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Objective Non-Invasive Assessment of the Inflammatory Process in the Parietal Peritoneum: An Experimental Study

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Abstract

Aims: To assess the feasibility of a non-invasive way for evaluating the inflammatory process occurring in the parietal peritoneum.

Methodology: A total of 50 albino non-pedigree female rats were employed in this study. The subjects were divided into two distinct groups: a control group consisting of 10 intact rats and a research group comprising 40 rats that exhibited models of peritoneal inflammation. Peritoneal inflammation was induced via the intra-abdominal administration of a 10% autologous fecal mixture at a dosage of 10 ml per 100 g of body weight. The width of the laser beam scattering zone at wavelengths of λ=0.63 and λ=0.4 μm was measured (in millimeters) in both the intact and inflammatory parietal peritoneum at 6, 12, 24, and 48 hours following the induction of peritoneal inflammation. Histological examination of peritoneal sections was conducted post-measurement.

Results: Signs of serous peritoneal inflammation were observed at the 6-hour mark, with indications of progression to purulent inflammation noted subsequently. At 6 hours post-simulation of peritoneal inflammation, the width of the scattering zone for the laser beam with a wavelength of λ=0.63 μm exhibited a statistically significant increase, which continued to rise significantly over time. Similarly, the width of the scattering zone for the laser beam with a wavelength of λ=0.4 μm demonstrated comparable trends. However, by the 48-hour mark, the measurements were consistent with those recorded at 24 hours. Notably, the ratio of the scattering zone widths for the laser beams at wavelengths of λ=0.63 μm and λ=0.4 μm showed a significant increase 6 hours following the simulation of peritoneal inflammation, with no substantial changes observed thereafter.

Conclusion: An objective criterion for assessing inflammatory changes in the parietal peritoneum is the ratio of the scattering zone width of laser beams at wavelengths of 0.63 μm and 0.4 μm, which exceeds 2 units.

Keywords: Abdominal Surgery; Peritoneum; Inflammation of the Peritoneum; Laser Beams; Diagnosis.

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Introduction

Evaluating inflammatory changes in the peritoneum is a routine responsibility for general surgeons, given the high prevalence of conditions that lead to peritoneal inflammation [1-4]. Common etiologies include acute appendicitis,

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acute cholecystitis, acute pancreatitis, and perforated gastroduodenal ulcers, among others [5-11]. Accurately determining the extent of peritoneal inflammation is crucial, as it significantly influences the scope of surgical intervention required for various pathologies [1-4]. Greater involvement of peritoneal tissues in the inflammatory process typically correlates with a reduced volume of surgical intervention, particularly in cases of perforated gastroduodenal ulcers, perforated diverticula, and acute colonic obstruction [8, 9,12-14]. Furthermore, assessing the distribution of inflammation within the peritoneum is essential for predicting treatment outcomes, as this factor is integral to many prognostic models for complicated intra-abdominal infections, abdominal sepsis, and acute peritonitis [1, 2, 3, 15].

Inflammatory changes in the peritoneum are typically evaluated through visual inspection. Common indicators of inflammation include edema, hyperemia, and the presence of exudate and fibrin [1-4]. However, these visual signs may lack reliability. Notably, the absence of significant visual alterations does not necessarily imply the absence of inflammation. Conversely, exudate in a specific anatomical region does not automatically indicate inflammation, as exudate may be present due to mechanical movement. Additionally, it is important to consider the subjective nature of visual assessments, which can vary based on individual interpretations by different physicians.

The evaluation of inflammatory changes in the peritoneum presents significant challenges, particularly in patients with comorbidities such as diabetes mellitus and chronic conditions affecting the liver, lungs, and kidneys [16]. These underlying health issues can lead to substantial alterations in immune function, which in turn influences the progression of inflammation [17-19]. Consequently, clinical manifestations in these patients may be ambiguous, and visual assessments of abdominal organs and structures may deviate from typical presentations. As a result, there is an increased risk of diagnostic errors in comorbid surgical patients, which can lead to complications [16].

The only definitive objective for evaluating inflammatory changes in the peritoneum is histological examination; however, this evaluation can only be conducted post-surgery. During the surgical procedure, physicians must depend on subjective visual evaluations.

Some researchers advocate for applying laser irradiation to the peritoneum to enhance the objectivity of evaluation, subsequently determining the ratio of the laser beam scattering zone width [20, 21]. However, one way relies on subjective visual evaluations of the unaltered peritoneum, compromising the findings' reliability [20]. Conversely, another way offers a more objective approach; however, it is hindered by considerable variability in absolute indicators, which inevitably impacts the relative indicators [21]. Nonetheless, the concept introduced by the researchers merits further investigation and consideration.

Research Problem

The primary objective of this research is to evaluate the viability of employing laser radiation as a means to investigate inflammatory alterations in the parietal peritoneum.

Research Focus

This study aims to conduct an experimental comparative assessment of laser beam scattering across various wavelengths on both intact and inflamed parietal peritoneum. The objective is to assess the feasibility of a non-invasive way for evaluating the inflammatory process occurring in the parietal peritoneum.

Research Aim and Research Questions

1. To assess the scattering indices of laser beams on both intact and inflamed parietal peritoneum.

2. To analyze the variations in scattering indices of laser beams.

3. To explore the potential application of laser scattering indicators for evaluating inflammatory alterations in the parietal peritoneum.

Research Methodology

General Background

Inflammatory alterations in the parietal peritoneum can be precisely assessed through histological examination. Data obtained from laser beam scattering can be correlated with findings from histological analyses. It is essential that these investigations are conducted within an experimental framework.

Sample / Participants / Group

A total of 50 albino non-pedigree female rats were utilized for the study. All subjects were sexually mature, with an age of 6 months. The body mass of the rats ranged from 180 to 200 grams. Prior to the commencement of the experiment, the rats were housed in a vivarium, where they were maintained under uniform conditions. Throughout the experiment, the rats continued to be kept in identical environments and were provided with the same drinking resources.

The study involved the division of rats into two distinct groups: a control group consisting of 10 intact animals and a research group comprising 40 animals exhibiting models of peritoneal inflammation.

Instrument and Procedures

Peritoneal inflammation was induced through the intra-abdominal administration of a 10% autologous fecal mixture at a dosage of 10 ml per 100 g of body weight.

Laparotomy was conducted in both the control and research groups at intervals of 6, 12, 24, and 48 hours following the simulation of peritoneal inflammation. The parietal peritoneum was subjected to laser irradiation, and the extent of the scattering zone within the peritoneum was quantified. Measurements were obtained using a standard ruler during the irradiation process, with the width of the scattering zone recorded in millimeters. Data collection was performed independently across distinct quadrants of the abdominal wall.

Laser light-emitting diodes emitting at wavelengths of 0.63 μm (red) and 0.4 μm (blue) were utilized for irradiation purposes. These laser LEDs were operated using a standard direct current power supply with a voltage of 1.5 V.

Peritoneal samples were collected for histological analysis following measurements. The histological sections of the peritoneal tissues were stained using hematoxylin-eosin. The specimens were examined under a Delta Optical Evolution Pro 100 light-optical microscope.

During the course of the study, the researchers adhered to the fundamental principles outlined in the Vancouver Conventions (1979, 1994) regarding biomedical experiments, as well as the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (1986).

Data Analysis

The hypothesis regarding the normal distribution of data (Gaussian distribution) was evaluated using the Shapiro-Wilk test. The equality of means was assessed through the Wilcoxon and Mann-Whitney tests. A significance level (alpha) of 0.05 was established for the study. The statistical analysis of the results was conducted using Microsoft® Office Excel (build 11.5612.5703).

Research Results

The histological examination of the peritoneum and surrounding tissues in the control group revealed no significant alterations. Furthermore, no notable differences were observed in the width of the radiation scattering zone across various regions of the abdominal wall or at differing distances, as detailed in the accompanying table 1.

Table 1. Values of the scattering zone width of laser beams at various distances within the control group

Measurement location	Distance (cm)	Spreading zone width (mm)	
		λ=0.63 мкм	λ=0.4 мкм
Anterior quadrants	10	6,1±0,15	3,25±0,02
	20	6,2±0,12	3,25±0,07
	30	6,3±0,11	3,27±0,09
Posterior quadrants	10	5,9±0,15	3,25±0,05
	20	6,1±0,12	3,26±0,03
	30	6,3±0,11	3,28±0,09

The mean values of the scattering zone width at a wavelength of $\lambda = 0.63$ μm were recorded as 6.2 ± 0.12 mm, while at a wavelength of $\lambda = 0.4$ μm, the values were 3.27 ± 0.09 mm.

Histological examinations conducted on all animals within the research group at 6 hours post-simulation of peritoneal inflammation indicated the presence of serous inflammation. Notable findings included edema, areas of mesotheliocyte desquamation, infiltration of the peritoneum by polymorphonuclear leukocytes, and fibrin formation. By 12 hours, all subjects exhibited characteristics of purulent peritoneal inflammation, characterized by peritoneal infiltration and edema, an increase in mesotheliocyte desquamation foci, fibrin threads, and further infiltration by polymorphonuclear leukocytes. Progression of purulent inflammation was observed at 24 and 48 hours, with exacerbated edema in the peritoneum and surrounding musculature, increased fibrin deposits, and evidence of disintegration in both superficial and deep peritoneal structures, alongside a significant accumulation of polymorphonuclear leukocytes.

In the 6 hours following the simulation of peritoneal inflammation, a statistically significant increase in the scattering zone width of the beam, with a wavelength of λ=0.63 μm, was observed (Fig. 1). Subsequently, the scattering zone width continued to increase statistically significantly with each measurement.

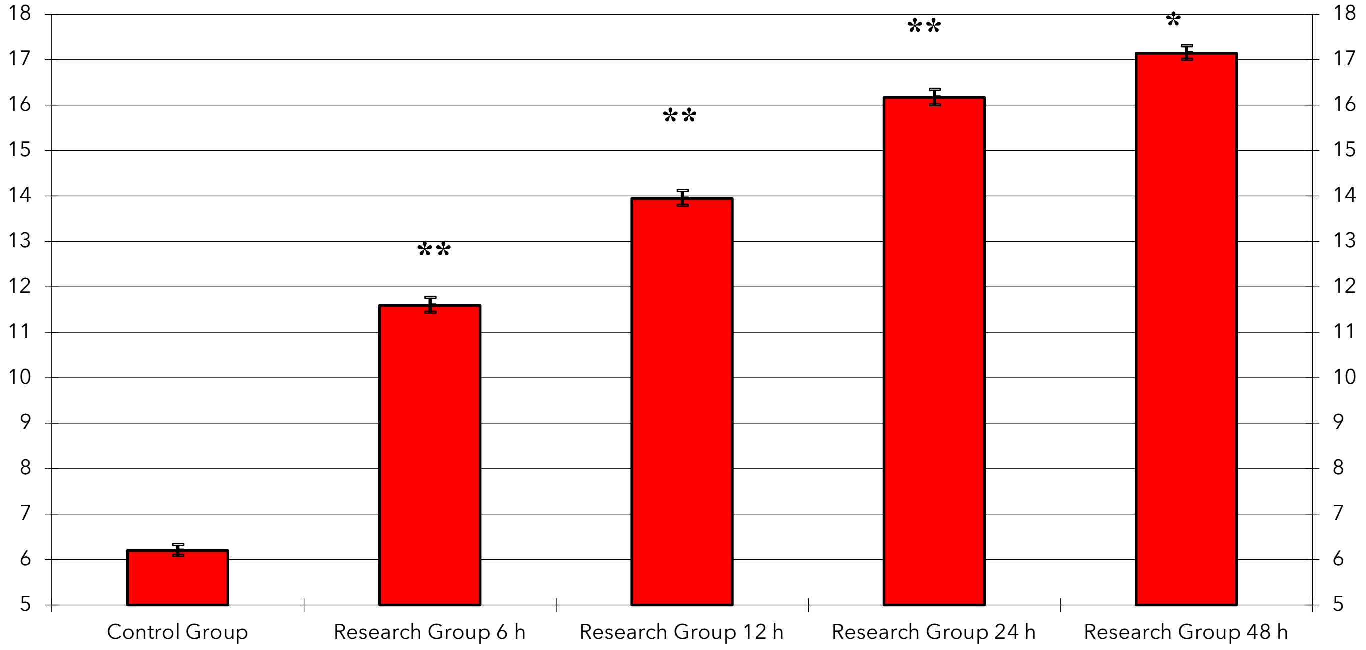

Figure 1. **Values of a laser beam with a wavelength of λ=0.63 μm scattering zone width in the parietal peritoneum (M±m)**

Note: *- p between adjacent observation periods < 0.05, **- < 0.01 (only statistically significant differences are given)

The width of the scattering zone for the beam with a wavelength of λ=0.4 μm exhibited comparable patterns (refer to Fig. 2). Notably, after 48 hours, the measurements were nearly indistinguishable from those recorded at 24 hours. Additionally, it is important to highlight that the data distribution within the samples conformed to a normal distribution, in contrast to the data distribution associated with the scattering zone width of the beam at a wavelength of λ=0.63 μm (Fig. 2).

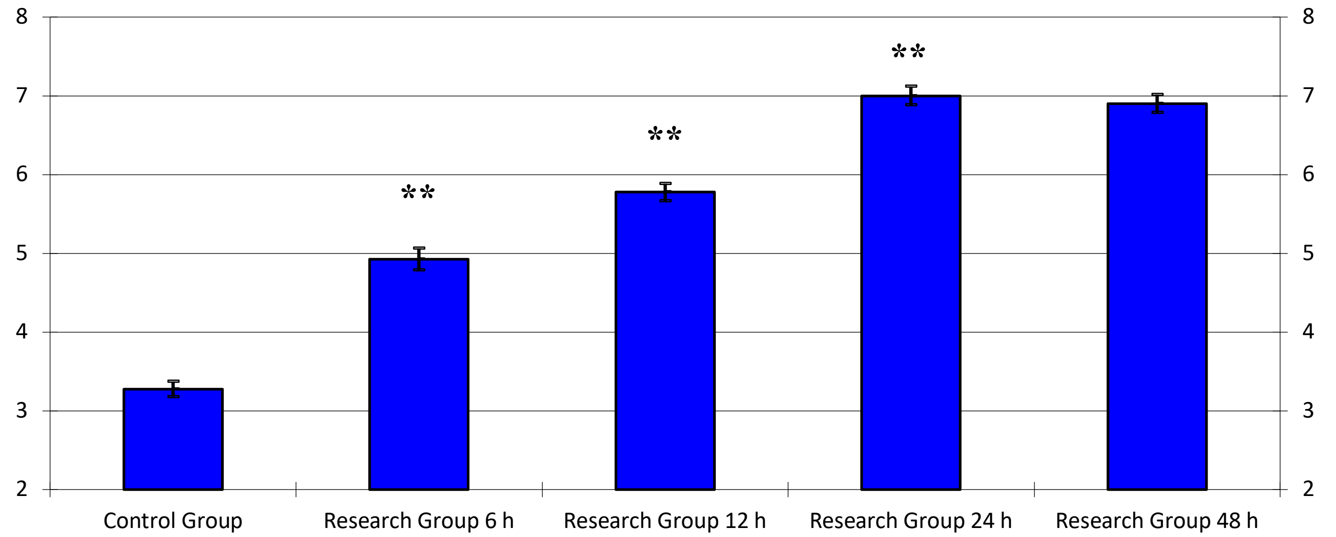

Figure 2. **Values of a laser beam with a wavelength of λ=0.4 μm scattering zone width in the parietal peritoneum (M±m)**

Note: **- p between adjacent observation periods < 0.01 (only statistically significant differences are given)

The analysis involved calculating the ratio of the scattering zone widths of laser beams at wavelengths of λ=0.63 μm and λ=0.4 μm. The findings indicate that the ratio parameters exhibit a statistically significant increase following the simulation of peritoneal inflammation, with no notable changes observed thereafter (Fig. 3).

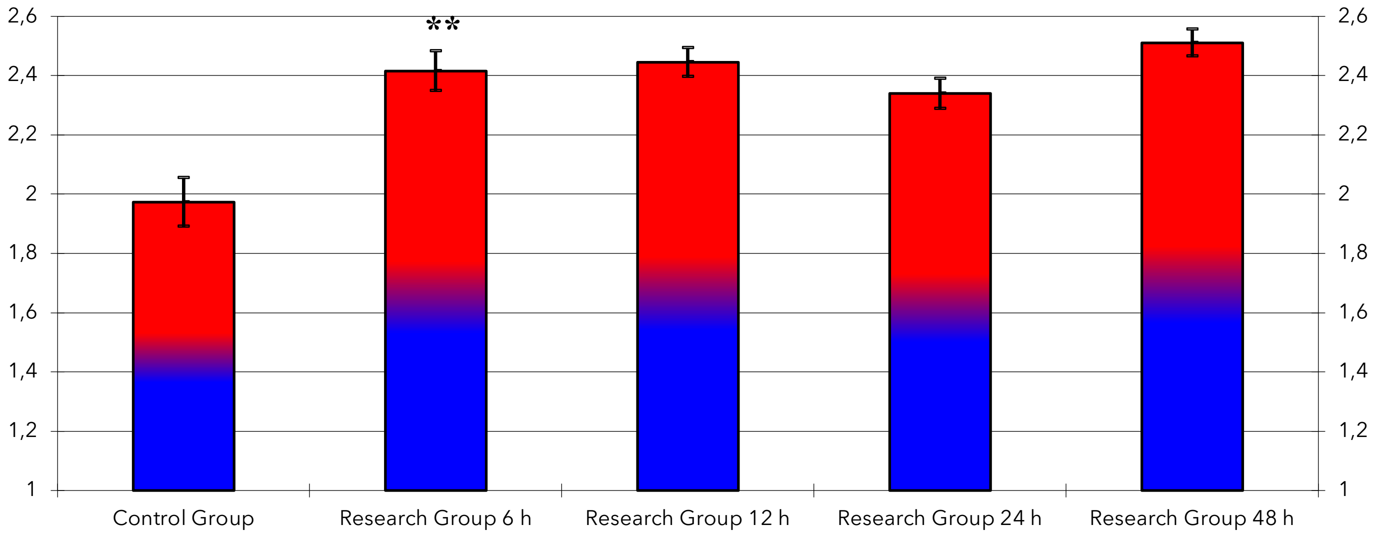

Figure 3. Ratio (units) of the scattering zone width of the scattering zone width of a laser beam with a wavelength of 0.63 μm to that of a laser beam with a wavelength of 0.4 μm.

Note: **- p between adjacent observation periods < 0.01 (only statistically significant differences are given)

Discussion

Laser beams are known to expand as they move away from their source; however, this phenomenon is typically noticeable only over significant distances [21, 22]. In our experiment, we determined that a distance of 20 cm was the most practical for our observations.

The analysis of the results regarding the width of the scattering zone, in conjunction with histological examination data from the research group, indicates that inflammation of the peritoneum leads to an increase in the scattering zone width compared to control data. This finding corroborates existing research that highlights the enlargement of the scattering zone width of laser beams in cases of peritonitis [20, 21].

The phenomenon of increased scattering of laser beams is attributed to the enlargement of the peritoneum's structural elements h, which exceed the wavelength of the laser beams λ. In a healthy state, the structures of the peritoneum exhibit characteristics closer to a solid state, resulting in smaller dimensions compared to those observed in inflamed peritoneum. Consequently, intact structures demonstrate reduced scattering of laser beams [22]. During serous inflammation, peritoneal tissues experience significant vascularization, becoming engorged with blood and plasma, leading to swelling. In cases of purulent inflammation, there is cellular destruction, with cellular contents breaching their boundaries. This results in a more homogeneous structure of the peritoneum, accompanied by an increase in size. Given that laser beam scattering occurs volumetrically, the width of the scattering zone expands. Thus, the widening of the scattering zone is a logical outcome of peritoneal inflammation.

The research confirms the limitations of utilizing absolute indicators for the width of the laser beam scattering zone when accurately assessing inflammatory changes in the peritoneum [20, 21]. This limitation arises from the multitude of factors that influence laser beam scattering, including age, gender, individual and local tissue characteristics, stress levels, time of day, seasonal variations, etc. Additionally, it is important to note the species-specific differences, as the histological structure of tissues in animals and humans can vary significantly.

Several technical factors are critical in this context. The absolute parameters, as previously noted, are influenced by the distance from the laser LED to the peritoneum. Additionally, the diameter of the laser beam plays a significant role; a larger diameter results in a broader scattering zone, while a smaller diameter has the opposite effect. The power of the laser beam, which is contingent upon both the laser LED's output and the source's power, is also a key consideration. A reduction in power will lead to a decrease in the scattering zone, and conversely, an increase in power will expand it. Furthermore, the illumination of the measurement environment is crucial; brighter lighting conditions can obscure the visibility of the scattering zone's boundaries, while dimmer lighting enhances visibility. Lastly, the precision of the measuring device is of paramount importance.

The technical factors discussed will uniformly influence the measurement results in any given scenario, resulting in consistent measurement errors. While these errors will impact the absolute parameters of the scattering zone width, they will not influence the relative parameters. Furthermore, these errors can be mitigated by developing a standardized and calibrated measuring device. The utilization of relative parameters effectively neutralizes the impact of these technical factors.

Our research indicates that the ratio of scattering zone width at wavelengths $\lambda=0.63/\lambda=0.4$ μm in the intact peritoneum (1.97±0.08 units) is significantly lower ($p<0.01$) than that observed in the inflamed peritoneum at various time points: 6 hours (2.41±0.07 units), 12 hours (2.44±0.05 units), 24 hours (2.34±0.05 units), and 48 hours (2.51±0.04 units) post-simulation of peritoneal inflammation. Notably, this ratio remained statistically unchanged during the 6 to 48-hour interval. In contrast, a similar study reported a significant increase in the ratio during the same timeframe following peritonitis simulation [21]. This discrepancy suggests that the indicators and ratios employed in our study are more reliable, as they accurately reflect the presence or absence of inflammatory changes in the peritoneum.

Thus, our study's findings indicate that evaluating the ratio of the scattering zone width of laser beams at wavelengths of $\lambda=0.63$ μm and $\lambda=0.4$ μm in the parietal peritoneum holds significant potential for assessing inflammatory changes. A ratio of less than 2 units suggests the absence of inflammation, whereas an increase in this ratio indicates inflammatory processes.

Limitations of the Study

The study presents several limitations that warrant consideration. It is based on prior research and investigates the potential of utilizing the scattering zone width of laser beam measurements to evaluate inflammatory changes in the parietal peritoneum. The experimental studies were conducted with non-standardized devices, highlighting the need for future research employing standardized equipment to validate the findings.

The sample size was limited to 50 rats, indicating the necessity for additional experiments involving a larger cohort to substantiate the results. The study exclusively utilized female rats, suggesting that future investigations should include male rats to enhance the robustness of the data.

Furthermore, while the previous research focused on rats, it is recommended to extend the experiments to other animal species for broader validation. Ultimately, the research findings must be tested on human subjects to definitively ascertain the validity of the experimental data.

Despite these limitations, it is important to note that the physiological and pathological principles shared among all warm-blooded mammals, along with the observed patterns in relative indicators, suggest that these constraints may not significantly impact the generalizability of the findings. It is anticipated that the fundamental principles will be corroborated, although some indicators may exhibit slight variations.

Prospects for Future Research

The research presented is an integral component of the scientific endeavors undertaken by the Department of Surgery No. 1 at Bukovinian State Medical University. A primary objective of this research is to prevent complications associated with abdominal surgery. This study is part of a broader series focused on evaluating inflammatory changes in the parietal peritoneum. Findings from additional research will be disseminated in subsequent publications. The overarching aim of this research series is to identify the most effective methods for assessing inflammatory changes in the parietal peritoneum. Future studies will involve a larger cohort of animal subjects, and the research will also extend to patient applications.

Conclusions and Implications

1. An objective criterion for assessing inflammatory changes in the parietal peritoneum is the ratio of the scattering zone width of laser beams at wavelengths of 0.63 μm and 0.4 μm, which exceeds 2 units.

2. The evaluation of this ratio can be utilized in patients to gauge its relevance and significance.

Declarations

Author Contributions

Conceptualization, F. Grynchuk; methodology, F. Grynchuk; validation, F. Grynchuk; formal analysis, F. Grynchuk and F. Grynchuk Jr.; investigation, F. Grynchuk Jr.; resources, F. Grynchuk Jr; data curation, F. Grynchuk Jr; writing-original draft preparation, F. Grynchuk Jr.; writing-review and editing, F. Grynchuk Jr and F. Grynchuk; visualization, F. Grynchuk Jr; supervision, F. Grynchuk; project administration, F. Grynchuk. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data sharing is not applicable: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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Institutional Review Board Statement

During the course of the study, the researchers adhered to the fundamental principles outlined in the WMA Declaration of Helsinki – Ethical principles for medical research involving human subjects, "Guide for the care and use of laboratory animals", ARRIVE (Animals in Research: Reporting In Vivo Experiments).

Informed Consent Statement

Not Applicable.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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