The study will be performed in 266 outpatients aged over 65 years, with prior consent, at high risk of developing PC-AKI undergoing a contrast-enhanced CT scan (CE-CT). The following risk factors where considered: diabetes mellitus, stable heart failure and renal insufficiency (estimated glomerular filtration rate calculated by the MDRD-4 formula between 30 and 59 mL/min).

In order to ensure that the amount, volume and type of contrast infusion is similar in all subjects, only patients who underwent CE-CT were selected.

4.7. Main objective

To compare the incidence of PC-AKI after oral vs. intravenous administration of NaCl in high-risk outpatients.

4.8. Secondary objectives

- To study and evaluate the clinical usefulness of various biomarkers of damage in PC-AKI, both for early diagnosis of renal damage and for prior stratification of the risk of PC-AKI.
- To elaborate a recommendation applicable to elderly outpatients at high risk of developing PC-AKI.

5. TRIAL TYPE AND DESIGN

5.1. Clinical trial phase

Clinical trial phase 2.

5.2. Main and secondary variables

Primary endpoint

PC-AKI is defined by the presence any of the following criteria in the first 48 hours after contrast administration.

- Increase in serum creatinine greater than 0.3 mg/dL from baseline.
- Reduction in glomerular filtration rate estimated by the MDRD* formula greater than 25% from baseline.

  *MDRD: 186 x (Cr)-(1.154) x (Age)-(0.203) x 0.742 (if female) x 21 (if African American).

Clinical variables

The following variables will be collected between 7 days and 24 hours before contrast administration:

- General data: patient code and date of inclusion.
- Demographic data: date of birth and gender.
- Personal history: smoking, hypertension, diabetes mellitus, heart failure, dyslipidemia, ischemic heart disease, atrial fibrillation, chronic kidney disease, peripheral arterial disease, cerebrovascular disease, anemia, COPD, hypoventilation (pCO_2>50 mmHg).
- Anamnesis and physical examination: NYHA functional class orthopnea, edema in extremities, ischemic chest pain, height, weight, BMI, HR, blood pressure. Pulmonary auscultation, hepatojugular reflux, hepatomegaly.
- Other analytical determinations: hemoglobin, basal glycemia, glycosylated hemoglobin, oxygen saturation by pulse oximetry, BNP.
- Imaging tests: electrocardiogram.
- Concomitant medication.
- Procedure data: volume of contrast administered.

Biological variables

Apart from creatinine measurement and glomerular filtration rate according to MDRD, which constitutes the primary response variable of the study, the following parameters will be measured immediately before the procedure, and at 24 and 48 hours after contrast administration: plasma cystatin C, serum biochemistry with ions, venous gasometry, urinary concentrations of: NGAL, KIM-1*, NAG*, t-gelsolin*, GM2AP*, sodium, microalbuminuria and protein.

* The biomarkers identified with an asterisk will be stored at -80 ºC until processing in the Laboratory of the Heart Failure and Vascular Risk Unit of the Internal Medicine Department of Hospital Universitario Ramón y Cajal. This Unit guarantee traceability, quality and safety in computer management, storage control and processing samples.

5.3. Study design

PNIC-Na is a phase 2, monocenter, randomized, open-label, non-inferiority trial. Two types of preventive treatment will be compared in patients undergoing a CE-CT scan:

- Oral prophylaxis with NaCl capsules and free water intake (250 mL of water with each capsule, ensuring a water intake of 750 mL/day) in the 48 hours prior to contrast exposure (n=133). It will be performed on an outpatient basis at a dose of 100 mg/kg body weight distributed in 60 hours, 48 hours prior to the procedure and 12 hours after. Appendix C describes the specific dose according to weight.
- Intravenous prophylaxis with 0.9% NaCl before and after contrast exposure (n=133). It will consist of in-hospital administration of 3 mL/kg in the hour before and 2 mL/kg in the 4 hours after the procedure.

The contrast used will be iodixanol (320 mg iodine/mL, infusion rate 2-5 mL/sec).

The corresponding treatment will be administered after medical prescription, validation by the pharmacist and confirmation. The nurse in charge of the patient will administer the treatment. The intravenous prophylaxis schedule for the study will begin in the Radiology Department of the Hospital, and will conclude in the Clinical Trials Unit of the same center.

The study will consist on the schedule of visits described in section 9.2.

5.4. Recruitment and randomization

Patients will be recruited from the Heart Failure and Vascular Risk Unit (UICARV) of the Internal Medicine Department of the Hospital Universitario Ramón y Cajal. Eligible patients will be informed at the time of requesting the contrast-enhanced CT scan and, after signing the consent, they will be selected. They may also be identified from the requests made to the Radiology department. In this case, the patient will be contacted prior to get the informed consent. After their definitive inclusion they will be randomized to one of the two arms. A 1:1 randomization will be performed. The randomization sequence will be generated by computer in variable blocks, ranging from 4 to 6. An automated allocation system will be used, so that the allocation sequence will be unknown to the researchers participating in the study. The patient will be considered as randomized as soon as a randomization number is assigned.

5.5. Treatment

Treatment under study

Oral prophylaxis with NaCl capsules and free water intake (250 mL of water with each capsule, ensuring a water intake of 750 mL/day) in the 48 hours prior to contrast exposure (n=133). It will be performed on an outpatient basis at a dose of 100 mg/kg body weight distributed in 60 hours, 48 hours prior to the procedure and 12 hours after. Appendix C describes the specific dose according to weight. Common salt will be used

in the experimental group. It will be encapsulated in the Pharmacy Department to ensure the exact dose administered to each subject in the trial.

Control treatment

Intravenous prophylaxis with 0.9% NaCl before and after contrast exposure (n=133). It will consist of in-hospital administration of 3 mL/kg in the hour before and 2 mL/kg in the 4 hours after the procedure. The NaCl solution used as a control drug is commercialized in our country and will be acquired by the promoter through the Hospital Pharmacy department, which will be responsible for its labeling and distribution.

Contrast used

The contrast used will be iodixanol (320 mg iodine/mL). A volume of 100 mL, at infusion rate 2-5 mL/s will be administered.

5.6. Schedule

Estimated duration of the clinical trial is 3 years.

Trial schedule

- Preparation of the study (3 months): Planning work for adequate information to patients, acquire the necessary material and schedule the laboratory determinations and the application of protocols.
- Development of the study: progressive inclusion and follow-up of patients, as well as sample collection (estimated at 8 patients per month). It is estimated that 30 months will be needed to include the 266 patients. This phase could be prolonged due to limitations in the recruitment. The protocol for the collection of samples will be applied to process and conserve the samples.
- Determinations of serum and urinary markers will be performed at the same time in an estimated period of 3 months. NAG, NGAL, KIM-1, GM2AP, t-gelsolin will be measured.
- Elaboration of the database for the inclusion of clinical and analytical variables. Collection and computerization of clinical data and analytical results (6 months).
- Statistical study of the results and their interpretation. Writing of the work and publications (5 months).

5.7. Completion criteria

Patients will terminate their participation in the study for the following reasons:

- Completion of treatment according to the study protocol.
- Medical safety reasons, at the discretion of the responsible Investigator.
- Serious adverse effect or adverse effect that the Researcher considers unacceptable.
- Voluntary abandonment by the patient.
- Death.
- Demonstration during follow-up that the patient did not meet the criteria for inclusion in the clinical trial.

5.8. Study medication accounting

Study medication should be used in accordance with the study protocol. The Principal Investigator, or the person he/she delegates, will be responsible for administering the study medication. The Principal Investigator will record used and unused medication for each trial patient on a Medication Record Sheet. This Record Sheet will be available for monitoring visits. To ensure traceability of intravenous NaCl the

following will be noted: trade name, quantity and lot number dispensed at each visit. This information will also appear in the electronic data capture system.

In the case of common salt (capsules), a record will be kept of those given to the patient and of the blisters returned by the patient at the 24h visit after the procedure. Accordingly, patient's adherence to the treatment will be assessed.

5.9. Randomization

The Clinical Biostatistics Unit of Hospital Universitario Ramon y Cajal will generate (by computer) a randomization list to assign each treatment. The ratio will be 1:1. This list will be sent to the Pharmacy Department of the hospital, which will be responsible for supplying the study researchers with the treatment corresponding to the patient number in the trial.

5.10. Data source

All procedures and variables related to this work will be collected in an electronic data capture system (EDC). There will be an EDC for each subject included, independently of the group he/she belongs, and all the variables related to the study will be recorded in this EDC. During the study, no personal data will be collected from patients as study participants that would allow identification of the subject. The data obtained will be kept under strict confidentiality (Organic Law 15/1999, on personal data protection) and only the research team of the project will have access to them. Radiological images and other complementary tests related to the study, as well as the EDC will be numbered with a code to guarantee the confidentiality of the sample and the data. During the study period EDC will be kept under lock, which will be held exclusively by the researcher responsible for the project. The coordinators of this study are committed to maintain absolute confidentiality and to protect the information provided to them.

All the information contained in the Clinical History and certified copies of the tests performed, necessary for the evaluation of the clinical trial.

Original documents, data and tests (e.g., medical history, medical charts, nursing charts, pharmacy records, laboratory tests, or radiological studies).

5.11. End of clinical trial

The end of the clinical trial will be the last visit of the last subject enrolled in the study.

6. SELECTION AND WITHDRAWAL OF SUBJECTS

6.1. Inclusion criteria

Patients are eligible to be included if they meet the following inclusion criteria: outpatients, aged over 65 years old, undergoing a contrast-enhanced CT scan, who are at high risk of developing PC-AKI, defined as having at least one of the following risk factors: diabetes mellitus, stable heart failure, chronic kidney disease (MDRD-4 between 30 and 59 mL/min), and capable of giving signed informed consent.

6.2. Exclusion criteria

- Glomerular filtration rate less than 30 mL/min.
- Presence of hypokalemia (serum potassium less than 3.5 mEq/L).
- Performance of other procedures with intravenous contrast in the previous fifteen days.
- Administration of nephrotoxic drugs (NSAIDs, aminoglycosides and/or potentially nephrotoxic chemotherapy) in the last 72 hours, or anticipation of receiving them in the following hours.
- Chronic decompensated pathology: acute heart failure, decompensated COPD or poorly controlled arterial hypertension.
- Allergy to iodinated contrasts.
- Patients diagnosed with hyperchloremia or hypernatremia.
- When assessing the inclusion of a patient in the study, all the specifications of the technical data sheet in relation to "special warnings and precautions for use" will be taken into account.

6.3. Sample size and justification

The study will be conducted in 266 individuals on an outpatient basis. Taking into account the non-inferiority design, for the determination of the sample size it has been considered acceptable to establish a priori a difference of no more than 5% in the incidence of PC-AKI of oral prophylaxis with respect to intravenous. Considering our high-risk population, we estimated an incidence of PC-AKI in the intravenous group of 7% and 12% in the oral group. With this expected response, a non-inferiority margin of 5%, a power of 80% and a degree of significance of 2.5%, the sample size will be 266 patients, 133 per group.

6.4. Withdrawal criteria and analysis

The patient may discontinue participation in the study at any time he/she wishes. The investigator/physician may also decide to withdraw a patient from the trial if the patient does not meet the protocol standards. Patients will terminate their participation in the study for the following reasons:

- Completion of treatment according to the study protocol.
- Medical safety reasons.
- Serious adverse effect or adverse effect that the Investigator considers unacceptable.
- Voluntary withdrawal by the patient.
- Death.
- Acknowledgement during the follow-up that the patient did not meet the selection criteria for the clinical trial.

6.5. Estimated duration of the recruitment period

The recruitment period is estimated to extend over 30 months.

7. DESCRIPTION OF TREATMENT

7.1. Treatment definition

Treatment A:
Sodium chloride, capsules of 500 mg.

- Dose per subject: 100 mg/kg total administered in several daily intakes during the 48 hours prior to the procedure and 12 hours after.
- Route of administration: oral.

Treatment B:
Sodium chloride, 0.9%.

- Dose per subject: 3 mL/kg of 0.9% sodium chloride in the hour before and 2 mL/kg in the 4 hours after the procedure.
- Route of administration: intravenous.

Concomitant treatments

Permitted: Diuretic and hypotensive medication will be maintained, as long as there have been no changes in the previous 10 days. No changes in these medications will be allowed, except in special cases, during the 48 hours following the CE-CT. Those patients in treatment with metformin, will suspend this medication from 48 hours before to 24 hours after the administration of the contrast, and it can be replaced by the introduction or temporary increase of insulin. During the 48 hours before and 24 hours after the procedure, free water and salt intake will be allowed.

Prohibited: Any nephrotoxic agent will be prohibited (NSAIDs, chemotherapy or immunosuppressants, nephrotoxic antibiotics) during the 10 days before and 48 hours after the procedure.

7.2. Compliance

Study medication should be used in accordance to the study protocol. The Principal Investigator, or a member of the team, will be responsible for administering the study medication. The researcher team will record used and unused medication for each trial patient on a Medication Record Sheet. This Record Sheet will be available for monitoring visits in accordance with RD 223/2004. To ensure traceability of the study, for NaCl intravenous, the following will be noted: trade name, quantity and lot number dispensed at each visit. This information will also appear in the EDC. In the case of common salt (capsules), a record will be kept of those given to the patient and of the blisters returned by the patient at the 24h visit after the contrast. In this way, the patient's adherence to the treatment will be assessed.

8. EFFICACY ASSESSMENT

8.1. Efficacy assessment criteria

- Increase in serum creatinine from baseline greater than 0.3 mg/dL.
- Reduction of the estimated glomerular filtration rate greater than 25% from baseline value.

8.2. Development of the study

Patients will undergo the following procedures:

Screening visit (from two months to 48 hours before the procedure): verification of inclusion and exclusion criteria. Signature of the informed consent. A laboratory blood test will be recorded within two months from the CE-CT scan. The screening visit may take place on the same day as randomization (visit 1).

Visit 1 (from two months to 48 hours before the procedure): review of inclusion and exclusion criteria. Serum creatinine, eGFR and ions will be checked. Registration of demographic and clinical data. The patient will be randomized and will receive therapeutic information to the assigned group. In the case of the oral sodium chloride arm, capsules will be provided.

Visit 2 (day of the procedure): Before the contrast administration, the investigator will ensure the patient has taken study medication. A physical examination will be performed. Furthermore, blood and urine samples will be collected for the specific analytical determinations and biomarkers. After that, contrast will be administered as well as the intravenous sodium chloride (intravenous arm). Adverse events will be reported.

Visit 3 (24 hours after contrast): The investigator will perform a physical examination, will ensure the compliance of study medication (oral NaCl arm), and will report the adverse events. Moreover, blood and urine samples will be collected.

Visit 4 (48 hours after contrast): same evaluation as visit 3, excluding compliance assessment.

Study Flowchart

	SCREENING (From two months to 48 hours before the procedure)	VISIT 1† (From two months to 48 hours before the procedure)	VISIT 2 (Day of the procedure; 0 hours)	VISIT 3 (24 hours after contrast)	VISIT 4 (48 hours after contrast)
Inclusion and exclusion criteria	X	X			
Informed consent	X				
Randomization		X			
Medical history		X			
Clinical variables		X	X	X	X
Contrast administration			X		
Study treatment administration		X*	X		
Evaluation of treatment compliance			X	X*	
Clinical laboratory assessments	X		X	X	X
Biomarkers			X	X	X
Adverse events evaluation		X	X	X	X

* Patients in oral NaCl arm.

† The screening visit may take place on the same day as randomization (visit 1).

8.3. Methods for efficacy assessment

The determinations of the study will be performed prior to contrast administration, and at 24 and 48 hours after the CE-CT scan.

- Plasma creatinine, ions, cystatin C, blood count and venous gasometry will be assessed.
- NGAL*, KIM-1*, NAG*, t-gelsolin*, GM2AP*, and urinary creatinine will be determined to homogenize the values of the different biomarkers. Sodium, pH, microalbuminuria and proteinuria will also be measured in urine.
-

These analyses will be performed in the laboratories of the Hospital Universitario Ramón y Cajal, as part of the clinical routine, except for the biomarkers identified with an asterisk. This samples will be stored at -80ºC in the Laboratory of the Heart Failure and Vascular Risk Unit of the Internal Medicine Dept. Working conditions guarantee traceability, quality and safety storage control and processing.

The methods for the different determinations are presented below:

- Creatinine in blood and urine: using the alkaline picrate colorimetric technique (kinetic Jaffé) on the Architect 16000 analyzer (Abbott Diagnostics).
- Cystatin C in blood: by turbidimetric technique on the Architect 16000 analyzer (Abbott Diagnostics).
- NAG: by commercial kit (Roche Diagnostics) based on spectrophotometric detection of the reaction product catalyzed by NAG.
- NGAL: by chemiluminescence detection techniques on the Architect 2000 immunoassay analyzer (Abbott Diagnostics).
- T- Gelsolin: by Western blot.
- KIM 1: by commercial ELISA kit (CUSABIO).
- Ganglioside M2 activating protein (GM2AP): by Western blot.

9. SAFETY

Safety assessments will be carried out in accordance with the Standards of Good Clinical Practice (GCP) and current legislation.

9.1. Definitions

Adverse Event (AE)

Any untoward health occurrence in a patient or clinical trial subject treated with a drug, even if not necessarily causally related to that treatment. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

Adverse Reaction (AR)

An unintended, harmful reaction to an investigational product, regardless of the dose administered. In this case there is a suspected causal relationship between the investigational product and the AR.

Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR)

Any adverse event or adverse reaction that, at any dose, results in death, threatens the life of the subject, requires hospitalization of the patient, or prolongs an existing hospitalization, causes permanent or significant disability or incapacity, results in a congenital anomaly or malformation.

Suspected AA or AR that are considered medically significant, even if they do not meet the above criteria, will also be treated as serious, including major medical events that require intervention to prevent the occurrence of one of the consequences described above. Likewise, all suspected transmission of an infectious agent should be reported as serious.

Unexpected Adverse Reaction (UAR)

Any AR whose nature, intensity or consequences do not correspond to the reference information for the medicinal product.

Minimum information to be specified

The researcher team will follow up on the possible adverse events that could arise throughout the study. Thus, analytical safety parameters will be determined periodically and the patient will be asked at each visit if he/she has experienced any type of symptomatology since the previous visit. If it exists, it will be reflected in the clinical history and in the data collection sheet. The information regarding the time of appearance, duration, intensity, course and outcome will be recorded. In addition, all patients in the study will be given a diary to record anything they consider of interest, including possible AEs. The reporting of serious or unexpected adverse events will be done according to Spanish legislation. All adverse events should be documented in the patient's medical record.

9.2. Causality assessment

Researches will take into account all aspects related to adverse reactions, contraindications, warnings and precautions for use, and possibilities of interactions with other drugs, as specified in the data sheets of the investigational drugs. Safety parameters to be measured are described in the section 6.2.

9.3. Safety assessment

Adverse events in this study will be classified on the basis of their causal relationship with the drug as:

- **Possibly/probably related**: if there is reasonable suspicion of a possible or probable relationship (reasonable temporal sequence between the administration of the investigational product and the occurrence of the adverse event and the AE cannot reasonably be attributed to any alternative cause).
- **Unrelated**: there is a reasonable time sequence between the administration of the investigational product and the occurrence of the adverse event, but the adverse event can be explained by alternative causes; or there is no reasonable time sequence between the administration of the drug and the occurrence of the adverse event.
-

The investigator should evaluate the causality of all serious adverse events. In the event that the investigator does not know causality, for reporting purposes the adverse event should be considered to be related to the investigational product.

9.4. Intensity assessment

For all AR observed the investigator will use the following definitions to rate the intensity of any AR collected during the study in the EDC:

- Mild: that which does not cause limitation in daily activities.
- Moderate: that which causes some limitation in the performance of daily activities.
- Severe: that which prevents the performance of daily activities.

9.5. Severity assessment

AR should also be classified on the basis of their severity. In general, if they meet any of the following criteria should be considered serious adverse reactions:

- Life-threatening.
- Requiring hospitalization or prolongation of an existing hospitalization.
- Causing permanent or significant disability or incapacity.
- Result in congenital anomaly or malformation.
- Considered medically important, understood as that which requires urgent intensive treatment to avoid one of the outcomes.

9.6. Expectancy assessment

An AE not listed in the drug label.

9.7. Safety recording procedure

Researcher team should record all AR specifying the date of initiation, intensity, severity, action taken and outcome. If any treatment is given for the AR, it should be recorded in the EDC. All ARs should be recorded in each subject's EDC.

9.8. Safety reporting procedure

The investigator will immediately report all AR to the sponsor (always within 24 hours of knowledge) on the form designed for this purpose (Reporting Form). This form, after being completed and signed by a member of the researcher team, will be sent in paper format by fax to the Clinical Trials Unit of the Hospital Universitario Ramón y Cajal (Fax number: +34 917 291 890, Unidad de Ensayos Clínicos, Hospital Universitario Ramón y Cajal, 7ª planta Izquierda, Carretera de Colmenar Viejo Km 9.100, 28034, Madrid). The initial communication will be followed by detailed written communication. The investigator must follow up all the AR until resolution, return to baseline or stabilization in case of permanent involvement. Initial and follow-up communications should identify trial subjects by a specific code number. The sponsor will keep a detailed record of all AR reported by the researchers. These records should be submitted to the AEMPS and the IRB/IEC upon request. The notification period will be from the moment the patient signs the informed consent until 30 days after the administration of the last dose. After this period, only those serious AR related to the study medication of which the investigator is aware should be reported to the sponsor.

Report of suspected serious AR and unexpected AR

The sponsor will notify all the suspected serious and unexpected adverse reactions associated with the product to the AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) Clinical Trials Area of the Department of Medicines for Human Use; the Ethics Committee involved and the Autonomous Communities delegates where the clinical trial is performed. Notification period will be 15 days, except when the suspected AR has caused the death of the subject, or endangered his life, which will be communicated within 7 days after its knowledge.

9.9. Follow-up plan

When an AR occurs, it will be recorded as specified in this protocol and the patient will be followed-up until it is considered resolved.

9.10. Notification of other safety information

The sponsor should also report any information that could change the benefit of the research product or that could lead to changes in the schedule of administration of the trial, such as:

- A qualitative change or an increase in the percentage of occurrence of unexpected and severe AR.
- Unexpected and severe AR occurring after completion of the trial and reported by the investigator to the sponsor.
- New events related to the conduct of the trial or the development of the research product and likely to affect patient safety such as: AR that may be associated with the trial procedures and may modify the conduct of the trial, lack of efficacy in a drug used for the treatment of a life-threatening disease, new safety findings from new animal studies (such as carcinogenicity), any premature termination or temporary halt of a clinical trial with the same research product for safety reasons, conducted in another country by the same sponsor, any recommendation from the Data Monitoring Committee that is relevant to patient safety. This information will be notified as soon as possible and no later than 15 days after the sponsor has become aware of it. Additional information will also be notified as soon as possible.

9.11. Annual safety reports

The annual safety reports, which will include the RAGIs collected in the study, will be sent by the sponsor to the AEMPS (Clinical Trials Area of the Sub-Directorate General for Medicinal Products for Human Use), the Autonomous Regions and the CEICs involved, within the deadlines established in the legislation in force.

9.12. Notification to investigators

The sponsor should notify the researcher of any information that could affect the safety of the trial subjects as soon as possible. The information on unexpected and severe AR will be sent annually in a list together with a brief analysis of the data provided. Researchers will also be informed of any safety issues impacting the conduct of the clinical trial or product development, including discontinuation of the development program or safety-related protocol modifications.

9.13. Access to the randomization code during the trial

In case of adverse events, the treatment assignment number from the randomization code list will be disclosed under special circumstances. If possible, the delegate should be contacted before the number is disclosed. Each patient will have a randomization code which contains the treatment assignment. It should be kept in a safe place throughout the clinical trial. The researcher is responsible for the integrity of the

randomization list. If a treatment assignment is unblinded, the researcher will record the date and reason in the EDC. Unblinding should not be a reason for withdrawal the product or the patient from the study.

10. STATISTICAL CONSIDERATIONS

10.1. Sample size calculation

The sample size of the trial was calculated to be able to show the non-inferiority of oral sodium chloride to intravenous prophylaxis with regard to the primary study endpoint in outpatients undergoing a contrast-enhanced CT scan. The calculation was based on an expected PC-AKI incidence of 7% in the intravenous arm and 12% in the oral arm. Accordingly, considering a non-inferiority margin of 5%, a power of 80% and a one-sided p-value of 0.025, the required sample size is 266 patients, 133 per arm.

10.2. Randomization and treatment assignment

The randomization process will be carried out by blocks of size six, in order to guarantee a balance in the study arms. Treatment assignment will be performed by opening numbered, sealed, opaque envelopes that describe inside whether the patient has been assigned to intravenous or oral NaCl prophylaxis

10.3. Statistical methods

A description of the study sample stratified by randomly assigned treatment will be made. Categorical variables will be described by absolute and relative frequencies. Quantitative variables will be described by the mean and standard deviation (or median and interquartile range in the case of highly asymmetric distributions). For the analysis of the primary endpoint of the study, the univariate comparison of the proportions of PC-AKI events within 48 hours after intravenous contrast administration in both arms will be carried out. The 95% confidence interval of the difference in proportions will be calculated and displayed graphically. All analyses will be intention-to-treat and per-protocol. For the main objective a p-value less than 0.025 (unilateral) will be considered as statistically significant. All other group comparisons will be of an exploratory nature. Subgroup analyses will be performed for the risk factors defined as inclusion criteria. For exploratory contrasts, a p-value less than 0.05 (two-sided) will be considered as statistically significant.

10.4. Missing data

The impact of missing data in the main outcome will be analyzed by sensitivity analysis. Participants whose main variable has not been collected will be assumed to have a PC-AKI event for both arms, only for the oral group, and only for the intravenous group.

10.5. Deviations from the statistical plan

Any deviation from the statistical plan will be communicated to the IRB and the AEMPS and its approval will be requested by means of an amendment.

10.6. Selection of subjects for analysis

All analyses will be by intention to treat and by protocol. Since the study is a non-inferiority study, the main analysis will be per protocol.

11. ACCESS TO SOURCE DATA AND DOCUMENTS

All data collected for the study will be kept in the participating departments, in paper and electronic format. Clinical histories will be filed according to regulations or procedures established in the hospital. Data collected for the study will be identified by a code and only the principal researcher will be able to associate these data to the patient. Data will be included in a database that complies with Law 15/1999 on Personal Data Protection. Likewise, the transmission of data will be done with the appropriate security measures in compliance with that law and R.D. 1720/2007. Only the information from clinical history that is related to the study will be subject to verification. The research team is responsible for maintaining confidentiality. Only the sponsor, study physician/collaborators, health authorities (Spanish Agency of Medicines and Medical Devices), Clinical Research Ethics Committee and personnel authorized by the sponsor may check information always maintaining confidentiality in accordance to current legislation. Patients' identity will not be revealed if the results of the study are published.

12. QUALITY CONTROL AND ASSURANCE

Monitoring visits

At least 2 monitoring visits will be made during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed verify the information recorded in the EDC. Source documents are the original documents, data, and records. The investigator will ensure that the sponsor or the sponsor's representative have access to source documents. Documentation and aspects of the trial that can be reviewed: researcher's file, trial medication, medical records, informed consent, EDC and associated source documents. It is important that the researcher and study personnel are available during monitoring visits.
Independent on-site monitoring will be performed by the Unit of Clinical Research and Clinical Trials (UICEC) of Hospital Universitario Ramón y Cajal, ISCIII National Network (SCReN).

Health Authorities audits

This study may be audited by the health authorities, AEMPS. If a health authority contacts the trial site for an inspection, the sponsor should be informed immediately. The researcher should guarantee the auditors access to all study documents for quality assurance.

13. ETHICAL ASPECTS

13.1. General and specific rules for the investigators

The investigators will strictly abide by the provisions of this protocol, filling out the data collection sheets, which will be sent in time to the sponsor or to the collaborating entity to analyze it. The trial will be carried out in accordance with the recommendations for clinical trials and evaluation of drugs in human, which appear in the Declaration of Helsinki (appendix A), revised in Tokyo, Venice, Hong Kong, South Africa, Edinburgh and Seoul (2008) and in the current Spanish legislation on clinical trials.

13.2. Informed consent

All participating subjects will be informed and give their written consent before starting the study.. The volunteer will be given a copy of this informed consent sheet.

13.3. Security and confidentiality arrangements

The information obtained by the implementation of the present study is considered confidential and should be treated as such at all times. The subjects of the study will be identified only with their subject code. In case of publication of the results of the study, the identity of the volunteers will not be revealed.

13.4. Content of the trial budget

The financial compensation to the trial researchers is specified in the financial report, which is included as an appendix.

13.5. Insurance

The Foundation for Biomedical Research of Hospital Universitario Ramón y Cajal has a civil liability insurance policy in accordance with Royal Decree 223/2004, which will provide compensation and indemnity to the participating subjects in case of damage to their health or injuries that may occur. In addition, this policy covers the responsibilities of the promoter, the research team and the Hospital Universitario Ramón y Cajal.

14. PRACTICAL CONSIDERATIONS

14.1. Responsibilities of the participants of the clinical trial

The investigators will follow the Good Clinical Practice guidelines and Service's standard operating procedures. All information collected during the trial should be recorded directly in the eDC. When a correction is made, date and initials of the person making the correction should be noted. Auxiliary personnel will follow the instructions given by the researcher regarding the extraction of blood samples and other complementary explorations. Patients should receive oral and written information regarding the design, purpose of the study and possible risks that may arise from it before enrolling in the trial. To participate in the study, they must sign their consent, although they can revoke it at any time and for any

reason. Patients will receive instructions to strictly respect the instructions. They will be informed of the need to contact if any incident arises during the study.

14.2. Deviations from the protocol

The researcher present in such circumstances will fully document the deviation and the reason in the EDC. When the deviation is related to inclusion/exclusion criteria, researchers will contact the clinical monitor by telephone to inform of the deviation.

14.3. Storage of the study file

All data will be kept on paper and in electronic form during 15 years after the end of the study.

This file should contain the following elements:

1. IRB/IEC approval of the protocol and informed consent form.
2. Copy of the written consent form and approved protocol with any amendments if applicable.
3. Any correspondence regarding the study with the sponsor, during the course of the study.
4. Any correspondence with the IRB/IEC.
5. List of IRB/IEC members who approved the study protocol.
6. Signed acceptance of the protocol.
7. Curriculum vitae of the research team.
8. Record of signatures of the members of the research team.
9. Serious AR communications.
10. Contract between the sponsor and the research team.
11. List of patients.
12. Copies of the EDC.

The documentation will be filed following the standard procedures of the Internal Medicine Department.

14.4. Sample identification

The study medication will be provided by the sponsor, and will be labeled according to recommendations of Royal Decree 223/2004.

14.5. Amendments to the protocol

Neither the investigator nor the monitor or the sponsor will modify this protocol without first obtaining the consent of the other parties. The modification should be documented in writing. Any changes in the research activity, except those necessary to eliminate an immediate apparent risk to the patient, should be reviewed and approved by the IRB/IEC prior to implementation. Amendments to the protocol should be submitted by the sponsor to the health authorities, and modifications may require review and approval by the IRB/IEC.

14.6. Acceptance by the investigator

The investigator commitment statement is included in section 2.

14.7. Conditions for publication

The results derived from the present study will be published; the approval of the investigators and the sponsor will be required. The monitor and the sponsor should have a copy of the manuscript to be published 15 days before it is sent to the editor. Likewise, the confidentiality of the patient's identity will always be respected. The study sponsor undertakes to publish the results, whether positive or negative.

15. APPENDICES

APPENDIX A. WMA DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975. 35th WMA General Assembly, Venice, Italy, October 1983. 41st WMA General Assembly, Hong Kong, September 1989. 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added). 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added). 59th WMA General Assembly, Seoul, October 2008.

INTRODUCTION

The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

In medical practice and in medical research, most interventions involve risks and burdens.

Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison

with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee.

Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

APPENDIX B. ADVERSE REACTION REPORTING

GENERAL INSTRUCTIONS

1. This form is to be used only for reporting suspected unexpected serious adverse reactions (ARs) occurring with investigational drugs. Both the specific investigational product and the control product are considered investigational drugs.

2. Suspected fatal or life-threatening adverse reactions (those that without immediate therapeutic intervention would have resulted in the death of the patient) should be reported within a maximum of 7 calendar days; if not all information is available, it may be completed within an additional 8 days. Other suspected serious and unexpected adverse reactions should be reported within a maximum of 15 days.

3. When the space available is insufficient, an additional information sheet should be added, correctly identified with the name of the sponsor and the number assigned to the notification. This additional information may include the causality assessment carried out by the reporting technician.

SPECIFIC INSTRUCTIONS

1. The protocol code is the code assigned by the sponsor to identify the assay. The sponsor's notification number is the one used by the sponsor for archiving. In the case of follow-up information, the same number should be used or, if changed, the number of the initial notification should be indicated. The shaded "Notification No." space should be left blank.

2. The age should be given in years, months, weeks or days as appropriate, but always indicating it. If the age is not known precisely, at least the age group to which it belongs should be stated (e.g., infant, child, adolescent, adult, elderly).

7. The adverse reaction should be described in full, indicating the date on which it ended and including the results of any complementary examinations or laboratory tests considered to be of interest. This notification may be accompanied by any reports deemed appropriate for the proper interpretation of the clinical picture suspected of being an adverse reaction.

8-13. The categories are not mutually exclusive. Attendance in an Emergency Department of a Hospital for less than 24 hours shall not be considered hospitalization.

14. Investigational medicinal products should be identified preferably by their generic name (DOE or INN), indicating when available the trade name, or alternatively, by the proposed name or laboratory code for the product.

15. In case the administration is not daily, it should be described with one of the following possibilities: cyclic, weekly, monthly, yearly or number of times it has been used (in this case, the dose of each intake, not the total).

17. The pathological process of the patient for whom the investigational product is intended, or "healthy volunteer" in case of such a patient, should be stated.

19. The duration of the treatment until the onset of the adverse reaction should be stated.

22. It should be explicitly indicated if no concomitant drugs have been taken. If any of the concomitant drugs are considered suspicious, they should be marked with an asterisk (e.g.: * AMOXICILLIN). Drugs used to treat the adverse reaction will be excluded.

SUSPECTED ADVERSE REACTION NOTIFICATION

NOTIFICATION OF SUSPECTED REACTION FOR INVESTIGATIONAL MEDICINAL PRODUCTS	PROTOCOL CODE (sponsor)	NOTIFICATION No. (Sponsor)
	PATIENT ID	NOTIFICATION No.

I. ADVERSE REACTION INFORMATION

1a. COUNTRY	2. DATE OF BIRTH			2a. AGE	3. SEX	3a. WEIGHT	3b. HEIGHT	4-6. START DATE OF THE REACTION		
	DAY	MONTH	YEAR		MALE FENALE			DAY	MONTH	YEAR

7. DESCRIPTION OF ADVERSE REACTION (Including relevant examination or laboratory results, and date of completion, if applicable).	8-13b. SEVERITY/OUTCOME CRITERIA
	DEATH THE PATIENT'S LIFE HAS BEEN HAS BEEN THREATENED HOSPITALIZATION PROLONGED HOSPITALIZATION PERMANENT OR SIGNIFICANT DISABILITY CLINICALLY RELEVANT ADVERSE REACTION PERSISTENCE OF ADVERSE REACTION RECOVERY

II. INVESTIGATIONAL DRUG INFORMATION

14. SUSPECTED DRUG	15. DAILY DOSE	16. VIA	17. DISEASE UNDER STUDY	18. START/END DATE		19. TREATMENT DURATION

20. DID THE REACTION RECUR WHEN THE MEDICATION WAS DISCONTINUED?	20a. DID THE REACTION REMIT ON REDUCTION OF DOSAGE? DOSE?	21. DID THE REACTION REAPPEAR WHEN THE MEDICATION WAS ADMINISTERED AGAIN?

YES NO NOT APPLICABLE	YES NO NOT APPLICABLE	YES NO NOT APPLICABLE

III. CONCOMITANT MEDICATIONS AND MEDICAL HISTORY

CONCOMITANT DRUGS (Mark with an asterisk the suspected drug(s))	22a. DAILY DOSE	22b. VIA	22c. START7END DATE		22d. INDICATION
23. IMPORTANT DATA FROM THE CLINICAL HISTORY (e.g., diagnoses, allergies, pregnancies, etc.)					

IV. SPONSOR AND INVESTIGATOR INFORMATION

24a. NAME AND ADDRESS OF SPONSOR		24b. NAME AND ADDRESS OF INVESTIGATOR
24c. LABORATORY CODE (AEM No.)	25a. REPORT TYPE □INITIAL □FOLLOW-UP	24c. SPONSOR'S TECHNICIAN REPORTING NAME: PHONE: SIGNATURE:
24e. REPORT DATE	24f. DATE OF ENTRY AT AEM	25b. SUPPLEMENTARY REPORT ATTACHED

APPENDIX C. SODIUM CHLORIDE ADMINISTRATION SCHEDULE ACCORDING TO WEIGHT

With each oral dose, 250 mL of water should be administered, ensuring a minimum daily intake of 750 ml of water.

WEIGHT (Kg) and total dose	**IV ADMINISTRATION (NaCl 0.9%)**	**ORAL ADMINISTRATION (NaCl capsules 500 mg)***
50 - <55	**550 mL**	**5 g (10 capsules)**
85 mEq	1 hour pre-test 150 mL 0-4 hours post-test 400 mL	48 hours pre-test 1 capsule 40 hours pre-test 1 capsule 32 hours pre-test 1 capsule 24 hours pre-test 1 capsule 16 hours pre-test 1 capsule 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 1 capsule
55 - <60	**605 mL**	**5.5 g (11 capsules)**
93,5 mEq	1 hour pre-test 165 mL 0-4 hours post-test 440 mL	48 hours pre-test 1 capsule 40 hours pre-test 1 capsule 32 hours pre-test 1 capsule 24 hours pre-test 1 capsule 16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 1 capsule
60 - <65	**660 mL**	**6 g (12 capsules)**
102 mEq	1 hour pre-test 180 mL 0-4 hours post-test 480 mL	48 hours pre-test 1 capsules 40 hours pre-test 1 capsules 32 hours pre-test 1 capsules 24 hours pre-test 2 capsules

		16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 1 capsule
65 - <70 110,5 mEq	**715 mL** 1 hour pre-test 195 mL 0-4 hours post-test 520 mL	**6.5 g (13 capsules)** 48 hours pre-test 1 capsule 40 hours pre-test 1 capsule 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 1 capsule
70 - <75 119 mEq	**770 mL** 1 hour pre-test 210 mL 0-4 hours post-test 560 mL	**7 g (14 capsules)** 48 hours pre-test 1 capsule 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 1 capsule
75 - <80 127,5 mEq	**825 mL** 1 hour pre-test 225 mL 0-4 hours post-test 600 mL	**7.5 g (15 capsules)** 48 hours pre-test 1 capsule 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 2 capsules

80 - <85	**880 mL**	**8 g (16 capsules)**
136 mEq	1 hour pre-test 240 mL 0-4 hours post-test 640 mL	48 hours pre-test 2 capsules 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 2 capsules 0 hours 2 capsules 12 hours post-test 2 capsules
85 - <90	**935 mL**	**8.5 g (17 capsules)**
144,5 mEq	1 hour pre-test 255 mL 0-4 hours post-test 680 mL	48 hours pre-test 2 capsules 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 3 capsules 0 hours 2 capsules 12 hours post-test 2 capsules
90 - <95	**990 mL**	**9 g (18 capsules)**
153 mEq	1 hour pre-test 270 mL 0-4 hours post-test 720 mL	48 hours pre-test 2 capsules 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 2 capsules 8 hours pre-test 3 capsules 0 hours 3 capsules 12 hours post-test 2 capsules
95 - <100	**1045 mL**	**9.5 g (19 capsules)**
161,5 mEq	1 hour pre-test 285 mL	48 hours pre-test 2 capsules

	0-4 hours post-test 760 mL	40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 2 capsules 16 hours pre-test 3 capsules 8 hours pre-test 3 capsules 0 hours 3 capsules 12 hours post-test 2 capsules
>100 177 mEq	**1100 mL** 1 hour pre-test 300 mL 0-4 hours post-test 800 mL	**10 g (20 capsules)** 48 hours pre-test 2 capsules 40 hours pre-test 2 capsules 32 hours pre-test 2 capsules 24 hours pre-test 3 capsules 16 hours pre-test 3 capsules 8 hours pre-test 3 capsules 0 hours 3 capsules 12 hours post-test 2 capsules