PLASMA AND TISSUE CONCENTRATION OF CEFAZOLIN FOR PREOPERATIVE PROPHYLAXIS IN PATIENTS UNDERGOING BARIATRIC SURGERY

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BACKGROUND

The growing obesity pandemic is a major public health issue. According to the World Health Organization (WHO), global obesity prevalence (BMI ≥ 30 kg/m²) affects about 650 million individuals. (1) In the U.S. (2014), 37.7% of the population was obese, with 7.7% of men and 9.9% of women classified as morbidly obese (BMI ≥ 35 kg/m²). (2) In Canada (2013), obesity prevalence was 22% in men and 20% in women. (3)

Obesity is associated with multiple comorbidities, including hypertension, coronary artery disease, type 2 diabetes, osteoarthritis, obstructive sleep apnea, and non-alcoholic fatty liver disease. Overweight (BMI ≥ 25 et < 30 kg/m^2) and obesity contributed to approximately 3.4 million deaths worldwide in 2010. (4) Bariatric surgery is an effective intervention for patients who fail conservative treatments, with over 100,000 procedures performed in North America in 2011. (5)

Surgical site infections (SSIs) significantly increase morbidity, mortality, hospital stay, and healthcare costs. (6) Reported SSI rates in bariatric surgery range from 1–20%, with laparoscopic approaches reducing infection risk. (7-11) Preoperative antibiotic prophylaxis administered within 60 minutes before incision is recommended. Cefazolin is typically the drug of choice due to its low cost, safety, duration of action, and activity against common SSI pathogens (staphylococci, streptococci, Enterobacteriaceae). (12)

Despite the high prevalence of obesity, limited evidence exists on whether cefazolin dosing should be adjusted for obese patients. U.S. guidelines (2013) recommend 2 g IV for patients weighing 80–120 kg and 3 g IV for those over 120 kg. (12)

This study aims to measure plasma and tissue concentrations of cefazolin administered per current recommendations and assess whether these levels are sufficient for effective prophylaxis.

1. CURRENT STATE OF KNOWLEDGE

It is well established that preoperative antibiotic prophylaxis with cefazolin reduces the rate of surgical site infections (SSIs) in bariatric surgery. (13) However, evidence regarding the appropriate dose for obese or severely obese patients is scarce, of low quality, and contradictory.

Unger *et al*., in a retrospective cohort study, suggest that obese patients can continue to receive a prophylactic dose of 2 g IV cefazolin, as they did not show a higher SSI rate compared to the non-obese population. (14) Peppard *et al*., in another retrospective cohort, found no significant difference in SSI rates between obese patients who received 2 g IV versus 3 g IV of cefazolin. (10)

Ho *et al*. conducted a prospective cohort study examining plasma concentrations of cefazolin by administering 2 g IV to patients with BMI between 40–50 kg/m² and 3 g IV to those above 50 kg/m². (15) They concluded that a single prophylactic dose of 2 g IV cefazolin was sufficient regardless of obesity level. (15) Van Kralingen *et al*. analyzed plasma concentrations after a 2 g IV dose and reported that this exceeded the minimum inhibitory concentration (MIC) for *Staphylococcus aureus.* (16) Hites *et al*. studied plasma concentrations after a prophylactic 2 g IV dose and found no difference between patients weighing <120 kg and those >120 kg. (17) Plasma concentrations at 180 minutes were all above the target MIC. (17)

Although these data are interesting, plasma concentration of the free fraction of cefazolin is only a limited pharmacokinetic marker. It is preferable to measure tissue concentration at the site of action. (18)

Using a microdialysis technique, Brill *et al*. measured cefazolin concentration in the interstitial fluid of adipose tissue in obese versus non-obese patients. They showed that tissue distribution of cefazolin was lower in obese patients and decreased with increasing weight, suggesting a need for higher doses in this population. (18)

However, more recently, Chen *et al*. analyzed a prospective cohort of patients undergoing gastric bypass or sleeve gastrectomy who received 2 g IV cefazolin, measuring plasma and tissue concentrations using High Performance Liquid Chromatography (HPLC). They concluded that the dose was adequate because tissue concentrations, although representing only 6–8% of plasma concentration, remained above the MIC for *Staphylococcus aureus.* (19)

The wide variability in current medical literature is, in our opinion, partly due to the lack of a clear definition of MIC, which represents the threshold for effective antibiotic prophylaxis. In clean-contaminated surgery, staphylococci, streptococci, and Enterobacteriaceae are the main pathogens likely to cause SSIs. According to EUCAST, the MIC of cefazolin for methicillin-sensitive *Staphylococcus aureus* is 2 mg/L, while for Enterobacteriaceae it is 8 mg/L. Several authors consider that effective antibiotic prophylaxis requires plasma and/or tissue concentrations of cefazolin of 8 mg/L or higher.

It should be noted that the question of cefazolin prophylaxis has been more extensively studied in obese pregnant women undergoing cesarean section. These studies, although methodologically interesting and hypothesis-generating, are not addressed here due to their limited external validity for our bariatric surgery population.

2. RESEARCH OBJECTIVES

PRIMARY OBJECTIVE:
The aim of this study is to determine whether the current cefazolin doses recommended by U.S. authorities for obese patients—2 g IV for those weighing <120 kg and 3 g IV for those ≥120 kg—achieve plasma and tissue concentrations equal to or greater than 8 mg/L[1].

SECONDARY OBJECTIVES:
We will also describe plasma and tissue concentrations of cefazolin by stratifying patients according to their weight:

- <120 kg
- ≥120 kg and <150 kg
- ≥150 kg

This stratification will allow us to identify whether any weight category is associated with insufficient plasma and/or tissue concentrations of cefazolin to provide effective antibiotic prophylaxis.

3. STUDY DESIGN

We will conduct a prospective analytical cohort study to examine plasma and tissue concentrations of cefazolin following administration of the standard doses recommended by U.S. practice guidelines. This will allow us to determine whether the current practice of surgical antibiotic prophylaxis with cefazolin is adequate.

4. POPULATION AND SAMPLE

TARGET POPULATION: All adults undergoing bariatric surgery at the *Institut Universitaire de Cardiologie et de Pneumologie de Québec–Université Laval* (IUCPQ-UL).

ACCESSIBLE POPULATION: All adults undergoing bariatric surgery of the sleeve gastrectomy type at IUCPQ-UL.

SAMPLE: A subgroup of adults undergoing sleeve gastrectomy at IUCPQ-UL who have given preoperative consent to participate in the project (convenience sample).

[1] Minimum inhibitory concentration for Enterobacteriaceae according to EUCAST criteria.

CONTROL: A sample of 10 patients undergoing sleeve gastrectomy at IUCPQ-UL who have not been exposed to cefazolin (patients allergic to penicillin) will be recruited to form a control group for the purpose of validating the analysis method.

PILOT SUBGROUP (BIOBANK): Ten subcutaneous adipose tissue biopsies from participants who have consented to the biobank will be obtained (8 from participants allergic to penicillin and 2 from participants exposed to cefazolin) to optimize cefazolin dosage measurement in this type of tissue.

5. SAMPLING

Patients will be invited to participate in the project during their preoperative consultation at the bariatric surgery clinic. All patients interested in participating will be included in the study if they do not meet any exclusion criteria (convenience sampling).

6. SAMPLE SIZE AND INCLUSION/EXCLUSION CRITERIA

SAMPLE SIZE:
A total of 60 patients will be recruited for the study, with 20 patients in each weight category. Additionally, 10 patients allergic to penicillin will be recruited for the purpose of standardizing the HPLC analysis.

INCLUSION CRITERIA:
- Age ≥18
- BMI ≥35 kg/m²
- undergoing laparoscopic sleeve gastrectomy at IUCPQ-UL

EXCLUSION CRITERIA:
- Age <18
- Weight >180 kg
- Penicillin allergy (except control group)
- Pregnancy or breastfeeding
- Chronic kidney disease (eGFR <60 mL/min)
- Liver cirrhosis
- Intraoperative blood loss ≥1 L

7. SUBJECT RECRUITMENT

Subjects will be recruited during their preoperative consultation at the bariatric surgery clinic of IUCPQ-UL. A nurse from the bariatric surgery clinical research team will present the project to candidates who meet the inclusion criteria. The nurse will also have participants sign an informed consent form approved by the IUCPQ-UL Research Ethics Committee if they agree to participate.

8. MEASUREMENT INSTRUMENTS

Plasma and tissue concentrations of cefazolin will be measured using high performance liquid chromatography (HPLC), a validated method widely used in similar studies. See the study procedure section for further details.

9. STUDY PROCEDURE

Each potential participant will be evaluated by a bariatric surgeon at IUCPQ-UL during their preoperative consultation. If the patient is eligible to participate in the study, they will meet with a research nurse on the same day. The nurse will explain the study procedure, answer any questions, and obtain informed consent if the patient wishes to participate. On this day, demographic data and routine preoperative blood tests will be collected. Renal and liver function data will be retrieved from the electronic medical record. To calibrate the HPLC technique, we will recruit 10 patients allergic to penicillin who will receive an alternative antibiotic prophylaxis for their sleeve gastrectomy.

Each participant scheduled for laparoscopic sleeve gastrectomy will be admitted to IUCPQ-UL the day before surgery. They will meet their surgeon in their room, who will review preoperative tests and blood work, ensure the patient is fit for surgery, answer questions, obtain written consent for the surgery, and reconfirm participation in the research project.

On the day of surgery, participants will receive an intravenous bolus of cefazolin at induction according to their weight:

- Patients weighing less than 120 kg will receive 2 g IV.
- Patients weighing 120 kg or more will receive 3 g IV.

To standardize the timing between antibiotic administration and the first incision, prophylaxis will be administered by the anesthesiologist or respiratory therapist during patient skin preparation with chlorhexidine.

Subcutaneous adipose tissue samples (~1 g) and skin samples (~0.6 cm^2) will be collected (at the incision site) at incision (t = 0 min), 30 minutes after the start of the procedure, and at the end of surgery. Samples will be rapidly frozen at -80°C until extraction and analysis of cefazolin concentrations by HPLC. Blood samples (2 mL in K_3 EDTA tubes) will also be collected at the same time points and kept on ice until centrifugation at 3,000 rpm for 20 minutes. Plasma will then be recovered and frozen at -80°C until extraction and analysis by HPLC.

Cefazolin Extraction Procedure

- **Adipose Tissue:** Samples will be prepared according to Waltrip *et al*. protocol (20):
    1. Precisely weighed the tissue;
    2. Add a cold extraction solution consisting of methanol/sodium acetate 1M (70/30, pH 5.2) in a 1:2 (w:v) ratio, containing a known amount of cefoxitin as an internal standard (21);
    3. Homogenize for 30 seconds;
    4. Cool for 10 min at -20°C;
    5. Centrifuge at 15,000 rpm for 10 min;
    6. Collect the supernatant and centrifuge again for 15 min at 15,000 rpm;
    7. Filter through 0.22 µm.

- **Dermis:** Skin samples will be heated with a hair dryer for 20 seconds, and the epidermis will be separated from the dermis. (22) Dermis samples will then be weighed precisely and extracted using the same procedure as adipose tissue.

- **Plasma:** 0.3 mL of plasma will be combined with an equal volume of methanol/sodium acetate 1M (70/30, pH 5.2) containing the internal standard, vortexed for 30 seconds, and incubated for 10 min at -20°C. Extracts will then be centrifuged for 10 min at 1,500 rpm, and supernatants will be collected and filtered through 0.22 µm. (20)

Sample Analysis

High-performance liquid chromatography will be performed at room temperature using a Shimadzu Prominence system (Columbia, MD, USA) consisting of an SIL-20ACHT autosampler, LC-20AT pump, and SPD-20A UV detector. The mobile phase will consist of 85% 0.01M sodium acetate (pH 5.2) and 15% of a solution composed of 96% acetonitrile and 4% methanol. (20) An Ultrasphere ODS 5 µm column (250 mm × 4.6 mm, Canadian Life Science, Peterborough, ON, Canada) and a µBondapak C18 pre-column (Waters, Mississauga, ON, Canada) will be used for separation at 1.5 mL/min of aliquots (20–100 µL) of the injected extracts. Cefazolin and cefoxitin (internal standard) peaks will be detected by UV absorbance at 254 nm. EZ-Start software (Columbia, MD, USA) will be used for data acquisition, storage, and analysis. Ratios of cefazolin/cefoxitin peak heights will be converted to µg of cefazolin detected using a standard curve.

10. DATA COLLECTION

The results of tissue and blood concentration measurements will be stored on the secure servers of CRIUCPQ-UL. Once the target of 60 patients is reached, data collection will be carried out by two medical residents. These data will be entered electronically in an Excel file and subsequently retained for five years for potential future verification.

11. DATA ANALYSIS

Descriptive statistical analyses will be performed to describe the baseline characteristics of our study population.

In addition, for each time point analyzed, we will report:

- the percentage of patients weighing <120 kg who do not reach the target tissue MIC
- the percentage of patients weighing <120 kg who do not reach the target plasma MIC
- the percentage of patients weighing ≥120 kg who do not reach the target tissue MIC
- the percentage of patients weighing ≥120 kg who do not reach the target plasma MIC

We will also determine the percentages of patients not reaching the tissue and plasma MIC for each weight category mentioned above.

12. TIMELINE

- June 2018: Project approval by the ethics committee
- June 2018 to October 2018: Patient recruitment and data collection
- September 2018: Application for research grant from the IUCPQ Foundation competition
- November 2018: Data entry into Excel file and analysis
- December 2018: Drafting of the scientific article
- September 2019: IFSO Congress, Madrid
- October 2019: IDWeek Conference

13. STUDY LIMITATIONS

The proposed study is exploratory in nature, meaning it is designed to verify the accuracy of current recommendations for preoperative antibiotic prophylaxis in surgery. We acknowledge that our study has limitations that may affect its external validity. First, it is a single-center study involving a sample of 60 patients undergoing sleeve gastrectomy—a relatively short procedure, mostly performed laparoscopically. Therefore, our results may not be generalizable to a broader population of patients undergoing clean-contaminated colorectal surgery. However, the relevance of our study remains undeniable, as current practice is based on very limited evidence and requires validation.

14. ETHICS

Participants will be approached by our research nurse during their preoperative consultation. For those interested, the purpose of the project, the participant's involvement, and the benefits and risks of the study will be clearly explained at the time of signing the informed consent form. At all times, participants will be informed that they may withdraw their consent without fear that this will affect the quality of their care.

Standard precautions will be taken to ensure patient confidentiality.

Research data will be stored on the secure server of CRIUCPQ-UL.

15. EXPECTED IMPACT

As previously stated, the study will generate data that we hope will validate or challenge current practices in preoperative antibiotic prophylaxis for morbidly obese patients. Depending on the results obtained, this study could highlight the need for a prospective cohort study to evaluate different dosing strategies based on weight. Furthermore, this study could generate hypotheses and encourage research into the appropriate dosing of other antimicrobial agents in the morbidly obese population.

16. DISSEMINTION OF RESULTS

The dissemination of these results will primarily take place through the writing of a scientific article. This article will then be published in a bariatric surgery or microbiology journal. It is also possible that these results will be presented at conferences in the two mentioned specialties, in front of colleagues from the medical field.

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