Impact of del-IMMUNE V® Treatment in Colon Cancer Surgery Patients

Authors: Gissel García, Josanne Soto, Lipsy Fernández, Mirka Bernal, Antonio Díaz, Luba Sichel, Raúl de Jesús Cano

Abstract

Introduction

del-IMMUNE V® is an FDA-registered nutritional supplement that contains the dried lysate of fermented cells of *Lactobacillus rhamnosus DV (NRRLB-68023)*, a gram-positive bacteria, of the DV strain. This metabiotic formulation is an immunomodulator that has been used to enhance the activation, regulation and balance of the immune system. It contains fragments of bacterial cell wall components such as muramyl peptides (a component of peptidoglycan) and DNA; it also has essential nucleotides and amino acids that are activators of the innate immune system. Muramyl peptide fragments act as microbial pathogen-associated molecular patterns (MAMPs), which are potent activators of innate immune cells such as phagocytes (macrophages) and NK cells. This compound also stimulates and regulates the synthesis of anti-inflammatory and immunoregulatory cytokines such as type I interferons (IFNs), IFN gamma, interleukin (IL)-1, IL-12, and IL-10, as well as B and T lymphocytes. Muramyl peptides have been demonstrated as an immunostimulant, both in acute conditions (such as viral and fungal infections of different types) and chronic conditions (such as asthma, fibromyalgia, and hepatitis C, among others). This compound also provides complementary support for patients undergoing chemotherapy or radiotherapy, as it stimulates the proliferation of stem cells. (1)

Colorectal cancer (CRC) is the second cause of cancer in women and the third in men worldwide, and approximately half of patients develop metastatic colon cancer (2). Substantial evidence indicates that an inflammatory microenvironment is a decisive factor in tumor progression to further stages. This environment is detected by monitoring inflammatory biomarkers such as IL-6 (3) and C-reactive protein (CRP) (4), among others, and detection allows therapeutic decisions to be made (5).

There are still many questions about the condition of the microbiota in the perioperative period; however, it is possible that, as a consequence of trauma, an imbalance of the microbiome occurs, which may contribute to the development of surgical complications (6, 7). This imbalance associated with surgical stress is intensified by intestinal cleansing of the colon, prophylactic use of antibiotics, type and duration of surgical intervention, hypoxia, and a lack of nutrients in the intestinal lumen (8).

Considering the anti-inflammatory and immunoregulatory properties of del-IMMUNE V®, this compound could have a protective effect if applied as a complementary therapy during the CRC patient's perioperative period (from prehabilitation to mid-postoperative). Its

effects could impact the quality of life of patients suffering from this disease, both short- and long-term.

How have gastroenterologists and surgeons contributed to reducing the occurrence of dysbiosis in their patients? In the preoperative period, we avoid colon preparation and reconsider the use of prophylactic antibiotics due to their harmful consequences for the resident microbiota. In the intraoperative period, a refined surgical technique is recommended, avoiding bleeding and blood transfusions, manipulating the tissues delicately, performing digestive anastomoses within the highest technical level, and opting, whenever possible, for less traumatic access routes with less inflammatory impact (9). But we can go even further. Among the different ways to modify the composition of the intestinal microbiota, prebiotics, probiotics, and synbiotics—the consumption of which has increased exponentially in the last decade—particularly stand out (10). **The general objective of this work is:** to demonstrate the del-IMMUNE V® formulation's effects on microbiome turnover and quality of life in individuals with colon cancer during the perioperative period. We hypothesized that, since the level of inflammation determined by CRP is a criterion for medical discharge after surgery, the main variables of the study should be CRP and IL-6 levels—a decrease in which would indicate better survival and possible improvement in quality of life for patients who receive subsequent chemotherapy. Microbiota assessment will be taken under consideration after comparison before and after microbiota profile status.

Materials and Methods

This study evaluated the metabiotic compound del-IMMUNE V® as a proof of concept for its use as a complementary therapy in the perioperative period of patients with colon cancer at any stage of the disease. For this purpose, a randomized, controlled, double-blind, phase I clinical trial was carried out.

ETHICAL ASPECTS

General ethical considerations of research

This is a randomized, controlled, double-blind, phase I trial with the investigational product del-IMMUNE V®, which has been registered as a nutritional supplement by the Regulatory Agency of the Cuban National Registry (Institute of Nutrition and Food Hygiene (INHA) belonging to the National Institute of Hygiene, Epidemiology, and Microbiology (INHEM)) (Health License No. Pl-30490/22). The clinical trial will be carried out in accordance with the provisions of the Declaration of Helsinki of the World Medical Association, with the last update at the 64th General Assembly, in Fortaleza, Brazil, October 2013 (11), and with the current state regulations for nutritional supplements of the Institute of Nutrition and Food Hygiene (INHA).

Inclusion, Exclusion, and Exit Criteria
The following are criteria for inclusion, exclusion, or exit from the study.

Inclusion criteria:

- Patients of any sex who are residents of Cuba, aged 18 years or older.
- Patients who meet the diagnostic criteria for colon or rectal resection.
- Patients with general health status according to Karnofsky ≥70%.
- Patients who have signed the informed consent.

Exclusion criteria:

- Pregnant or breastfeeding patients.
- Patients undergoing emergency surgery.
- Patients receiving another investigational product.
- Patients with brain metastases.
- Patients with mental disorders that could limit adherence to the requirements of the clinical trial and make information collection, treatment, or follow-up difficult.

Exit or study withdrawal criteria:

- Patients who have undergone a colostomy procedure.
- Patients who experience serious adverse events related to the nutritional supplement at any point during the trial which, in medical opinion, require the interruption of treatment.
- Voluntary withdrawal.
- The appearance of any condition that, in the opinion of the investigator, prevents continuation of the study treatment.
- Irregularities in treatment with the nutritional supplement (more than 15 days without ingesting it).
- Death.

Patients who exit treatment will be part of the study and will be taken into account when analyzing the final data.

TREATMENT:
Randomized patients were divided into 2 groups of 25 individuals each. The groups were classified as Group A and Group B. Each group received the formulations as follows:
Group A (placebo): Received 2 capsules daily (LOT 425). Each capsule was administered orally, 1 every 12 hours, uninterrupted, from a minimum of 7 days to a maximum of 15 days before surgical intervention, throughout the perioperative period, and 15 days afterwards. The placebo was 3.53 g of inert compound rice flour.
Group B (treated with del-IMMUNE V® (DIV)): Received 2 capsules daily (LOT 426). Each capsule was administered orally, 1 every 12 hours, and in the same way as Group A. DIV is a nutritional supplement containing the dried lysate of fermented cells of

Lactobacillus rhamnosus DV. Each capsule contained 100mg of DIV.
The capsules were delivered 7 days before surgery by the pharmacy specialist who controls the temperature and storage of the products. The capsules were delivered the same day that the patient went to the general surgery clinic and received the admission order with a previously coordinated admission date. The capsules were in well-identified envelopes.

Sample Collection, Processing, and Data Management

Sample collection, supplement delivery, and clinical evaluation were conducted at the start of the study (week 1 prior to surgery) and at the end of the surgical treatment (60 days). Blood samples were collected via venipuncture, properly identified with the participant's inclusion number, processed, and aliquoted within 1 hour for storage and future analyses. All participant records were maintained in a secure, dedicated database. Access to these records was restricted to the study and clinical staff responsible for participant care. The Health and Hospital Administration (HHA) was responsible for managing the security of the information technology infrastructure.

Clinical Determinations

For the evaluation of IL-6, carcinoembryonic antigen (CEA), and CRP biomarkers, a Cobas® 600 modular immunochemical autoanalyzer (Roche Diagnostics) was employed. The analysis was performed on serum samples, following the manufacturer's recommended protocols.

Results

The measurements of the studied variables—weight, body mass index (BMI), IL-6, CRP, and CEA—were compared at the beginning of and 60 days after treatment with del-IMMUNE V® using the Wilcoxon test for related samples.

The median CRP significantly decreased from 58.61 mg/L (IQR: 15.14–147.54) to 17.20 mg/L (IQR: 2.97–25.14) after the treatment ($W = 52$, $Z = -2.497Z$, $p = 0.013$), with a large effect size ($r = 0.45$). No significant differences were found in weight, BMI, IL-6, and CEA. However, it is important to note that, although not significant, there was a trend towards reduction in IL-6 values (post-treatment median = 1.92 mg/dL; pre-treatment median = 3.66 mg/dL) (see Table 1).

Table 1. Comparison of the studied variables at the beginning and 60 days according to assigned treatment group.

Variables	Median Day Zero	Median 60 Days	Statistic (W; Z)	p-value	Effect Size (Cohen's r)
Weight (kg)	60.50 (56.75–65.25)	60.35 (54.30–63.15)	38; -1.071	0.284	0.23
BMI (kg/m^2)	23.95 (21.70–26.10)	23.10 (20.40–24.47)	38; -1.070	0.285	0.23
IL-6 (mg/dL)	3.66 (1.50–17.00)	1.95 (1.50–6.37)	18; -0.676	0.499	0.15
CRP (mg/L)	58.61 (15.14–147.54)	17.20 (2.97–25.14)	52; -2.497	**0.013**	0.56
CEA (ng/mL)	4.27 (1.79–27.41)	4.44 (1.85–8.11)	32; -0.459	0.646	0.14

Discussion

The study involved a comparison of several variables, including weight, BMI, IL-6, C-CRP, and CEA, both at the beginning of and 60 days after treatment with del-IMMUNE V®. The most notable result was the significant reduction in median CRP levels after treatment. This result suggests that del-IMMUNE V® treatment substantially reduces inflammation as reflected by CRP levels. CRP is a highly sensitive marker of systemic inflammation and is primarily produced by liver hepatocytes in response to elevated inflammatory cytokines such as IL-6. The exact mechanism by which CRP is related to the prognosis of patients with CRC remains obscure, but mechanistic studies have suggested that elevated CRP correlates with increased expression of oncogenes, resulting in DNA damage. Consequently, elevated circulating CRP has been widely reported as a marker of poor prognosis. There is also a positive association between post-operative serum CRP levels and disease relapse (4).

Although not statistically significant, there was a declining trend in IL-6 values, implying a potential positive impact of the treatment on reduction of inflammatory cytokines. The small number of samples analyzed may explain the non-significance of the decrease in IL-6 values. However, this declining trend is a very important result, as a high serum IL-6

level has been associated with a poor prognosis for both CRC overall survival and disease-free survival (3).

These findings suggest that del-IMMUNE V® has a pronounced effect in decreasing systemic inflammation, as evidenced by the significant reduction in CRP levels. Even though other variables did not show significant changes, the trend in IL-6 reduction could indicate further benefits that might emerge with prolonged treatment or in a larger sample. This highlights the potential therapeutic benefits of del-IMMUNE V® in managing systemic inflammation in patients undergoing surgery for colon cancer and in improving their quality of life with positive prognosis.

References

1. Sichel L, Timoshok NA, Pidgorskyy VS, 107. SN. Study of Interferonogenous Activity of the New Probiotic Formulation Del-Immune V®. J Prob Health. 2013;1(170).
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA: a cancer journal for clinicians. 2024;74(1):12-49.
3. Xu J, Ye Y, Zhang H, Szmitkowski M, Mäkinen MJ, Li P, et al. Diagnostic and Prognostic Value of Serum Interleukin-6 in Colorectal Cancer. Medicine (Baltimore). 2016;95(2):e2502.
4. Gwenzi T, Schrotz-King P, Anker SC, Schöttker B, Hoffmeister M, Brenner H. Post-operative C-reactive protein as a strong independent predictor of long-term colorectal cancer outcomes: consistent findings from two large patient cohorts. ESMO open. 2024;9(4):102982.
5. Cotan HT, Emilescu RA, Iaciu CI, Orlov-Slavu CM, Olaru MC, Popa AM, et al. Prognostic and Predictive Determinants of Colorectal Cancer: A Comprehensive Review. Cancers [Internet]. 2024; 16(23).
6. Koliarakis I, Athanasakis E, Sgantzos M, Mariolis-Sapsakos T, Xynos E, Chrysos E, et al. Intestinal Microbiota in Colorectal Cancer Surgery. Cancers (Basel). 2020;12(10).
7. Cong J, Zhu H, Liu D, Li T, Zhang C, Zhu J, et al. A Pilot Study: Changes of Gut Microbiota in Post-surgery Colorectal Cancer Patients. Front Microbiol. 2018;9:2777.
8. Cronin P, Murphy CL, Barrett M, Ghosh TS, Pellanda P, O'Connor EM, et al. Colorectal microbiota after removal of colorectal cancer. NAR Cancer. 2022;4(2):zcac011.
9. Tweed TTT, Sier MAT, Daher I, Bakens MJAM, Nel J, Bouvy ND, et al. Accelerated 23-h enhanced recovery protocol for colon surgery: the CHASE-study. Scientific Reports. 2022;12(1):20707.
10. Pitsillides L, Pellino G, Tekkis P, Kontovounisios C. The Effect of Perioperative Administration of Probiotics on Colorectal Cancer Surgery Outcomes. Nutrients [Internet]. 2021; 13(5).
11. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama. 2013;310(20):2191-4.