CHARACTERIZATION OF CHANGES IN PERITONEAL CELLS GENE EXPRESSION AFTER STANDARD VERSUS LOW PRESSURE LAPAROSCOPIC CHOLECYSTECTOMY AND ITS CLINICAL CORRELATION.

PROTOCOL

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TITLE OF THE RESEARCH PROJECT: Characterization of changes in peritoneal cells gene expression after standard versus low pressure laparoscopic cholecystectomy and its clinical correlation.

HYPOTHESES

We hypothesized that applying a low intraperitoneal pressure of pneumoperitoneum (≤ 8mmHg) during laparoscopic cholecystectomy, the adverse impact on the surgical peritoneal environment (measured as gene expression of extracellular matrix adhesion and inflammatory cytokine and apoptotic index), can be minimized and probably clinical outcomes might be better.

OBJECTIVES

Primary Objective.

To characterize the changes produced in gene expression (mRNA) levels of remodelling and inflammatory markers as well as to measure apoptotic index, in peritoneal tissue, after laparoscopic cholecystectomy with low and standard pneumoperitoneum pressures (≤ 8mmHg vs ≥ 12mmHg).

The primary aim of this study is to analyse differences in those variables between both intraperitoneal pressure groups at different times: Before starting surgery (T0) and 60 minutes after surgery started.

Secondary Objectives.

To analyse within groups (high and low pressure) differences between times T0 and T1 in the same remodelling and inflammatory physiological parameters. To analyse the effect of intraperitoneal pressure in outcomes (see below).

STUDY DESIGN

This is a prospective randomized and blinded controlled trial to assess the potential adverse impact of pneumoperitoneum during laparoscopic cholecystectomy on peritoneal tissue at two different working pressures and at two different times and to correlate it with clinical outcomes as well.

STUDY GROUPS

A. **Intervention group**: in all patients allocated to the intervention group laparoscopic cholecystectomy will be performed with pneumoperitoneum pressure (PnPr) at or below 8 mm Hg. To ease this goal deep neuromuscular block will be provided with

rocuronium till the end of surgery. Neuromuscular block will be reversed with sugammadex at a dose of 4mg/kg.

B. **Control group**: In patients allocated to this group laparoscopic cholecystectomy with abdominal pressure at 12 mm Hg or higher will be performed. In this group the level of neuromuscular block and its reversion will be done in the same way as in the intervention group.

POPULATION

Reference population: Patients undergoing elective laparoscopic cholecystectomy.

Eligibility: Patients older than 18 years, signed informed consent, undergoing

laparoscopic cholecystectomy for symptomatic cholelithiasis or gallbladder polyps.

Exclusion criteria:

- Emergency surgery.

- Previous surgery at supramesocolic compartment.

- Previous peritoneal inflammatory process.

- Pregnancy or breastfeeding.

- Patient refusal to participate in the study.

RANDOMIZATION

Patients will be randomly assigned to the control or intervention group according to a simple randomization list generated by Query v.7. Allocation will be concealed till the surgical intervention date using sealed opaque numbered envelopes.

MAIN VARIABLES IN THIS STUDY ARE:

- **Inflammatory peritoneal markers:** (gene expression (mRNA) levels of):

  - IL-1, IL-6, IL-10.
  - VEGF-A “vascular endotelial growth factor A”.
  - TNFα “tumor necrosis factor α”.
  - CXCL-2 “chemokine CXC lingand 2”.

- **Remodeling peritoneal markers:** (gene expression (mRNA) levels of):

  - CTGF “connective tissue growth factor”.
  - MMP-9 “matrix metalloproteinase-9”.
  - PAI-I “plasminogen activator inhibitor-I”.
  - E-selectine.

- **Apoptotic index.**

SECONDARY VARIABLES ARE:

During the intraoperative period:

- Surgical Rating Score (SRS) to measure the working space during this laparoscopic surgery (optimal, good, acceptable, poor or very poor conditions).
- The need to change initial intraperitoneal PnPr (yes/no).
- Subjective sensation of the PnPr performed (standard or low) reported by the main surgeon (consistent vs inconsistent).
- Peak inspiratory pressure and tidal volume (mmHg).
- Total Co2 volume administered during pneumoperitoneum (L).
- Total dose of sugammadex (mg).

During postoperative period:

- Pain measured by visual analogue scale (VAS) from 0 to 10.
- Time to start oral intake (in hours).
- Time to start ambulation (in hours).
- Long of hospital stay (in days).

STATISTICAL ANALYSIS

Biomarker data will be log-transformed before data analysis to make these variables to follow a normal distribution. Continuous variables will be expressed as mean and standard deviation. Categorical variables will be expressed as numbers and proportions. Between groups comparisons at T0 and T1 will be made by means of t-Student test. In case of non-normal distribution despite log-transformation of data we used a non-parametric alternative (U-Mann–Whitney test) instead. We will also compare within groups means differences between times T1 and T0. For this comparison we will use paired t-student test or McNemar test for normal or non-normal variable distributions respectively. Covariance analysis will be used to compare the effect of high and low pressure on parameter analysed adjusting for baseline value of the biomarker. Normality tests will be based on Kolmogorov- Smirnov. All analysis will be performed using STATA 13 (STATACorp.2013.Stata Statistical Software: Release13. College Station, TX: StataCorpLP).