OFFICIAL STUDY TITLE: Effects of Remote Ischemic Preconditioning on Contrast-Induced Nephropathy in Diabetic Patients: Relationship with Oxidative Stress and Inflammatory Status.

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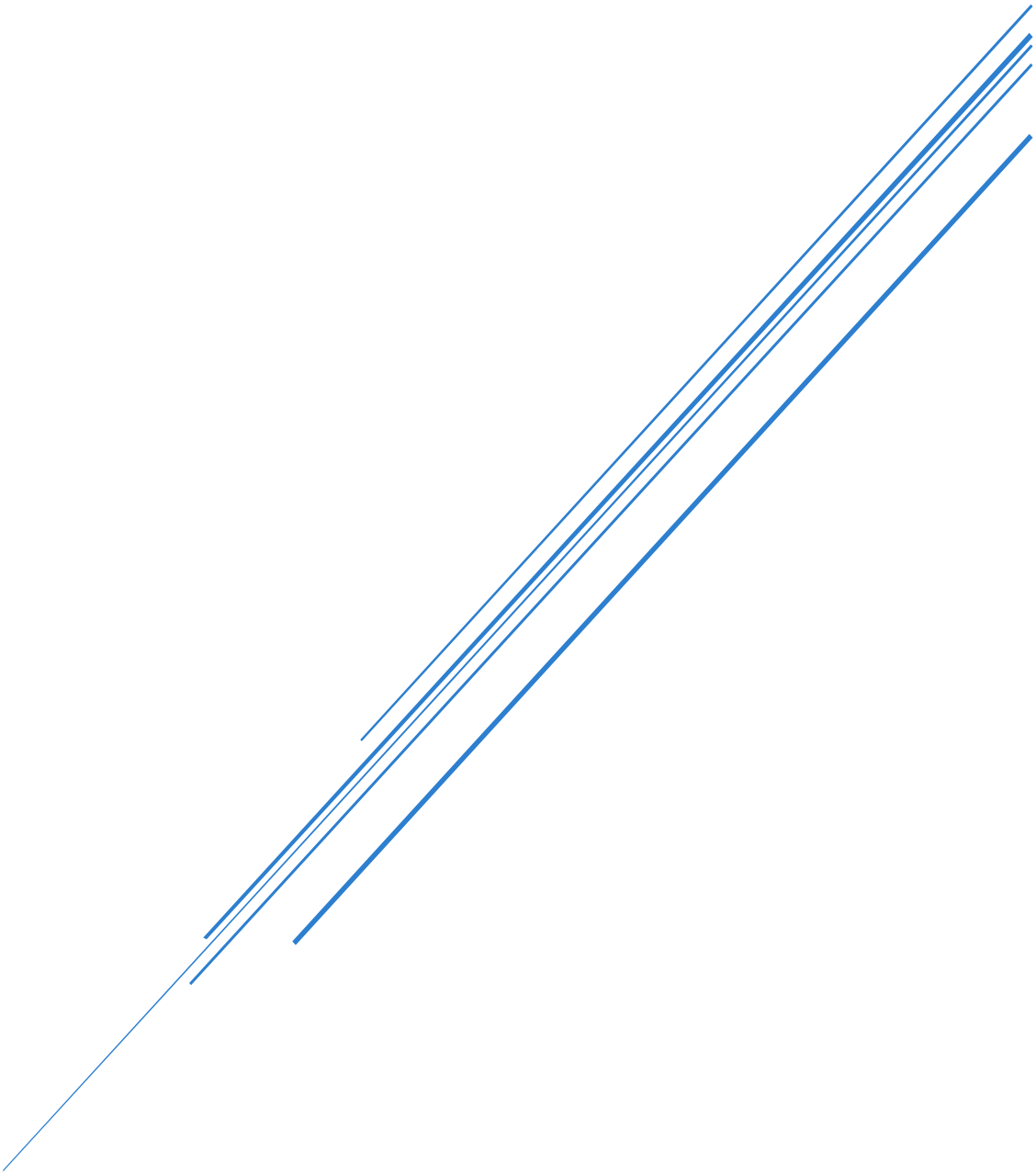

Study Protocol with SAP and ICF

Study Title: Effects of Remote Ischemic Preconditioning on Contrast-Induced Nephropathy in Diabetic Patients: Relationship with Oxidative Stress and Inflammatory Status.
Study Acronym: PRINCES Study.

Location: Hospital General Universitario Santa Lucía, Cartagena (Murcia, Spain)

INVESTIGATORS.

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1. Scientific Background.

Contrast-induced nephropathy (CIN) is a frequent cause of acute kidney injury (AKI), particularly among hospitalized patients with diabetes mellitus. Individuals with diabetes are especially vulnerable due to metabolic disturbances, oxidative stress, and endothelial dysfunction, which heighten the risk of CIN following contrast exposure. Mechanistically, CIN is driven by reactive oxygen species (ROS), impaired nitric oxide (NO) bioavailability, and inflammatory processes (Tumlin et al., 2002; Heyman et al., 2008).

Remote ischemic preconditioning (RIPC)—a procedure involving brief episodes of non-lethal ischemia and reperfusion applied to a limb—has emerged as a potential non-invasive strategy to mitigate ischemia-reperfusion injury. Preclinical and clinical studies suggest that RIPC activates endogenous protective pathways, including the induction of heme oxygenase-1 (HO-1), modulation of NO signaling, activation of K+-ATP channels, and reduction of systemic inflammation (Zarbock et al., 2015; Er et al., 2012).

However, the efficacy of RIPC in diabetic populations remains controversial. Experimental data indicate that diabetes may interfere with key molecular signaling pathways responsible for RIPC-mediated protection. Impaired humoral signaling, autonomic dysfunction, increased oxidative stress, and diabetic neuropathy have all been implicated as potential mechanisms of attenuation (Li et al., 2010; Hausenloy et al., 2013). Approximately 20–50% of individuals with diabetes mellitus develop peripheral neuropathy, which may compromise neurogenic components of RIPC signaling (Pop-Busui et al., 2017).

Despite these concerns, some clinical trials have reported preserved protective responses in diabetic patients, particularly in the context of contrast administration. For instance, the RenPro Trial found that RIPC significantly reduced the incidence of CIN in both diabetic and non-diabetic populations (Er et al., 2012). These contradictory results may

reflect differences in glycemic control, duration of diabetes, antidiabetic pharmacotherapy, and the presence of comorbidities.

Although early evidence pointed toward a protective effect of RIPC in preventing CIN, especially via activation of cytoprotective pathways, growing data suggests that such benefits may be attenuated in diabetic populations due to disease-related metabolic and neurovascular alterations. The present study was initially designed to test the protective efficacy of RIPC, while also specifically addressing the possibility that these mechanisms might be disrupted in patients with diabetes mellitus. This dual focus reflects the urgent need to clarify the clinical utility of RIPC in this high-risk population.

2. Objectives

Primary Objective

- To evaluate the impact of RIPC on the incidence of CIN in diabetic patients undergoing coronary catheterization, using serum creatinine, as biomarkers.

Secondary Objectives

- To assess HO-1 levels and other oxidative stress markers (malondialdehyde (MDA) before and after RIPC.
- To identify predictors of RIPC failure.
- To evaluate ICU/hospital stay duration, clinical status and survival at discharge.

3. Study Design

- **Type**: Randomized, parallel-group clinical trial.
- **Setting**: ICU, Hospital General Universitario Santa Lucía, Cartagena (Murcia, Spain)
- **Randomization method**: Based on sealed envelopes, using block randomization into two groups: **RIPC** and non- **RIPC**.
- **Groups**:
    - **Intervention Group**: RIPC + hydration.
    - **Control Group**: Hydration only.
- **Planned sample Size**: The sample size was determined based on data from the ARIAM registry, a Spanish national database focused on patients with acute coronary syndrome (ACS) admitted to intensive care units. According to ARIAM estimates, approximately 35–40 patients per year would meet the eligibility criteria for recruitment. It was therefore decided that the sample size would be defined by the number of patients admitted to our department with the specified characteristics during a fixed time period, establishing a convenience sampling strategy over a two-year period.
- Actual **enrolled patients:** 71.
- **Analysis population:** Both Intention-to-Treat (ITT) and Per Protocol (PP).

4. Eligibility Criteria

Inclusion

- Adults aged ≥18 years with diabetes mellitus.
- Hospitalized for acute coronary syndrome and scheduled for coronary angiography.

Exclusion

- Non-diabetic.
- Pregnant or lactating.
- Kidney transplant recipients.
- Recent exposure to contrast agents.
- End-stage renal disease.
- Participation in other clinical trials.

5. Intervention

RIPC Procedure: Four cycles of 5-minute ischemia (inflation of a blood pressure cuff to 50 mmHg above systolic BP) followed by 5-minute reperfusion. The procedure was performed within 45 minutes prior to catheterization. **Contrast Agent**: Visipaque® (low-osmolar)

6. Outcome Measures

Primary Endpoint

- CIN defined as either a 25% increase in serum creatinine (SCr) from baseline or a 0.5 mg/dL increase in absolute SCr value—within 72 hours after intravenous contrast administration.

Secondary Endpoints

- Plasma, urinary, and intracellular HO-1 levels.
- Oxidative stress markers (MDA).
- Cardiac stress biomarkers (NT-proBNP).
- Inflammatory and hematological indices: C-reactive protein (CRP), Total leukocyte count, Neutrophil and lymphocyte counts Neutrophil-to-lymphocyte ratio (NLR).
- Duration of ICU/hospital stay duration
- Mortality, readmission, clinical status and need for dialysis.

7. Statistical Analysis Plan (SAP)

7.1 Descriptive Statistics

- **Qualitative variables**: Presented as frequencies and percentages per group/subgroup.
- **Quantitative variables**: Described using minimum, maximum, mean, and standard deviation.

7.2 Univariate Analysis

- **Qualitative comparisons**: Chi-square test.
- **Quantitative comparisons**: Student's T-test, after verifying:
  - **Normality**: Shapiro-Wilk test.
  - **Homogeneity of variances**: Levene test.
- If assumptions are violated: Mann-Whitney U test will be used.

7.3 Repeated Measures

- **Two-factor ANOVA with repeated measures** under General Linear Model (GLM).
- **Post hoc tests**: Bonferroni or Tukey, depending on variance structure.

7.4 Multivariate Analysis

- **Binary logistic regression** to predict CIN occurrence, adjusted for covariates.

7.5 Assumption Verification

Assumption	Test Used
Normality	Shapiro-Wilk
Equal variances	Levene

7.6 Missing Data

- ≤5% Complete case.
- ≥5%: Multiple imputation considered
- Sensitivity analysis may be performed

7.7 Multiple Comparisons

- Bonferroni or False Discovery Rate (FDR) correction applied where appropriate

7.8 Analysis Populations

- **Intention-to-Treat (ITT)**
- **Per Protocol (PP)**
  Both populations will be analyzed and compared

7.9 Software

- **SPSS Statistics v25.0** for Windows

7.10 Significance Threshold

- All tests two-sided
- **$p < 0.05$** considered statistically significant

8. Ethical Considerations

- Approval granted by Ethics Committees of Área Sanitaria II and the University of Murcia.
- Informed consent obtained and signed by all participants.
- Data management adhered to Spanish data protection legislation
- Absence of prospective registry is acknowledged and will be transparently disclosed.

9. Study Limitations and Early Termination

Data collection was conducted over a three-years period. The final patient was enrolled and data collection for all primary and secondary outcomes, as well as adverse event monitoring, was completed in January 2017. Due to multiple logistical and operational constraints, no further patients were included after that date. Subsequently, with the emergence of the COVID-19 pandemic in December 2019, further recruitment was permanently halted. Additionally, preliminary analyses revealed that the primary outcome—the incidence of contrast-induced nephropathy (CIN) following remote ischemic preconditioning (RIPC)—did not show statistically significant differences between the intervention and control groups. These findings, combined with logistical constraints, led to the decision to halt the study before reaching the originally planned sample size. Thus, only 71 patients were enrolled—below the planned sample size. The reduced statistical power and lack of effect signal were key reasons for early termination.

Note: The study was not prospectively registered in a clinical trial database prior to patient enrollment. At the time of trial initiation, Spain did not have a clearly defined legal or regulatory framework mandating prospective registration for investigator-initiated interventional studies of this nature.

While Spain's Biomedical Research Law (Law 14/2007) established ethical principles and data protection standards, it did not impose a legal obligation to register non-pharmacological trials in platforms such as ClinicalTrials.gov or EudraCT. Furthermore, the regulatory provisions introduced by Royal Decree 1090/2015—which require prospective registration—apply specifically to clinical trials involving medicinal products and did not extend to procedural interventions such as remote ischemic preconditioning (RIPC). This regulatory gap, coupled with the academic nature of the study and its non-commercial scope, contributed to the lack of prospective registration.

This limitation is acknowledged and must be considered when interpreting results and assessing risk of reporting bias. The available data were analyzed and will be presented with full transparency regarding these limitations.