RESEARCH PROTOCOL

STUDY OF PERIOPERATIVE PLASMA LEVELS OF ENDOTHELIAL GLYCOCALIX MARKERS IN PATIENTS UNDERGOING COLON SURGERY.

Principal Investigator: NURIA GARCIA DEL OLMO. NIF: 26748077C Telephone: 619660434. Email: nuriagarciadelolmo@gmail.com

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SUMMARY: The glycocalyx is a layer of macromolecules that covers the luminal surface of the endothelium, playing an important role in vascular homeostasis. Several studies have shown that in conditions of sepsis there is a degradation of the glycocalyx mediated by inflammatory mechanisms, releasing its components into the bloodstream. These glycocalyx fragments can be measured in blood plasma, showing a correlation between these markers and organ dysfunction, severity and mortality in sepsis situations. However, despite the great interest in glycocalyx biomarkers, their values in patients undergoing colorectal surgery are not well known. Therefore, verifying whether plasma levels of glycocalyx damage markers increase in the perioperative period of colorectal surgery is of great interest at the pathophysiological and clinical level, since the correlation of these markers with postoperative clinical evolution is unknown. In this study we want to determine the plasma levels of Heparan-sulphate and Syndecan-1, two of the most important constituents of the glycocalyx, and perform several measurements to form a "time course" of the plasma levels of these markers, in order to compare the different subgroups of patients undergoing colorectal surgery based on their clinical and surgical characteristics.

BACKGROUND:

The endothelial glycocalyx (EG) is a complex layer of macromolecules that lines the luminal surface of the vascular endothelium. This concept was proposed more than 70 years ago and its composition is well studied as detailed in two reviews (1, 2). However, its role in the mechanisms of endothelial protection and injury, and its subsequent clinical implications, have recently become evident. The GE consists of a variety of molecules, including glycoproteins and proteoglycans, that provide the basis for plasma-endothelial cell interaction. The structure of GE, although well characterized in vitro, is poorly defined in vivo because its composition changes dynamically by self-assembly and shear-dependent enzymatic degradation or detachment of its elements. Its major components are hyaluronic acid, proteoglycans (PG) such as syndecan-1, glycoproteins, plasma proteins and glycosaminoglycans (GAG), whose main component is Heparan Sulfate. The GE as a whole forms an endothelial surface layer that acts as a barrier to circulating cells and large molecules. Considerable prognostic-therapeutic promise lies in the emergence of the EG as a key mediator of endothelial dysfunction in pathogenic states, particularly with respect to vascular permeability and edema formation. Several studies have demonstrated the role of GE in plasma/interstitial fluid balance (3,4,5), mechano-transduction that couples intravascular pressure, and endothelial cell responses, i.e., biochemical signals, (6) to the inflammatory response cascade (7,8,9). EG damage affects a number of important endothelial cell functions leading to impaired mechano-transduction with changes in fluid passage (6,10), activation of

coagulation pathways (11), leukocyte adhesion (7,11) and platelets (12) to the surface of endothelial cells, and leakage of fluid and plasma proteins into the interstitium (13,14), resulting in tissue edema (15). The specific pathophysiological triggers that lead to EG damage are still actively investigated and remain poorly understood. Nevertheless, EG protection appears to be a promising target in many clinical scenarios, especially since its degradation is closely associated with the pathophysiology of inflammation, capillary leak, and edema formation in various injuries and disease states; including ischemia/reperfusion, hypoxia, inflammation, trauma, hypervolemia, atherosclerosis, diabetes, and hypertension (16). In patients undergoing major abdominal surgery (including digestive, urological, gynecological or other surgery) there is very little information on the potential endothelial damage secondary to surgery (19). There are some reviews that have studied the damage of the SG in septic patients of medical and surgical origin (17, 18). However, in relation to digestive abdominal surgery, and specifically in colorectal surgery, only one study has been carried out, with only 18 patients undergoing colectomy (without mentioning the pathologies) and they are included with other patients of major abdominal surgery of the pancreas, liver, gynecology and urology (19).

EXPECTED RESULTS:

In this study, it is expected that in the patients who underwent elective surgery, the markers of glycocalyx damage will increase their values in the postoperative laboratory tests compared to the values of the baseline (control) laboratory tests. It is equally expected that in patients undergoing urgent surgery, generally in clinical states of occlusion, perforation or sepsis, with activation of the inflammatory response, dehydration with decreased oncotic pressure and generation of tissue edema and third space, the markers of damage of glycocalyx are already elevated in the baseline analysis, with a maintenance or greater elevation of its values in the postoperative period. Likewise, a correlation would be expected between glycocalyx damage markers and other markers involved in the inflammatory response, such as leukocytes, C-reactive protein, procalcitonin, and lactate; as well as with associated patient comorbidities that lead to endothelial degeneration, such as high blood pressure, obesity, diabetes, atherosclerosis, etc. If the results of this study were as expected, glycocalyx damage markers (Heparan sulfate and Syndecan-1) could be used together with other inflammatory markers (CRP, procalcitonin, etc.) and be very useful in postoperative follow-up. of surgical patients, being able to anticipate possible postoperative complications. However, if elevations in glycocalyx markers were not observed in the perioperative period, it could be due to a lack of the number of Time-course determinations; that is to say, that an elevation of markers did occur, but we would not have detected it in the measurement times; or a larger

study population might also be needed. In either case, the study could be completed in subsequent research projects.

OBJECTIVES

Primary Objectives: To estimate the plasma levels of the 2 most representative markers of damage to the endothelial glycocalyx (Heparan-sulfate and Syndecan-1) in patients undergoing colorectal surgery during the perioperative period ("time-course").

Secondary Objectives:

- To analyze a possible correlation of the glycocalix damage markers with other analytical parameters (PCR, procalcitonin, lactate, complete blood count, coagulation and basic biochemistry).
- To analyze the possible relationship of glycocalyx damage markers with different parameters depending on the patient (age, sex and associated comorbidities) and the surgical intervention (surgical time, approach, type of surgery...).
- To analyze other clinical parameters that may indicate a possible postoperative complication and their relationship with markers of endothelial glycocalyx damage will also be studied.

METHODOLOGY DESIGN: The present work is about a clinical, prospective, observational, and longitudinal study of single center groups (Hospital Lluís Alcanyís, Xàtiva). Always after the approval of the Research Ethics Committee of said Hospital.

STUDY POPULATION: The study population (value of n) has been calculated on a population of 100 patients who will undergo surgery approximately one year. With a confidence level of 95% and a margin of error of 5%, a sample size of 80 patients has been calculated. These 80 patients will be of legal age, men and women, undergoing elective or emergency colorectal surgery (right hemicolectomy, extended right hemicolectomy, left hemicolectomy, sigmoidectomy, anterior resection of the rectum or abdominoperineal amputation).

CHOICE OF PATIENTS AND INFORMED CONSENT: The selection of patients and the drafting of the informed consent documents will be carried out in a reasoned manner as established in article 32 of the Declaration of Helsinki and in article 58.2 of the Research Law. Biomedical. It should be considered taking into account the Declaration of Helsinki of the World Medical Association in its latest revision. And according to its latest version of the Helsinki Declaration of Fortaleza (Brazil) October

2013. Patients will be included in the study upon preoperative hospital admission, after verifying compliance with the inclusion criteria. To participate, it will be necessary to sign the informed consent.

INCLUSION CRITERIA:

To be included in the study, the following criteria must be met:

- Signature of the informed consent document.
- Age equal to or greater than 18 years.
- ASA surgical risk ≤ III.
- Being operated on for colorectal surgery

EXCLUSION CRITERIA:

The presence of any of the following criteria will be excluding:

- Younger than 18 years old
- Pregnant women

WITHDRAWAL FROM THE STUDY:

Patients will be withdrawn from the study when any of the following events occur:

- Non-compliance inclusion criteria
- Due to voluntary abandonment of the patient

METHOD:

Blood samples will be drawn from an intravenous line, in BD Vacutainer® LH PST™ II tubes with lithium heparin at different times: 1st) basal time (preoperative), at hospital admission; 2nd) 2 hours after the end of the surgery, 3rd) 6 hours after the end of the surgery, 4th) 24 hours after the end of the surgery and 5th) 48 hours after the surgery. The blood samples will be centrifuged at 3000 rpm/12 min and the supernatants will be collected and stored at -80ºC until the determination of the markers. Plasma levels of Syndecan-1 and Heparan Sulfate will be determined by ELISA (Enzyme-Linked Immunosorbent Assay), PCR (C-reactive protein), procalcitonin, lactate, blood count, renal function, basic coagulation and general biochemistry techniques. The blood samples will be drawn by the nursing team of the general surgery hospitalization ward, and will be transported to the laboratory by the auxiliary staff of said hospitalization ward. The laboratory staff will be in charge of centrifuging the samples and storing the supernatants in the pertinent conditions mentioned above. Later, when all the samples have been collected, the determination of the markers will be carried out, and the result will be displayed through the Orion clinic computer program or in paper format, which will be delivered to me personally. In addition, all the usual anesthetic, surgical and

postoperative care protocols of the Institution (General and Digestive Surgery Department. Lluís Alcanyís Hospital) will be maintained for these patients.

STATISTICAL ANALYSIS PLAN (SAP): Continuous data are expressed as mean ± standard deviation. The normality of the data obtained was determined with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences between baseline values and different moments after the "time course" of plasma levels of glycocalyx damage markers and other parameters will be analyzed by one-way analysis of variance (ANOVA). Data sets in which F is significant will be examined for multiple comparisons by Tukey's test. The Pearson correlation coefficient (r) will be calculated between the values of the EG damage markers and other parameters. The possible differences between the different population subgroups will be examined with the Student's t-test. $P < 0.05$ will be considered statistically significant. All statistical calculations will be performed using the SPSS program.

MEDIA: We have sufficient infrastructure and biochemical laboratory reagents, as well as an ELISA reader. We also have all the consumable material necessary for the determination of glycocalyx damage markers, as well as all other biochemical determinations.

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