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Efecto sobre la colitis por diversión tras estimulación del asa eferente con probióticos previo al cierre de la ileostomía de protección en pacientes intervenidos de carcinoma colorrectal. Estudio prospectivo, randomizado, doble ciego

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Ángela del Rocío Rodríguez Padilla

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Carlos Ruiz Frutos

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Presentada por:

Ángela del Rocío Rodríguez Padilla

Director:

Dr. Carlos Ruíz Frutos

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compartimos durante 10 días, hablando de la enfermedad y lo que es más importante, ¿qué hacer tras superarla?

A mi madre, mi padre, mi hermana y a Germán.

ABREVIATURAS

- CD: colitis por diversión
- GE: grupo estimulado
- GC: grupo control
- IP: ileostomía de protección
- CCR: cáncer colorrectal
- IPP: íleo paralítico postoperatorio
- SNG: sonda nasogástrica
- RAB: resección anterior baja
- IMC: índice de masa corporal
- EII: enfermedad inflamatoria intestinal
- ASA: American Society of Anaesthesiologist
- IHQ: inmuninohistoquímica
- HE: hematoxilina-eosina
- CRP: C-reactive protein
- NLR: ratio neutrófilos/linfocitos
- LMR: ratio linfocitos/monocitos
- PLR: ratio plaquetas/linfocitos
- AGCC: ácidos grasos de cadena corta

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Introducción

La creación de una ileostomía temporal en pacientes intervenidos de carcinoma colorrectal mediante resección anterior baja con escisión total del mesorrecto, permite disminuir la morbilidad asociada a la fístula anastomótica. Aunque la reconstrucción del tránsito intestinal no está exenta de morbilidad. Entorno al 18-40% presentarán complicaciones tales como colitis por diversión, obstrucción de intestino delgado, infección de la herida quirúrgica, íleo postoperatorio, fuga anastomótica, fístula, perforación, absceso, hemorragia o eventración.

La colitis por diversión es la inflamación crónica producida en un segmento de colon desfuncionalizado tras la realización de un estoma temporal. Esta inflamación se asocia a un cambio en la flora microbiota, cambios endoscópicos, cambios histológicos y una alteración de marcadores séricos inflamatorios. Estas alteraciones provocan una disbacteriosis y una atrofia vellositaria en el segmento de colon excluido, provocando un deterioro de la capacidad de absorción y contracción luminal, con pérdida de contractilidad y motilidad. Para evitarlo se han propuesto dos líneas de actuación; el cierre precoz de la ileostomía de protección, que sería la mejor opción, o en su defecto, un cierre tardío previa estimulación del asa eferente.

Basándonos en la necesidad de devolver al intestino excluido su funcionalidad, prehabilitándolo antes de la reconstrucción del tránsito y la disbiosis bacteriana demostrada, decidimos administrar probióticos a través del extremo distal de la ileostomía. Los probióticos se están aplicando cada vez en la patología gastrointestinal. Son bacterias vivas no patógenas como Lactobacillus, Bifidobacterium y Estreptococcus, que presentan actividad antimicrobiana, inmunomoduladora y mejoran la barrera intestinal. Suministrados en las concentraciones adecuadas, promueven beneficios en la salud del organismo huésped.

Hipótesis

La estimulación preoperatoria del intestino con sustancias probióticas a través del asa eferente de la ileostomía en pacientes intervenidos de carcinoma colorrectal disminuye la colitis por diversión y, por tanto, disminuye la clínica asociada a la colitis por derivación tras la cirugía de reconstrucción del tránsito.

Objetivos

Evaluar la eficacia y seguridad de la estimulación del asa eferente de la ileostomía de protección con probióticos previa a la reconstrucción del tránsito intestinal en pacientes intervenidos de carcinoma colorrectal y su efecto sobre la colitis por diversión, evaluando los cambios endoscópicos, histológicos, en los biomarcadores serológicos así como la aparición de íleo paralítico postoperatorio.

Metodología

Se diseñó un estudio prospectivo, aleatorizado, doble ciego, controlado. Se incluyeron pacientes intervenidos de carcinoma colorrectal con creación de ileostomía de protección entre enero de 2017 y diciembre de 2018; pendientes de cirugía de reconstrucción del tránsito que presentaban diagnóstico endoscópico e histológico de colitis por diversión. Fueron aleatorizados y divididos en dos grupos: un grupo estimulado con probióticos (GE) y otro grupo control (GC). Completaron el estudio 34 casos y 35 controles. Se determinaron los cambios endoscópicos, histológicos y serológicos pre y post-estimulación con probióticos, así como las complicaciones postoperatorias y en el seguimiento a corto plazo.

Resultados

La incidencia de íleo postoperatorio fue similar en ambos grupos. Igualmente no hubo diferencias significativas en cuanto a la necesidad de sonda nasogástrica, restablecimiento del tránsito ni estancia hospitalaria. En el análisis comparativo se demostró un relación directa entre la aparición de íleo tras la cirugía oncológica y tras la reconstrucción del tránsito independiente de la estimulación p=0,005.

Se observó una reducción del grado de severidad histológica y endoscópicos en el grupo estimulado preestimulación/postestimulación respecto al grupo control. Estos resultados fueron estadísticamente significativos, con una p<0,001 y un coeficiente de asociación intensa. Se observó una reducción de los hallazgos endoscópicos (friabilidad de la mucosa, úlceras, pólipos, edema de mucosa, eritema y estenosis) e histológicos (hiperplasia folicular, infiltración de la lámina propia por eosinófilos, abscesos crípticos, infiltración por linfocitos, infiltración por células plasmáticas y distorsión de la arquitectura) en el grupo estimulado preestimulación/postestimulación respecto al grupo control. Estos resultados fueron estadísticamente significativos, con una p<0,001 y un coeficiente de asociación intensa.

En el estudio de biomarcadores inflamatorios serológicos, se observó una disminución significativa de los valores de PCR, cociente NLR y cociente LMR y un aumento significativo de los valores de transferrina y del cociente PLR en el GE frente al GC tras la estimulación con probióticos con una p<0,001. En el seguimiento a los 3 meses tras la reconstrucción del tránsito se normalizan las cifras de PCR y transferrina, y se igualan los cocientes NLR, LMR y PLR en ambos grupos.

Conclusiones

1) La estimulación del asa eferente con probióticos es un método seguro y eficaz; 2) Provoca una disminución o desaparición del grado de colitis por diversión macroscópica y microscópica; 3) Disminuye la clínica a corto plazo tras la reconstrucción del tránsito intestinal; 4) Puede ser una alternativa para pacientes que no son candidatos a cirugía de reconstrucción del tránsito; 5) El grado de severidad endoscópica e histológica de la colitis por diversión se relaciona con una mayor alteración de los biomarcadores inflamatorios en sangre; 6) La estimulación con probióticos previa a la reconstrucción del tránsito produce una normalización de estos parámetros de forma precoz; 7) La aparición de íleo paralítico secundario al cierre de la ileostomía de protección es independiente de la estimulación del asa eferente con probióticos; 8) Parece existir relación entre la aparición de íleo tras la reconstrucción y la existencia previa en el postoperatorio de la cirugía del cáncer colorrectal.

Abstract

Introduction

The performance of a temporary ileostomy in patients operated on for colorectal carcinoma, by means of an anterior resection with total mesorectal excision, reduces the morbidity associated with anastomotic leak. However, reconstructive surgery is not risk-free. The incidence of complications following ileostomy closure, such as diversion colitis, small bowel obstruction, surgical wound infections, postoperative ileus, anastomotic leak, fistula, perforation, abscess, bleeding or hernia, ranges from 18% to 40%.

Diversion colitis is a non-specific inflammation of a defunctionalised segment of the colon after a temporary stoma has been performed. This inflammation is associated with a change in the colonic flora, endoscopic changes, histological changes and an alteration of certain inflammatory serum biomarkers. This changes produces dysbacteriosis and atrophy of its villi and muscular layers in the excluded colon segment, as well as a loss of segmentary contractility that could produce a reduction in the ability for absorption. To avoid this, two lines of studies have been proposed; early closure of the loop ileostomy, which would be the best option, or late closure after stimulation of the efferent loop.

Based on the need to return the excluded intestine to its function, pre-enabling it before surgery reconstruction and demonstrated bacterial dysbiosis, we decided to administer probiotics through the efferent loop. Probiotics are increasingly being applied in gastrointestinal pathology. They are non-pathogenic live bacteria, such as Lactobacillus, Bifidobacterium and Estreptococcus, which present antimicrobial and immunomodulatory activity and improve the intestinal barrier. Supplied in adequate amounts, they promote health benefits on the host.

Hypothesis

Preoperative stimulation of the efferent loop with probiotics prior to closure of the protective ileostomy in patients operated on colorectal carcinoma decreases the diversión colitis and, therefore, reduces the symptons associated with diversión colitis after restorative surgery.

Objectives

To evaluate the efficacy and safety of preoperative stimulation of the efferent loop with probiotics prior to closure of the protective ileostomy in patients operated on colorectal carcinoma and its effect on diversion colitis. Endoscopic and histological changes, biomarkers serological changes and postoperative ileus were evaluated.

Results

The incidence of postoperative ileus was similar in both groups. There were no significant differences with regard to need of a nasogastric tube, restoration of bowel function and duration of hospital stay. The comparative analysis showed a direct relationship between postoperative ileus after oncological surgery and postoperative ileus after reconstruction surgery, independently of stimulation with $p = 0.005$.

A decrease in endoscopic and histological severity were observed in stimulated group. These results are statistically significant with a $p<0,001$ and a intense level association. A decrease in endoscopic findings (mucosal friability, mucous erosions, polyps, edema, erythema and stenosis) and in histological findings (follicular hyperplasia, eosinophils, cryptic abscesses, lymphocyte infiltration, plasma cell infiltration and architecture distortion) were observed in stimulated group. These results are statistically significant with a $p<0,001$ and a intense level association.

In serological biomarkers study, a significant decrease in C-reactive protein (CRP), Neutrophil/lymphocyte ratio (NLR ratio) and monocyte/lymphocyte ratio (MRL ratio) and a significant increase in transferrin values and in the platelet/lymphocyte ratio (PLR) was observed in the stimulated group versus control group after stimulation with probiotics with a $p < 0.001$. A normalization of CRP and transferrin levels was observed in the third month of follow-up after closure ileostomy, and NLR, MRL and PLR ratios were equal in both groups.

Conclusions

1) Probiotic stimulation of the efferent loop is a safe and effective method; 2) It produces a decrease of the endoscopic and histological severity of colitis; 3) As well as, a decrease in symptoms in the short term after reconstructive surgery; 4) This procedure can be an alternative treatment in patients who surgical option is not feasible or available; 5) The endoscopic and histological severity of DC is associated with a greater alteration of inflammatory biomarkers in the blood; 6) The stimulation with probiotics prior to reconstructive surgery produces an early normalization of these parameters; 7) Postoperative ileus after closure ileostomy is independent of stimulation of the ileostomy with probiotics through the efferent loop; 8) There seem to be a relationship between PI after reconstruction and the previous existence of postoperative ileus after colorectal cancer surgery.

Contextualización de la colitis por diversión y su abordaje terapéutico

La presente tesis doctoral, se enmarca en un proyecto de investigación que analiza si mejora la sintomatología asociada a la colitis por diversión tras el cierre de la ileostomía de protección en pacientes intervenidos de carcinoma colorrectal. Estos pacientes, tras la cirugía de reconstrucción del tránsito intestinal, experimentan una clínica exacerbada relacionada con la inflamación crónica del segmento de colon excluido que mantiene una marcada ausencia de funciones, lo que conlleva a una notable disminución en la calidad de vida.

El proyecto de investigación que ha servido de base para desarrollar esta tesis doctoral ha sido aprobado por el Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, España, y registrado con el apoyo de la Fundación Andaluza Beturia para la Investigación en Salud (FABIS), con el número de Proyecto 2017/331191354. Para este proyecto se estableció una colaboración entre la Universidad de Huelva y los tres hospitales de la provincia de Huelva, el Hospital Universitario Juan Ramón Jiménez, el Hospital Comarcal Infanta Elena y el Hospital General de Riotinto.

1.1. Cáncer colorrectal e ileostomía de protección

En los países occidentales, el cáncer de recto representa el 28-35% de todos los cánceres colorectales, con una incidencia de 15-25 nuevos pacientes por cada 100000 habitantes/año en países europeos (Glynne-Jones R, 2017). Presenta una mayor incidencia en el sexo masculino y a partir de los 60 años de edad, aunque en más de un 5% de los casos esta enfermedad debuta en individuos menores de 40 años, por lo que debemos analizar los factores que influyen en la génesis de la enfermedad (Dekker E, 2019). Numerosos estudios han demostrado la implicación de la obesidad, la dieta, consumo carne roja, grasas saturadas y disminución de la ingesta de fibra y fruta (Gao R, 2017; Lucas C, 2017; Thanikachalam K, 2019; Morze J, 2021).

Una gran parte de los cánceres de recto no dan sintomatología hasta estadíos avanzados (Glynne-Jones R, 2017). La rectorragia es el síntoma más frecuente y muchas veces es atribuido de forma incorrecta a patología proctológica (Dekker E, 2019). Cuando la enfermedad progresa, el paciente refiere cambio en el hábito intestinal asociado a la obstrucción parcial por progresión del tumor. Cuando la enfermedad se sitúa en el recto bajo (<5cm del margen anal), el paciente puede referir sensación de evacuación incompleta o tenesmo rectal. Las molestias abdominales suelen aparecer sobre todo a nivel de hipogastrio. Cuando el tumor se encuentra en una etapa avanzada y provoca infiltración de estructuras osteomusculares o nerviosas puede provocar dolor a nivel del sacro o rectal que muchas veces es de gran intensidad y de difícil manejo farmacológico (Zielińska A, 2021). Si el cáncer invade vejiga, síntomas como cistitis o fecaluria pueden aparecer. En un estudio de Beard et al (Beart RW, 1995), dónde se analizaba la sintomatología de 5696 pacientes con cáncer de recto, la presencia de síntomas por orden de frecuencia fue: rectorragia (60,4%), cambios en el hábito intestinal (43,3%), sangrado oculta en heces (25,8%), dolor abdominal (20,9%), malestar general (9,1%), obstrucción intestinal (9%) y dolor pélvico (5%) (Glynne-Jones R, 2017; Dekker E, 2019).

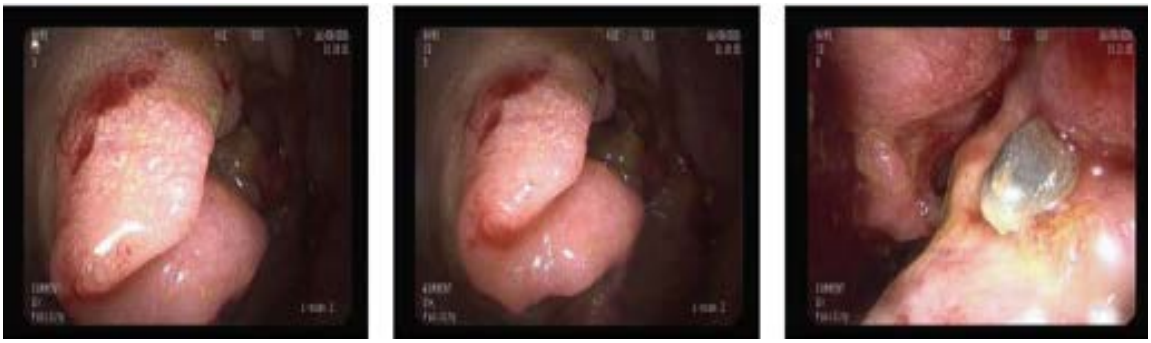


Figura 1. Colonoscopia: lesión estenosante, ulcerada a unos 6cm del margen anal, compatible con neoplasia de recto medio.

La exploración física en el cáncer de recto es esencial para determinar la extensión local de la enfermedad. La presencia de una masa protuberante con una ulceración central es característica del cáncer de recto. Esta exploración deberá llevarse siempre a cabo ante la presencia de síntomas de sospecha a la hora de establecer un adecuado diagnóstico diferencial. El estudio deberá ser completado mediante analítica de sangre incluyendo hemograma, función hepática, CEA, Ca 19.9, test de sangre oculta (TSOH) en heces, y estudios de imagen como rectocolonoscopia, ecografía endorrectal, tomografía axial computerizada torácica y abdominal (TAC) y resonancia magnética (RNM) (Glynne-Jones R, 2017; Dekker E, 2019; Montminy EM, 2020).

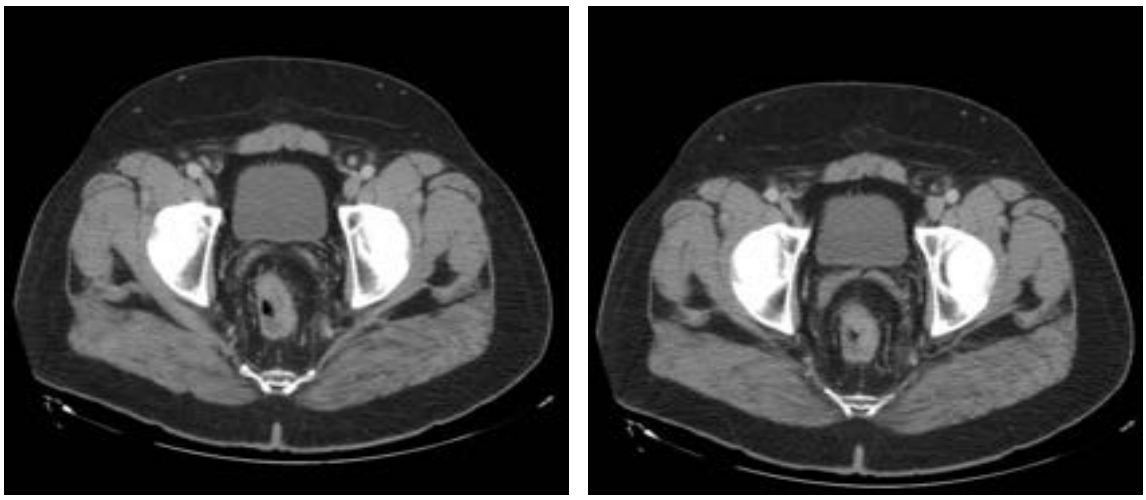


Figura 2.Tomografía axial computerizada: engrosamiento mural mamelonado, de 20mm de grosor, que afecta a márgenes izquierdo y anteroposterior, estenosando la luz del recto medio. Presenta especulaciones hacia la grasa perirrectal, en relación con neoplasia rectal. Asocia reticulación de la grasa perirrectal, en la que existen varias imágenes nodulares compatibles con adenopatías de aspecto patológico. Clasificación TNM – T3b, N1, Mx.

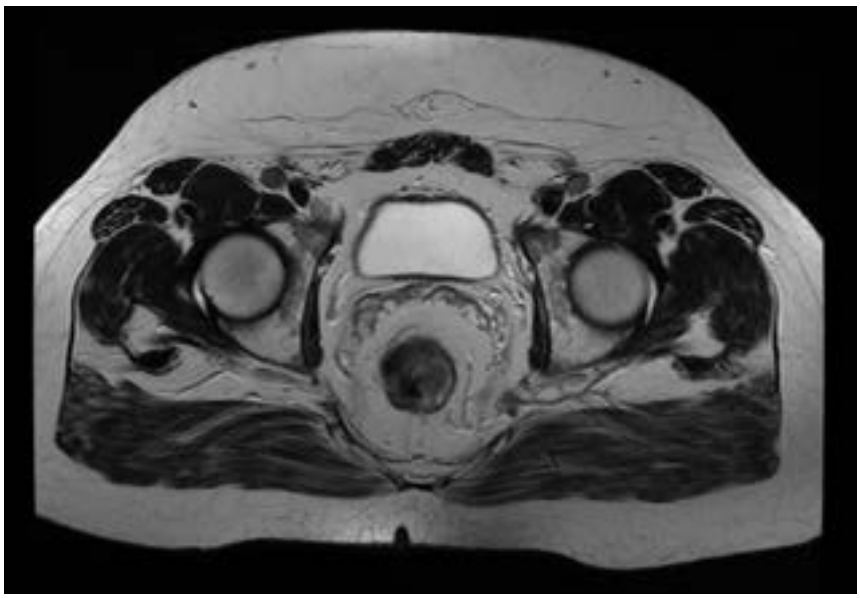


Figura 3. Resonancia Nuclear Magnética. Secuencias para estadificación locorregional del cáncer de recto T1 y T2 con contraste iv. Se apercia un engrosamiento mural casi circunferencial , mamelonado, con señal intermedia en T1/T2 de aspecto neoplásico que involucra la pared anterior, lateral izquierda y posterior del recto superior, ocasionando una estenosis no obstructiva de la luz rectal.

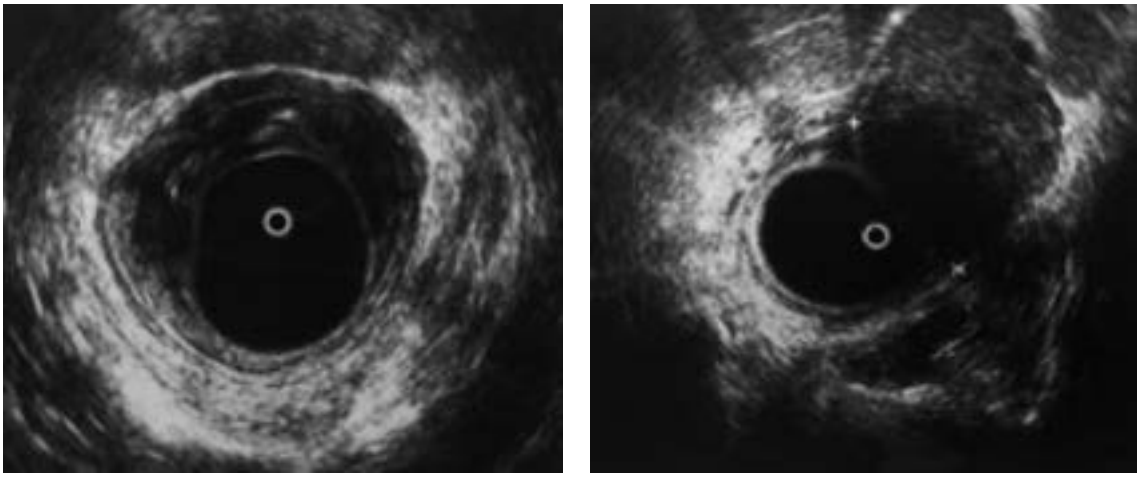


Figura 4. Ecografía endoanal: canal rectal, se aprecia tumoración submucosa uT2 a las 3-9 horas. Tumor rectal que incluye la capa muscular de la pared.

La radioterapia preoperatoria es el tratamiento inicial de elección en el caso de tumores rectales localmente avanzados, generalmente acompañada de tratamiento sistémico con quimioterapia (QT) (Glynne-Jones R, 2017; Dekker E, 2019; Keller DS, 2020). Se ha demostrado la disminución de probabilidad de recidiva local en pacientes tratados de esta forma. Los tumores de recto localmente avanzados son aquellos que se extienden más allá de la capa muscular, o aquellos en los que se demuestra la presencia de metástasis ganglionares, serían candidatos a tratamiento neoadyuvante previo a la cirugía del cáncer colorrectal (Glynne-Jones R, 2017; Dekker E, 2019; Arredondo J, 2020).

En cuanto al tratamiento quirúrgico, desde la primera intervención exitosa para el tratamiento del cáncer de recto, realizada por Lisfranc en 1826, las técnicas quirúrgicas han ido mejorando de forma progresiva, permitiendo resecciones cada vez más complejas (Breen RE, 1983). La primera cirugía que combinó abordaje perineal y abdominal fue descrita por Czerny en 1884, y modificada por Miles en 1908, para incluir la resección de los nódulos linfáticos regionales junto con una completa extirpación del recto y del ano (Graney MI, 1980; Aaron R, 2020). Pero no fue hasta 1982, cuando Heald introdujo el concepto de escisión total del mesorrecto (ETM), que incluiría la resección colorrectal junto con todo el tejido graso que rodea al recto y que contiene la grasa perirrectal, nódulos linfáticos y pequeños vasos sanguíneos implicados en la extensión tumoral (Heald RJ, 1982; Heald RJ, 1988; Heald RJ, 2017). Progresivamente, y gracias al abordaje combinado laparoscópico, se fueron realizando resecciones de recto más complejas, incluyendo resecciones ultrabajas y la creación de reservorios o “pouch” con la intención de disminuir el grado de incontinencia tras la cirugía (Parc R, 1986; Lazorthes F, 1986; Leong AF, 2000). Junto al desarrollo de estas técnicas y la realización de anastomosis cada vez más bajas, se observó un aumento en la tasa de dehiscencias o fístula anastomóticas, demostrando en varios trabajos que el factor más importante en la dehiscencia tras la resección anterior baja o ultrabaja era la distancia de la anastomosis respecto al margen anal. Como medida para solucionar

este problema se comenzaron a realizar de forma conjunta ileostomías derivativas o de protección (IP) (Jacobs M, 1991; Luján J, 2009; Matthiessen P, 2017).

La complicación más frecuente y más temida tras la cirugía del cáncer de recto es la fístula anastomótica (FA), con una incidencia del 25%. Su presencia condiciona entre el 6-30% de la mortalidad (Matthiessen P, 2017; Dekker E, 2019). Esta grave complicación no sólo tiene efectos sobre la morbilidad y la mortalidad de los pacientes, sino también sobre los resultados oncológicos, describiéndose en pacientes con FA un incremento en la recurrencia local de la enfermedad y una disminución del periodo libre de enfermedad. Se consideran factores predisponentes de FA el sexo masculino, la localización ultrabaja de la anastomosis, la quimioterapia preoperatoria, estadios tumorales avanzados, la transfusión perioperatoria y los disparos múltiples en la línea de grapado se han asociado con un riesgo aumentado de FA (Heald RJ, 2017; Dekker E, 2019). Entre las medidas adoptadas para reducir la morbimortalidad derivada de la FA, la creación de ostomías de protección ha demostrado tener efectividad, sugiriéndose, que la ausencia de una ileostomía derivativa es un factor de riesgo para la aparición de una dehiscencia sintomática después de la práctica de una ETM en pacientes con cáncer rectal (Heald RJ, 2017; Matthiessen P, 2017; Farag S, 2017).

Como parte final del tratamiento combinado del cáncer de colorrectal se administra tratamiento adyuvante. La quimioterapia adyuvante está indicada en pacientes con un estadio tumoral III-IV tras el análisis de la pieza quirúrgica. En pacientes en los que se ha realizado una ileostomía de protección, el papel de la quimioterapia adyuvante cobra protagonismo, ya que suele ser necesario retrasar el cierre de la ileostomía hasta después de finalizar el tratamiento, pudiendo esto condicionar un aumento en las complicaciones asociadas al cierre de ileostomía y la aparición de colitis por diversión (Sharma A, 2013; Bhalla A, 2015; Danielsen AK, 2017; Glynne-Jones R, 2017; Dekker E, 2019; Keane C, 2019; Arredondo J, 2020).

La primera ileostomía fue realizada por Baum en Alemania en 1879 (Hardy KJ, 1989), a partir de ese momento la ileostomía empezó a practicarse con más frecuencia sobre todo debido a la ausencia de colon en pacientes con colitis ulcerosa. En las últimas décadas, movido por los avances en la cirugía laparoscópica de recto y por las graves consecuencias de la fuga de anastomosis, el papel de la IP ha cobrado especial importancia. La IP en la cirugía del cáncer de recto ha sido un tema muy debatido, sin embargo, los estudios prospectivos randomizados que evalúan su utilidad son escasos y los datos aportados en la literatura son contradictorios (Huser N, 2008; Warschkow R, 2011; Sharma A, 2013; Matthiessen P, 2017; Farag S, 2017). En un estudio prospectivo multicéntrico observacional, el "Working Group Colon/Rectum Carcinoma" (WGCRC) demostró que la incidencia de FA que requería reintervención quirúrgica fue significativamente menor en pacientes portadores de una IP tras una RAB frente a los pacientes sin IP (Marusch F, 2002). Metaanálisis recientes apoyan estos resultados, demostrando que la IP disminuye la incidencia de FA y la tasa de reintervenciones (Chen J, 2012; Tan WS, 2019; Qiu XY, 2021). A pesar de los beneficios demostrados por la IP, su utilización sistemática no cuenta con una total aceptación, ya que el cierre de ileostomía representa una cirugía adicional, que requiere un ingreso hospitalario y que no está exenta de complicaciones y mortalidad (Sharma A, 2013). Además ser portador de una IP representa para el paciente una serie de complicaciones en su manejo diario y una clara disminución de la calidad de vida hasta que se produce el cierre de la misma. Por todo ello parece indicado individualizar la realización de una IP en función de las características del paciente y de los factores de riesgo de desarrollar una FA. Se recomienda la creación de una IP tras una RAB en tumores de localización baja con anastomosis a menos de 5 cm del margen anal, complicaciones durante la realización de la anastomosis, disección dificultosa del recto en varones tras una ETM, pacientes que ha recibido tratamiento con quimioradioterapia neoadyuvante, cirugía de urgencias, tiempo operatorio prolongado

y pacientes con mala situación basal (Glynne-Jones R, 2017; Keller DS, 2020; Arredondo J, 2020; Qiu XY, 2021).

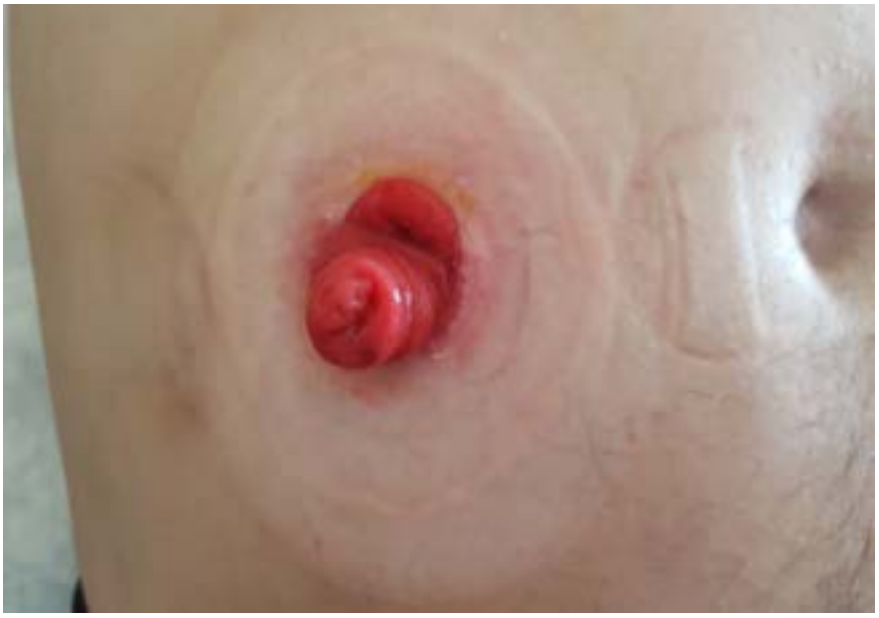


Figura 5. Ileostomía de protección en paciente intervenido de carcinoma colorrectal. Se aprecia tetón prolapsado en región caudal característico del cabo proximal o asa aferente y tetón invaginado en región craneal característico del cabo distal o asa eferente. El asa aferente presenta debito intestinal y el asa eferente es por donde se realiza la estimulación preoperatoria.

Por otro lado, se ha demostrado que la creación de un estoma temporal provoca una disbacteriosis y una atrofia vellositaria en el segmento de colon excluido, provocando un deterioro de la capacidad de absorción y contracción luminal, con pérdida de contractilidad y motilidad (Williams L, 2007; Beamish EL, 2017). Williams estudió los cambios intestinales producidos tras la creación de una ileostomía derivativa en humanos, demostrando mediante un exhaustivo estudio histológico una significativa pérdida de contractilidad de la capa muscular así como una atrofia de las

vellosidades intestinales (Williams L, 2007). Para evitar esta complicación se han propuesto dos líneas de actuación; el cierre precoz de la ileostomía de protección, que sería la mejor opción, o en su defecto, un cierre tardío previa estimulación del asa eferente (Keane C, 2019). Existen múltiples publicaciones sobre la estimulación del asa eferente previa al cierre del estoma. Todas presentan diferentes protocolos de actuación y diferentes sustancias administradas, desde líquido fecal autólogo a suero fisiológico con espesantes, pasando por ácidos grasos de cadena corta, dieta líquida y sacarosa (Harig JM, 1989; Rombey T, 2019).

La estimulación del asa eferente previa a la reconstrucción del tránsito, la idea nace tras plantear la hipótesis de que la aparición de íleo postoperatorio se debe a los cambios estructurales y funcionales que tienen lugar en el intestino desfuncionalizado, como la atrofia muscular y vellositaria o la disminución de la capacidad de absorción (Williams L, 2007; Abrisqueta J, 2014). Este intestino delgado distal y colon funcionalmente no preparados para reestablecer el tránsito provoca una parálisis intestinal. Al respecto se han publicado varios artículos (Menéndez P, 2013; Garfinkle R, 2017; Vázquez-Melero A, 2018; Fernández López F, 2019), pero la primera revisión sistemática fue publicada por Rombey y col**.** (Rombey T, 2019), incluyendo 8 estudios con un total de 267 pacientes. En ella, a pesar de los prometedores resultados iniciales, no existen pruebas de calidad suficiente como para recomendar la implementación rutinaria de la estimulación intestinal preoperatoria en la práctica clínica. Esto se debe a la heterogeneidad de los estudios. En su mayoría son estudios con bajo poder estadístico y con resultados difíciles de comparar entre sí, ya que algunos comparan pacientes sometidos a proctocolectomía con anastomosis ileoanal en J y otros pacientes con resección anterior baja, sólo uno fue aleatorizado, tres eran casos clínicos únicos y había diversidad en cuanto a la sustancia usadas durante la estimulación y duración de la misma.

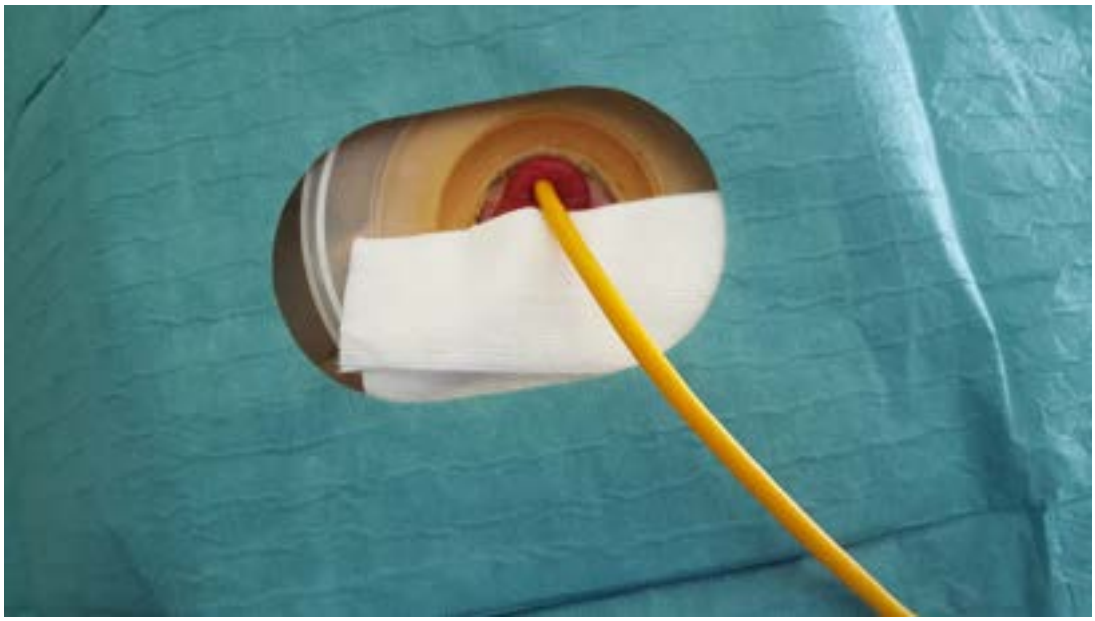


Figura 6. Estimulación preoperatoria del asa eferente previa a la cirugía de reconstrucción. Canalizamos mediante sonda de Foley de 14 Fr el extremo distal o asa eferente, generalmente locaclizado en la región craneal.

Por todo lo anterior, decimos que la reconstrucción del tránsito intestinal no está exenta de morbilidad (Matthiessen P, 2017). Entorno al 18-40% presentarán complicaciones tales como colitis por diversión, obstrucción de intestino delgado, infección de la herida quirúrgica, íleo postoperatorio, fuga anastomótica, fístula, perforación, absceso, hemorragia o eventración (Sharma A, 2013; Bhalla A, 2015; Keane C, 2019). De todas ellas, la complicación más frecuente sigue siendo el íleo postoperatorio, con una incidencia 13-20% (Garfinkle R, 2019). Esto implica un importante impacto socioeconómico, con afectación de la calidad de vida del paciente (mayor malestar, aumento del riesgo de infecciones nosocomiales y de morbilidad asociada a la cirugía, y un aumento de la mortalidad postoperatoria), condicionando todo ello una estancia hospitalaria prolongada y un mayor gasto sanitario (Danielsen AK, 2017).

1.2. Colitis por diversión

La colitis por diversión (CD) es la inflamación producida en un segmento de colon desfuncionalizado tras la realización de un estoma temporal (Szczepkowski M, 2017; Sartor RB, 2017). Descrita por Glotzer et al. en 1981 (Glotzer DJ, 1981), se caracteriza por una inflamación crónica de la mucosa del intestino grueso que puede simular una enfermedad inflamatoria intestinal (Basso PJ, 2018; Cohen LJ, 2019), con cambios macroscópicos y microscópicos característicos asociados a la disminución de las bacterias en el área desfuncionalizada. El 52% de los pacientes con CD presentan formas leves, el 44% moderada y el 4% inflamación severa. Su prevalencia oscila en torno al 95-100%, con una incidencia anual cercana a 120000 pacientes/año, representando un problema clínico frecuente con un importante deterioro significativo en la calidad de vida de los pacientes afectos (Kabir SI, 2014).

En cuanto a su patogénesis existen varias teorías. Se ha planteado su asociación a un sobrecrecimiento excesivo de bacterias, presencia de determinadas cepas bacterianas, déficit nutricional, aumento de toxinas o la alteración de la relación simbiótica entre bacterias luminales y la capa mucosa del colon (Kabir SI, 2014; Szczepkowski M, 2017). Existen estudios que relacionan la reducción de las concentraciones de carbohidratos fermentadores de bacterias anaerobias y bacterias patógenas en el colon excluido con un aumento de bacterias reductoras de nitratos (Beamish EL, 2017). Éstas están involucradas en la producción de óxido nitroso (NO), que en concentraciones altas es tóxico para la mucosa colónica, originando la colitis por derivación. Otra teoría se apoya en la isquemia asociada al aumento de la resistencia arteriolar provocada por disminución de los ácidos grasos de cadena corta (Williams L, 2007; Szczepkowski M, 2017). También la disminución de la capa de mucina de colon excluido conlleva a la disminución de la barrera entre la luz y la submucosa, que se convierte en una capa delgada y anormal en condiciones

inflamatorias. Finalmente se ha relacionado con metabolitos bacterianos como el butilo, aumentando la respuesta inmunitaria e inflamatoria del huésped; sin embargo el papel de la inmunidad en la patogenia de la colitis por derivación no ha sido aún investigado (Kabir SI, 2014; Szczepkowski M, 2017; Morgan DM, 2020).

La sintomatología suele comenzar entre el primer mes y el tercer año tras la creación de un estoma derivativo. Puede manifestarse como dolor abdominal o pélvico (75%), descarga mucosa (40%), tenesmo (15%), y fiebre, distensión y sangrado rectal en los casos más graves (3%); aunque hasta un 30% de los pacientes pueden permanecer asintomáticos. Los síntomas se resuelven tras la cirugía de reconstrucción del tránsito intestinal, consiguiendo la restauración completa de la arquitectura colónica entre los 3 y los 6 meses en la mayoría de los pacientes. Tras el cierre del estoma derivativo, puede persistir síntomas como el estreñimiento o la diarrea, distensión abdominal, dolor cólico y dispepsia leve. Diversos estudios asocian estos síntomas con la atrofia vellositaria, la disminución de la motilidad y el adelgazamiento de la muscularis del intestino excluido (Kim KH, 2011; Son DN, 2013; Kabir SI, 2014; Szczepkowski M, 2017).

La colitis por diversión se diagnostica en base a la historia clínica, la sintomatología y los hallazgos endoscópicos e histológicos. Los cambios macroscópicos de la CD pueden involucrar a segmentos aislados o al colon excluido por completo. Estos hallazgos incluyen mucosa friable (90%), eritema (80%), edema (60%), estenosis (35%) aparición de pólipos (20%) y úlceras (10%) (Kabir SI, 2014; Szczepkowski M, 2017). El hallazgo microscópico más característico es la hiperplasia folicular linfoide, que suele acompañarse de infiltración de la lámina propia por linfocitos, eosinófilos, aparición de células plasmáticas, desestructuración de la arquitectura y aparición de abscesos en las criptas (Harig JM, 1989; Komorowski RA, 1990). Sin embargo, estas características histológicas no son específicas y hasta la fecha no se han detectado hallazgos patognomónicos de CD; por lo que, para llegar al diagnóstico, debemos de reunir

tanto alteraciones macroscópicas como microscópicas en pacientes con antecedentes de creación de un estoma derivativo (Kabir SI, 2014; Szczepkowski M, 2017; Knox NC, 2019; Morgan DM, 2020).

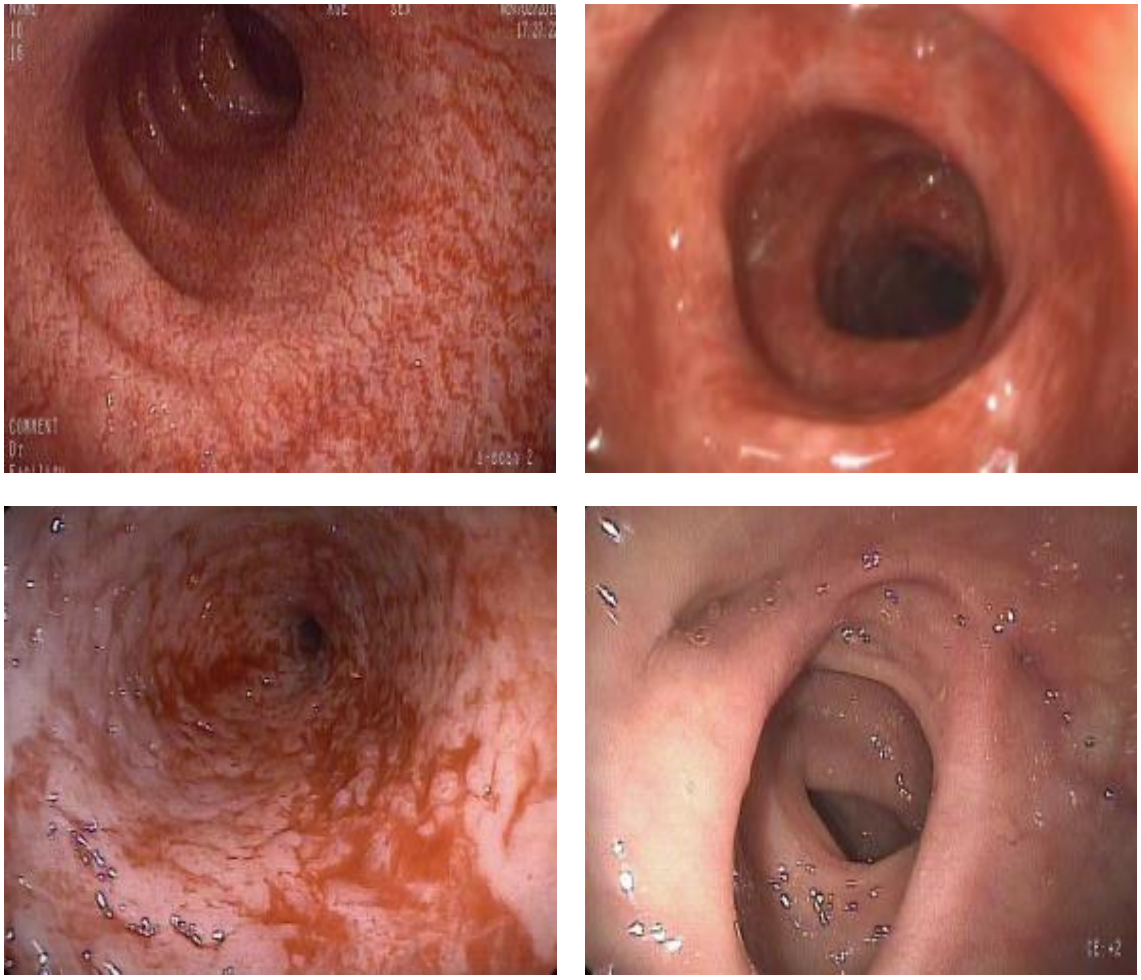


Figura7. Cambios macroscópicos asociados a colitis por diversión en pacientes intervenidos de carcinoma colorrectal. Mediante colonoscopia se aprecia una mucosa friable al roce, con importante edema y eritema. También podemos encontrar pólipos, estenosis inflamatoria y en estadíos avanzados úlceras.

No existe abundante bibliografía sobre la colitis por diversión y su relación con la alteración microbiota. En los dos últimos años se han publicados dos estudios sobre el microbioma intestinal, Mishima (Mishima, 2020) y Knox (Knox NC, 2019); a los que hay que sumar, por un lado la última revisión sistemática publicada sobre la colitis por diversión por Kabir y col. (Kabir, 2014) que revisa un total de 3305 artículos, incluyendo finalmente 35 estudios, que analiza la fisiopatología, presentación clínica y tratamiento de la colitis por diversión que concluye que hay una gran variabilidad de formas de presentación y un único tratamiento definitivo que es la reconstrucción del tránsito.

El tratamiento definitivo de la CD es el restablecimiento del tránsito intestinal, que restaura el flujo luminal, resolviendo de forma progresiva las lesiones asociadas al proceso inflamatorio crónica (Kabir SI, 2014). Szczepkowski propuso una estrategia de manejo de la CD basada en la clasificación de la severidad según hallazgos endoscópicos e histológicos (Szczepkowski M, 2017):

- Grupo 1: sin evidencia clínica, morfológica o endoscópica de colitis por diversión. Subsidiario de tratamiento conservador.

- Grupo 2: signos leves o moderados de CD. Candidatos a tratamiento conservador de inicio y posteriormente tratamiento quirúrgico.

- Grupo 3: CD severa. Candidatos a tratamiento quirúrgico.

En aquellos pacientes no candidatos a cirugía de reconstrucción del tránsito se han estudiado diversos tratamientos farmacológicos con resultados variables, en su mayoría basados en los estudios preeliminares de Harig (Harig JM, 1989), con un tamaño muestral reducido y con limitaciones en la duración del tratamiento y seguimiento. Se han probado irrigaciones con ácidos grasos de cadena corta, fibra, mesalazina y corticoides, bien como tratamiento alternativo o como tratamiento para estimulación del asa eferente previa a la cirugía (Guillemot F, 1991; Schauber J, 2000; Pacheco RG, 2012; Martinez CA, 2013; Alvarenga V, 2014; Buanaim RP, 2019; Keane C,

2019; Rombey T, 2019), Nuestro estudio es el primero en realizar la estimulación con probióticos a través del asa eferente de la IP para aprovechar los beneficios antiinflamatorios y reforzar la barrera intestinal en el segmento de colon desfuncionalizado, consiguiendo la reducción de la CD y mejoría de la sintomatología asociada (Zhao-hua Shen, 2018).

Basándonos en estudios como el publicado por Morgan et al. (Morgan, 2020), que demuestra el papel fundamental del microbioma intestinal en el desarrollo de la colitis microscópica y la regresión de la inflamación tras la reconstrucción del tránsito, nos planteamos que la manipulación microbiana mediante la estimulación de probióticos previa al cierre de la ileostomía de protección permitiría normalizar la función autoinmune alterada, restaurar la homeostasis y disminuir la inflamación de la mucosa consiguiendo la disminución o la desaparición de los cambios macroscópicos y microscópicos asociados a ésta. Esta hipótesis inicial parece correlacionarse con los resultados obtenidos, consiguiendo una marcada reducción de los característicos hallazgos endoscópicos e histológicos de la colitis por diversión en el GE, dónde se aprecia una significativa disminución en el GE frente al GC tras la estimulación del asa eferente previa al cierre de la ileostomía de protección. Esta reducción de los hallazgos endoscópicos e histológicos, ya se había buscado en estudios previos, en modelos experimentales y estudios fase III, mediante la estimulación con diversas sustancias como soluciones nutricionales, trasplante fecal, enemas de ácidos grasos de cadena corta (SCFA) como el butirato, ácido 5 aminosalicilato (5-ASA), glucocorticoides, sucralfato y N-acetilcisteína entre otros con diferentes resultados (Harig 1989; Guillemot F, 1991; Schauber J, 2000; Williams L, 2007; Pacheco RG, 2012; Martinez CA, 2013; Alvarenga V, 2014; Kabir SI, 2014; Szczepkowski M, 2017; Beamish EL, 2017; Kleinwort A, 2017; Buanaim RP, 2019). En su mayoría mostraron resultados incosistentes, aunque parece existir una disminución de la inflamación de la mucosa

intestinal, reduciendo las lesiones epiteliales y la infiltración de neutrófilos de forma generalizada.

Por otro lado, en ninguno de estos estudios se mencionan si estos pacientes o animales de experimentación presentan de forma concomitante elevación de los biomarcadores inflamatorios. Estas alteraciones serológicas sí han sido descritas y estudiadas en patologías como la enfermedad inflamatoria intestinal, buscando la corrección de estos parámetros inflamatorios asociados a la disbiosis con resultados también variables (Kabir SI, 2014; Williams L, 2007; Szczepkowski M, 2017). Por ello, nuestros resultados son esperanzadores, ya que demuestran una reducción de los parámetros inflamatorios previa a la reconstrucción del tránsito, por lo que puede ser una alternativa para aquellos pacientes que no son candidatos al tratamiento quirúrgico.

1.3. Probióticos

Los probióticos se definen como microorganismos vivos que administrados en la dosis adecuada confieren un beneficio para la salud del huésped (Hill C, 2014; Biagioli M, 2019). El término probiótico fue introducido en 1953 por el alemán Werner Kollath y significa "sustancia activa esencial para una vida sana" (Liu Y, 2018). Los probióticos se están aplicando cada vez con una mayor frecuencia en la patología gastrointestinal. Son bacterias vivas no patógenas como Lactobacillus, Bifidobacterium y Enterococcus, que presentan actividad antimicrobiana, inmunomoduladora y mejoran la barrera intestinal (Rossi M, 2010; Beamish EL, 2017; Morgan DM, 2020). Éstos y sus productos metabólicos se han propuesto como suplementos alimenticios para conseguir una homeostasis intestinal más saludable y también como tratamiento en patologías con importante componente inflamatorio huésped (Corridoni D, 2012; Hill C, 2014).

Los probióticos se han utilizado para mejorar los síntomas gastrointestinales desde la antigüedad. Existen datos del consumo de leche fermentada en Oriente Medio desde el 10000 a. de C. (Liu Y, 2018). Alrededor del año 8000 a. de C., nómadas tibetanos que poblaban altitudes de más de 4000 metros, mantuvieron buena salud a pesar de la ausencia de frutas y verduras en su dieta por el consumo de leche fermentada de yak y productos derivados de ésta (Guo X, 2014). Unos 250 ml de leche fermentada de yak al día podían proporcionarles más de 200 mil millones de lactobacillus, fundamentalmente L. fermentum y L.casei; con propiedades antiinflamatorias y eliminación de radicales libres (McFarland LV, 2015; Gasbarrini G, 2016). Existen también jeroglíficos egipcios datados del año 4000 a. de C. en los que se consumía leche fermentada para mejorar la salud (Liu Y, 2018). Del mismo modo, en la antigua Grecia y Roma, alrededor del año 400 a. de C., existía un condimento llamado garum, derivado del intestino del pescado, que era (y todavía es) fermentado durante 12-18 meses en vasijas de barro, consumiéndose a diario, con poderosas propiedades antioxidantes e importante beneficio para la salud. Los turcos nómadas usaban el "yogurmak" para tratar la diarrea, los calambres y a piel quemada por el sol, como lo demuestran escritos del siglo XI; y más tarde, Gengis Kan, el gran conquistador mongol, alimentaba a su ejército con "su yogur militar" que les infundía valentía en la batalla (Fisberg M, 2015).

Pero no es hasta el siglo XX, cuando comienza la inquietud por estos microorganismos y su interés por aislarlos y conocer verdaderamente sus efectos beneficiosos (Liu Y, 2018). En 1905, Elie Metchnikoff, buscó la respuesta a la longevidad de los búlgaros, relacionándola con el consumo excesivo de yogur fermentado y desmostando posteriormente que se podía cultivar un bacilo a partir de este yogur, que era idéntico al bacilo encontrado en sus heces llamado L. bulgaricus Gasbarrini G, 2016). Al mismo tiempo, Henry Tissler en París, aisló un organismo con forma de Y que llamó Bifidobacterium, capaz de neutralizar bacterias patógenas in

vitro. Todo ello contribuyó al inicio de la investigación en la década de 1940 de las bacterias patógenas y desarrollo de las terapias antimicrobianas; y posteriormente, en 1950, a la identificación de bacterias con propiedades probióticas que proporcionen resistencia a la colonización por estos patógenos (McFarland LV, 2015; Liu Y, 2018). La investigación comenzó a centrarse en bacterias del género Lactobacillus y Bifidobacterium en el combate de las enfermedades diarreicas, concluyendo que pueden ayudar a prevenir y tratar infecciones virales, salmonelosis, shigelosis, cólera y facilitar la cicatrización en pacientes con úlcera péptica (Liu Y, 2018).

Durante los últimos 40 años se ha demostrado que los probióticos afectan al sistema inmunológico, tanto in vivo como in vitro. Esta interacción está relacionada con los microorganismos intestinales, sus antígenos polisacáridos y los metabolitos producidos por estas bacterias. Varias vías metabólicas se han implicado en estos mecanismos de acción: producción y señalización de ácidos grasos de cadena corta, metabolismo del triptófano, activación de los receptores de hidrocarburos, señalización de nucleósidos en el intestino y la activación de los receptores intestinales de histamina entre otros (Liu Y, 2018). En estos mecanismos de acción se han basado múltiples estudios sobre el uso de probióticos y su efecto sobre la flora microbiota aplicados a diferentes patologías como la diabetes mellitus, obesidad, enfermedad inflamatoria intestinal, colitis e incluso alteraciones psicológicas (Rajkumar H, 2014; Kumar M, 2016; Zhang Y, 2016; Zhao-hua Shen, 2018; Baldassarre ME, 2018; Callaway LK, 2019; Michima Y, 2020; Amoroso C, 2020). En ellos se evalúan in vitro e in vivo el efecto modulador de la inflamación dependiente de las cepas incluidas en los mismos, con diferentes propiedades bioquímicas e inmunológicas (Marteau P, 2003). Estos efectos biológicos están íntimamente relacionados con el metabolismo microbiota y la capacidad de colonizar el tracto gastrointestinal (Baldassarre ME, 2018). Los probióticos interactúan con la mucosa intestinal disminuyendo la producción molecular de sustancias proinflamatorias (Biagioli M, 2019). Actualmente los

probióticos disponibles dirigidos a otras patologías con afectación inflamatoria ejercen un efecto modulador de forma transitoria y limitada (Sartor RB, 2017; Basso PJ, 2018; Cohen LJ, 2019).

El microbioma intestinal se compone de bacterias que residen en el intestino y puede ser alterado por la dieta, el estilo de vida, la exposición a toxinas y el uso de antibióticos. Existe relación entre la enfermedad, la salud, la inmunidad sistémica y los cambios en el microbioma. La mayoría de estas bacterias viven en la superficie de la mucosa intestinal, adheridas a la superficie de las células epiteliales del intestino formando un biofilm o capa bacteriana implicada en el metabolismo intestinal de los nutrientes, permeabilidad y función del sistema inmunológico (Zhao-hua Shen, 2018). La evidencia publicada refuerza el papel de la microflora intestinal en patologías como la colitis asociada a antibióticos, enteritis inducida por quimioterapia, enteritis por radiación, síndrome de dificultad respiratoria del adulto, síndrome de intestino irritable, colitis ulcerosa y eficacia de la inmunoterapia antitumoral entre otras patologías (Hill C, 2014; Guarner F, 2017; Wilkins T, 2017).

Los probióticos promueven la secreción de factores antiinflamatorios, mejorando la barrera de la mucosa intestinal y la función del sistema inmunológico, así como inhibir el crecimiento de bacterias dañinas en el intestino. Los patógenos pueden debilitar la barrera de la mucosa intestinal, dañarla y debilitarla (Michima Y, 2020; Amoroso C, 2020). Los probióticos pueden prevenir o reparar estos daños. Los principales mecanismos de acción de los probióticos incluyen competir con estas bacterias patógenas por los receptores de adhesión tisular y los nutrientes, manteniendo el equilibrio de la flora microbiótica intestinal normal, mejorando la función barrera de la mucosa intestinal, promoviendo la inmunidad e interfiriendo con la respuesta inflamatoria intestinal y la inhibición de la apoptosis de las células epiteliales (Zhang Y, 2016; Louis P, 2017). A pesar del aumento de datos, no existe consenso ni directrices claras sobre el uso de probióticos, prebióticos o simbióticos, así

como su dosis y duración del tratamiento. Sabemos que son seguros para bebés, niños, adultos y pacientes mayores, pero deben ser administrados con cuidado en poblaciones con vulnerabilidad inmunológica. Están disponibles como suplemento alimenticio y dietético, requeridos por la Agencia Española de Seguridad Alimentaria y Nutrición (Wilkins T, 2017).

Los probióticos contienen microorganismos, la mayoría de los cuales son bacterias similares a las bacterias beneficiosas que se encuentran naturalmente en el intestino humano (Beamish EL, 2017). Se ha descubierto que ejercen su efecto en todas las capas del intestino, desde la serosa a la luminal, pasando por el epitelio, la lámina propia, la muscular y la submucosa; ejerciendo su efecto en el microbioma, la barrera mucosa, células del epitelio, linfocitos y células plasmáticas de la lámina propia, elementos neurales y vasculares, motilidad del músculo liso y ganglios linfáticos mesentéricos como comunicación con el sistema inmunológico sistémico (Guillemot F, 1991; Schauber J, 2000; Williams L, 2007; Pacheco RG, 2012). Los metabolitos locales y sistémicos modulados por los probióticos son capaces de producir AGCC en el colon, relacionados con la regulación de moléculas antiinflamatorios y principal sustrato energético (Guillemot F, 1991). Específicamente el acetato, el propionato y el butirato, son producidos tanto por probióticos como por bacterias comensales como Facecalibacterium prausnitizii, Eubacterium rectale, Eubacterium hallii y Ruminococcus bromii (Louis P, 2017). La producción de AGCC tiene lugar a través de la fermentación, principalmente de acetato y lactato. El butirato protege la integridad del epitelio intestinal, promueve la respuesta inmune del intestino, inhibe el crecimiento de células tumorales y protege la pared intestinal reduciendo la inflamación. El ácido láctico producido por las cepas de Lactobacilos, y en menor medida por Bifidobacterias, es un regulador negativo de mediadores proinflamatorios tales como metaloproteasas, TNF alfa y monocitos, a la vez que inducen la expresión de antinflamatorios como la IL-17, IL-22, IL-23 y EGR 2, aumentando las células CD4 y

macrófagos en la lámina propia del colon (Corridoni D, 2012; Biagioli M, 2019). Estos efectos se deben a la capacidad de los probióticos de interactuar con la mucosa intestinal disminuyendo la producción molecular de sustancias proinflamatorias (Szczepkowski M, 2017), y con ello disminuyendo la capacidad de migración de células inflamatorias a la lámina propia, tales como linfocitos, eosinófilos y células plasmáticas (Guarner F, 2017; Lopes RCSO, 2018).

Las bacterias probióticas mas estudiadas pertenecen al género Bifidobacterium, Lactobacillus y Saccharomyces spp. Éstas disminuyen moléculas proinflamatorias y aumentan moléculas que inhiben la inflamación, protegiendo además contra el estrés oxidativo en humanos y demostrando un papel importante sobre la disbiosis intestinal (Rossi M, 2010; Beamish EL, 2017; Sartor RB, 2017). Actualmente la evidencia de la que disponemos nos demuestra que los probióticos tienen un efecto positivo sobre sobre la inflamación sistémica y oxidativa, sobre todo a nivel gastrointestinal. L. acidophilus, uno de los probióticos administrados durante nuestra estimulación, he demostrado su papel en la regulación inflamatoria en múltiples estudios experimentales (Rossi M, 2010; Yang YJ, 2012; Borthakur A, 2013; Stenman LK, 2014; Li H, 2016; Biagioli M, 2017, Hod K, 2017, Hajifaraji M, 2018; Tenorio-Jiménez C, 2019; Buanaim RP, 2019). Un ensayo clínico controlado aleatorizado realizado por Jafarnejad et al. (Jafarnejad S, 2016). Describe el efecto de la suplementación con probióticos, en este caso, con VSL3, compuesto probiótico similar al administrado en nuestro estudio, durante 8 semanas, sobre el estado glucémico y los marcadores inflamatorios entre mujeres embarazadas. Los suplementos probióticos contenían ocho cepas de bacterias del ácido láctico (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L.acidophilus, L.plantarum, L.paracasei y L. delbrueckii subsp. Bulgaricus). Encontraron una disminución estadísticamente significativa en los niveles de TNF alpha y PCR en el grupo de los probióticos en comparación con el placebo. Resultados superponibles a otros estudios (Hajifaraji M, 2018). Otro ensayo clínico publicado

posteriormente, donde se evalúa la eficacia de los probióticos VSL3 administrados durante 8 semanas en 24 pacientes con Síndrome de Intestino Irritable, encontrando una mejora significativa de la sintomatología, pero sin diferencias significativas entre los grupos (Michail S, 2019).

Además del efecto de los probióticos sobre los procesos inflamatorios, existe evidencia de su efecto sobre el dolor abdominal. Un metaanálisis con 23 ensayos clínicos en los que participaban niños y adultos demostró que los probióticos mejoraban de forma significativa los síntomas globales, la hinchazón y la flatulencia en comparación con el grupo placebo (Ford AC, 2014). Y otro metaanálisis con 21 ensayos clínico encontró en el síndrome de intestino irritable una mejoría significativa de los síntomas y la calidad de vida en comparación con el grupo placebo (Zhang Y, 2016). Pero aunque se han investigado los efectos de los probióticos in vitro, en modelo animal y humano, todavía hoy se sabe poco sobre la interacción exacta entre la suplementación probiótica y los cambios en la microbiota intestinal, así como su efecto sobre las transformaciones neoplásicas de la mucosa gastrointestinal. Por ello la mayoría de autores concluyen que los probióticos y sus efectos suponen un reto científico y un camino aún por descubrir (Wilkins T, 2017).

Basándonos en los cambios endoscópicos e histológicos asociados a la distorsión producida en el segmento de colon obstruido, nos planteamos que la repoblación de estas bacterias podría disminuir o resolver la colitis por diversión, y por consiguiente, la sintomatología asociada y la disminución en la calidad de vida de estos pacientes. Pudiendo ser, de confirmarse nuestra hipótesis, una alternativa para aquellos pacientes que por morbilidad o negativa, no son candidatos a cirugía de reconstrucción del tránsito. En este sentido, esta tesis doctoral, en convergencia con el proyecto de investigación, tiene como objetivo analizar la eficacia y seguridad de la estimulación del asa eferente con probióticos previa al cierre de la ileostomía de protección en

pacientes intervenidos de carcinoma colorrectal y los cambios histológicos, endoscópicos y serológicos en pacientes con colitis por diversión.

Hipótesis y objetivos

2.1. Hipótesis

La hipótesis principal de nuestro trabajo es que la estimulación preoperatoria del intestino con probióticos a través del asa eferente de la ileostomía en pacientes intervenidos de carcinoma colorrectal disminuye la colitis por diversión.

2.2. Objetivo general

Evaluar el efecto de los probióticos sobre la colitis por diversión o colitis por derivación tras la estimulación del asa eferente de la ileostomía de protección en pacientes intervenidos de carcinoma colorrectal previo a la cirugía de reconstrucción del tránsito intestinal.

2.3. Objetivos específicos

- Evaluar la eficacia y seguridad de la estimulación del asa eferente con probióticos previa al cierre de la ileostomía de protección en pacientes intervenidos de carcinoma colorrectal

- Evaluar los cambios macroscópicos y microscópicos asociados a la estimulación del asa eferente de la ileostomía de protección con probióticos, valorando las alteraciones endoscópicas e histológicas asociadas a la colitis por diversión y su disminución o desaparición en el grupo estimulados con probióticos frente al grupo control

- Evaluar el efecto de la estimulación con probióticos sobre el grado de colitis por diversión histológico y endoscópico

- Evaluar la persistencia de la clínica de colitis por diversión tras la cirugía de reconstrucción del tránsito, al mes y durante el seguimiento a corto plazo

- Evaluar los efectos de la estimulación con probióticos sobre la emisión de gas y heces tanto en la fase prequirúrgica como postquirúrgica y durante el seguimiento a corto plazo.

- Evaluar la incidencia de íleo paralítico postoperatorio, inicio de la tolerancia oral, presencia de deposiciones y emisión de gas, restablecimiento del tránsito intestinal, complicaciones postquirúrgicas y estancia hospitalaria entre pacientes estimulados con probióticos y pacientes del grupo control

- Evaluar los cambios serológicos asociados a la estimulación del asa eferente de la ileostomía de protección con probióticos, evaluando la correlación entre colitis por diversión, histológica y endoscópica, y la alteración de los biomarcadores proinflamatorios en sangre.

Metodología de investigación

3.1. Diseño del estudio

Diseñamos un estudio experimental, prospectivo, aleatorizado, multicéntrico, doble ciego, comparando dos grupos de pacientes intervenidos de carcinoma colorrectal libres de enfermedad y portadores de ileostomía de protección. Un grupo de intervención formado por pacientes sometidos a estimulación del asa eferente con probióticos previo a la cirugía de reconstrucción del tránsito, y un grupo control sometidos a estimulación sin administrar ninguna sustancia.

3.2. Selección de pacientes

Entre enero 2017 y diciembre de 2018, se evaluaron de forma consecutiva todos los pacientes de los centros participantes incluidos en lista de espera quirúrgica para cierre de estoma temporal tras carcinoma colorrectal (codificados según CIE-10), para determinar su inclusión en el estudio. En el Anexo 7 podemos ver el Diagrama de flujo de selección de pacientes del estudio.

Los criterios de inclusión fueron: tener más de 18 años, ser portadores de ileostomía de protección tras intervención de carcinoma colorrectal libre de enfermedad, con confirmación endoscópica e histológica de colitis por diversión y firma del consentimiento informado. Como criterios de exclusión: ser menor de 18 años, historia clínica y confirmación histológica de enfermedad inflamatoria intestinal con afectación colorrectal y negativa a participar en el estudio.

Los criterios de abandono: pérdida durante el seguimiento, exitus y dehiscencia de anastomosis tras cirugía de reconstrucción del tránsito.

3.3. Tamaño muestral

El tamaño muestral se calculó de acuerdo con la incidencia de colitis por diversión publicada en revisiones sistemáticas, buscando una supuesta reducción del 50% (100-50%). Con un ajuste de pérdida del 15%, fue necesario tener 30 pacientes por grupo. Reclutamos 34 pacientes en el grupo estimulado y 35 pacientes en el grupo control para un nivel estadístico del 95% y una potencia de 0,8.

3.4. Examen endoscópico, histológico y determinación serológica

Se realizó una colonoscopia con toma de biopsias para estudio histológico a todos los pacientes candidatos a participar para excluir aquellos que no tuvieran colitis por derivación y establecer el grado de la misma. Las endoscopias fueron realizadas únicamente por tres especialistas con un colonoscopio estándar serie Silver Scope de Storz®. Los hallazgos endoscópicos fueron cuantificados según el sistema de puntuación de Harig (Harig JM, 1989), valorando la aparición de mucosa friable, edema, eritema, presencia de pólipos, úlceras y estenosis. Estos hallazgos fueron puntuados en una escala de 0 a 1 o de 0 a 3, obteniendo el grado de colitis mediante su suma. El grado de colitis por diversión fue establecido desde ausencia a leve, moderado o severo. La colonoscopia con toma de biopsias se repitió tras la estimulación y a los 3 meses de la reconstrucción del tránsito.

El estudio anatomopatológico se realizó con las biopsias obtenidas mediante colonoscopia. La evaluación de las muestras fue realizada únicamente por tres anatomopatólogos. Del mismo modo que en la endoscopia se diagnosticó la colitis por diversión valorando la aparición de hiperplasia folicular linfoide, infiltración de la lámina propia por linfocitos, eosinófilos, células plasmáticas, desestructuración de la arquitectura y abscesos en las criptas. Estos hallazgos fueron puntuados en una escala de 0 a 1 o de 0 a 3, obteniendo el grado de colitis mediante su suma. El grado

histológico de colitis por diversión fue establecido desde ausencia a leve, moderado o severo. El estudio anatomopatológico se repitió tras la estimulación y a los 3 meses de la reconstrucción del tránsito.

Se realizó extracción sanguínea tras firma del consentimiento informado del estudio. Se determinó PCR y albúmina sérica basales para establecer el mGPS según tabla1. Como apoyo para establecer el IRB se realizó un recuento de glóbulos blancos y determinación de transferrina en un recuento sanguíneo de rutina en el laboratorio de cada hospital, estableciendo los valores de corte en función de curvas ROC. Estas determinaciones se volvieron a realizar tras completar la fase de estimulación, de forma preoperatoria, y posteriormente al finalizar la fase de seguimiento a los 3 meses de la cirugía, correlacionando los valores pre y postoperatorios con las alteraciones endoscópicas e histológicas y persistencia o no de colitis por derivación.

3.5. Aleatorización e intervención

Se realizó una colonoscopia con toma de biopsias para estudio histológico a todos los pacientes candidatos a participar en el estudio. Tras confirmar el diagnóstico, grado de colitis (basado en el sistema de puntuación de Harig), determinación de biomarcadores séricos y excluir los pacientes que no cumplían criterios, se realizó la aleatorización. Los pacientes fueron asignados al azar mediante el uso de una secuencia generada mediante computadora (software estadístico EPIDAT versión 4.2. Consellería de Sanidade, Xunta de Galicia, España; Organización Panamericana de la Salud (OPS-OMS); Universidad CES, Colombia) estableciendo 2 grupos:

- **Grupo estimulación (GE):** se realizó la estimulación preoperatoria del asa eferente con probióticos durante los 20 días previos a la intervención quirúrgica en días alternos. Durante el proceso y tras el mismo el propio paciente registra la aparición de síntomas tras cada sesión de estimulación:

dolor abdominal, emisión de gas y heces. Se introdujo a través del cabo desfuncionalizado una sonda estéril de Foley de 14 Ch conectado a un sistema de suero para permitir la infusión lenta, durante 20-30 minutos, de una solución con 4.5mg de probióticos diluidos en 250ml de suero fisiológico 0.9% (Figura 2). Cada preparado se realiza en condiciones de esterilidad y manteniendo la cadena de frío. Vivomixx®, comercializado por MENDES, S.A., contiene 450 millones de bacterias vivas liofilizadas en cada preparación, incluyendo:

- Cuatro cadenas de Lactobacillus:
  - Lactobacillus acidophilus DSM 24735®
  - Lactobacillus plantarum DSM 24730®
  - Lactobacillus paracasei DSM 24733®
  - Lactobacillus delbrueckii subsp. bulgaricus DSM 24734®
- Tres cadenas de Bifidobacterium:
  - Bifidobacterium breve DSM 24732®
  - Bifidobacterium longum DSM 24736®
  - Bifidobacterium infantis DSM 24737®
- Una cadena de Streptococcus
  - Streptococcus thermophilus DSM 24731®

- **<u>Grupo control (GC):</u>** se realizó exactamente el mismo procedimiento, pero con el sistema de suero cerrado. Durante el proceso y tras el mismo el propio paciente registra la aparición de síntomas tras cada sesión de estimulación: dolor abdominal, emisión de gas y heces.

Tras completar las 10 sesiones de estimulación, 24 horas antes de la cirugía, se realizó nueva colonoscopia diagnóstica con toma de muestras para Anatomía Patológica a todos los pacientes, volviendo a cuantificar el grado de colitis por

diversión endoscópico e histológico. Del mismo modo se procedió a la extracción de sangre para determinar los biomarcadores séricos.

3.6. Cirugía y seguimiento

Todos los pacientes ingresan el día previo a la intervención quirúrgica, permaneciendo en ayunas, administrando profilaxis antitrombótica (enoxaparina 40mg subcutánea) y premedicación según instrucciones de la hoja de preanestesia. La reconstrucción del tránsito se llevó a cabo por tres cirujanos expertos de las Unidades de Cirugía Colorrectal. Se realizó el cierre de la ileostomía de protección mediante abordaje abierto por laparotomía lateral periileostomía. La anastomosis fue latero-lateral manual o mecánica según la decisión personal de cada cirujano, así mismo cada cirujano podía decidir cambiar la vía de abordaje a laparotomía media. Las complicaciones o eventos registrados durante la cirugía se recogieron en el protocolo de la intervención quirúrgica. La anestesia fue general en todos los pacientes y tras la extubación y estabilización en la sala de reanimación postquirúrgica fueron directamente a la planta de hospitalización.

El seguimiento durante la hospitalización fue realizado por el equipo de la Unidad de Cirugía Colorrectal de cada centro, registrando cualquier complicación postoperatoria; con especial vigilancia del dolor abdominal, inicio de expulsión de gas y heces, control y cuantificación de los mismos, inicio de la tolerancia oral, aparición de íleo paralítico y necesidad de colocación de sonda nasogástrica (SNG). Se procedió al alta domiciliaria tras restablecimiento del tránsito intestinal, tolerancia oral adecuada y control de las deposiciones, registrando los días de estancia hospitalaria.

Se realizó una revisión en consultas externas de Cirugía Colorrectal tras el primer mes de la cirugía y posteriormente a los 3 meses. Dichas revisiones fueron realizadas por el cirujano colorrectal que intervino a cada paciente. Se registró cualquier sintomatología relacionada con la intervención, con especial vigilancia a la

clínica relacionada con la colitis por diversión; dolor abdominal, número de deposiciones, descarga mucosa, tenesmo y rectorragia. Tras completar los 3 meses de seguimiento se realizó nueva colonoscopia de control con toma de muestras para estudio histológico, volviendo a cuantificar la presencia y grado de colitis por diversión endoscópico e histológico si la hubiese. Del mismo modo se procedió a la extracción de sangre para determinar los biomarcadores séricos.

3.7. Enmascaramiento

Para asegurar el enmascaramiento de los pacientes, a todos se les realizará el mismo procedimiento diagnóstico. Posteriormente, durante las sesiones de estimulación, tanto la solución con probióticos como el sistema de suero estarán envueltos por un envoltorio protector opaco que impedirá visualizar las características de color y transparencia de su contenido y la apertura o cierre del sistema de suero. Las sesiones de estimulación fueron realizadas a puerta cerrada por un único cirujano que además era el encargado de preparar la dilución.

Tanto el endoscopista, como el anatomopatólogo, como el especialista en análisis clínicos, como los cirujanos que interactuaron con los pacientes desconocían si habían recibido probióticos o no.

3.8. Criterios de valoración

El criterio de valoración principal fue el efecto causado por la estimulación del asa eferente con probióticos sobre la persistencia y el grado de colitis por diversión, endoscópico e histológico, al compararlo con el grupo control tras la estimulación, tras la intervención quirúrgica, al mes y durante el seguimiento a corto plazo. Así como la correlación de la severidad endoscópica e histológica con la aparición de clínica derivada por la colitis por diversión y la disminución o desaparición de la misma en un

primer tiempo tras la estimulación con probióticos, y en un segundo tiempo, en el seguimiento a corto plazo tras la reconstrucción del tránsito.

Los criterios de valoración secundarios fueron:

- El efecto causado por la estimulación del asa eferente con probióticos sobre la aparición de íleo paralítico postoperatorio, necesidad de sonda nasogástrica, tiempo en tolerancia oral, reestablecimiento del tránsito intestinal y estancia hospitalaria al compararlos con el grupo tras la intervención quirúrgica. Se definió el íleo postoperatorio como la disfunción gastrointestinal postoperatoria medida mediante la escala validada I-FEED (incapacidad de tolerar la ingesta oral o la interrupción de la misma con una duración igual o superior a 72 horas, ruidos intestinales ausentes y distensión abdominal o necesidad de colocar una sonda nasogástrica).
- La estancia hospitalaria medida como días de ingreso y la aparición de íleo postoperatorio durante la cirugía colorrectal con creación de la ileostomía derivativa previa medido mediante las evoluciones registradas durante la primera cirugía, aplicando igualmente mediante la escala I-FEED.
- La relación entre la colitis por diversión y la elevación de biomarcadores proinflamatorios séricos y su modificación tras estimulación del asa eferente con probióticos previa al cierre de la ileostomía de protección en pacientes intervenidos de carcinoma colorrectal, al comparar con el grupo control, los niveles basales, tras la estimulación y a corto plazo, al tercer de mes de seguimiento. También se valoró la correlación entre el grado de la colitis por diversión, endoscópico e histológico, y la alteración de los biomarcadores proinflamatorios en sangre.

3.9. Análisis estadístico

Se realizó un análisis descriptivo univariante de las variables sociodemográficas y clínicas. Para la comprobación de la normalidad de las variables cuantitativas se utilizó la prueba de Kolmogorov-Smirnov. Para describir las variables cuantitativas se usó la media y desviación típica y la mediana y el rango intercuartílico para aquellas variables que no siguieron una distribución normal. Para las variables cualitativas utilizamos frecuencias y porcentajes. Posteriormente, para comprobar los objetivos principales, se realizó un análisis bivariado. Se utilizó una prueba de contraste de proporciones basada en el test de Chi-cuadrado con el fin de determinar si la estimulación del asa eferente con probióticos previa al cierre de la ileostomía de protección disminuye el grado de colitis por diversión, sus hallazgos macro y microscópicos, así como su relación con la aparición de clínica derivada por la colitis por diversión. Para cuantificar su nivel de asociación se calculó el coeficiente Phi y V de Cramer.

Con el fin de determinar si la estimulación del asa eferente con probióticos previa al cierre de la ileostomía de protección disminuye el íleo postoperatorio, así como la necesidad de SNG, el tiempo que tarda en tolerar la alimentación oral, restablecimiento del tránsito intestinal y la posible disminución de tiempo de estancia hospitalaria, se utilizó una prueba de contrate de proporciones basada en el test de Chi-cuadrado. Para la correlación de variables cuantitativas se utilizó el Spearman´s rank correlation coefficient.

La precisión de cada biomarcador serológico fue evaluada mediante curvas ROC (Receiver Operating Characteristic) para determinar el punto de corte óptimo de NLR, PLR y LMR. Las comparaciones entre los grupos en cuanto a tasas de respuesta, niveles de PCR y parámetros bioquímicos, se realizaron utilizando la prueba exacta de Fisher o la prueba de chi-cuadrado para las variables categóricas y la prueba de Mann-Whitney para las que no tienen distribución normal. Las comparaciones dentro de cada grupo

para valorar modificación del grado de severidad histológico y endoscópico, PCR y parámetros bioquímicos se analizaron usando Wilcoxon y prueba t pareada.

En todos los casos se requirió una significación estadística del 5%. El análisis estadístico se realizó mediante el programa estadístico SPSS versión 24.0, con el apoyo de complementos de cálculo proporcionado por el programa Microsoft Excel o R.

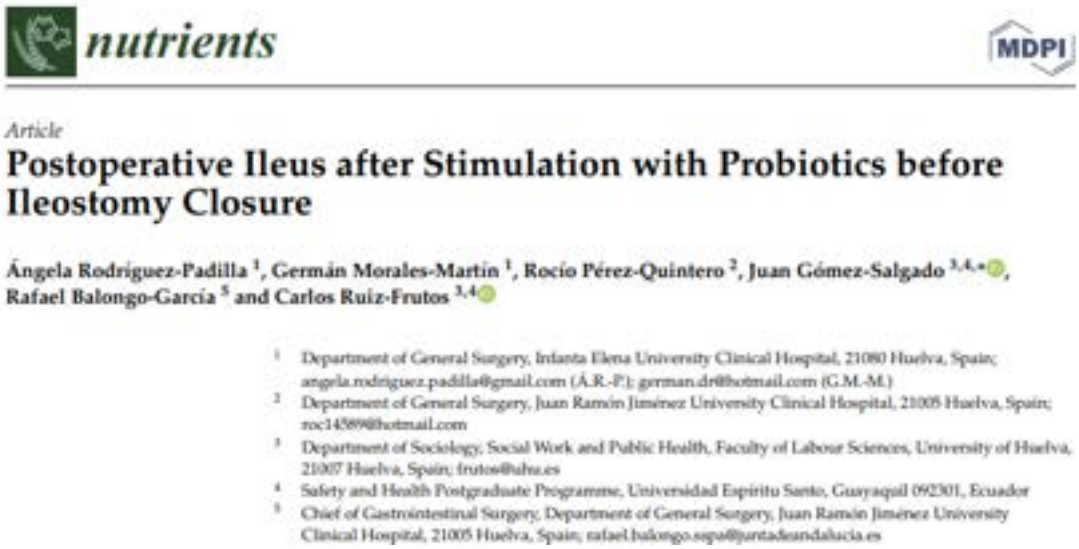
3.10. Aspectos éticos

El proyecto fue aprobado por el Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, España, y registrado con el número de Proyecto 2017/331191354 (Anexo 1). Se solicitó el consentimiento informado por escrito a participar en el estudio, dándole detalles tanto de los objetivos del estudio como de la metodología a seguir (Anexo 2). Los datos se guardaron de forma anónima manteniendo la confidencialidad y anonimato de los participantes.

Resultados

4.1. Postoperative ileus after stimulation with probiotics before ileostomy closure

El estudio que se presenta a continuación ha sido publicado con la siguiente referencia: Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez-Salgado J, Balongo-García R, Ruiz-Frutos C. Postoperative Ileus after Stimulation with Probiotics before Ileostomy Closure. Nutrients. 2021 Feb 15; 13(2):626. doi: 10.3390/nu13020626.



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Postoperative Ileus after Stimulation with Probiotics before Ileostomy Closure

Ángela Rodríguez-Padilla [1], Germán Morales-Martín [1], Rocío Pérez-Quintero [2], Juan Gómez-Salgado [3,4,*], Rafael Balongo-García [5] and Carlos Ruiz-Frutos [3,4]

1 Department of General Surgery, Infanta Elena University Clinical Hospital, 21080 Huelva, Spain; angela.rodriguez.padilla@gmail.com (Á.R.-P.); german.dr@hotmail.com (G.M.-M.)
2 Department of General Surgery, Juan Ramón Jiménez University Clinical Hospital, 21005 Huelva, Spain; roc145898@hotmail.com
3 Department of Sociology, Social Work and Public Health, Faculty of Labour Sciences, University of Huelva, 21007 Huelva, Spain; frutos@uhu.es
4 Safety and Health Postgraduate Programme, Universidad Espíritu Santo, Guayaquil 092301, Ecuador
5 Chief of Gastrointestinal Surgery, Department of General Surgery, Juan Ramón Jiménez University Clinical Hospital, 21005 Huelva, Spain; rafael.balongo.sspa@juntadeandalucia.es

4.1.1. Abstract

Loop ileostomy closure after colorectal surgery is often associated with Postoperative ileus, with an incidence between 13–20%. The aim of this study is to evaluate the efficacy and safety of preoperative stimulation of the efferent loop with probiotics prior to ileostomy closure in patients operated on for colorectal carcinoma. For this, a prospective, randomized, double-blind, controlled study is designed. All patients who underwent surgery for colorectal carcinoma with loop ileostomy were included. Randomized and divided into two groups, 34 cases and 35 controls were included in the study. Postoperative ileus, the need for nasogastric tube insertion, the time required to begin tolerating a diet, restoration of bowel function, and duration of hospital stay were evaluated. The incidence of Postoperative ileus was similar in both groups, 9/34 patients stimulated with probiotics and 10/35 in the control group (CG) with a $p = 0.192$. The comparative analysis showed a direct relationship between Postoperative ileus after oncological surgery and Postoperative ileus after reconstruction surgery, independently of stimulation. Postoperative ileus after closure ileostomy is independent of stimulation of the ileostomy with probiotics through the efferent loop. There seem to be a relationship between Postoperative ileus after reconstruction and the previous existence of Postoperative ileus after colorectal cancer surgery.

Keywords: probiotics; postoperative ileus; efferent loop stimulation; ileostomy closure; protective ileostomy

4.1.2. Introduction

The performance of a temporary ileostomy in patients operated on for colorectal carcinoma, by means of an anterior resection with total mesorectal excision, reduces the morbidity associated with anastomotic leak [1]. However, reconstructive surgery is not risk-free. The incidence of complications following ileostomy closure, such as diversion colitis, small bowel obstruction, surgical wound infections, postoperative ileus, anastomotic leak, fistula, perforation, abscess, bleeding, or hernia [2,3], ranges from 18% to 40%. The most common complication is postoperative ileus, with an incidence of 13%–20% [4]. As a result, this can lead to a significant socioeconomic impact, the worsening of a patients' quality of life due to greater discomfort, increased risk of nosocomial infections and morbidity associated with surgery, and an increase in postoperative mortality; all of this conditioning a prolonged hospital stay and higher healthcare costs [5].

On the other hand, previous studies have shown that the colon defunctionalization produces dysbacteriosis and atrophy of its villi and muscular layers in the excluded colon segment, as well as a loss of segmentary contractility that could produce a reduction in the ability for absorption [6,7]. To avoid this, two lines of studies have been proposed; early closure of the loop ileostomy, which would be the best option [8], or late closure after stimulation of the efferent loop. There are many publications on stimulation of the efferent loop prior to stoma closure. Different protocols exist, with different substances administered, from autologous fecal fluid to physiological serum with thickeners, passing through short-chain fatty acids, liquid diet, and sucrose [9].

Based on the need to return the excluded intestine to its function, pre-enabling it before surgery reconstruction, and demonstrated bacterial dysbiosis, we decided to administer probiotics through the efferent loop. Probiotics are increasingly being applied in gastrointestinal pathology. They are non-pathogenic live bacteria, such as

Lactobacillus, Bifidobacterium, and Enterococcus, which present antimicrobial and immunomodulatory activity and improve the intestinal barrier. Supplied in adequate amounts, they promote health benefits on the host [10].

4.1.3. Materials and methods

Study Design

We conducted a prospective, randomized, multicenter, double-blind experimental study, comparing two groups of patients operated on for colorectal carcinoma with loop ileostomy. One group included patients treated with stimulation of the efferent loop with probiotics prior to transit reconstruction surgery; the other control group was stimulated without giving any substance.

Simple Size

The sample size was calculated according to the incidence of postoperative complications (PI) obtained after ileostomy closure published in a recent systematic review. The assumed reduction in PI was 50% (20%–10%). With a loss adjustment of 15%, it was necessary to have 30 patients per group. We recruited 34 patients in the stimulated group and 35 patients in the control group for a statistical level of 95% and a power of 0.8.

Selection of Patients

Between January 2017 and December 2018, all the patients from the three participating centers included in the surgical waiting list for temporary stoma closure after colorectal carcinoma were consecutively evaluated to determine their inclusion in the study. The selection flowchart of the study patients can be seen in Figure 1.

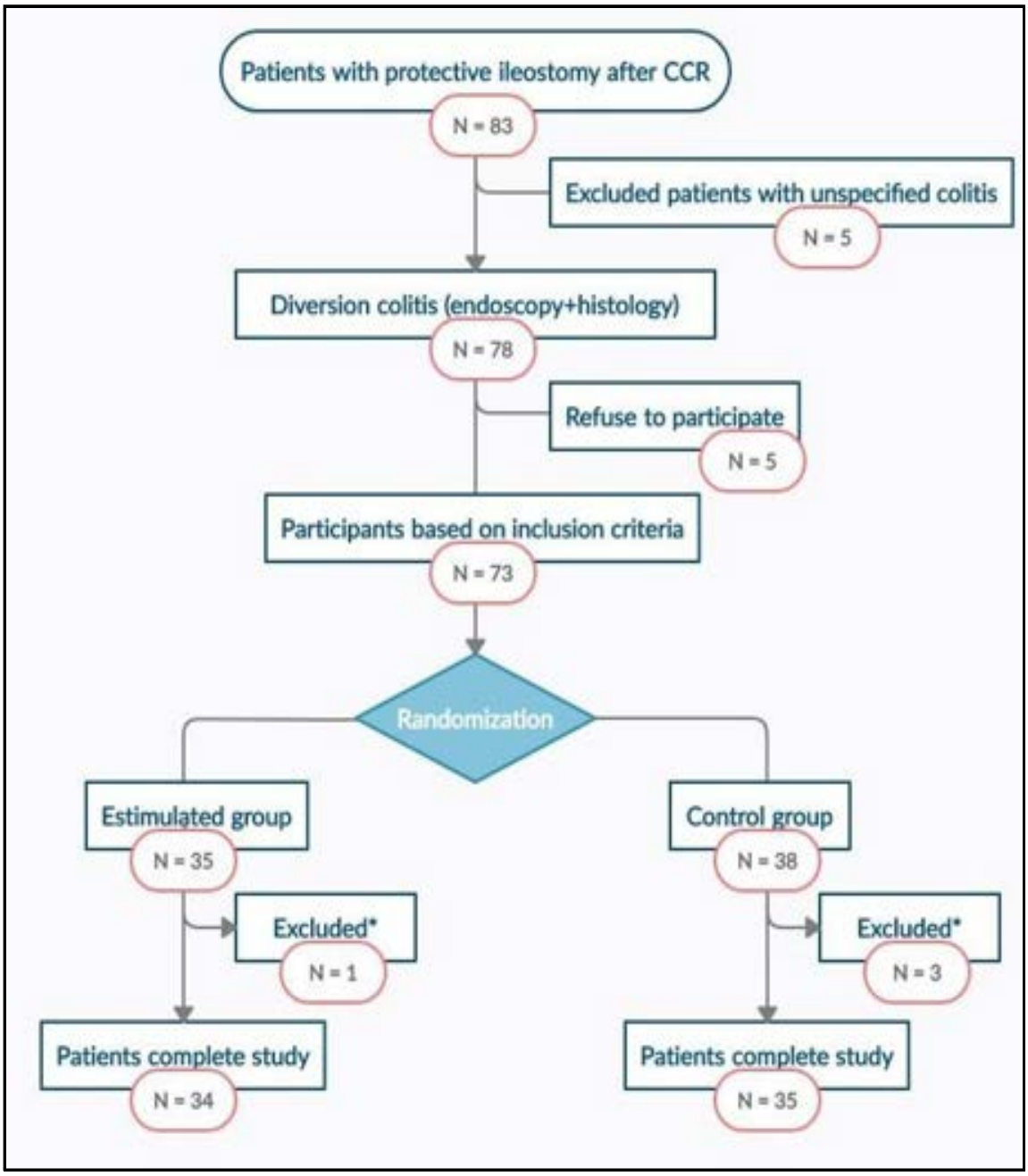

Figure 1. Study flowchart. CRC: Colorectal Cancer. *: excluded patients with an anastomotic leak.

The inclusion criteria were being over 18 years of age, having protective ileostomy after colorectal carcinoma surgery free of disease, with endoscopic and histological confirmation of diversion colitis, and having signed the informed consent.

The exclusion criteria were being under 18 years of age, clinical history, and histological confirmation of inflammatory bowel disease with colorectal involvement and refusal to participate in the study. Abandonment criteria were loss during follow-up, exitus, and anastomotic dehiscence after reconstruction surgery.

Randomisation and Intervention

A colonoscopy including biopsies for histological study, was performed on all patients selected to participate in the study. After confirming the diagnosis, level of colitis (based on the Harig scoring system), and excluding patients who did not meet the selection criteria, randomisation was performed. Randomisation was performed by using a computer-generated sequence (Statistical software EPIDAT 4.1) into 2 groups:

- Stimulation group (SG): preoperative stimulation of the distal limb of the ileostomy loop with probiotics was performed during the 20 days prior to surgery every second day. During the process and after it, the patient registers the appearance of symptoms after each stimulation session: abdominal pain, emission of gas, and stool. A sterile Foley catheter No.14 Ch connected to an infusion set was introduced through the de-functioned bowel to allow the slow infusion of a solution with 4.5 mg of probiotics diluted in 250 ml of 0.9% physiological saline for 20–30 min. Each preparation was made under sterile conditions and maintaining the cold chain. Vivomixx® lyophilised live bacteria, marketed by MENDES, S.A, contained 4.5 × 10 11 of live bacteria in each preparation:

◦ Four strains of Lactobacillus:

Lactobacillus acidophilus DSM 24735®

Lactobacillus plantarum DSM 24730®

Lactobacillus paracasei DSM 24733®

Lactobacillus delbrueckii subsp. bulgaricus DSM 24734®

◦ Three strains of Bifidobacterium:

Bifidobacterium breve DSM 24732®

Bifidobacterium longum DSM 24736®

Bifidobacterium infantis DSM 24737®

◦ One strain of Streptococcus

Streptococcus thermophilus DSM 24731®

• Control group (CG): exactly the same procedure was carried out, but with the infusion set closed. During the process and after it, the patient himself registers the appearance of symptoms after each stimulation session: abdominal pain, emission of gas, and stool.

After ten stimulation sessions, 24 h before surgery, a colonoscopy with biopsy was performed on all patients, re-quantifying the nendoscopic and histological index of severity of diversion colitis.

Surgery and Follow-up

All patients were admitted to the hospital the day before surgery, fasting, receiving antithrombotic prophylaxis (enoxaparin 40 mg subcutaneous), and premedication according to the pre-anesthesia instruction sheet. The reconstruction surgery was carried out by three expert surgeons from the Colorectal Surgery department. A parastomal incision was made and carried out sharply into the peritoneal cavity. The anastomosis was lateral-lateral, either manual or mechanical, according to the decision of the surgeon. Every surgeon was allowed to decide as to whether to change to a median laparotomy procedure. Complications or events that happened during surgery were recorded in the surgical procedure protocol. General anesthesia was given to all patients and, after extubation and stabilization in the postoperative resuscitation room, they went directly to the hospitalization ward.

Follow-up during hospitalization was carried out by the team of the Colorectal Surgery department of each center, recording any postoperative complications, with special vigilance of abdominal pain, the passage of flatus or stool with correct quantification and initiation of oral tolerance, postoperative ileus, and the need of nasogastric tube insertion. Patients were discharged from the hospital after re-establishing intestinal transit, adequate oral tolerance, and stool control, and recording the length of stay in the hospital.

Masking

To ensure masking of the patients, all underwent the same diagnostic procedure. During the stimulation sessions, both the solution with probiotics and the serum system was covered by an opaque protective envelope, which prevented observing the color and transparency of the fluid, or whether the system was open or

closed. The stimulation sessions were performed by a single surgeon, who was also in charge of preparing the dilution.

The endoscopist, the pathologist, and the surgeon who performed the surgical intervention and the follow-up, as well as surgeons who participated, after the surgery, in the hospitalization process, did not know whether the patient had received probiotics or not.

Assessment Criteria

The main endpoint was the effect caused by stimulation of the efferent loop with probiotics on the appearance of postoperative ileus, the need for nasogastric tube insertion, the time required to begin tolerating a diet, restoration of bowel function, and the duration of hospital stay when compared with CG after surgery. Postoperative ileus was defined as impaired gastrointestinal motility leading to a delayed return of bowel function, measured using the validated I-FEED scale (intolerance to oral intake or interruption of oral diet for more than 72 hours, absence of bowel sounds, abdominal distention, or need for inserting a nasogastric tube).

Secondary evaluation criteria were the duration of hospital stay, measured as days of admission, and the appearance of postoperative ileus after colorectal surgery with a protective ileostomy, measured by the evolutions recorded during the first surgery, also applying the I-FEED scale[11,12].

Statistical Analysis

A descriptive univariate analysis of sociodemographic and clinical variables was performed. The Kolmogorov-Smirnov test was used to verify the normality of the quantitative variables. To describe the quantitative variables, the mean and standard

deviation were used, and the median and interquartile range for those variables that did not follow a normal distribution. For qualitative variables, frequencies and percentages were used. Afterward, to verify the main objectives, a bivariate analysis was performed. A contrast test of proportions based on the Chi-square test was used in order to determine whether stimulation of the efferent loop with probiotics prior to the closure of the protective ileostomy reduces the appearance of postoperative ileus, the need for nasogastric tube insertion, the time required to begin tolerating a diet, restoration of bowel function and the possibility of reducing the duration of hospital stay. For the correlation of quantitative variables, Spearman´s rank correlation coefficient was used.

In all cases, a statistical significance of 5% was required. Statistical analyses were performed using the statistical program SPSS version 24.0 (IBM: Armonk, NY, USA), with the support of calculation tools provided by the software Microsoft Excel or R (Microsoft: Redmond, WA, USA).

Ethical Aspects

The project was performed with the consent of the Ethics Coordinating Committee for Biomedical Research of Andalusia, Spain, and registered with the project number 2017/331191354. Written informed consent was requested to participate in the study, giving details of both the study objectives and the methodology to be followed. The data was kept anonymous, maintaining the confidentiality and anonymity of the participants. The study was conducted under the "Ethical Principles for Medical Research Involving Humans"; contained in the latest version of the Helsinki Declaration (Fortress Amendment, Brazil, October 2013). The CONSORT Statement criteria for the design of clinical trial were followed (standard trial methodology).

4.1.4. Results

Study Population

Between January 2017 and December 2018, 83 disease-free patients with protective ileostomy after colorectal carcinoma resection were reviewed and included in the surgical waiting list for intestinal transit reconstruction. Seventy-eight of them met the endoscopic and histological criteria for diversion colitis diagnosis, and 73 patients were finally randomized into two groups, intervention (n = 35) and control (n = 38). Sixty-nine patients completed the study, one of them from SG and three from the CG abandoning the study because of anastomotic fistula. The study flowchart is presented in Figure 1.There were no significant differences between SG and CG in terms of sociodemographic, clinical, or surgical variables (Table 1).

Abdominal pain was the only symptom observed during stimulation, which appeared in 20.5% of patients SG (n = 7) compared to 14.3% CG (n = 5). There were no statistically significant differences between the two groups. Abdominal pain was evaluated using the visual analog scale (VAS). Pain was moderated in SG, only present in the first stimulation sessions and disappearing afterward. Pain was mild-moderate in CG in all stimulation sessions and disappeared after their completion.

Main Assessment Criteria

The results of the main endpoint and postoperative evolution are described in Table 2. The incidence of postoperative ileus was similar in both groups, present in 10/34 (29.4%) SG and 11/35 (31.4%) CG with p = 0.192. There were no significant differences regarding the need for a nasogastric tube, the time required to begin tolerating a diet, restoration of bowel function, and the duration of hospital stay. After closure ileostomy, SG showed initiation of oral tolerance 24-hour earlier than CG (2 vs 3 days), which is considered statistically non-significant. The passage of gases and

Table 1. Demographics, clinics and surgical characteristics.

	Stimulated Group (n = 34)	**No Stimulated Group (n = 35)**	p
Demographics			
Age (years)	65 (45–81)	68 (41–80)	0.130
Sex ratio (Male:Female)	23:11	25:10	0.733
BMI (kg/m^2)	23.5 (21.6–32.6)	27.6 (18.8–40.2)	0.091
ASA			0.483
ASA I-II	31	30	
ASA III	3	5	
Smoker/nonsmoker	20/14	23/12	0.826
Colorectal surgery			
Surgical procedure:			0.129
LAR	25	25	
uLAR	9	10	
Surgical approach:			0.551
Laparoscopic	24	24	
Open	10	11	
Type of anastomosis:			0.430
Stapled EEA	31	33	
Coloanal anastomosis	3	2	
Neoadjuvant therapy	26	25	0.239
Adjuvant treatment	26	28	0.256
Ileostomy closure			
Time between surgeries (months)	12 (8–37)	9 (6–32)	0.813
Surgery:			0.690
Small bowel resection	33	34	
Ileocecal resection	1	1	
Surgical approach:			0.291
Peri-ileostomy	30	28	
Midline laparotomy	4	7	
Type of anastomosis:			0.355
Sewn	29	29	
Stapled	5	6	
Time (minutes)	50 (30–70)	65 (50–120)	0.053

BMI: body mass index. ASA: American Society of Anesthesiologists Classification. LAR/uLAR: low anterior resection/ultralow anterior resection. EEA: end to end anastomosis stapler.

restoration of bowel function appeared 2 days after surgery in both groups. SG had an interval between 1–20 days to the emission of gases and 1–21 days to the restoration of bowel function compared to CG, which had an interval of 1–48 days and 1–48 days, respectively. The hospital stay was also shorter in SG but without statistical significance. In SG, nine patients needed a nasogastric tube (26%) to manage postoperative ileus. In CG, all the patients who presented postoperative ileus required a nasogastric tube as part of their treatment (31.4%).

Table 2. Postoperative results.

	Stimulated Group (n = 34)	**No Stimulated Group (n = 35)**	p
Postoperative ileus, n (%)	10 (29.4%)	11 (31.4%)	0.192
Nasogastric tube, n (%)	9 (26%)	11 (31.4%)	0.116
Time to tolerating a diet, days-mean (range)	2 (1–24)	3 (2–50)	0.619
Start of the passage of flatus, days-mean (range)	2 (1–20)	2 (1–48)	0.173
Start of the passage of stool, days-mean (range)	3 (1–21)	3 (1–48)	0.184
Postoperative stay, days-mean (range)	4 (4–26)	5 (4–56)	0.105

The median has been used as a measure of central tendency for the evaluation of variables as the initiation of oral tolerance, gas emission, restoration of transit, and the duration of hospital stay.

Secondary Assessment Criteria: Postoperative Ileus and Colorectal Sur*gery*

From the comparative analysis, the incidence of postoperative ileus after closure ileostomy was found to be similar to the one that occurred in primary colorectal surgery. In other words, patients who presented ileus after reconstruction surgery also presented it in the previous colorectal surgery. In the SG, postoperative ileus after colorectal surgery happened in 11/23 patients, and after reconstruction, it appeared in 10 of these 11 cases. In the same way, in the CG postoperative ileus after colorectal surgery happened in 11/24 patients, and after reconstruction, the same 11 patients presented it. Therefore, there is a direct relationship between ileus after colorectal surgery and after intestinal transit reconstruction, independent of stimulation with p <0.001 (Figure 2).

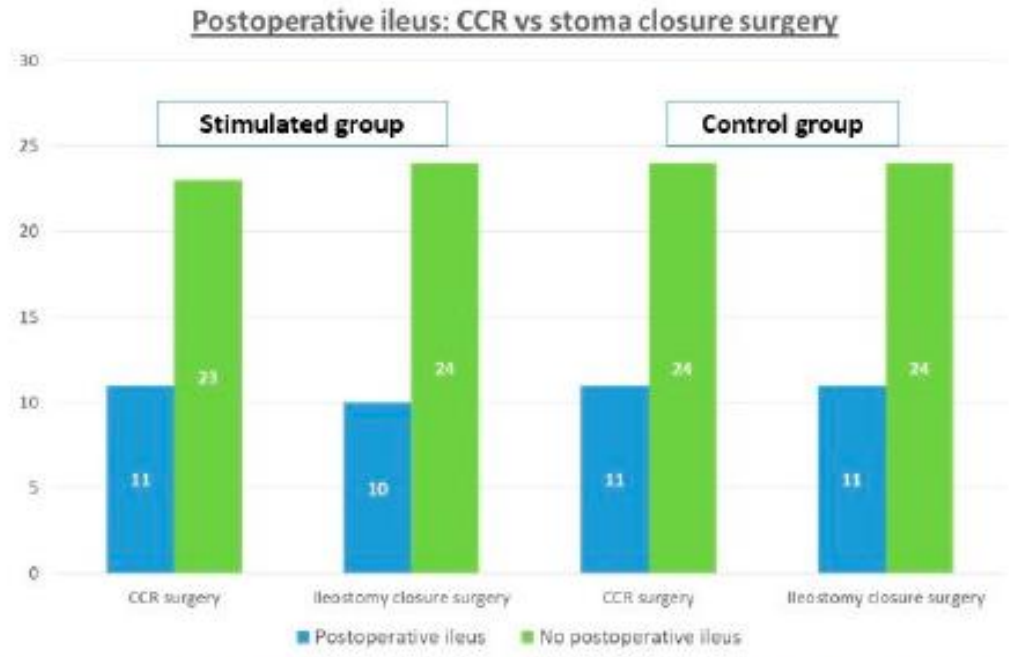

Figure 2. Comparison of postoperative ileus and the need for a nasogastric tube (NT) in colorectal surgery (CCR) and after reconstructive surgery.

4.1.5. Discussion

The appearance of postoperative ileus after closure ileostomy has been analyzed in several studies [4,5,8,13,14]. Garfinkle et al. published the first systematic review in 2019 [4]. It includes 9.528 patients in 67 studies, most of them retrospective record. The authors point out that its incidence depends on the definition that we use about ileus and that these variations cause great heterogeneity in the included studies, so we will need to continue investigating and defining it with homogeneous criteria. In fact, more than half of the studies in this meta-analysis (39/67) did not have defined criteria for postoperative ileus (studies with a lower incidence), and among those, there is no coincidence in the definition.

In order to find a common denominator to compare different studies, we relied on the study published by the ASER (American Society of Enhanced Recovery) and POQI (Perioperative Quality Initiative) working group [11], which establishes definitions based on the Symptom score for postoperative gastrointestinal dysfunction (using terms such as ileus, paralytic ileus, postoperative ileus, or prolonged ileus) including oral tolerance, nausea, vomiting, bloating, and duration of these symptoms. This validated scoring system called I-FEED [12] was used in our study because, in our opinion, it appears to be the most objective and extrapolated way to determine the appearance of postoperative ileus, defined as postoperative gastrointestinal dysfunction, which is different from postoperative gastrointestinal intolerance that is resolved in 24–48 hours, and allows it to be compared with other studies in the future.

The idea of the stimulation of the efferent loop prior to reconstructive surgery was born after raising the hypothesis that the appearance of postoperative ileus is due to the structural and functional changes that take place in the dysfunctional intestine, such as muscle and villous atrophy or decreased absorption capacity. [7,14]. Distal bowel and colon, which are unready to re- establish transit, cause intestinal paralysis. In this regard, several articles have been published [13–17], but the first systematic

review was published by Rombey et al. [9] in 2019, including eight studies with 267 patients. Despite the excellent initial results, there is not sufficient evidence to recommend the routine implementation of preoperative bowel stimulation in clinical practice. This is due to the heterogeneity of the studies, which have not enough statistical significance, and whose results are difficult to compare with each other since some compare patients undergoing proctocolectomy with ileoanal J anastomosis and other patients with low anterior resection. Only one study was randomized, three were unique clinical cases, and there was diversity in terms of the substance used during stimulation and its duration. Only two comparative studies found that preoperative bowel stimulation reduced the time to restoration of bowel function when compared to standard preoperative care [14,16]. The reduction was statistically significant with regards to the mean time to the tolerance of solid food. The mean time to passing flatus or stool was significantly lower, but the mean time to tolerance of liquids did not significantly differ between the intervention and control groups in the two studies reporting this outcome. The case reports results matched the comparative studies results [15,17]. Preoperative bowel stimulation significantly reduced the mean postoperative ileus in two comparative studies [14,16], by 2.1 and by 2.6 days, respectively. In the two case reports [15,17], the postoperative ileus was of two and four days, respectively, which is consistent with the RCT result [14].

The hypothesis of the present study was motivated by the absence of clear scientific evidence on the benefit of stimulation of the efferent loop. We used a double-blind design, maintained until the end of the study, a standardized definition of postoperative gastrointestinal dysfunction, which had not been used up to now, and based our trial on comparative studies and published meta-analyzes on intestinal pathophysiological changes caused by the loss of intestinal flora in the excluded colon segment [6–9,14]. We decided to carry out our study to compare results after stimulation of the efferent loop with probiotics in patients previously operated on for colorectal carcinoma. Based on systematic reviews about the use of probiotics as

treatment of other gastrointestinal pathologies [18–20], we considered that this stimulation would achieve the repopulation of the excluded colon, activating the cellular absorption mechanisms, increasing motility, and decreasing efferent loop atrophy, so that after closure ileostomy, the return to normality would be faster, with a shorter time required to re-establish oral transit and tolerance, reducing the onset of postoperative ileus and the duration of hospital stay. We chose the formulation of probiotics with the highest number of strains, which had an adequate synergistic and complementary effect between them, and the highest concentration. Another positive point is the absence of allergens such as gluten, lactose, or soy.

In our study, after the inclusion and randomization of 73 patients, no statistically significant differences were detected in terms of sociodemographic, clinical, or surgical variables. A notable reduction in surgical time was observed in the SG, which we associate with the thickening of the efferent loop, resulting from the stimulation, and which facilitates the performance of anastomosis. We also did not find differences between the incidence of paralytic ileus in the SG (n = 10; 29.4%) and the CG (n = 11; 31.4%), with a slight increase in it, compared to studies collected in systematic reviews published by Rombey and Garfinkle [4,9], that we associate to the standardization of the definition of postoperative gastrointestinal dysfunction. The difference in terms of duration of hospital stay was 1 day, with a median of 4 days in the SG and 5 days in the CG. This was associated with a 24-hour difference in oral tolerance in the SG, in which 58% of patients tolerated a liquid diet in the first 24–48 hours, compared to the CG, which took 48–72 hours. These results are similar to those reported by other studies [9,14,15]. Moreover, it was observed that the incidence of postoperative ileus was exactly the same as that presented in colorectal surgery and that this was due to the fact that all the patients who presented ileus after reconstruction surgery also did in the previous colorectal surgery. This fact is important, as the closuring of ileostomy does not present the traditional risk factors of

colorectal surgery, such as surgical time, increased level of fluids, and loss of blood, among others [4,21–23].

4.1.6. Conclusions

Therefore, given the results obtained in our study, we have not been able to demonstrate the benefit of stimulating the efferent loop with probiotics on the appearance of postoperative ileus. Scientific evidence is insufficient to incorporate this procedure into our protocol prior to closure ileostomy. We will need more studies to establish its impact on operating results. We have indeed observed a direct relationship between the onset of ileus after reconstruction surgery and the fact that these patients had previously presented the same affection after colorectal carcinoma surgery, a finding that may represent a new line of research.

4.1.7. References

1. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg. 2007, 246: 207–14. DOI: 10.1097/sla.0b013e3180603024
2. Sharma A, Deeb AP, Rickles AS, Iannuzzi JC, Monson JRT, Fleming FJ. Closure of defunctioning loop ileostomy is associated with considerable morbidity. Colorectal Dis. 2013, 15: 458–62. Doi: 10.1111/codi.12029
3. Bhalla A, Peacock O, Tierney GM, Tou S, Hurst NG, Speake WJ et al. Day-case closure of ileostomy: feasible, safe and efficient. Colorectal Dis. 2015; 17: 820–823. Doi: 10.1111/codi.12961
4. Garfinkle R, Savage P, Boutros M, Landry P, Reynier P, Morin N et al. Incidence and predictors of postoperative ileus after loop ileostomy closure: a systematic

review and meta-analysis. Surg Endosc 2019; 33(8):2430-2443. doi: 10.1007/s00464-019-06794-y

5. Danielsen AK, Park J, Jansen JE, Bock D, Skullman S, Wedin A, et al. Early Closure of a Temporary Ileostomy in Patients With Rectal Cancer: A Multicenter Randomized Controlled Trial. Ann Surg 2017; 265: 284 -90. DOI: 10.17116/hirurgia201906135
6. Beamish EL, Johnson J, Shaw EJ, Scott NA, Bhowmick A, Rigby RJ. Loop ileostomy -mediated fecal stream diversion is associated with microbial dysbiosis. Gut Microbes 2017; 8: 467 -78. doi: 10.1080/19490976.2017.1339003
7. Williams L, Armstrong MJ, Finan P, Sagar P, Burke D. The effect of faecal diversion on human ileum. Gut 2007; 56: 796 -801. doi: 10.1136/gut.2006.102046
8. Keane C, Park J, Öberg S, Wedin A, Bock D, O'Grady G, et al. Functional outcomes from a randomized trial of early closure of temporary ileostomy after rectal excision for cancer. Br J Surg. 2019 Apr; 106 (5):645-652. doi: 10.1002/bjs.11092
9. Rombey T, Panagiotopoulou IG, Hind D, Fearnhead NS, Preoperative bowel stimulation prior to ileostomy closure to restore bowel function more quickly and improve postoperative outcomes: a systematic review. Colorectal Dis. 2019; 21 (9): 994-1003. doi: 10.1111/codi.14636
10. Shen ZH, Zhu CX, Quan YS, Yang ZY, Wu S, Luo WW, Tan B, Wang XY. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J Gastroenterol 2018 January 7; 24(1): 5-14. doi: 10.3748/wjg.v24.i1.5.
11. Hedrick TL, McEvoy MD, Mythen MMG, et al. American Society for Enhanced Recovery and Perioperative Quality Initiative Joint Consensus Statement on Postoperative Gastrointestinal Dysfunction Within an Enhanced Recovery

Pathway for Elective Colorectal Surgery. Anesth Analg 2018;126:1896–907. doi: 10.1213/ANE.0000000000002742

12. Alsharqawi N, Alhashemi M, Kaneva P, et al. Validity of the I-FEED score for postoperative gastrointestinal function in patients undergoing colorectal surgery. Surg Endosc. 2020 May;34(5):2219-2226. doi: 10.1007/s00464-019-07011-6
13. Garfinkle R, Trabulsi N, Morin N, et al. Study protocol evaluating the use of bowel stimulation before loop ileostomy closure to reduce postoperative ileus: a multicenter randomized controlled trial. Colorectal Disease 2017; 19: 1024–1029. doi: 10.1111/codi.13720
14. Abrisqueta J, Abellan I, Lujan J, Hernandez Q, Parrilla P. Stimulation of the efferent limb before ileostomy closure: a randomized clinical trial. Dis Colon Rectum 2014; 57: 1391–6. DOI: 10.1097/dcr.0000000000000237
15. Menéndez P, Garcia A, Lozano E, Pelaez R. Effectiveness of afferent loop stimulation prior to ileostomy closure. Cir Esp 2013; 91: 547-8. doi: 10.1016/j.ciresp.2013.02.021
16. Vázquez-Melero A, Loizate Totoricaguena A, García Alonso P. Stimulation of the Efferent Loop Before Loop Ileostomy Closure. National Library of Medicine, Bethesda (Maryland), U.S., 2018. ClinicalTrials.gov Identifier: NCT03424447.
17. Fernández López F, González López J, Paz Novo M, Ladra González MJ, Paredes Cotoré J. Estimulación preoperatoria del asa eferente de la ileostomía con ácidos grasos de cadena corta. Cir Esp. 2019; 97: 54-6. DOI: 10.1016/j.ciresp.2018.06.018
18. Zhang Y, Li L, Guo C, et al. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. BMC Gastroenterol. 2016;16(1):62. doi: 10.1186/s12876-016-0470-z.

19. Redman MG, Ward EJ, Phillips RS. The efficacy and safety of probiotics in people with cancer: a systematic review. Ann Oncol. 2014;25(10): 1919-1929. doi: 10.1093/annonc/mdu106
20. Yoon BJ, Oh HK, Lee J, Cho JR, Kim MJ, Kim DW, Kang SB. Effects of probiotics on bowel function restoration following ileostomy closure in rectal cancer patients: a randomized controlled trial. Colorectal Dis 2020; Online ahead of print. DOI: 10.1111/codi.15463.
21. Wolthuis AM, Bislenghi G, Fieuws S, de Buck van Overstraeten A, Boeckxstaens G, D'Hoore A. Incidence of prolonged postoperative ileus after colorectal surgery: a systematic review and meta-analysis. Colorectal Dis 2016; 18: O1–9. doi: 10.1111/codi.13210
22. Sugawara K, Kawaguchi Y, Nomura Y, Suka Y, Kawasaki K, Uemura Y et al. Perioperative factors predicting prolonged postoperative ileus after major abdominal surgery. J Gastrointest Surg 2018; 22: 508–515. doi: 10.1007/s11605-017-3622-8.
23. Rybakov EG, Shelygin YA, Khomyakov EA, Zarodniuk IV. Risk factors for postoperative ileus after colorectal cancer surgery. Colorectal Dis 2018; 20: 189–194. doi: 10.1111/codi.13888

4.2. Diversion colitis and Probiotic stimulation: Effects of bowel stimulation prior to ileostomy closure

El estudio que se presenta a continuación ha sido publicado con la siguiente referencia: Rodríguez-*Padilla Á, Morales-Martín G, Pérez-Quintero R, Rada-Morgades R, Gómez-Salgado J, Ruiz-Frutos C. Diversion Colitis and Probiotic Stimulation: Effects of Bowel Stimulation Prior to Ileostomy Closure. Front. Med. 2021 Jun 8:654573. doi: 10.3389/fmed.2021.654573.*

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Diversion colitis and Probiotic stimulation: Effects of bowel stimulation prior to ileostomy closure

Provisionally accepted The final, formatted version of the article will be published soon. Notify me

Ángela Rodríguez-Padilla[1], Germán Morales-Martín[1], Rocío Pérez-Quintero[2], Ricardo Rada-Morgades[2], Juan Gómez-Salgado[3, 4*] and Carlos Ruiz-Frutos[1, 4]

[1]Department of General Surgery, Infanta Elena University Clinical Hospital, Huelva, Andusian Health Service, Spain
[2]Colorectal Area, Department of General Surgery, Hospital Juan Ramón Jiménez, Spain
[3]Department of Sociology, Social Work and Public Health, Faculty of Labour Sciences, University of Huelva, Spain
[4]Safety and Health Postgraduate Programme, Espíritu Santo University, Ecuador

4.2.1. Abstract

Background: Diversion colitis is a non-specific inflammation of a defunctionalised segment of the colon after a temporary stoma has been performed. This inflammation is associated with a change in the colonic flora.

Aim: to evaluate the efficacy and safety of preoperative stimulation of the efferent loop with probiotics prior to closure of the protective ileostomy in patients operated on colorectal carcinoma and its effect on diversion colitis. A prospective, randomised, double-blind, controlled study is carried out.

Methods: Patients who underwent surgery for colorectal carcinoma with protective ileostomy pending reconstructive surgery and with diversion colitis as diagnosis are included. Randomised and divided into two groups. Histological and endoscopic changes were evaluated after stimulation, after restorative surgery and during the short-term follow-up after surgery.

Results: Patients in CG were distributed according to the endoscopic index of severity in pre-stimulation/post-stimulation as follows: severe n = 9/9 (25.7%), moderate n = 23/23 (65.7%) and mild n = 3/3 (8.6%); compared to the distribution in SG, severe n = 9/0 (26.5% / 0%), moderate n = 23/3 (67.6% / 8.8%), mild n = 2/19 (5.9% / 55.9%) and normal colonoscopy in 0/12 patients (0% / 35.3%).

Conclusion: Probiotic stimulation of the efferent loop is a safe and effective method, managing to reduce both macroscopic and microscopic colitis, as well as a decrease in symptoms in the short term after reconstructive surgery.

Keywords: Diversion colitis, efferent loop stimulation, Ileostomy closure, Inflammatory Bowel Diseases, IBD management, Probiotics

4.2.2. Introduction

In Western countries, rectal cancer represents 28–35% of all colorectal cancers, with an incidence of 15–25 new patients per 100,000 inhabitants/year. Its greatest complication is anastomotic leakage, with an incidence of 25% (1). The creation of a temporal ileostomy in patients operated on for colorectal carcinoma by means of a low anterior resection with total excision of the mesorectum, reduces the morbidity associated with anastomotic leakage (2). Around 18–40% of patients will present complications, such as diversion colitis, small bowel obstruction, surgical wound infection, postoperative ileus, anastomotic leak, fistula, perforation, abscess, bleeding, or hernia (3).

Diversion colitis (DC) is an inflammation produced in a defunctionalised segment of the colon after a temporary stoma has been performed (4). Described by Glotzer et al. (5), it is characterised by an inflammation of the large bowel mucosa that mimics idiopathic inflammatory bowel disease (4). It can manifest symptoms such as abdominal or pelvic pain, mucous discharge, tenesmus, fever, and rectal bleeding in the most severe cases, although up to 30% of patients remain asymptomatic (6). There are several hypotheses to explain the pathogenesis. One of them relates chronic inflammation with a decrease of bacteria in the dysfunctional area (7, 8). This inflammation causes endoscopic findings such as mucosal friability, oedema, erythema, appearance of polyps, ulcers, stenosis, and microscopic findings such as lymphoid follicular hyperplasia, infiltration of the lamina propria by lymphocytes, eosinophils, the appearance of plasma cells, architectural disruption, and the appearance of crypt abscesses (4).

The definitive treatment is the restoration of the continuity of the digestive tract (4, 6). Pharmacological treatments using instillations with short-chain fatty acids, mesalazine fibre, or corticosteroids are reserved for patients who are not candidates for surgical treatment or for stimulation of the efferent loop prior to surgery (9, 10).

Stimulation with probiotics prior to closing the protective stoma would allow the dysfunctional colon segment to be repopulated, which would reduce diversionary colitis (11).

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (12, 13). These and their metabolic products have been proposed as food supplements to achieve a healthier intestinal homeostasis and also as a treatment for pathologies with an important inflammatory component. Probiotics interact with the intestinal mucosa, reducing the molecular production of pro-inflammatory substances (12, 14). This immunomodulatory effect is what is needed in order to reduce the level of diversion colitis. Currently available probiotics, aimed at other pathologies with inflammatory conditions, produce a modulating effect in a transitory and limited way. This time-limited effect is what is needed to reduce the level of diversion colitis, since after reconstruction this tends to disappear.

4.2.3. Materials and methods

Study Design

Prospective, randomised, multicentre, double-blind experimental study, comparing two groups of patients operated on for colorectal carcinoma with protective ileostomy. One group includes patients treated with stimulation of the efferent loop with probiotics prior to transit reconstruction surgery; the other control group is stimulated without giving any substance.

Simple Size

The sample size was calculated according to the incidence of diversion colitis published in systematic reviews. Assumed reduction in PI was 50% (100–50%). With a

loss adjustment of 15%, it was necessary to have 30 patients per group. We recruited 34 patients in stimulated group and 35 patients in control group for a statistical level of 95% and a power of 0.8.

Selection of Patients

Between January 2017 and December 2018, all the patients from participating centres included in the surgical waiting list for temporary stoma closure after colorectal carcinoma were consecutively evaluated to determine their inclusion in the study. The selection flowchart of the study patients can be seen in Figure 1.

The inclusion criteria were being over 18 years of age, having protective ileostomy after colorectal carcinoma surgery free of disease, with endoscopic and histological confirmation of diversion colitis and having signed the informed consent. The exclusion criteria being under 18 years of age, clinical history and histological confirmation of inflammatory bowel disease with colorectal involvement and refusal to participate in the study. Abandonment criteria: loss during follow-up, exitus, and anastomotic leakage after stoma closure.

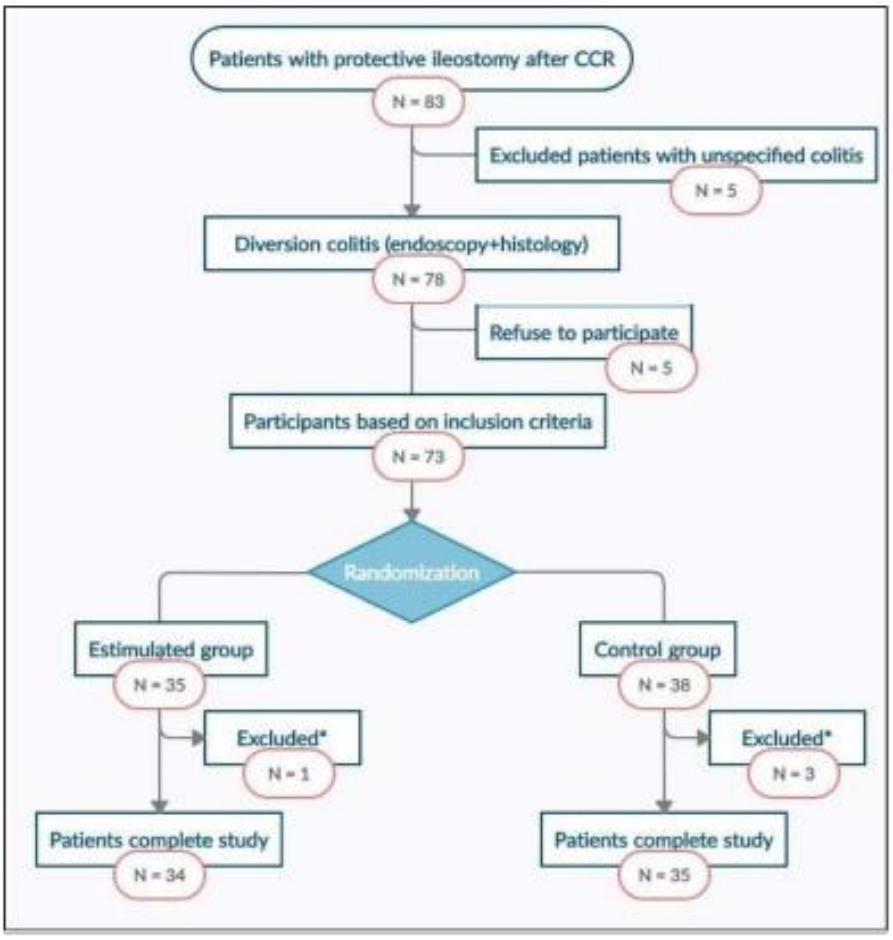


FIGURE 1 | Study flowchart. CRC, Colorrectal cancer. *Excluded patients with anastomic leak.

Randomisation and Intervention

A colonoscopy including biopsies for histological study, was performed on all patients selected to participate in the study. After confirming the diagnosis, level of colitis (based on the Harig scoring system (15)), and excluding patients who did not meet the selection criteria, randomisation was performed. Randomisation was performed by using a computer-generated sequence (Statistical software EPIDAT 4.1) into 2 groups:

- Stimulation group (SG): preoperative stimulation of the distal limb of the ileostomy loop with probiotics was performed during the 20 days prior to

surgery every second day. During the process and after it, the patient registers the appearance of symptoms after each stimulation session: abdominal pain, emission of gas, and stool. A sterile Foley catheter No.14 Ch connected to an infusion set was introduced through the de-functioned bowel to allow the slow infusion of a solution with 4.5 mg of probiotics diluted in 250 ml of 0.9% physiological saline for 20–30 min. Each preparation was made under sterile conditions and maintaining the cold chain. Vivomixx® lyophilised live bacteria, marketed by MENDES, S.A, contained 4.5 × 10 11 of live bacteria in each preparation:

- Four strains of Lactobacillus:
  - Lactobacillus acidophilus DSM 24735®
  - Lactobacillus plantarum DSM 24730®
  - Lactobacillus paracasei DSM 24733®
  - Lactobacillus delbrueckii subsp. bulgaricus DSM 24734®
- Three strains of Bifidobacterium:
  - Bifidobacterium breve DSM 24732®
  - Bifidobacterium longum DSM 24736®
  - Bifidobacterium infantis DSM 24737®
- One strain of Streptococcus
  - Streptococcus thermophilus DSM 24731®

• Control group (CG): exactly the same procedure was carried out, but with the infusion set closed. During the process and after it, the patient himself registers the appearance of

symptoms after each stimulation session: abdominal pain,

emission of gas, and stool.

After ten stimulation sessions, 24 h before surgery, a colonoscopy with biopsy was performed on all patients, re-quantifying the nendoscopic and histological index of severity of diversion colitis.

Surgery and Follow-Up

All patients were admitted in the hospital the day before surgery, fasting, receiving antithrombotic prophylaxis (enoxaparin 40 mg subcutaneous) and premedication according to the pre-anaesthesia's instructions sheet. The reconstruction surgery was carried out by three expert surgeons from the Colorectal Surgery department. A parastomal incision was made and carried out sharply into the peritoneal cavity. The anastomosis was lateral-lateral, either manual or mechanical, according to the decision of the surgeon. Every surgeon was allowed to decide as well whether to change to a median laparotomy procedure. Complications or events happened during surgery were recorded nin the surgical procedure protocol. General anaesthesia was given to all patients and, after extubation and stabilisation in the postoperative resuscitation room, they went directly to the hospitalisation ward.

Follow-up during hospitalisation was carried out by the staff of the Colorectal Surgery department of each centre, recording any postoperative complications, with special vigilance of abdominal pain, passage of flatus, or stool with correct quantification and initiation of oral tolerance. Patients were discharged from the hospital after re-establishing intestinal transit, adequate oral tolerance and stool control, recording the length of stay in hospital.

Follow-up after hospitalisation was carried out by Colorectal Surgery team in the first, third, and sixth postoperative months. These evaluations were performed by

the colorectal surgeon who intervened in each patient. Any symptomatology related to the intervention was recorded, with special monitoring of abdominal pain and number and control of stools.

Blinding

To ensure blinding of the patients, all underwent the same diagnostic procedure. During the stimulation sessions, both the solution with probiotics and the infusion set were covered by an opaque protective envelope, which prevented observing the colour and transparency of the fluid, or whether the system was open or closed. The stimulation sessions were performed by a single surgeon, who was also in charge of preparing the dilution.

The endoscopist, the pathologist and the surgeon who performed the surgical intervention and the follow-up, as well as surgeons who participated, after the surgery, in the hospitalisation process, did not know whether the patient had received probiotics or not.

Assessment Criteria

The main evaluation criterion was the effect caused by stimulation of the efferent loop with probiotics on the persistence and severity index of diversion colitis, both endoscopic and histological, comparing SG and CG after stimulation, after surgery, in the first post-operative month and during short-term follow-up (third and sixth postoperative month).

Secondary evaluation criteria were passage of gas and stool during the stimulation period and after surgery, the initiation of oral tolerance, restoration of intestinal transit (gas and stool), and hospital stay.

Statistical Analysis

A descriptive univariate analysis of sociodemographic and clinical variables was performed. The Kolmogorov-Smirnov test was used to verify the normality of the quantitative variables. To describe the quantitative variables, the mean and standard deviation were used, and the median and interquartile range for those variables that did not follow a normal distribution. For qualitative variables frequencies and percentages will be used. Afterwards, to verify the main objectives, a bivariate analysis was performed. A contrast test of proportions based on the Chi- square test was used in order to determine whether stimulation of the efferent loop with probiotics prior to the closure of the protective ileostomy reduces the level of diversion colitis, as well as its effects on the passage of gas and stool, the initiation of oral tolerance, re-establishing intestinal transit and hospital stay. To quantify its level of association, Cramer's Phi and V coefficients were calculated. In order to correlate quantitative variables, the Spearman's rank correlation coefficient was used. A $p < 0.05$ was considered to be significant. Statistical analysis was performed using the statistical program SPSS version 24.0, with the support of calculation tools provided by the software Microsoft Excel or R.

Ethical Aspects

The project was performed with the consent of the Ethics Coordinating Committee for Biomedical Research of Andalusia, Spain, and registered with the project number 2017/331191354. Written informed consent was requested to participate in the study, giving details of both the study objectives and the methodology to be followed. The data was kept anonymous, maintaining the

confidentiality and anonymity of the participants. The CONSORT Statement criteria for the design of clinical trial were followed (standard trial methodology).

4.2.4. Results

Study Population

Between January 2017 and December 2018, 83 disease free patients with protective ileostomy after colorectal carcinomaresection were reviewed and included in the surgical waiting list for intestinal transit reconstruction. Seventy eight of them met the endoscopic and histological criteria for diversion colitis diagnosis and 73 patients were finally randomised into two groups, intervention (n = 35) and control (n = 38). Sixty nine patients completed the study, 1 of them from SG and 3 from CG abandoning the study because of anastomotic leakage. Study flowchart is presented in Figure 1. There were no significant differences between SG and CG in terms of sociodemographic, clinical, or surgical variables (Table 1).

Main Assessment Criteria

The effect of stimulation with probiotics on diversion colitis, identified by endoscopic, is showed in Figure 2. It shows a homogeneous distribution in the pre-stimulation phase between SG and CG (p = 0.911), with n = 9 patients with severe diversion colitis in both groups (26.5% SG vs. 25.7% CG), n = 23 patients with moderate diversion colitis in both groups (67.6% SG vs.65.7% CG) and n = 2 patients with mild diversion colitis in SG (5.9%) vs. n = 3 patients in CG (8.6%). In the post-stimulation phase, CG maintains its distribution with n = 9 patients with severe diversion colitis (25.7%), n = 23 patients with moderate diversion colitis (65.7% CG), and n = 3 patients with mild diversion colitis (8.6%); on the other hand, SG shows no patients with severe diversion colitis (n = 0), n = 3 patients with moderate diversion colitis (8.8% CG), n = 19

patients with mild diversion colitis (55.9%) and n = 12 patients with normal endoscopic findings (35.3%), achieving a $p < 0.001$ and a Phi and V Cramer coefficient of 0.883.

	Stimulated group (n = 34)	Non-stimulated group (n = 35)	p
Demographics			
Age (years)	65 (45 – 81)	68 (41 – 80)	0.130
Sex ratio (Men/Women)	23:11	25:10	0.733
IMI (kg/m^2)	23.5 (21.6 – 32.6)	27.6 (18.8 – 40.2)	0.091
ASA			0.483
ASA I-II	31	30	
ASA III	3	5	
Smoker / Non-smoker	20/14	23/12	0.826
Colorectal surgery			
Surgical procedure:			0.129
LAR	25	25	
uLAR	9	10	
Surgical approach:			0.551
Laparoscopic	24	24	
Open	10	11	
Type of anastomosis:			0.430
Stapled EEA	31	33	
Coloanal anastomosis	3	2	
Neoadjuvant therapy	26	25	0.239
Adjuvant treatment	26	28	0.256
Ileostomy closure			
Time between surgery	12 (8 – 37)	9 (6 – 32)	0.813
Surgery:			0.690
Small bowel resection	33	34	
Ileocecal resection	1	1	
Surgical approach:			0.291
Peri-ileostomy	30	28	
Midline laparotomy	4	7	
Type of anastomosis:			0.355
Sewn	29	29	
Stapled	5	6	
Time (minutes)	50 (30 – 70)	65 (50 – 120)	0.053

ASA, American Society of Anaesthesiologists Classification; BMI, body mass index; EEA, end to end anastomosis stapler; LAR/uLAR, low anterior resection/ultralow anterior resection.

Table 1. Demographics, clinics, and surgical characteristics.

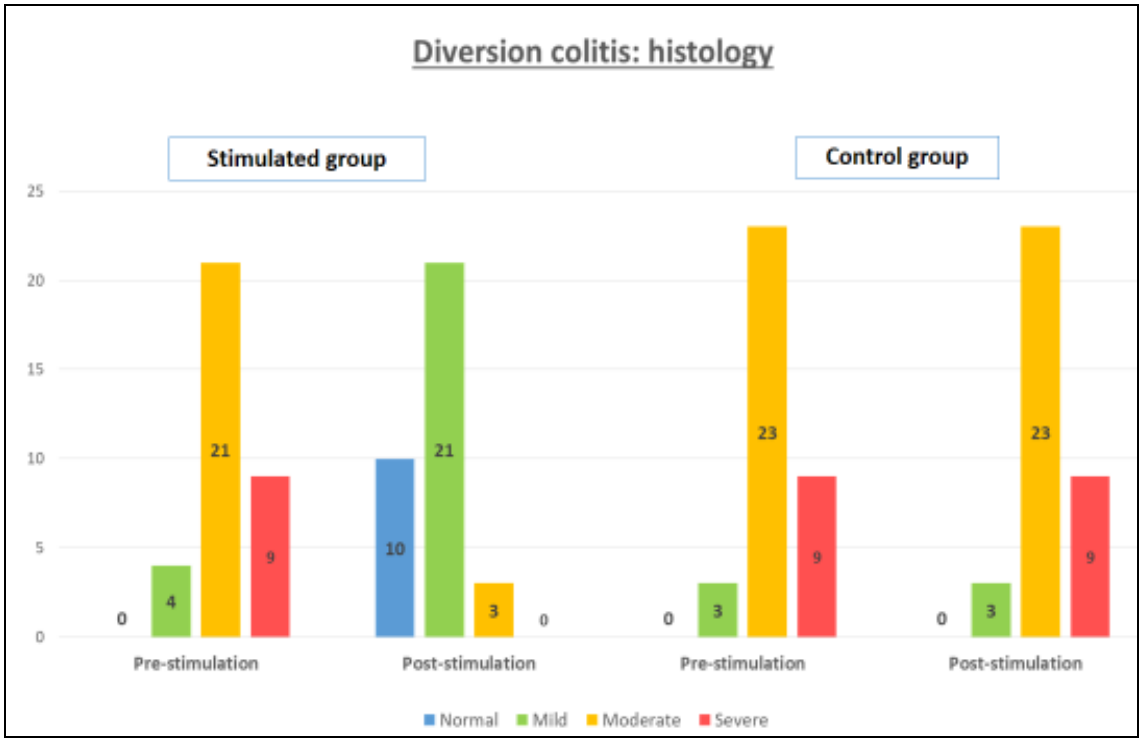
The effect of stimulation with probiotics identified by histology is showed in Figure 3. It shows a homogeneous distribution in the pre-stimulation phase between SG and CG ($p = 0.896$), with n = 9 patients with severe diversion colitis in both groups (26.5% SG vs. 25.7% CG), n = 23 in CG and n = 21 patients with moderate diversion colitis in both groups (61.8% SG vs. 65.7 % CG) and n = 4 patients with mild diversion colitis in SG (11.7%) vs. n = 3 patients in CG (8.6%). In the post-stimulation phase, the CG maintains its distribution with n = 9 patients with severe diversion colitis (25.7%), n = 23 patients with moderate diversion colitis (65.7% CG), and n = 3 patients with mild diversion colitis (8.6%), on the other hand, SG shows no patients with severe diversion colitis (n = 0), n = 3 patients with moderate diversion colitis (8.8% CG), n = 21 patients with mild diversion colitis (61.8%) and n = 10 patients with normal histologic findings (29.4%), getting a $p < 0.001$ and a Phi and V Cramer coefficient of 0.843.

Abdominal pain was the only symptom observed during stimulation, which appeared in 20.5% of patients SG (n = 7) compared to 14, 3% CG (n = 5). There were no statistically significant differences between the two groups. Abdominal pain was evaluated using the visual analogue scale (VAS). Pain was moderated in SG, only present in the first stimulation sessions and disappearing afterwards. Pain was mild-moderate in CG in all stimulation sessions and disappeared after their completion.

The symptoms of diversion colitis after reconstruction surgery appeared in 100% (n = 35) of the patients in CG, vs. 23.5% in SG (n = 8), the remaining 76.5% being asymptomatic after surgery (n = 26), with $p < 0.001$ and a Phi and V Cramer coefficient 0.789. This clinical scenario is maintained 1 month after reconstructive surgery in CG, with 94.3% (n = 33) of patients showing symptoms of diversion colitis, compared to 5.7% who are asymptomatic (n = 2). SG shows 97.1% of asymptomatic patients after surgery (n = 33) compared to 2.9% of patients with diversion colitis (n = 1), with $p < 0.001$ and a Phi and V coefficient of Cramer 0.913. Finally, during the short-term follow-up after reconstructive surgery, symptoms are present in CG with 28.6% (n =

10) compared to 71.4% who are asymptomatic (n = 25). SG shows 100% asymptomatic patients after surgery, with p < 0.001 and a Phi and V Cramer coefficient of 0.406.

Figure 2. Grade of macroscopic diversion colitis, measured by endoscopy, in the stimulated group and control group, in the pre-stimulation and post-stimulation phases.



Secondary Endpoints

In the comparative analysis, we found a direct relationship between passage of gas and stool during the stimulation period and the decrease in the level of diversion colitis in SG with $p < 0.001$ and a Phi and V Cramer coefficient of 0.683 for stool and 0.971 for gas. There were no statistically significant differences among the initiation of oral tolerance, restoration of intestinal transit, or length of hospital stay, as shown in Table 2. The incidence of postoperative ileus was similar in both groups, appearing in 10/34 (29, 4%) for SG and 11/35 (31.4%) for CG with $p = 0.192$. After reconstructive surgery, SG started oral tolerance 24 h earlier than CG, but it was statistically non-significant (2 days vs. 3). The emission of gases and the restoration of intestinal transit happened 2 days after surgery in both groups. SG had an interval between 1 and 20 days in the emission of gases and between 1 and 21 days in the intestinal transit, vs. 1–48 days in both cases in CG. The hospital stay was also shorter in the SG, but without statistical significance.

TABLE 2 | Postoperative results.

	Stimulated group ($n = 34$)	Non-stimulated group ($n = 35$)	p
Postoperative ileus, n (%)	10 (29.4%)	11 (31.4%)	0.192
Nasogastric tube, n (%)	9 (26%)	11 (31.4%)	0.116
Time to tolerating a diet, days-mean (range)	2 (1 – 24)	3 (2 – 50)	0.619
Start of the passage of flatus, days-mean (range)	2 (1 – 20)	2 (1 – 48)	0.173
Start of the passage of stool, days-mean (range)	3 (1 – 21)	3 (1 – 48)	0.184
Postoperative stay days-mean (range)	4 (4 – 26)	5 (4 – 56)	0.105

The median has been used as a measure of central tendency for the evaluation of variables as initiation of oral tolerance, gas emission, restoration of transit and duration of hospital stay.

4.2.5. Discussion

As mentioned in the introduction, colorectal cancer surgery has progressively evolved. More and more complex resections are performed both by abdominal and perineal approach, increasing their quality and achieving a more radical surgery with increasingly inferior anastomoses and without the need for a permanent stoma, even if still requiring a temporary stoma (16). To protect low colorectal anastomosis from possible complications, we must create an ileostomy, which also will lead to morbidity. Derivative stomas are not exempt from complications, the most feared being the anastomotic leak however the most common is diversion colitis (2, 3, 17). In patients that require a protective ileostomy, dysbiosis happens in the excluded colon segment that leads to diversion colitis (6). This dysbiosis activates metabolic products that produce an immune response, causing an attack on the colonic mucosa and affecting the function of the regulatory immune cells that normally promote its homeostasis (18). This causes structural changes, such as atrophy of villi in the defunctionalised limb, and in consequence produce loss of smooth muscle area and reduced isometric contractility with loss of the intestinal absorption capacity (7). Intestinal dysbiosis has been linked to the pathogenesis of numerous chronic diseases, such as inflammatory bowel disease, and has been studied in order to identify its correction, with variable results (19, 20).

The bibliography about diversion colitis and its relationship with microbiota alteration is not extensive. In the last 2 years, two studies have been published about intestinal microbiota (19, 21); to which we must add, on the one hand, the latest systematic review on diversion colitis published by Kabir et al. (6) that reviews a total of 3,305 articles, eventually including 35 studies, and analyses the pathophysiology, clinical presentation and treatment of diversion colitis which concludes that there is a great variability of forms of presentation and a single definitive treatment, which is the reconstruction of the transit. Results on preoperative stimulation prior to the closure

of the protective ileostomy published by Rombey et al. (10), including 8 studies with a total of 267 patients, despite the promising initial results, show that there is no sufficient quality evidence to recommend routine implementation of preoperative bowel stimulation in clinical practise. That is why we propose that microbial manipulation by stimulating probiotics prior to closing the protective ileostomy would allow the altered autoimmune function to be normalised, restore homeostasis, and reduce mucosal inflammation.

After completing the stimulation phase with probiotics, no statistically significant differences were detected in terms of sociodemographic, clinical or surgical variables. On the contrary, there is indeed a notable decrease in surgical time in SG, which we associate with the thickening of the distal end caused by stimulation and which facilitates the performance of the anastomosis. In the pre-stimulation phase, no differences were found in terms of index of severity in both groups, either by endoscopy or histology. After stimulation, a decrease of the index of severity was observed in SG compared to CG, both by endoscopy and histology, as shown in Figures 2,3.

A reduction in the level of diversion colitis was achieved by 100% of SG patients, with the disappearance of macroscopic diversion colitis in 35.3%, obtaining normal endoscopic findings in 12 patients ($p < 0.001$ and a Phi and V Cramer coefficient of 0.883) and the disappearance of microscopic sign in 29.4%, obtaining normal histological findings in 10 patients ($p < 0.001$ and a Phi and V Cramer coefficient of 0.843). Both cases showed a strong correlation between stimulation with probiotics and the decrease of diversion colitis, with the appearance of colicky abdominal pain in 20.5% of the patients (n = 7) as the only side-effect, valued as moderate pain using the visual analogue scale (VAS) and only associated with the first stimulation sessions, disappearing afterwards. This effect has already been described in studies with stimulation of the efferent loop with short chain fatty acids (6, 10, 15).

At the same time as stimulation, an increase in stool and gas emission was observed during this phase in SG compared to CG, which we directly relate to a decrease or disappearance of diversion colitis after stimulation with probiotics with a value of $p < 0.001$ and a Phi and V Cramer coefficient of 0.683 for stool and 0.971 for gas, both showing the association with a strong correlation. Initiation of intestinal transit prior to reconstruction allows the identification of functional obstructions distal to the ileostomy that can cause mechanical ileus after surgery and that can be solved by endoscopic dilations or, on the contrary, it allows the identification of a level of incontinence associated with the anterior resection syndrome, which allows for early toilet training(22, 23).

Regarding the effect on bowel function after transit reconstruction, no differences were observed between the two groups, with a non-statistically significant time to recovery of transit and oral tolerance. The difference in terms of hospital stay was 1 day, with a median of 4 days in SG and 5 days in CG, associating it with 24-h difference in oral tolerance between SG, in which 58% of patients tolerate liquid diet in the first 24–48 h, compared to the CG, which requires 48–72 h. There were no differences in postoperative ileus between SG (n = 10, 29.4%) and CG (n = 11, 31.4%), highlighting a disagreement with systematic reviews published by Rombey and Garfinkle and other studies on post-operative ileus that seem to improve after stimulation of the efferent loop prior to closure of the ileostomy (7, 23–28). Nonetheless, both conclude with a lack of evidence to recommend the stimulation of the efferent loop in a routine way and emphasise the need to carry out multicentre studies with higher scientific quality, in order to verify their validity.

The observations presented here demonstrate that the decrease in endoscopic and histological findings associated with the decrease in inflammation means that stimulation of the efferent loop of the ileostomy with probiotics can be an alternative treatment in patients with symptomatic DC who are not candidates for reconstructive

surgery as a treatment to resolve the colonic inflammation (29, 30). Finally, the stimulation of the efferent loop with probiotics can be an alternative treatment to resolve the inflammation in patients whose surgical option is not feasible.

The most studied probiotic bacterias are Bifidobacterium and Lactobacillus spp. These two produce a decrease of pro‐inflammatory molecules and an increase of molecules that inhibit inflammation, also protecting against oxidative stress in humans and demonstrating an important role in intestinal disbiosis (7, 31, 32). Currently, the evidence shows that probiotics have a positive effect on systemic and oxidative inflammation, especially at the gastrointestinal level. L. acidophilus, one of the probiotics administered during our stimulation, has demonstrated its role in inflammatory regulation in multiple experimental studies (32–41). A randomised controlled clinical trial conducted by Jafarnejad et al. (42) describes the effect of supplementation with probiotics for 8 weeks, in this case, with VSL3 (a probiotic similar to the one administered in our study), on glycaemic status and inflammatory markers among pregnant women. The probiotic supplements contained eight strains of lactic acid bacteria (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L. acidophilus, L. plantarum, L. paracasei, and L. delbrueckii subsp. Bulgaricus). They found a statistically significant decrease in TNF alpha and CRP levels in the probiotic group compared to the placebo. Results are superimposable to other studies (41).

Finally, it was possible to reduce diversion colitis symptoms after reconstructive surgery in 76.5% of SG (n = 26) with $p < 0.001$ and observing correlation between the absence of symptoms and stimulation with a V Cramer coefficient of 0.789, compared to CG, in which 100% of patients presented symptoms. This is maintained in the follow-up to the first month after closure ileostomy with 94.3% of symptomatic patients in CG vs. 2.9% in SG, with $p < 0.001$ and an association of 0.913. However, in the short-term follow-up, both tend to equalise, achieving 100% asymptomatic

patients in SG and 71.4% in CG, with $p < 0.001$ and a Phi and V Cramer coefficient of 0.406. This correlation disappears between the third and sixth month, when diversion colitis becomes non-existent both endoscopically and microscopically. This fact coincides with the transitory and limited modulatory effect that probiotics produces on the intestinal mucosa (43–47) and with the recovery curve of intestinal function published in other studies (7, 9). However, as the main limitation of our study has been the sample size we cannot make definitive statements about the effectiveness of this technique. We will need additional multicentre studies on bowel function and preoperative stimulation prior to closure of the protective ileostomy to confirm these conclusions.

4.2.6. Conclusions

The pathogenesis of diversion colitis is related with a chronic inflammation and decrement of flora in the dysfunctional area. The definitive treatment is the restoration of the continuity of the digestive tract, and the stimulation of the efferent loop with probiotics would allow patients to reduce complications in the pre/postoperative process. The stimulation with probiotics is a safe and feasible procedure with minimal adverse effects, being an option for patients who are not candidates for surgical treatment.

4.2.7. References

1. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, et al. ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. (2017) 28(Suppl 4):vi22–40. doi: 10.1093/annonc/mdx224

2. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum

for cancer: a randomized multicenter trial. Ann Surg. (2007) 246:207–14. doi: 10.1097/SLA.0b013e3180603024

3. Sharma A, Deeb AP, Rickles AS, Iannuzzi JC, Monson JRT, Fleming FJ. Closure of defunctioning loop ileostomy is associated with considerable morbidity. Colorectal Dis. (2013) 15:458–62. doi: 10.1111/codi.12029

4. Szczepkowski M, Banasiewicz T, Kobus A. Diversion colitis 25 years later: the phenomenon of the disease. Int J Colorectal Dis. (2017) 32:1191–6. doi: 10.1007/s00384-017-2802-z

5. Glotzer DJ, Glick ME, Goldman H. Proctitis and colitis following diversion of the fecal stream. Gastroenterology. (1981) 80:438–41. doi: 10.1016/0016-5085(81)90003-2

6. Kabir SI, Kabir SA, Richards R, Ahmed J, MacFie J. Pathophysiology, clinical presentation and management of diversion colitis: a review of current literature. Int J Surg. (2014) 12:1088–92. doi: 10.1016/j.ijsu.2014.08.350

7. Beamish EL, Johnson J, Shaw EJ, Scott NA, Bhowmick A, Rigby RJ. Loop ileostomy - mediated fecal stream diversion is associated with microbial dysbiosis. Gut Microbes. (2017) 8:467–78. doi: 10.1080/19490976.2017.1339003

8. Williams L, Armstrong MJ, Finan P, Sagar P, Burke D. The effect of faecal diversion on human ileum. Gut. (2007) 56:796–801. doi: 10.1136/gut.2006.102046

9. Keane C, Park J, Öberg S, Wedin A, Bock D, Grady G, et al. Functional outcomes from a randomized trial of early closure of temporary ileostomy after rectal excision for cancer. Br J Surg. (2019) 106:645–52. doi: 10.1002/bjs.11092

10. Rombey T, Panagiotopoulou IG, Hind D, Fearnhead NS. Preoperative bowel stimulation prior to ileostomy closure to restore bowel function more quickly and improve postoperative outcomes: a systematic review. Colorectal Dis. (2019) 21:994–1003. doi: 10.1111/codi.14636

11. Shen Z, Zhu C, Quan Y, Yang Z, Wu S, Luo W, et al. Relationship between intestinal microbiota and ulcerative colitis: mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J Gastroenterol. (2018) 24:5–14. doi: 10.3748/wjg.v24.i1.5

12. Biagioli M, Capobianco D, Carino A, Marchianò S, Fiorucci C, Ricci P, Distrutti E, et al. Divergent effectiveness of multispecies probiotic preparations on intestinal microbiota structure depends on metabolic properties. Nutrients. (2019) 11:325. doi: 10.3390/nu11020325

13. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. (2014) 11:506–

14. doi: 10.1038/nrgastro.2014.66 14. Corridoni D, Pastorelli L, Mattioli B, Locovei S, Ishikawa D, Arseneau KO, et al. Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. PLoS ONE. (2012) 7:e42067. doi: 10.1371/journal.pone.0042067

15. Harig JM, Soergel KH, Komorowsky RA, Wood CM. Treatment of diversion colitis with short-chain-fatty acid irrigation. N Eng J Med. (1989) 320:23–8. doi: 10.1056/NEJM198901053200105

16. Heald RJ, Santiago I, Pares O, Carvalho C, Figueiredo N. The perfect total mesorectal excision obviates the need for anything else in the management of most rectal cancers. Clin Colon Rectal Surg. (2017) 30:324–32. doi: 10.1055/s-0037-1606109

17. Farag S, Rehman S, Sains P, Baig MK, Sajid MS. Early vs delayed closure of loop defunctioning ileostomy in patients undergoing distal colorectal resections: an integrated systematic review and meta-analysis of published randomized controlled trials. Colorectal Dis. (2017) 19:1050–7. doi: 10.1111/codi.13922

18. Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. J AOAC Int. (2012) 95:50–60. doi: 10.5740/jaoacint.SGE_Macfarlane

19. Mishima Y, Sartor RB. Manipulating resident microbiota to enhance regulatory immune function to treat inflammatory bowel diseases. J Gastroenterol. (2020) 55:4–14. doi: 10.1007/s00535-019-01618-1

20. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in bowel diseases. Nature. (2019) 569:655–62. doi: 10.1038/s41586-019-1237-9

21. Knox NC, Forbes JD, Van Domselaar G, Bernstein CN. The gut microbiome as a target for IBD treatment: are we there yet? Curr Treat Options Gastroenterol. (2019) 17:115–26. doi: 10.1007/s11938-019-00221-w

22. Kim KH, Yu CS, Yoon YS, Yoon SN, Lim SB, Kim JC. Effectiveness of biofeedback therapy in the treatment of anterior resection syndrome after rectal cancer surgery. Dis Colon Rectum. (2011) 54:1007–13. doi: 10.1097/DCR.0b013e318221a934

23. Abrisqueta J, Abellan I, Lujan J, Hernandez Q, Parrilla P. Stimulation of the efferent limb before ileostomy closure: a randomized clinical trial. Dis Colon Rectum. (2014) 57:1391–6. doi: 10.1097/DCR.0000000000000237

24. Garfinkle R, Savage P, Boutros M, Landry P, Reynier P, Morin N, et al. Incidence and predictors of postoperative ileus after loop ileostomy closure: a systematic review and meta-analysis. Surg Endosc. (2019) 33:2430–43. doi: 10.1007/s00464-019-06794-y

25. Menéndez P, Garcia A, Lozano E, Pelaez R. Effectiveness of afferent loop stimulation prior to ileostomy closure. Cir Esp. (2013) 91:547–8. doi: 10.1016/j.cireng.2013.11.007

26. Vázquez-Melero A, Loizate-Totoricaguena A, García-Alonso P. Stimulation of the Efferent Loop Before Loop Ileostomy closure. U.S. National Library of Medicine, Bethesda (Maryland, USA). ClinicalTrials.gov Identifier: NCT03424447 (2018).

27. Fernández-López F, González López J, Paz Novo M, Ladra González MJ, Paredes Cotoré J. Estimulación preoperatoria del asa eferente de la ileostomía con ácidos grasos de cadena corta. Cir Esp. (2019) 97:54–6. doi: 10.1016/j.ciresp.2018.06.018

28. Garfinkle R, Trabulsi N, Morin N, Phang T, Liberman S, Feldman L, et al. Study protocol evaluating the use of bowel stimulation before loop ileostomy closure to reduce postoperative ileus: a multicenter randomized controlled trial. Colorectal Dis. (2017) 19:1024–9. doi: 10.1111/codi.13720

29. Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez□Salgado J, Rada-Morgades R, Ruiz-Frutos C. Diversion Colitis: macro and microscopic findings after probiotics stimulation. Biology. (2021) 10:303. doi: 10.3390/biology10040303

30. Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez□Salgado J, Balongo-García R, Ruiz-Frutos C. Postoperative Ileus after stimulation with probiotics before ileostomy closure. Nutrients. (2021) 13:626. doi: 10.3390/nu13020626

31. Morgan DM, Cao Y, Miller K, McGoldrick J, Bellavance D, Chin SM, et al. Microscopic colitis is characterized by intestinal dysbiosis. Clin Gastroenterol Hepatol. (2020) 18:984–6. doi: 10.1016/j.cgh.2019.06.035

32. Rossi M, Amaretti A. Bifidobacteria: genomics and molecular aspects. In: Q16 Mayo B, van Sinderen D, editors. Probiotic Properties of Bifidobacteria. Horizon Scientific Press. (2010). p. 97–123.

33. Buanaim RP, Pereira JA, Campos FG, Kotze PG, Goto EFK, Mendonça RLS, et al. Effects of anti-TNF-α in experimental diversion colitis. Acta Cir Bras. (2019)

34: e201901004. doi: 10.1590/s0102-865020190100000004 34. Biagioli M, Laghi L, Carino A, Cipriani S, Distrutti E, Marchianò S, et al. Metabolic variability of a multispecies probiotic preparation impacts on the anti-inflammatory activity. Front Pharmacol. (2017) 8:505. doi: 10.3389/fphar.2017.00505

35. Hod K, Sperber AD, Ron Y, Boaz M, Dickman R, Berliner S, et al. A double‐blind, placebo-controlled study to assess the effect of a probiotic mixture on symptoms and inflammatory markers in women with diarrhea-predominant IBS. Neurogastroenterol Motil. (2017) 29:e13037. doi: 10.1111/nmo.13037

36. Tenorio-Jiménez C, Martínez-Ramírez MJ, Del Castillo-Codes I, Arraiza‐Irigoyen C, Tercero-Lozano M, Camacho J, et al. Lactobacillus reuteri V3401 reduces inflammatory biomarkers and modifies the gastrointestinal microbiome in adults with metabolic syndrome: the PROSIR study. Nutrients. (2019) 11:1761. doi: 10.3390/nu11081761

37. Yang YJ, Chuang CC, Yang HB, Lu CC, Sheu BS. Lactobacillus acidophilus ameliorates H. pylori-induced gastric inflammation by inactivating the Smad7 and NFkappaB pathways. BMC Microbiology. (2012) 12:38. doi: 10.1186/1471-2180-12-38

38. Borthakur A, Bhattacharyya S, Kumar A, Anbazhagan AN, Tobacman JK, Dudeja PK. Lactobacillus acidophilus alleviates platelet-activating factor‐induced inflammatory responses in human intestinal epithelial cells. PLoS ONE. (2013) 8:e75664. doi: 10.1371/journal.pone.0075664

39. Li H, Zhang L, Chen L, Zhu Q, Wang W, Qiao J. Lactobacillus acidophilus alleviates the inflammatory response to enterotoxigenic Escherichia coli K88 via inhibition of the NF-kappaB and p38 mitogen-activated protein kinase signaling pathways in piglets. BMC Microbiol. (2016) 16:273. doi: 10.1186/s12866-016-0862-9

40. Stenman LK, Waget A, Garret C, Klopp P, Burcelin R, Lahtinen S. Potential probiotic Bifidobacterium animalis ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. Benef Microbes. (2014) 5:437– 45. doi: 10.3920/BM2014.0014

41. Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Abbasi MM, Dolatkhah N. Effect of probiotic supplements in women with gestational diabetes

mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial. Asia Pac J Clin Nutr. (2018) 27:581–91. doi: 10.6133/apjcn.082017.03

42. Jafarnejad S, Saremi S, Jafarnejad F, Arab A. Effects of a multispecies probiotic mixture on glycemic control and inflammatory status in women with gestational diabetes: a randomized controlled clinical trial. J Nutr Metab. (2016) 2016:5190846. doi: 10.1155/2016/5190846

43. Sartor RB, Wu GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. Gastroenterology. (2017) 152:327–39. doi: 10.1053/j.gastro.2016.10.012

44. Cohen LJ, Cho JH, Gevers D, Chu H. Genetic factors and the intestinal microbiome guide development of microbe-based therapies for inflammatory bowel diseases. Gastroenterology. (2019) 156:2174–89. doi: 10.1053/j.gastro.2019.03.017

45. Kang SI, Oh HK, Kim MJ, Son IT, Kim DW, Kang SB. The effect of probiotics (CJLP243) on bowel function restoration after ileostomy closure in patients Q17 with rectal cancer: a pilot randomised controlled trial. Colorectal Dis. (2016) 18(Suppl 1):127.

46. Basso PJ, Câmara NOS, Sales-Campos H. Microbial-based therapies in the treatment of inflammatory bowel disease—an overview of human studies. Front Pharmacol. (2018) 9:1571. doi: 10.3389/fphar.2018.01571

47. Amoroso C, Perillo F, Strati F, Fantini M, Caprioli F, Facciotti F. The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. Cells. (2020) 9:1234. doi: 10.3390/cells9051234

4.3. Diversion colitis: macro and microscopic findings after probiotics stimulation

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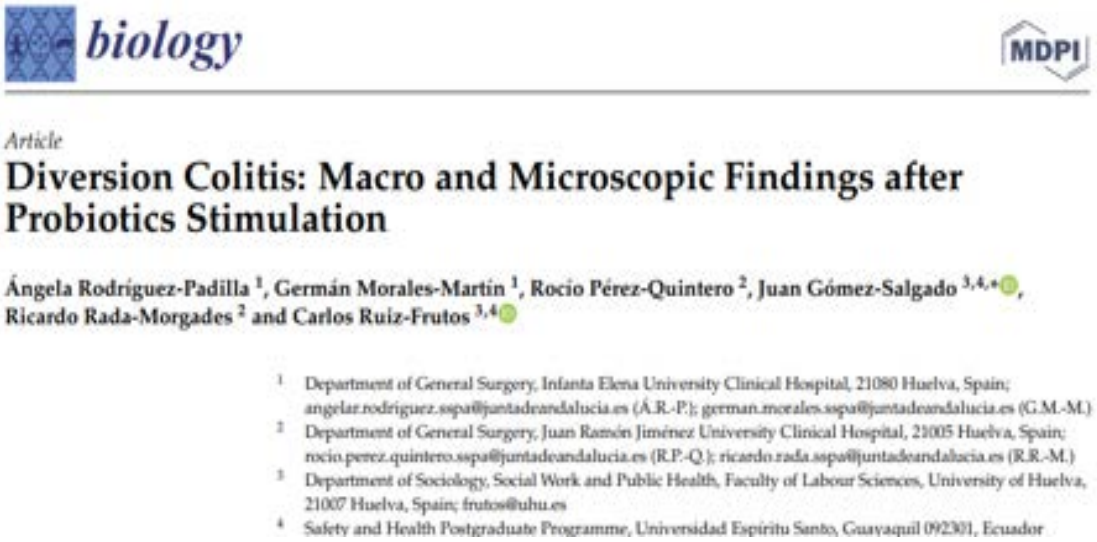

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Diversion Colitis: Macro and Microscopic Findings after Probiotics Stimulation

Ángela Rodríguez-Padilla [1], Germán Morales-Martín [1], Rocío Pérez-Quintero [2], Juan Gómez-Salgado [3,4,*], Ricardo Rada-Morgades [2] and Carlos Ruiz-Frutos [3,4]

1 Department of General Surgery, Infanta Elena University Clinical Hospital, 21080 Huelva, Spain; angelar.rodriguez.sspa@juntadeandalucia.es (Á.R.-P.); german.morales.sspa@juntadeandalucia.es (G.M.-M.)
2 Department of General Surgery, Juan Ramón Jiménez University Clinical Hospital, 21005 Huelva, Spain; rocio.perez.quintero.sspa@juntadeandalucia.es (R.P.-Q.); ricardo.rada.sspa@juntadeandalucia.es (R.R.-M.)
3 Department of Sociology, Social Work and Public Health, Faculty of Labour Sciences, University of Huelva, 21007 Huelva, Spain; frutos@uhu.es
4 Safety and Health Postgraduate Programme, Universidad Espíritu Santo, Guayaquil 092301, Ecuador

4.3.1. Abstract

Simple Summary: The observations presented in this study conclude that the preoperative stimulation with probiotics of the efferent loop through the dysfunctional bowel, to allow the slow infusion, can have a reducing effect on the endoscopic and histopathological alterations of diversion colitis. This procedure may be an alternative treatment to resolve the inflammation in patients where the surgical option is not feasible or available.

Abstract: The use of a loop ileostomy as the defunctioning procedure of choice to protect a distal colonic anastomosis causes histological and endoscopic changes in the intestinal mucosal architecture, which have been related to chronic inflammation and changes in the microflora that consequently impact the intestinal structure and function following fecal stream diversion. The aim of this study was to evaluate the histological and endoscopic changes on the colonic mucosa in patients with diversion colitis after stimulation of the efferent loop with probiotics prior to closure of the protective ileostomy. A prospective, randomized, double-blind, controlled study was designed. All patients who underwent surgery for colorectal carcinoma with protective ileostomy between January 2017 and December 2018 were included. These patients were pending reconstructive surgery and were diagnosed with endoscopic and histological diversion colitis. Divided into two groups, a group stimulated with probiotics (SG) and a control group (CG). 34 cases and 35 controls were included in the study. Histological and endoscopic changes were evaluated after stimulation, after restorative surgery and during the short-term follow-up after surgery. A decrease in endoscopic pathological findings (mucosal friability, mucous erosions, polyps, edema, erythema and stenosis) and in histological findings (follicular hyperplasia, eosinophils, cryptic abscesses, lymphocyte infiltration, plasma cell infiltration and architecture distortion) was observed in SG. These results were statistically significant with a $p < 0.001$. The stimulation of the efferent loop of the ileostomy in patients with diversion

colitis produced a decrease of the endoscopic and histological severity of colitis in the short term.

Keywords: diversion colitis; colonoscopy; efferent loop stimulation; ileostomy closure; probiotics; microbiome therapeutics.

4.3.2. Introduction

Probiotics were defined as live microorganisms that improve the host's health when administered in adequate amounts [1,2]. These microorganisms and their metabolic products have been suggested as food supplements to achieve a healthier intestinal homeostasis and, also, as a treatment for pathologies with an important inflammatory component. In recent years, multiple studies have been published which explain the use of probiotics and their effect on the microbiota flora when applied to different pathologies such as diabetes mellitus, obesity, inflammatory bowel disease, colitis and even psychological disorders[3–10]. Depending on the strains included in probiotics, with different biochemical and immunological properties, the modulating effect of inflammation was evaluated in vitro and in vivo [11]. These biological effects are closely related to the microbiota metabolism and the ability to colonize the gastrointestinal tract [7]. Probiotics interact with the intestinal mucosa, reducing the molecular production of proinflammatory substances [1]. Live bacteria, such as Lactobacillus, Bifidobacterium, and Enterococcus, present antimicrobial and immunomodulatory activity and improve the intestinal barrier [3]. This immunomodulatory effect is what is needed in order to reduce the level of diversion colitis [12–14]. Currently available probiotics, aimed at other pathologies with inflammatory conditions, produce a modulating effect in a transitory and limited way. This time-limited effect is also essential to reduce the level of diversion colitis, since after reconstruction the effect of probiotics tends to disappear [15,16].

In patients suffering from colorectal cancer who have undergone partial colonic resection, the creation of a temporary ileostomy causes a defunctionalization of the colon [15]. Consequently, the microflora within the defunctioned colon is less diverse and convergence between defunctioned microbiota profiles was observed; additionally, a chronic inflammation appears, with endoscopic and histological changes compatible with diversion colitis (DC) [17,18].

Diversion colitis was described by Glotzer et al. in 1981 [19]. It is characterized by an inflammation of the large bowel mucosa that mimics idiopathic inflammatory bowel disease [15]. It has an incidence between 50–91% [15,16]. It can manifest symptoms such as abdominal or pelvic pain, mucous discharge, tenesmus, fever, and rectal bleeding in the most severe cases, although up to 30% of patients remain asymptomatic [16]. 52% of patients with DC show mild inflammation, 44% moderate and 4% severe [3,20], which represents an annual incidence close to 120,000 patients/year. DC is still a problem and probably will remain so in the future, which impairs the quality of life of a significant number of patients [15,16]. DC shows a wide spectrum of endoscopic and histopathological changes, most importantly in the distal segments of the colon.

The endoscopic findings described are: friable mucosa, edema, erythema, appearance of polyps, ulcers, stenosis and histological findings such as lymphoid follicular hyperplasia, infiltration of the lamina propria by lymphocytes, eosinophils, the appearance of plasma cells, architectural disruption, and the appearance of crypt abscesses [15,21].

The definitive treatment is the restoration of the continuity of the digestive tract [15,16]. Pharmacological treatments using instillation techniques with short-chain fatty acids, mesalazine, fiber or corticosteroids are reserved for patients who are not candidates for surgical treatment or for stimulation of the efferent loop prior to surgery [22–24]. Stimulation with probiotics prior to closing the protective stoma

would allow the dysfunctional colon segment to be repopulated, which would reduce diversion colitis [3,15,16]. The objective of this study was to evaluate the histological and endoscopic changes in the colonic mucosa in patients with diversion colitis after stimulation of the efferent loop with probiotics and prior to closure of the protective ileostomy.

4.3.3. Materials and methods

Study Design

Prospective, randomized, multicenter, double-blind study, comparing two groups of patients operated for colorectal carcinoma with protective ileostomy. One group includes patients treated with probiotic stimulation of the efferent loop prior to transit reconstruction surgery; the other control group was stimulated without any substance.

Sample Size

The sample size was calculated according to the diversion colitis endoscopic and histological findings published in previous studies [16,24]. Assumed reduction in macroscopic and microscopic pathological findings was 50% (100–50%). With a loss adjustment of 15%, it was necessary to have 30 patients per group. We recruited 34 patients for the stimulated group and 35 patients for the control group for a statistical level of 95% and a power of 0.8.

Selection of Patients

Between January 2017 and December 2018, all the patients, from the three participating centers, that were included in the surgical waiting list for temporary stoma closure (coded according to CIE-10) after colorectal carcinoma, were consecutively evaluated to determine their inclusion in the study. The flowchart for the selection of the study patients is depicted in Figure 1.

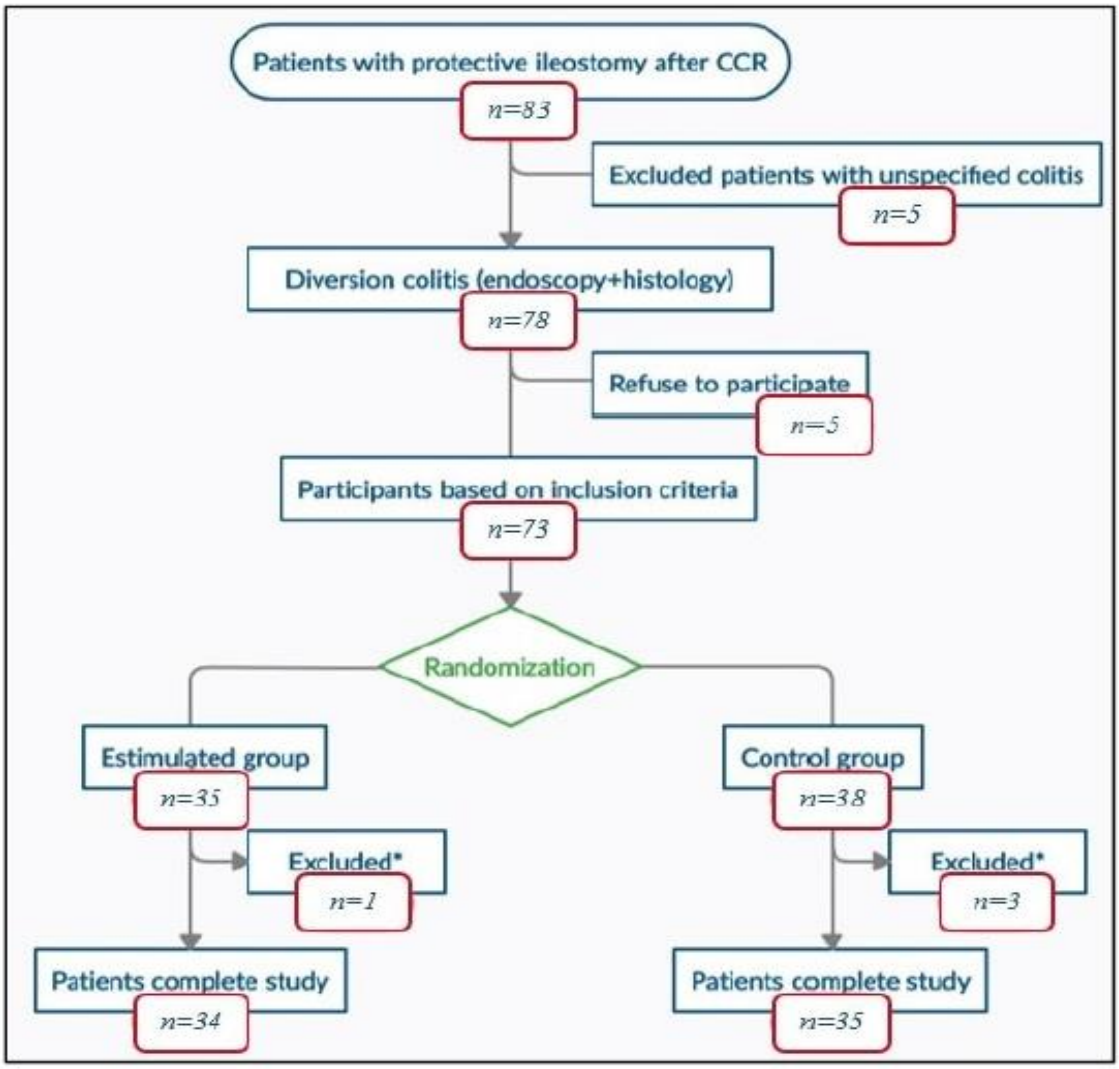


Figure 1. Study flowchart; CRC: Colorectal Cancer; *: excluded patients with anastomotic leak.

The inclusion criteria were being over 18 years of age, having protective ileostomy after colorectal carcinoma surgery free of disease, with endoscopic and histological confirmation of diversion colitis and having signed the informed consent. As exclusion criteria: being under 18 years of age, clinical history, and histological confirmation of inflammatory bowel disease with colorectal involvement, and refusal to participate in the study. Abandonment criteria: loss during follow-up, exitus, and anastomotic dehiscence after reconstruction surgery.

Endoscopic and Histopathological Examination

Colonoscopies with random biopsies were performed on all patients to exclude those who did not have colitis and to establish the severity index. Three endoscopists performed the endoscopies with a Silver Scope Storz® colonoscope (Karl Storz SE; Co. KG, Tuttlingen, Germany). The endoscopic findings were expressed according to the scoring system of Harig et al. [20] including erythema, edema, friability, polypus, granularity, stenosis and erosions. Every finding was scored in a scale from 0 to 1 or from 0 to 3, and the grade of colitis was obtained by adding these scores. The total score was validated as absent, mild, moderate, or severe. The histopathological analysis nwas assessed by three independent histopathologists. Samples of colon were fixed in buffered formalin and stained with hematoxylin and eosin (H&E). In the same way as in the endoscopy, DC was diagnosed by evaluating the appearance of follicular lymphoid hyperplasia, eosinophilic, lymphocytes and plasma cells infiltrations and crypt narchitecture distortion. Every finding was scored in a scale from 0 to 1 or from 0 to 3, obtaining the grade of colitis by adding the results. The total score was validated as absent, mild, moderate, or severe.

Both endoscopic and histopathological studies were performed after stimulation and 3 months after intestinal continuity reconstruction.

Randomization and Intervention

After confirming the diagnosis and excluding patients who did not meet the selection criteria, randomization was performed creating two groups by using a computer-generated sequence on EPIDAT (Statistical software EPIDAT version 4.2. Consellería de Sanidade, Xunta de Galicia, España; Organización Panamericana de la Salud (OPS-OMS); Universidad CES, Colombia):

Stimulation group (SG): preoperative stimulation with probiotics of the distal limb of the ileostomy loop was performed during the 20 days prior to surgery every second day. During the process and after it, the patient himself registered the appearance of symptoms after each stimulation session: abdominal pain, emission of gas and stool. A sterile Foley catheter No.14 Ch connected to an infusion set was introduced through the defunctioned bowel. This was done to allow slow infusion of a solution of 4.5 mg of probiotics diluted in 250 mL of 0.9% physiological saline for 20–30 min. Each preparation was made under sterile conditions and maintaining the cold chain. Vivomixx ® lyophilized live bacteria, marketed by MENDES, S.A, contained 4.5 × 10 11 of live bacteria in each preparation:

- o Four strains of Lactobacillus:
  - ▪ Lactobacillus acidophilus DSM 24735 ®
  - ▪ Lactobacillus plantarum DSM 24730 ®
  - ▪ Lactobacillus paracasei DSM 24733 ®
  - ▪ Lactobacillus delbrueckii subsp. bulgaricus DSM 24734 ®
- o Three strains of Bifidobacterium:
  - ▪ Bifidobacterium breve DSM 24732 ®
  - ▪ Bifidobacterium longum DSM 24736 ®

- ▪ Bifidobacterium infantis DSM 24737 ®

- o One strain of Streptococcus

  - ▪ Streptococcus thermophilus DSM 24731 ®

Control group (CG): exactly the same procedure was carried out, but with the closed infusion set. During the process and after it, the patient himself registered the appearance of symptoms after each stimulation session: abdominal pain, emission of gas and stool.

After ten stimulation sessions, 24 h before surgery, a colonoscopy with biopsy was performed on all patients, re-quantifying the index of severity of endoscopic and histological diversion colitis.

Surgery and Follow-Up

The reconstruction surgery was carried out by three expert surgeons from the Colorectal Surgery Department. A parastomal incision was made and carried out sharply into the peritoneal cavity. The anastomosis was lateral-lateral, either manual or mechanical, according to the decision of the surgeon. Every surgeon could decide whether to change to a median laparotomy procedure or not. Complications or events happened during surgery were recorded in the surgical procedure protocol. General anesthesia was given to all patients and, after extubation and stabilization in the postoperative recovery room, they went directly to the hospitalization ward.

Follow-up during hospitalization was carried out by the team of the Colorectal Surgery department of every center, recording any postoperative complications. Patients were discharged from the hospital after re-establishing intestinal transit, adequate oral tolerance and stool control, recording the length of their hospital stay.

Follow-up after hospitalization was carried out by the Colorectal Surgery team in the first, third and sixth postoperative months. These evaluations were performed by the colorectal surgeon who had performed the surgical intervention in each patient. Any symptomatology related to the intervention was recorded, with special monitoring of abdominal pain, number and control of stools, mucous discharge, tenesmus and rectorrhagia. After completing a three-month follow-up, a colonoscopy with biopsies was performed to evaluate the presence and grade of DC.

Masking

To ensure masking of the patients, all underwent the same diagnostic procedure. During the stimulation sessions, both the solution with probiotics and the infusion set were covered by an opaque protective envelope, which prevented from observing the color and transparency of the fluid, or whether the system was open or closed. The stimulation sessions were performed by a single surgeon, who was also in charge of preparing the dilution.

The endoscopist, the pathologist and the surgeon who performed the surgical intervention and the follow-up, as well as the surgeons who participated in the hospitalization process after the surgery, did not know whether the patient had received probiotics or not.

Assessment Criteria

The main endpoint was the decrease or disappearance of endoscopic and histological findings in the short-term follow-up, caused by stimulation of the efferent loop with probiotics. The secondary endpoints were the correlation of endoscopic and histological severity with DC symptoms and its decrease or disappearance after

stimulation with probiotics and in the short-term follow-up after reconstructive surgery (3 months).

Statistical Analysis

A descriptive univariate analysis of sociodemographic and clinical variables was performed. The Kolmogorov-Smirnov test was used to verify the normality of the quantitative variables. To describe the quantitative variables, the mean and standard deviation were used, and the median and interquartile range for those variables that did not follow a normal distribution. For qualitative variables frequencies and percentages were used. Afterwards, to verify the main objectives, a bivariate analysis was performed. A contrast test of proportions based on the Chi-square test was used in order to determine whether stimulation of the efferent loop with probiotics prior to the closure of the protective ileostomy reduced the level of diversion colitis, macroscopic and microscopic findings, as well as its relationship with the appearance of symptoms of diversion colitis.

To quantify its level of association, Cramer's Phi and V coefficients were calculated. In order to correlate quantitative variables, the Spearman's rank correlation coefficient was used; 0.05 was considered to be significant. Statistical analyses were performed using the statistical program SPSS ® version 24.0 (IBM, Armonk, NY, USA), with the support of calculation tools provided by the software Microsoft Excel and R.

Ethical Aspects

The project was performed according to the guidelines of the Declaration of Helsinki, with the authorization of the Ethics Coordinating Committee for Biomedical Research of Andalusia, Spain, registered with the project number 2017/331191354.

Written informed consent was requested to participate in the study, giving details of both the study objectives and the methodology to be followed. The data was kept anonymous, maintaining the confidentiality and anonymity of the participants.

4.3.4. Results

Study Population

Between January 2017 and December 2018, 83 disease free patients with protective ileostomy after colorectal carcinoma resection were reviewed and included in the surgical waiting list for intestinal transit reconstruction. 78 of them met the endoscopic and histological criteria for diversion colitis diagnosis and 73 patients were finally randomized into two groups, intervention (n = 35) and control (n = 38). 69 patients completed the study, 1 of them from SG and 3 from CG abandoning the study due to anastomotic leak.

There were no significant differences between SG and CG in terms of sociodemographic, clinical or surgical variables (Table 1). Symptoms of DC were observed in 70.6% of patients in SG (abdominal pain in 44.1%, mucous rectal discharge in 61.7%, rectal tenesmus in 14.7% and bleeding in 5.9%) and in 60% of patients in CG (abdominal pain in 51.4%, mucous rectal discharge in 40%, rectal tenesmus in 5.7% and bleeding in 11.4%).

Table 1. Demographics and clinics characteristics in Stimulated and Control group.

	Stimulated Group (n = 34)	Non Stimulated Group (n = 35)	p
Demographics			
Age (years)	65 (45–81)	68 (41–80)	0.421
Sex ratio (M:F)	23:11	25:10	0.170
BMI (kg/m^2)	23.5 (21.6–32.6)	27.6 (18.8–40.2)	0.091
ASA			0.483
ASA I-II	31	30	
ASA III	3	5	
Smoker/nonsmoker	20/14	23/12	0.826
Time between surgeries (months) *	12 (8–37)	9 (6–32)	0.813
Clinic:			
Asymptomatic	10 (29.4%)	14 (40%)	0.309
Abdominal pain	15 (44.1%)	18 (51.4%)	0.402
Tenesmus	5 (14.7%)	2 (5.7%)	0.702
Mucous Discharge	21 (61.7)	14 (40%)	0.117
Rectorrhagia	2 (5.9%)	4 (11.4%)	0.668

BMI: body mass index; ASA: American Society of Anesthesiologists Classification; * Time from creation of stoma to the closure of the protective ileostomy.

Main Endpoints

Macroscopic Findings

The effects of probiotic stimulation are shown in Figure 2. A homogeneous distribution between SG and CG was observed in the pre-stimulation phase. Friability of the colonic mucosa was seen in 100% of patients in both CG (n = 35) and SG (n = 34). Differences were found in the post-stimulation phase where friability of the colonic mucosa was seen in 100% of patients in CG compared to 58.8% in SG (n = 20), where this symptom disappeared in 41.2% of patients (n = 14) with $p < 0.001$ and a Phi and V Cramer coefficient of 0.948. Friability of the colonic mucosa had already disappeared in both groups after reconstructive surgery in the short-term follow-up.

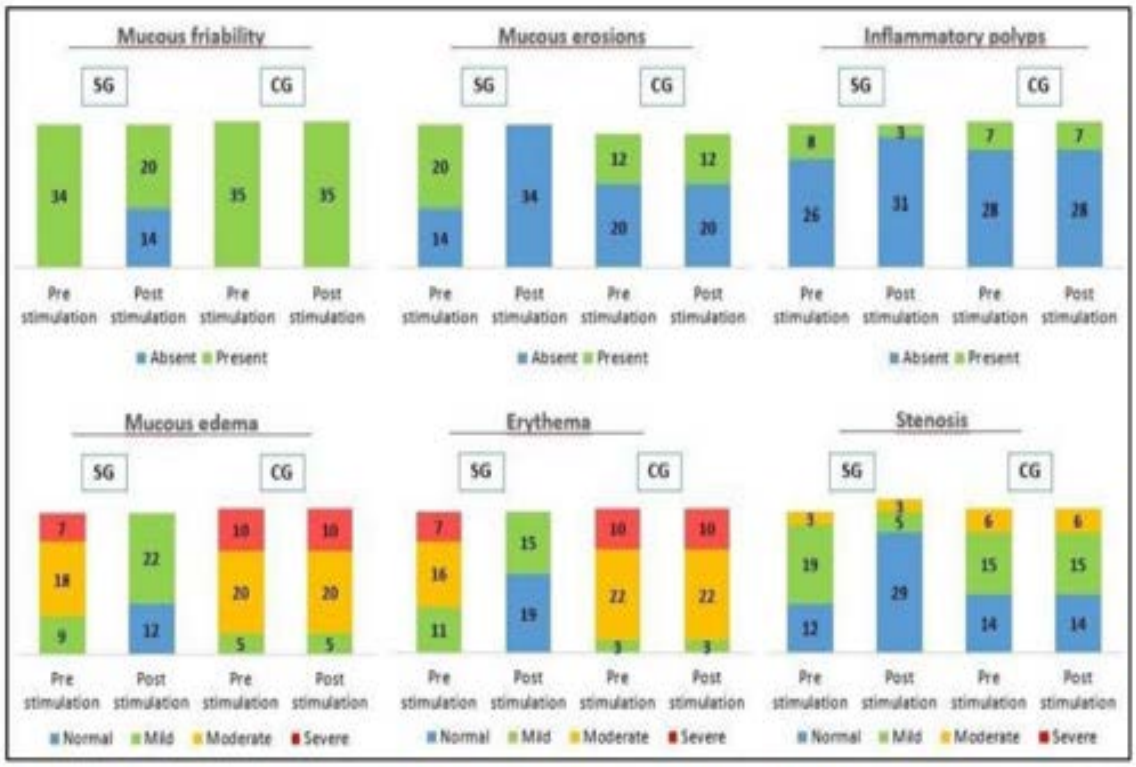


Figure 2. Macroscopic findings by endoscopy before and after stimulation in CG and SG in diversion colitis. A statistically significant decrease in endoscopic pathological findings (mucosal friability, mucous erosions, polyps, edema, erythema and stenosis) were observed in SG, with a $p < 0.001$.

The presence of ulcers in the colonic mucosa was found in 34.3% of patients in CG (n = 12) and 58.8% in SG (n = 20). These lesions were observed in colonoscopy after stimulation with probiotics in 34.4% of patients in CG, unlike SG, in which it disappears with p < 0.001 and a Phi and V Cramer coefficient of 0.693. The ulcers disappeared in both groups after reconstructive surgery in the short-term follow-up.

Inflammatory polyps were present in 20% of patients in CG (n = 7) and 23.5% in SG (n = 8). After stimulation with probiotics polyps were still present in 20% of CG whether they reduced to 8.8% of SG (n = 3) with p < 0.001 and a Phi and V coefficient of Cramer 0.781. The inflammatory polyps disappeared in both groups after reconstructive surgery in the short-term follow-up.

Mucosal edema was present in 100% in both CG (n = 35) and SG (n = 34). The distribution of CG according to endoscopic severity was 5 mild (14.3%), 20 moderate (57.1%) and 10 severe (28.6%); contrasting with SG, 9 mild (36.5%), 18 moderate (52.9%), and 7 severe (20.6%). Mucosal edema was maintained in colonoscopy after stimulation with probiotics in 100% of CG with the same distribution of severity, compared to SG, 22 mild (64.7%) and no edema in 12 patients (35.3%), with a $p < 0.001$ and a Phi and V Cramer coefficient of 0.670. The edema had disappeared in 85.7% in CG (n = 30) and 91.2% in SG (n = 31). In the short-term follow-up, after reconstructive surgery, we observed mild edema in 14.3% in CG (n = 5) and 8.8% SG (n = 3), with a $p = 0.479$.

Mucosal erythema was present in 100% of both, CG (n = 35) and SG (n = 34). The distribution of CG according to endoscopic severity was 3 mild (8.6%), 22 moderate (62.9%) and 10 severe (28.6%); in contrast to SG, 11 mild (32.3%), 16 moderate (47.1%), and 7 severe (20.6%). Mucosal erythema was maintained in colonoscopy after stimulation with probiotics in 100% of CG with the same distribution of severity, compared to SG, 15 patients (44.1%) and no erythema in 19 patients (55.9%), with a $p < 0.001$ and a Phi and V Cramer coefficient of 0.976. In the short-term follow-up, after reconstructive surgery, we observed erythema had disappeared in both groups.

Bowel stenosis was present in 60% in CG (n = 21) and 64.7% SG (n = 22). The distribution of CG according to endoscopic severity was 15 mild (42.9%) and 6 moderate (17.1%), compared to SG, 19 mild (55.9%) and 3 moderate (8.8%). Bowel stenosis was maintained in colonoscopy after stimulation with probiotics in 60% in CG with the same distribution of severity, compared to SG, 5 patients (14.7%) and no stenosis in 29 patients (85.3%), with a p < 0.001 and a Phi and V Cramer coefficient of 0.842. In the short-term follow-up, after reconstructive surgery, we observed

stenosis had disappeared in 97.1% in CG (n = 34) and in 100% in SG (n = 34), with a p = 0.321.

Microscopic Findings

The effects of probiotic stimulation were depicted in Figure 3. A homogeneous distribution between SG and CG was observed in the pre-stimulation phase. Lymphoid follicular hyperplasia was present in 71.4% of patients in CG (n = 25) and 88.2% in SG (n = 30) and was maintained after stimulation with probiotics in 71.4% in CG compared to 23.5% in SG (n = 8), being absent in 76.5% in SG (n = 26), with a p < 0.001 and Phi and V Cramer coefficient of 0.683. In the short-term follow-up lymphoid follicular hyperplasia had already disappeared after reconstructive surgery in both groups. Eosinophils were present in 85.7% in CG (n = 30) and 76.5% in SG (n = 26) and it was maintained after stimulation with probiotics in 85.7% in CG compared to 20.6% in SG (n = 7), being absent in 79.4% in SG (n = 27), with a p < 0.001 and Phi and V Cramer coefficient of 0.518. In the short-term follow-up eosinophils were only present in 2.9% after reconstructive surgery in both groups, with a p = 0.182. Crypt abscesses were present in 20% in CG (n = 7) and 20.6% in SG patients (n = 7) and it was maintained after stimulation with probiotics in 20% in CG, less when compared to SG in which were absent in 100% of patients (n = 34), with a p < 0.001 and Phi and V Cramer coefficient of 0.666. In the short-term follow-up crypt abscesses had already disappeared after reconstructive surgery in both groups. Lymphocytic infiltration of the lamina propria was present in 94.3% of patients in CG (n = 33) and 91.2% in SG (n = 31). The distribution of CG according to endoscopic severity was 17 mild (48.6%), 14 moderate (40%) and 2 severe (5.7%) while in SG it was 11 mild (32.4%) and 20 moderate (58.8%). Lymphocytic infiltration of the lamina propria was maintained in 94.3% in CG with the same distribution after stimulation with probiotics, compared to SG with 18 mild (52.9%) and absence in 16 patients (47.1%), with a p < 0.001 and Phi

and V coefficient of Cramer 0.692. In the short-term follow-up lymphocytic infiltration of the lamina propria had disappeared in 85.7% in CG (n = 30) and 100% in SG (n = 34) after reconstructive surgery, persisting in a mild form in 14.3% in CG (n = 5), with a p = 0.004 and Phi and V Cramer coefficient of 0.442. Plasma cell infiltration was present in 94.3% in CG (n = 33) and 76.5% in SG (n = 26). The distribution of CG according to endoscopic severity was 14 mild (40%) and 19 moderate (54.3%). However, it was observed in SG 11 mild (32.4%) and 15 moderate (44.1%). Plasma cell infiltration was maintained with the same distribution after stimulation with probiotics in 94.3% in CG compared to SG with 9 mild (26.5%) being absent in 25 in SG (73.5%), with a p < 0.001 and Phi and V Cramer coefficient 0.771. In the short-term follow-up plasma cell infiltration had disappeared in 94.3% in CG (n = 33) and 97.1% in SG (n = 33) after reconstructive surgery, persisting mild infiltration by lymphocytes in 5.7% in CG (n = 2) and 2.9% in SG (n = 1), with a p = 0.190. Destructuring of the crypt architecture was present in 100% in CG (n = 35) and 91.2% in SG (n = 31). The distribution of CG according to endoscopic severity was 8 mild (22.9%), 19 moderate (54.3%) and 8 severe (22.9%), however in SG 4 mild (11.8%), 20 moderate (58.8%) and 7 severe (20.6%) was observed. Destructuring of the crypt architecture was maintained, after stimulation with probiotics, in 100% in CG with the same distribution, compared to SG with 23 mild (67.6%), 2 moderate (5.9%) and absence in 9 patients (26.5%), with a p < 0.001 and Phi and V Cramer coefficient of 0.911. In the short-term follow-up destructuring of the crypt architecture had disappeared in 85.7% in CG (n = 30) and 100% in SG (n = 34), persisting mild alteration of the architecture in 14.3% in CG (n = 5) after reconstructive surgery, with a p = 0.002 and Phi and V Cramer coefficient of 0.471.

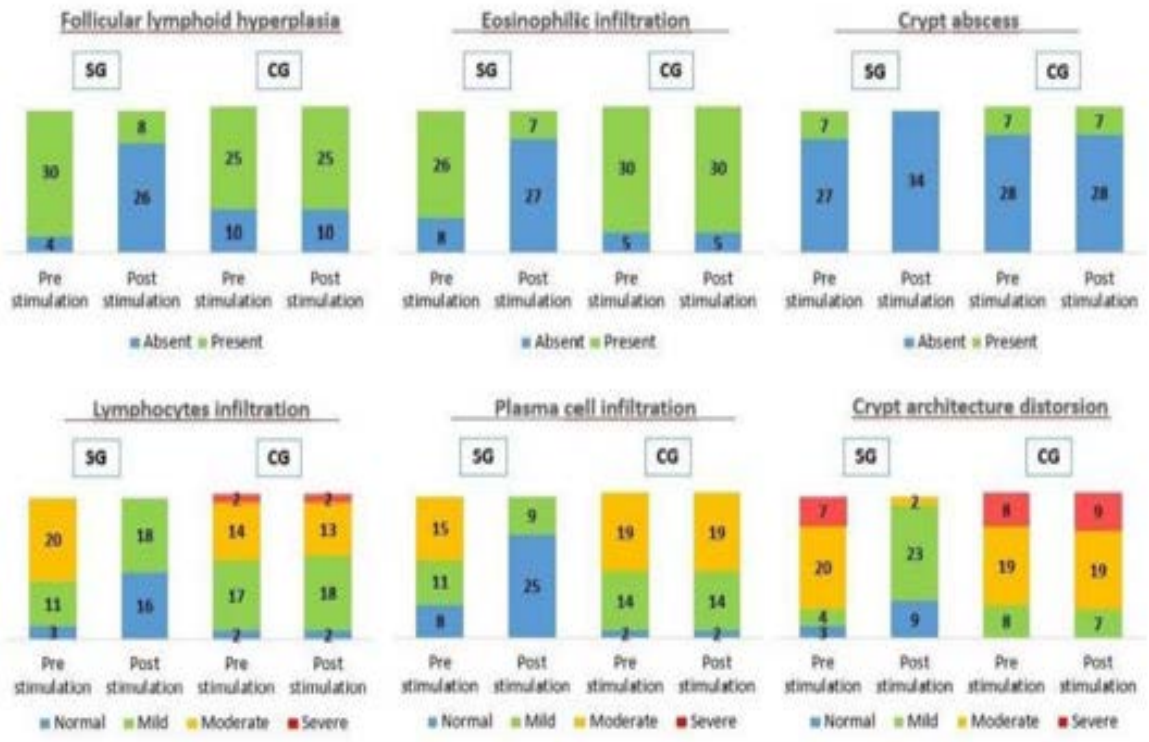


Figure 3. Microscopic findings by histological study before and after stimulation in CG and SG in diversion colitis. A statistically significant decrease in histologic pathological findings (follicular hyperplasia, eosinophils, cryptic abscesses, lymphocyte infiltration, plasma cell infiltration and architecture distortion) were observed in SG, with a $p < 0.001$.

Secondary Endpoints

We found a homogeneous distribution in the diversion colitis degree in pre-stimulation phase between SG and CG (p = 0.911), with 9 patients with severe diversion colitis in both groups (26.5% SG vs 25.7% CG), 23 patients with moderate diversion colitis in both groups (67.6% SG vs 65.7% CG) and 2 patients with mild diversion colitis in SG (5.9%) versus 3 patients in CG (8.6%). The associated symptoms were abdominal pain, mucous discharge, urgency, and rectal bleeding (Table 1). Abdominal pain was present in 44.1% of patients in SG (n = 15) and 51.4% in CG (n = 18). Mucous discharge was present in 61.7% of patients in SG (n = 21) and 40% in CG (n = 14). Tenesmus appeared in 14.7% in SG (n = 5) and 5.7% in CG (n = 2). Rectorrhagia was observed in 5.9% in SG (n = 2) and 11.4% in CG (n = 4).

In the post-stimulation phase, CG maintains its distribution with 9 patients with severe diversion colitis (25.7%), 23 patients with moderate diversion colitis (65.7% CG) and 3 patients with mild diversion colitis (8.6%); on the other hand, SG shows no patients with severe diversion colitis (n = 0), 3 patients with moderate diversion colitis (8.8% CG), 19 patients with mild diversion colitis (55.9%) and 12 patients with normal endoscopic and histological findings (35.3%), achieving a $p < 0.001$ and a Phi and V Cramer coefficient of 0.834. After stimulation and reconstructive surgery, DC symptoms were present in 100% in CG (n = 35) compared to 14.7%in SG (n = 5) with a $p < 0.001$ mand Phi and V coefficient of Cramer 0.789. The associated symptoms in CG were diarrhea in 85.7% (n = 30), abdominal pain in 57.1% (n = 20), urgency in 34.3% (n = 12) and rectal bleeding in 5.7% (n = 2). The associated symptoms in SG were abdominal pain in 11.7% (n = 4) and tenesmus in 5.9% (n = 2). In the post-stimulation phase, CG maintains its distribution with 9 severe DC (25.7%), 23 moderate DC (65.7% CG) and 3 mild DC (8.6%); on the other hand, SG shows no patients with severe DC (n = 0), 3 patients with moderate DC (8.8% CG), 19 patients with mild DC (55.9%) and 12 patients with normal endoscopic findings (35.3%), achieving a $p < 0.001$ and Phi and V Cramer coefficient of 0.883.

Finally, during the short-term follow-up after reconstructive surgery, symptoms were present in CG with 28.6% (n = 10) compared to 71.4% who were asymptomatic (n = 25). SG showed 100% asymptomatic patients after surgery, with p < 0.001 and a Phi and V mCramer coefficient of 0.406. The persistent symptoms in CG were diarrhea 28.6% (n = 10), abdominal pain 22.8% (n = 8) and tenesmus 5.7% (n = 2)

4.3.5. Discussion

In our study, macroscopic and microscopic alterations associated with DC dysbiosis were observed in both CG and SG without observing a statistically significant relationship between severity, symptoms, or time since the creation of the stoma,

findings similar to those published in previous studies [15,16,25–28]. There does seem to be a significantly strong relationship between stimulation with probiotics and the decrease in endoscopic and histological severity on one hand, and between, stimulation with probiotics and the decrease in symptoms after reconstructive surgery on the other. In our series, symptoms associated with DC were presented in 100% of patients in CG compared to 14.7% in SG with a Phi and V Cramer coefficient that indicates a relatively strong relationship between stimulation with probiotics and the appearance of symptoms after the closure of the ileostomy; a fact that we believe is related to the decrease in dysbiosis in the defunctionalized segment of the colon and which was maintained in the short term. This dysbiosis activates metabolic products that produce an immune response, causing an attack on the colonic mucosa and affecting the function of the regulatory immune cells that normally promote its homeostasis [25]. Its decrease causes an improvement in structural changes, such as atrophy of villi in the defunctionalized limb, and in consequence produces a loss of smooth muscle area and a reduced isometric contractility with loss of the intestinal absorption capacity [18]. Intestinal dysbiosis has been linked to the pathogenesis of numerous chronic diseases, such as inflammatory bowel disease, and has been studied in order to identify its correction obtaining variable results [4,26].

Most of the clinical research on DC was carried out during the 90's and early 2000's with the aim of identifying the molecular mechanisms that triggered this disease, its endoscopic and histological characteristics, and its relationship with the microbiota alteration [21,29–32]. In the last two years, two studies have been published regarding intestinal microbiota, Mishima [4] and Knox [33]; to which we must add, on the one hand, the latest systematic review on diversion colitis published by Kabir et al. [16] in 2014 that reviews a total of 3305 articles, eventually including 35 studies, and analyses the pathophysiology, clinical presentation and treatment of diversion colitis which concludes that there is a great variability of forms of presentation and a single definitive treatment, which is the reconstruction of the

transit. Results on preoperative stimulation prior to the closure of the protective ileostomy published by Rombey et al. [23] in 2019, including 8 studies with a total of 267 patients, despite the promising initial results, show that there is no sufficient quality evidence to recommend routine implementation of preoperative bowel stimulation in clinical practice.

Based on studies such as the one published by Morgan et al. in 2020 [34], which demonstrated the important role of the intestinal microbiome in the development of microscopic colitis and the regression of inflammation after reconstructive surgery, we considered that stimulation with probiotics prior to closure of the protective ileostomy would allow the altered autoimmune function to be normalized, restore homeostasis, and reduce mucosal inflammation, achieving the reduction or disappearance of the macroscopic and microscopic changes associated with it. This initial hypothesis seems to correlate with the results we have obtained, achieving a remission in endoscopic and histological findings of DC in SG, as we can see in Figure 2 and Figure 3, where a significant remission in SG was observed compared to CG after stimulation with probiotics prior to closing the protective stoma. This remission in endoscopic and histological findings had already been studied in previous clinical trials, in experimental models and phase III studies, through stimulation with different substances such as nutritional solutions, fecal transplantation, short chain fatty acid enemas (SCFA) such as butyrate, 5-aminosalicylate acid (5-ASA), glucocorticoids, sucralfate and N-acetylcysteine among others, with different results [15–18,35–40]. Most of them showed inconsistent results, although there seems to be a decrease in intestinal mucosa inflammation, remission epithelial lesions and neutrophils.

The results of this study, at the macroscopic level, demonstrated a significant decrease in all endoscopic findings in SG compared to CG, with a strong correlation between stimulation of the efferent loop with probiotics and a decrease in macroscopic findings on colitis. It was worth highlighting a notable decrease in the

severity of the edema and mucous erythema, turning from severe or moderate to mild or simply disappearing, a fact that we relate to the anti-inflammatory effect of probiotics, and a complete disappearance of the ulcers in the SG after the stimulation phase, also with an intense correlation, as we can see in Figure 2 and Figure 4. Ulcers associated with DC in humans were an inconsistent finding. There seems to be an association with late structural changes on the superficial epithelium [15,16]. In our study, the patients with mucous ulcers were those living more time with the stoma. These findings were consistent with previous observations made in humans and animal models [37,38].

Morphological studies about colonic biopsies in patients with ileostomy have shown chronic inflammation in more than 50% of the samples, with an increase in lymphoid tissue in 100% of the cases [15,40]. The most significant histologic finding in DC is lymphoid follicular hyperplasia, which was present in experimental models and patients 60 days after the creation of the stoma [20], with infiltration of the mucosa by lymphocytes that can be from mild to severe. Pacheco et al. described lymphoid follicular hyperplasia in 100% of the cases in animal models, and crypt atrophy in approximately 24% after six weeks of the stoma creation and 42% after 17 weeks [37,38]. In our study, histopathological changes were characterized, as in previous studies, mainly by lymphoid follicular hyperplasia and lymphocytic infiltration of the lamina propria, with destructuring of the crypt architecture and cryptitis in those patients with a longer time since the stoma was created. A significant remission in microscopic alterations in SG compared to CG was seen, with an intense correlation between stimulation of the efferent loop with probiotics and the decrease or disappearance of histological alterations, as we can see in Figure 3 and Figure 4. As we have commented previously, these findings could be explained by the ability of probiotics to interact with the intestinal mucosa, decreasing the molecular production of pro-inflammatory substances, and thereby reducing the migration capacity of

inflammatory cells to the lamina propria, such as lymphocytes, eosinophils, and plasma cells.

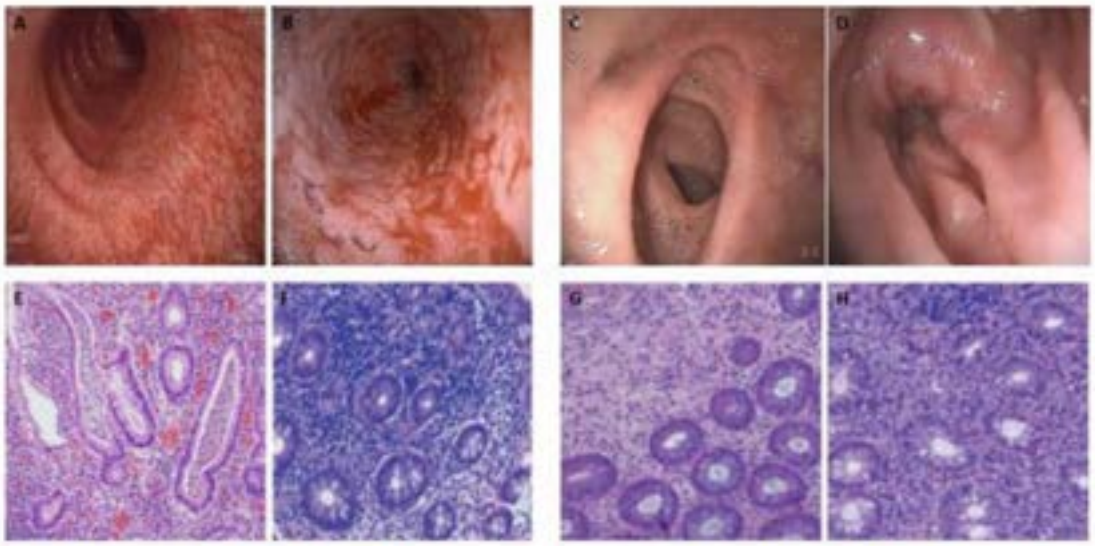


Figure 4. Endoscopic and histopathological findings of diversion colitis. (**A–B**): Pre-stimulation colonoscopy showed easily hemorrhagic mucosa, with edema, spontaneous bleeding, erythema and stenosis (**B**). (**C–D**): Biopsies after probiotics stimulation demonstrated only low-grade inflammation and edema, with diffuse erythema (**D**), without erosions and friability mucous. (**E–F**): Pre-stimulation histopathological evaluation of colon biopsies with hematoxylin and eosin staining showed inflammation with lymphoplasmocytic infiltration, follicular lymphoid hyperplasia, crypt abscesses, and crypt architecture distortion. (**G–H**): Biopsies after probiotics stimulation demonstrated a low-grade inflammation with reduced crypt abscesses, lymphoplasmocytic infiltration, follicular lymphoid hyperplasia, and crypt architecture distortion.

The observations presented here demonstrate that the decrease in endoscopic and histological findings associated with the decrease in inflammation means that stimulation of the efferent loop of the ileostomy with probiotics can be an alternative treatment in patients with symptomatic DC who are not candidates for reconstructive surgery as a treatment to resolve the colonic inflammation. Finally, the stimulation of the efferent loop with probiotics can be an alternative treatment to resolve the inflammation in patients whose surgical option is not feasible or available.

There are few studies on endoscopic and histopathological DC after reconstructive surgery, with limited information on follow-up. A Korean study published by Son et al. [27] found an incidence of symptomatic DC in 63.3% of patients with ileostomy, remaining in 46.6% six months after its closure. Szczepkowski et al. [15] observed in their 2017 study a persistence of macroscopic and microscopic inflammation in patients with a diagnosis of DC at three months and five years after the reconstructive surgery. In this study, limited by a small sample size, the endoscopic and histological manifestations of DC decreased after reconstructive surgery, observing reappearance of the inflammation in the colonic mucosa in the long term. In the short-term follow-up after reconstructive surgery, a significant correlation was observed between stimulation of the efferent loop with probiotics and the disappearance of the symptoms associated with DC in SG compared to CG, observing a persistence of DC in the CG of around 30% after closure of the ileostomy.

4.3.6. Conclusions

Therefore, we can conclude that stimulation of the efferent loop with probiotics seems to have a reducing effect on the endoscopic and histopathological alterations in DC and consequently, this procedure can be an alternative treatment for patients where surgical option is not feasible or available. Nevertheless, we will need more multicenter studies based on macroscopic and microscopic changes after stimulation prior to closure ileostomy to support these conclusions.

4.3.7. References

1. Biagioli, M.; Capobianco, D.; Carino, A.; Marchianò, S.; Fiorucci, C.; Ricci, P.; Distrutti, E.; Fiorucci, S. Divergent Effectiveness of Multispecies Probiotic

Preparations on Intestinal Microbiota Structure Depends on Metabolic Properties. Nutrients 2019, 11, 325, doi:10.3390/nu11020325.

2. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 2014, 11, 506–514, doi:10.1038/nrgastro.2014.66.
3. Shen, Z.-H.; Zhu, C.-X.; Quan, Y.-S.; Yang, Z.-Y.; Wu, S.; Luo, W.-W.; Tan, B.; Wang, X.-Y. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J. Gastroenterol. 2018, 24, 5–14, doi:10.3748/wjg.v24.i1.5.
4. Michima, Y.; Sartor, R.B. Manipulating resident microbiota to enhance regulatory immune function to treat inflammatory bowel diseases. J. Gastroenterol. 2020, 55, 4–14, doi:10.1007/s00535-019-01618-1.
5. Rajkumar, H.; Mahmood, N.; Kumar, M.; Varikuti, S.R.; Challa, H.R.; Myakala, S.P. Effect of Probiotic (VSL#3) and Omega-3 on Lipid Profile, Insulin Sensitivity, Inflammatory Markers, and Gut Colonization in Overweight Adults: A Randomized, Controlled Trial. Mediat. Inflamm. 2014, 2014, 1–8, doi:10.1155/2014/348959.
6. Callaway, L.K.; McIntyre, H.D.; Barrett, H.L.; Foxcroft, K.; Tremellen, A.; Lingwood, B.E.; Tobin, J.M.; Wilkinson, S.; Kothari, A.; Morrison, M.; et al. Probiotics for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Women Diabetes Care. Diabetes Care 2019, 42, 364–371, doi:10.2337/dc18-2248.
7. Baldassarre, M.E.; Di Mauro, A.; Tafuri, S.; Rizzo, V.; Gallone, M.S.; Mastromarino, P.; Capobianco, D.; Laghi, L.; Zhu, C.; Capozza, M.; et al. Effectiveness and Safety of a Probiotic-Mixture for the Treatment of Infantile Colic: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial with Fecal Real-Time

PCR and NMR-Based Metabolomics Analysis. Nutrient 2018, 10, 195, doi:10.3390/nu10020195.

8. Kumar, M.; Hemalatha, R.; Nagpal, R.; Singh, B.; Parasannanavar, D.; Verma, V.; Kumar, A.; Marotta, F.; Catanzaro, R.; Cuffari, B.; et al. Probiotic Approaches for Targeting Inflammatory Bowel Disease: An Update On Advances and Opportunities in Managing the Disease. Int J Probiotics Prebiotics 2016, 11, 99–116.
9. Amoroso, C.; Perillo, F.; Strati, F.; Fantini, M.; Caprioli, F.; Facciotti, F. The Role of Gut Microbiota Biomodulators on Mucosal Immunity and Intestinal Inflammation. Cells 2020, 9, 1234. doi:10.3390/cells9051234.
10. Zhang, Y.; Li, L.; Guo, C.; Mu, D.; Feng, B.; Zuo, X.; Li, Y. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: A meta-analysis. BMC Gastroenterol. 2016, 16, 1–11, doi:10.1186/s12876-016-0470-z.
11. Marteau, P.; Shanahan, F. Basic aspects and pharmacology of probiotics: An overview of pharmacokinetics, mechanisms of action and side-effects. Best. Pract. Res. Clin. Gastroenterol. 2003, 17, 725–740, doi:10.1016/s1521-6918(03)00055-6.
12. Sartor, R.B.; Wu, G.D. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology 2017, 152, 327–339.e4, doi:10.1053/j.gastro.2016.10.012.
13. Cohen, L.J.; Cho, J.H.; Gevers, D.; Chu, H. Genetic Factors and the Intestinal Microbiome Guide Development of Microbe-Based Therapies for Inflammatory Bowel Diseases. Gastroenterol. 2019, 156, 2174–2189, doi:10.1053/j.gastro.2019.03.017.
14. Basso, P.J.; Câmara, N.O.S.; Sales-Campos, H. Microbial-Based Therapies in the Treatment of Inflammatory Bowel Disease – An Overview of Human Studies. Front. Pharmacol. 2019, 9, 1571, doi:10.3389/fphar.2018.01571.

15. Szczepkowski, M.; Banasiewicz, T.; Kobus, A. Diversion colitis 25 years later: The phenomenon of the disease. Int. J. Color. Dis. 2017, 32, 1191–1196, doi:10.1007/s00384-017-2802-z.
16. Kabir, S.; Richards, R.; Ahmed, J.; MacFie, J. Pathophysiology, clinical presentation and management of diversion colitis: A review of current literature. Int. J. Surg. 2014, 12, 1088–1092, doi:10.1016/j.ijsu.2014.08.350.
17. Williams, L.; Armstrong, M.J.; Finan, P.; Sagar, P.; Burke, D. The effect of faecal diversion on human ileum. Gut 2007, 56, 796–801, doi:10.1136/gut.2006.102046.
18. Beamish, E.L.; Johnson, J.; Shaw, E.J.; Scott, N.A.; Bhowmick, A.; Rigby, R.J. Loop ileostomy-mediated fecal stream diversion is associated with microbial dysbiosis. Gut Microbes 2017, 8, 467–478, doi:10.1080/19490976.2017.1339003.
19. Glotzer, D.J.; Glick, M.E.; Goldman, H. Proctitis and colitis following diversion of the fecal stream. Gastroenterology 1981, 80, 438–441, doi:10.1016/0016-5085(81)90003-2.
20. Kleinwort, A.; Döring, P.; Hackbarth, C.; Heidecke, C.-D.; Schulze, T. Deviation of the Fecal Stream in Colonic Bowel Segments Results in Increased Numbers of Isolated Lymphoid Follicles in the Submucosal Compartment in a Novel Murine Model of Diversion Colitis. BioMed Res. Int. 2017, 2017, 1–10, doi:10.1155/2017/5265969.
21. Harig, J.M.; Soergel, K.H.; Komorowski, R.A.; Wood, C.M. Treatment of Diversion Colitis with Short-Chain-Fatty Acid Irrigation. New Engl. J. Med. 1989, 320, 23–28, doi:10.1056/nejm198901053200105.
22. Keane, C.; Park, J.; Öberg, S.; Wedin, A.; Bock, D.; O'Grady, G.; Bissett, I.; Rosenberg, J.; Angenete, E. Functional outcomes from a randomized trial of early closure of temporary ileostomy after rectal excision for cancer. Br. J. Surg. 2019, 106, 645–652, doi:10.1002/bjs.11092.
23. Rombey, T.; Panagiotopoulou, I.G.; Hind, D.; Fearnhead, N.S. Preoperative bowel stimulation prior to ileostomy closure to restore bowel function more

quickly and improve postoperative outcomes: A systematic review. Colorectal Dis. 2019, 21, 994–1003, doi:10.1111/codi.14636.

24. Garfinkle, R.; Trabulsi, N.; Morin, N.; Phang, T.; Liberman, S.; Feldman, L.; Fried, G.; Boutros, M. Study protocol evaluating the use of bowel stimulation before loop ileostomy closure to reduce postoperative ileus: A multicenter randomized controlled trial. Colorectal Dis. 2017, 19, 1024–1029, doi:10.1111/codi.13720.
25. Macfarlane, G.T.; Macfarlane, S. Bacteria, Colonic Fermentation, and Gastrointestinal Health. J. AOAC Int. 2012, 95, 50–60, doi:10.5740/jaoacint.sge_macfarlane.
26. Lloyd-Price, J.; IBDMDB Investigators; Arze, C.; Ananthakrishnan, A.N.; Schirmer, M.; Avila-Pacheco, J.; Poon, T.W.; Andrews, E.; Ajami, N.J.; Bonham, K.S.; et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nat. Cell Biol. 2019, 569, 655–662, doi:10.1038/s41586-019-1237-9.
27. Son, N.N.; Choi, D.J.; Woo, S.U.; Kim, J.; Keom, B.R.; Kim, C.H.; Baek, S.J.; Kim, S.H. Relationship between diversion colitis and quality of life in rectal cancer. World J. Gastroenterol. 2013, 19, 542–549, doi:10.3748/wjg.v19.i4.542.
28. Baek, S.-J.; Kim, S.-H.; Lee, C.-K.; Roh, K.-H.; Keum, B.; Kim, C.-H.; Kim, J. Relationship between the Severity of Diversion Colitis and the Composition of Colonic Bacteria: A Prospective Study. Gut Liver 2014, 8, 170–176, doi:10.5009/gnl.2014.8.2.170.
29. Ferguson, C.M.; Siegel, R.J. A prospective evaluation of diversion colitis. Am. Surg. 1991, 57, 46–9.
30. Orsay, C.P.; Kim, D.O.; Pearl, R.K.; Abcarian, H. Diversion colitis in patients scheduled for colostomy closure. Dis. Colon Rectum 1993, 36, 366–367, doi:10.1007/bf02053940.
31. Frisbie, J.; Ahmed, N.; Hirano, I.; Klein, M.; Soybel, D. Diversion Colitis in Patients with Myelopathy: Clinical, Endoscopic, and Histopathological Findings. J. Spinal Cord Med. 2000, 23, 142–149, doi:10.1080/10790268.2000.11753522.

32. Komorowski, R.A. Histologic Spectrum of Diversion Colitis. Am. J. Surg. Pathol. 1990, 14, 548–554, doi:10.1097/00000478-199006000-00005.

33. Knox, N.C.; Forbes, J.D.; Van Domselaar, G.; Bernstein, C.N. The Gut Microbiome as a Target for IBD Treatment: Are We There Yet? Curr. Treat. Options Gastroenterol. 2019, 17, 115–126, doi:10.1007/s11938-019-00221-w.

34. Morgan, D.M.; Cao, Y.; Miller, K.; McGoldrick, J.; Bellavance, D.; Chin, S.M.; Halvorsen, S.; Maxner, B.; Richter, J.M.; Sassi, S.; et al. Microscopic Colitis Is Characterized by Intestinal Dysbiosis. Clin. Gastroenterol. Hepatol. 2020, 18, 984–986, doi:10.1016/j.cgh.2019.06.035.

35. Schauber, J.; Bark, T.; Jaramillo, E.; Katouli, M.; Sandstedt, B.; Svenberg, T. Local short-chain fatty acids supplementation without beneficial effect on inflammation in excluded rectum. Scand J Gastoenterol 2000, 35,184–189, doi:10.1080/003655200750024371.

36. Guillemot, F.; Colombel, J.F.; Neut, C.; Verplanck, N.; LeComte, M.; Romond, C.; Paris, J.C.; Cortot, A. Treatment of diversion colitis by short-chain fatty acids. Dis. Colon Rectum 1991, 34, 861–864, doi:10.1007/bf02049697.

37. Pacheco, R.G.; Esposito, C.C.; Müller, L.C.; Castelo-Branco, M.T.; Quintella, L.P.; Chagas, V.L.; De Souza, H.S.P.; Schanaider, A. Use of butyrate or glutamine in enema solution reduces inflammation and fibrosis in experimental diversion colitis. World J. Gastroenterol. 2012, 18, 4278–4287, doi:10.3748/wjg.v18.i32.4278.

38. Alvarenga, V Jr.; Pacheco, R.G.; Esposito, C.C.; Buongusto, F.; Castelo-Branco, M.T.; Madi, K.; Belmiro, C.R.; Pavão, M.S.; de Souza, H.S.; Schanaider, A. Ascidian (chordate-tunicate) and mammalian heparin enemas attenuate experimental diversion colitis. Surgery 2014, 155, 217–227, doi:10.1016/j.surg.2013.06.057.

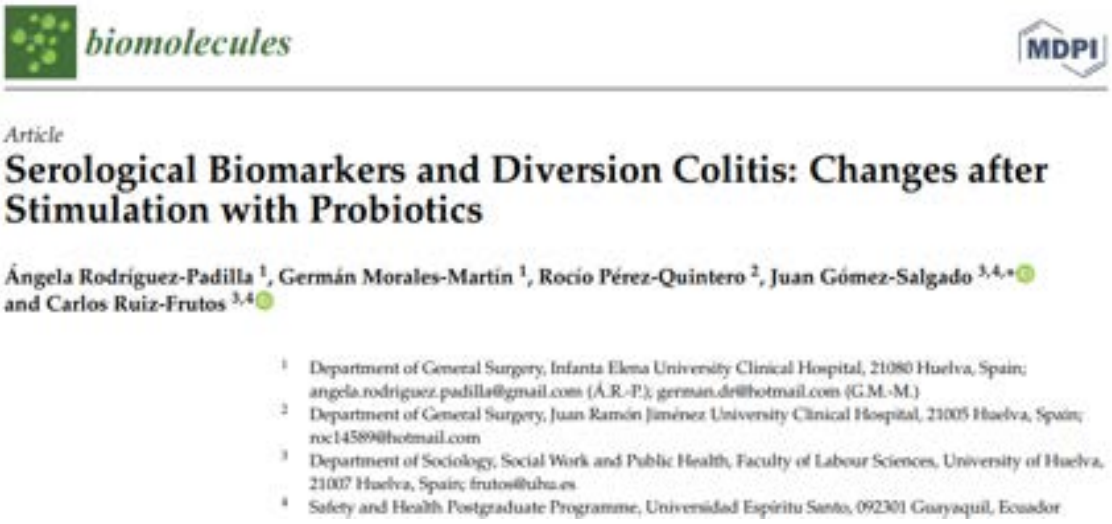
39. Martinez, C.A.R.; De Almeida, M.G.; Da Silva, C.M.G.; Ribeiro, M.L.; Da Cunha, F.L.; Rodrigues, M.R.; Sato, D.T.; Pereira, J.A. Enemas with n-Acetylcysteine Can Reduce the Level of Oxidative Damage in Cells of the Colonic Mucosa Diverted

from the Faecal Stream. Dig. Dis. Sci. 2013, 58, 3452–3459, doi:10.1007/s10620-013-2768-9.

40. Buanaim, R.P.; Pereira, J.A.; Campos, F.G.; Kotze, P.G.; Goto, E.F.K.; Mendonça, R.L.S.; Kanno, D.T.; Martinez, C.A.R. Effects of anti-TNF-α in experimental diversion colitis. Acta Cir. Bras. 2019, 34, e201901004, doi:10.1590/s0102-865020190100000004.

4.4. Serological biomarkers and diversion colitis: changes after stimulation with probiotics

El estudio que se presenta a continuación ha sido publicado con la siguiente referencia: Rodríguez-*Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez-Salgado J, Ruiz-Frutos C. Serological Biomarkers and Diversion Colitis: Changes after Stimulation with Probiotics. Biomolecules. 2021 May 2;11(5):684. doi: 10.3390/biom11050684.*



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Serological Biomarkers and Diversion Colitis: Changes after Stimulation with Probiotics

Ángela Rodríguez-Padilla [1], Germán Morales-Martín [1], Rocío Pérez-Quintero [2], Juan Gómez-Salgado [3,4,*] and Carlos Ruiz-Frutos [3,4]

[1] Department of General Surgery, Infanta Elena University Clinical Hospital, 21080 Huelva, Spain; angela.rodriguez.padilla@gmail.com (Á.R.-P.); german.dr@hotmail.com (G.M.-M.)
[2] Department of General Surgery, Juan Ramón Jiménez University Clinical Hospital, 21005 Huelva, Spain; roc145898@hotmail.com
[3] Department of Sociology, Social Work and Public Health, Faculty of Labour Sciences, University of Huelva, 21007 Huelva, Spain; frutos@uhu.es
[4] Safety and Health Postgraduate Programme, Universidad Espíritu Santo, 092301 Guayaquil, Ecuador

4.4.1. Abstract

Diversion colitis is a non-specific inflammation of a defunctionalised segment of the colon after a temporary stoma has been performed. This inflammation is associated with an alteration of certain inflammatory serum markers. The aims of this study were, firstly, to evaluate the modification of inflammatory biomarkers after stimulation with probiotics prior to closure of the protective ileostomy. Secondly, to identify if a relationship could be established between the severity of diversion colitis and the alteration of inflammatory biomarkers in the blood. A prospective, randomized, double-blind, controlled study was conducted. Patients who underwent surgery for colorectal carcinoma with protective ileostomy between January 2017 and December 2018 were included, pending reconstructive surgery and with diversion colitis as diagnosis. The sample was randomly divided into a group stimulated with probiotics (SG) (n = 34) and a control group (CG) (n = 35). Histological and endoscopic changes were evaluated after stimulation, after restorative surgery and during the short-term follow-up after surgery, including the correlation with pro-inflammatory biomarkers in blood. As main findings, a significant decrease in C-reactive protein (CRP), Neutrophil/lymphocyte ratio (NLR ratio), and monocyte/lymphocyte ratio (LMR ratio) was observed in the SG versus the CG with a $p < 0.001$. A significant increase in transferrin values and in the platelet/lymphocyte ratio (PLR) was observed in the SG versus CG after stimulation with probiotics with a $p < 0.001$. A normalisation of CRP and transferrin levels was observed in the third month of follow-up after closure ileostomy, and NLR, LMR and PLR ratios were equal in both groups. Decreased modified Glasgow prognostic score was found in SG compared to CG after probiotic stimulation ($p < 0.001$). The endoscopic and histological severity of diversion colitis is associated with a greater alteration of blood inflammatory biomarkers. The stimulation with probiotics prior to reconstructive surgery promotes an early normalization of these parameters.

Keywords: diversion colitis; probiotics; efferent loop stimulation; inflammatory bowel disease; C-reactive protein; Glasgow Pronostic Score; serological biomarkers

4.4.2. Introduction

Diversion colitis (DC) is an inflammation produced in a defunctionalised segment of the colon after a temporary stoma has been performed [1]. Described by Glotzer et al. in 1981 [2], it is characterized by endoscopic findings such as mucouse friability, edema, erythema, appearance of polyps, ulcers, stenosis and microscopic findings such as lymphoid follicular hyperplasia, infiltration of the lamina propria by lymphocytes, eosinophils, the appearance of plasma cells, architectural disruption, and the appearance of crypt abscesses [1]. Chronic inflammation produces an increase of serum biomarkers, like other systemic inflammatory diseases such as gastrointestinal tumor, systemic lupus erythematosus, inflammatory bowel disease and cardiovascular disease [3-6]. The Glasgow Pronostic Score (GPS) and its modified scale (mGPS) were used to quantify the inflammatory state, based on serum levels of C-reactive protein (CRP) and albumin [7]. High levels of CRP, acute phase reactant, and low levels of albumin, related to malnutrition and intestinal disorders, reflect a systemic inflammatory response. These can be used as prognostic predictors, already available in daily practice [8], together with other inflammatory response biomarkers (IRB) such as transferrin, the neutrophil/lymphocyte ratio (NLR), the platelet/lymphocyte ratio (PLR) and the lymphocyte/monocyte ratio (LMR).

The definitive treatment for diversion colitis is the restoration of bowel continuity [1,9]. Pharmacological treatments using instillations with short-chain fatty acids, mesalazine fiber or corticosteroids are reserved for patients who are not candidates for surgical treatment or for stimulation of the efferent loop prior to surgery [10-12]. Continuing on this line of research, the efferent loop has been

stimulated through probiotics, which interact with the intestinal mucosa, decreasing the production of pro-inflammatory substances [13].

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [13-14]. Probiotics and their metabolic products have been proposed as food supplements to achieve a healthier intestinal homeostasis and also as a treatment for pathologies with an important inflammatory component. Currently available probiotics, aimed at other pathologies with inflammatory conditions, produce a modulating effect in a transitory and limited way [15-18].

This study has two objectives. Firstly, to evaluate the modification of inflammatory biomarkers after stimulation with probiotics prior to closure of the protective ileostomy. Secondly, to identify if a relationship could be established between the severity of diversion colitis and the alteration of inflammatory biomarkers in the blood.

4.4.3. Materials and methods

Study Design

Prospective, randomized, multicenter, double-blind experimental study, comparing two groups of patients operated for colorectal carcinoma with protective ileostomy. The intervention group included patients treated with stimulation of the efferent loop with probiotics prior to transit reconstruction surgery; the control group was not treated with any substance.

Sample Size

The sample size was calculated according to the cut-off values of inflammatory biomarkers ROC curves published in previous studies [3-8]. 50% of reduction/normalisation of pathological biomarkers (100–50%) was assumed. With an adjustment loss of 15%, 30 patients per group were required. 34 patients were recruited for the stimulated group (SG) and 35 patients for the control group (CG) for a confidence level of 95% and a power of 0.8.

Selection of Patients

Between January 2017 and December 2018, all the patients from the three participating centres included in the surgical waiting list for temporary stoma closure after colorectal carcinoma were consecutively assessed to determine their inclusion in the study. The inclusion criteria were being over 18 years of age, disease-free having protective ileostomy after colorectal carcinoma surgery, with endoscopic and histological confirmation of diversion colitis and having signed the informed consent. The exclusion ncriteria being clinical history and histological confirmation of inflammatory bowel disease with colorectal involvement and refusal to participate in the study. Abandonment criteria were loss during follow-up, exitus, and anastomotic leakage after stoma closure.

During the study, 83 patients with protective ileostomy after colorectal carcinoma resection were assessed and included in the surgical waiting list for intestinal transit reconstruction. 78 of them met the endoscopic and histological criteria for diversion bcolitis diagnosis and 73 patients were finally randomized into two groups, intervention (n = 35) and control (n = 38). 69 patients completed the study, 1 of them from SG and 3 from CG abandoning the study because of anastomotic leakage. The selection flowchart of the study patients is shown in Figure 1.

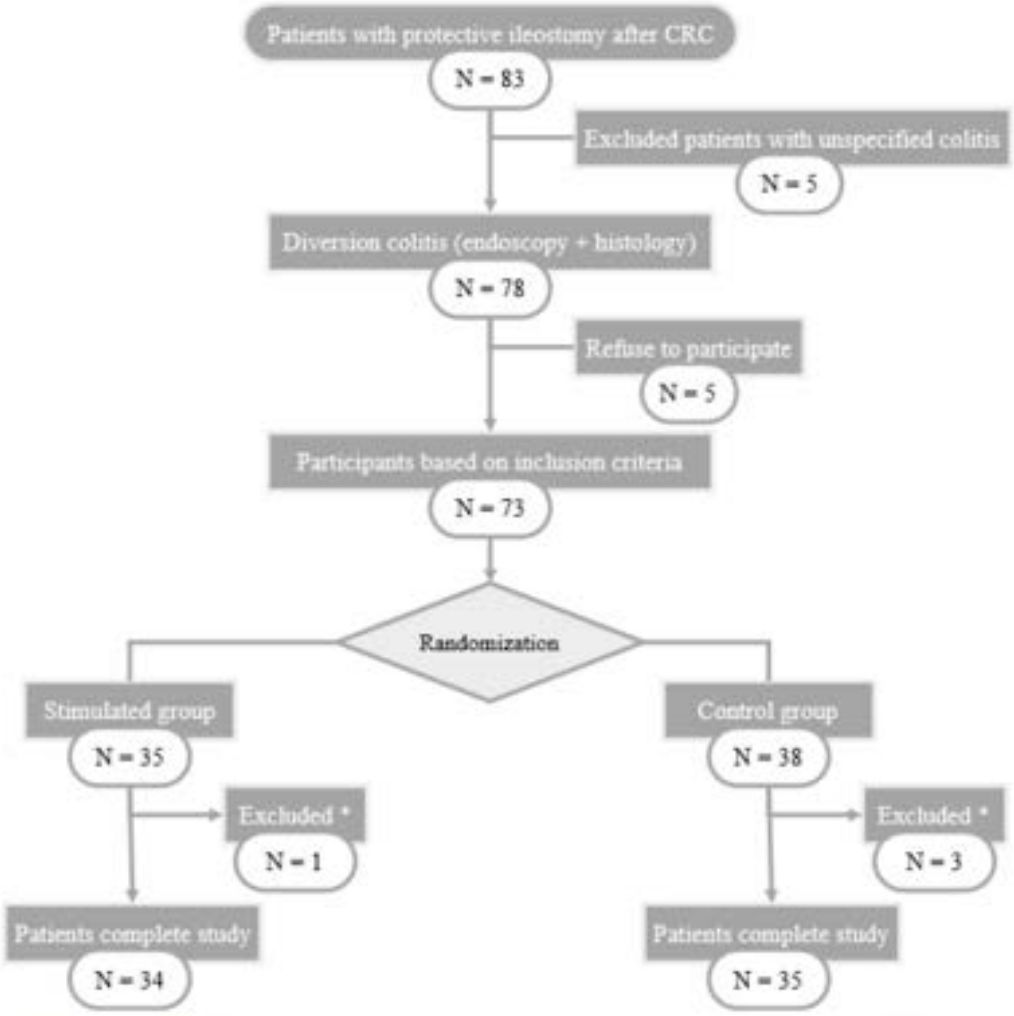


Figure 1. Flow-chart of patient selection. CRC: Colorectal Cancer; *: excluded patients with anastomotic leak.

Randomization

A colonoscopy, including biopsies for histological study, was performed on all patients selected to participate in the study. After confirming the diagnosis, level of colitis (based on the Harig scoring system) [19], and excluding patients who did not meet the selection criteria, randomization was performed. Randomization was performed by using a computer-generated sequence (Statistical software EPIDAT version 4.2. Consellería de Sanidade, Xunta de Galicia, España; Organización Panamericana de la Salud (OPS- OMS); Universidad CES, Colombia).

Endoscopic and Histopathological Examination

Colonoscopies with random biopsies were performed on all patients to exclude those who did not have diversion colitis. Three endoscopists performed the endoscopies with a Silver Scope Storz ® colonoscope. The endoscopic findings were expressed according to the scoring system by Harig et al., including erythema, oedema, friability, polyps, granularity, stenosis, and erosions. Every finding was scored in a scale from 0 to 1 or from 0 to 3, obtaining the colitis degree by adding them. The total score was categorised as absent, mild, moderate, or severe.

The histopathological analysis was assessed by three independent histopathologists. Samples of colon were fixed in buffered formalin and stained with Haematoxylin and eosin (H&E). In the same way as in the endoscopy, DC was diagnosed by evaluating the appearance of follicular lymphoid hyperplasia, eosinophilic, lymphocytes and plasma cells infiltrations and crypt architecture distortion. Every finding was scored in a scale from 0 to 1 or from 0 to 3, obtaining the grade of colitis by adding them. The total score was categorised as absent, mild, moderate or severe.

Both endoscopic and histopathological studies were performed after stimulation and 3 months after reconstruction of intestinal continuity.

Determination of Serum Biomarkers

Blood extraction was performed. Basal levels of CRP and serum albumin were determined to calculate mGPS. A white blood cell count and transferrin determination were performed in a routine blood analysis in each hospital to establish the IRB, establishing cut-off values based on ROC curves.

These determinations were repeated after the completion of the stimulation phase, preoperatively, and in the third month of follow-up after surgery, correlating the pre and postoperative values with the endoscopic and histological alterations and persistence of diversion colitis.

Randomization and Intervention

After confirming the diagnosis and excluding patients who did not meet the selection criteria, randomization was performed creating two groups by using a computer-generated sequence on EPIDAT (Statistical software EPIDAT version 4.2. Consellería de Sanidade, Xunta de Galicia, España; Organización Panamericana de la Salud (OPS-OMS); Universidad CES, Colombia):

Stimulation group (SG): preoperative stimulation with probiotics of the distal limb of the ileostomy loop was performed during the 20 days prior to surgery every second day. During the process and after it, the patient himself registered the appearance of symptoms after each stimulation session: abdominal pain, emission of gas and stool. A sterile Foley catheter No.14 Ch connected to an infusion set was introduced through the defunctioned bowel. This was done to allow slow infusion of a solution of 4.5 mg of probiotics diluted in 250 mL of 0.9% physiological saline for 20–30 min. Each preparation was made under sterile conditions and maintaining the cold chain. Vivomixx ® lyophilized live bacteria, marketed by MENDES, S.A, contained 4.5 × 10 11 of live bacteria in each preparation:

- Four strains of Lactobacillus:
  - Lactobacillus acidophilus DSM 24735 ®
  - Lactobacillus plantarum DSM 24730 ®
  - Lactobacillus paracasei DSM 24733 ®

- ▪ Lactobacillus delbrueckii subsp. bulgaricus DSM 24734 ®

o Three strains of Bifidobacterium:

- ▪ Bifidobacterium breve DSM 24732 ®
- ▪ Bifidobacterium longum DSM 24736 ®
- ▪ Bifidobacterium infantis DSM 24737 ®

o One strain of Streptococcus

- ▪ Streptococcus thermophilus DSM 24731 ®

Control group (CG): exactly the same procedure was carried out, but with the closed infusion set. During the process and after it, the patient himself registered the appearance of symptoms after each stimulation session: abdominal pain, emission of gas and stool.

After ten stimulation sessions, 24 h before surgery, a colonoscopy with biopsy was performed on all patients, re-quantifying the index of severity of endoscopic and histological diversion colitis.

Surgery and Follow-Up

The reconstruction surgery was carried out by three expert surgeons from the Colorectal Surgery Department. A parastomal incision was made and carried out sharply into the peritoneal cavity. The anastomosis was lateral-lateral, either manual or mechanical, according to the decision of the surgeon. Every surgeon could decide whether to change to a median laparotomy procedure or not. Complications or events happened during surgery were recorded in the surgical procedure protocol. General anesthesia was given to all patients and, after extubation and stabilization in the postoperative recovery room, they went directly to the hospitalization ward.

Follow-up during hospitalization was carried out by the team of the Colorectal Surgery department of every center, recording any postoperative complications. Patients were discharged from the hospital after re-establishing intestinal transit, adequate oral tolerance and stool control, recording the length of their hospital stay.

Follow-up after hospitalization was carried out by the Colorectal Surgery team in the first, third and sixth postoperative months. These evaluations were performed by the colorectal surgeon who had performed the surgical intervention in each patient. Any symptomatology related to the intervention was recorded, with special monitoring of abdominal pain, number and control of stools, mucous discharge, tenesmus and rectorrhagia. After completing a three-month follow-up, a colonoscopy with biopsies was performed to evaluate the presence and grade of DC.

Blinding

To ensure blinding of the patients, all underwent the same diagnostic procedure. During the stimulation sessions, both the solution with probiotics and the infusion set were covered by an opaque protective envelope, which prevented observing the colour and transparency of the fluid, or whether the system was open or closed. The stimulation sessions were performed by a single surgeon, who was also in charge of preparing the dilution.

The endoscopist, the pathologist and the surgeon who performed the surgical intervention and the follow-up, as well as surgeons who participated, after the surgery, in the hospitalization process, did not know whether the patient had received probiotics or not.

Assessment Criteria

The aim was to determine the relationship between diversion colitis and the increase of serum biomarkers and their modification after stimulation of the efferent loop with probiotics prior to closure of ileostomy in patients operated on colorectal carcinoma. The results were compared with the control group in the following situations: basal levels, after stimulation and at the third month of follow-up. The study also aimed to determine whether there is a relationship between the levels of severity of diversion colitis, both endoscopic and histological, and the alteration of pro-inflammatory biomarkers in the blood.

Statistical Analysis

A descriptive univariate analysis of sociodemographic and clinical variables was performed. The Kolmogorov-Smirnov test was used to verify the normality of the quantitative variables. To describe the quantitative variables, the mean and standard deviation were used, along with the median and interquartile range for those variables that did not follow a normal distribution. For qualitative variables, frequencies and percentages will be used.

The precision of each biomarker was evaluated by ROC (Receiver Operating Characteristic) curves to determine the optimal cut-off point for NLR, PLR, and LMR. Subsequently, a univariate test was performed to verify the main objectives. The box-plot graphics allowed to visualise the results in terms of response rates and CRP levels. Biochemical parameters were performed using the Fisher's test or the chi-squared test for categorical variables, and the Mann–Whitney test for variables without normal distribution. Comparisons within each group to evaluate the modification of histological and endoscopic severity, CRP, and biochemical parameters were analysed using paired Wilcoxon and t-test. A p < 0.05 was considered to be significant.

Statistical analyses were performed using the statistical programme SPSS ® version 24.0 (IBM, Armonk, NY, USA), with the support of calculation tools provided by the Microsoft Excel and R Commander software.

Ethical Aspects

The project was performed with the consent of the Ethics Coordinating Committee for Biomedical Research of Andalusia, Spain, and registered with the project number 2017/331191354. Written informed consent was requested to participate in the study, giving details of both the study objectives and the methodology to be followed. The data was kept anonymous, maintaining the confidentiality and anonymity of the participants.

Table 1. Sociodemographic and clinical variables and endoscopic and histological severity index of diversion colitis between the stimulated group (SG) and the control group (CG).

	Stimulated Group (n = 34)	Control Group (n = 35)	*p*
Demographics			
Age (years)	65 (45–81)	68 (41–80)	0.421
Sex ratio (M:F)	23:11	25:10	0.170
BMI (kg/m^2)	23.5 (21.6–32.6)	27.6 (18.8–40.2)	0.091
ASA			0.483
ASA I-II	31	30	
ASA III	3	5	
Smoker/non-smoker	20/14	23/12	0.826
Time between surgery (months) *	12 (8–37)	9 (6–32)	0.813
Clinic			
Asymptomatic	10 (29.4%)	14 (40%)	0.309
Abdominal pain	15 (44.1%)	18 (51.4%)	0.402
Tenesmus	5 (14.7%)	2 (5.7%)	0.702
Mucous Discharge	21 (61.7)	14 (40%)	0.117
Rectorrhagia	2 (5.9%)	4 (11.4%)	0.668
Endoscopic severity			
Mild	2 (5.9%)	3 (8.6%)	0.511
Moderate	23 (67.6%)	23 (65.7%)	0.648
Severe	9 (26.5%)	9 (25.7%)	0.498
Histological severity			
Mild	4 (11.8%)	3 (8.6%)	0.479
Moderate	21 (61.7)	23 (65.7%)	0.321
Severe	9 (26.5%)	9 (25.7%)	0.518

BMI: body mass index. ASA: American Society of Anesthesiologists Classification. * Time from creation of stoma to the closure of the protective ileostomy.

4.4.4. Results

Sociodemographics

There were no significant differences between SG and CG in terms of sociodemographic, clinical or endoscopic and histological severity (Table 1).

Serological Biomarkers

The determination of the inflammatory markers was performed in different phases (Figure 2). No differences were found between SG and GC in the baseline determinations of CRP (p = 0.299), NLR (p = 0.457), LMR (p = 0.144), PLR (p = 0.150), or transferrin (p = 0.746).

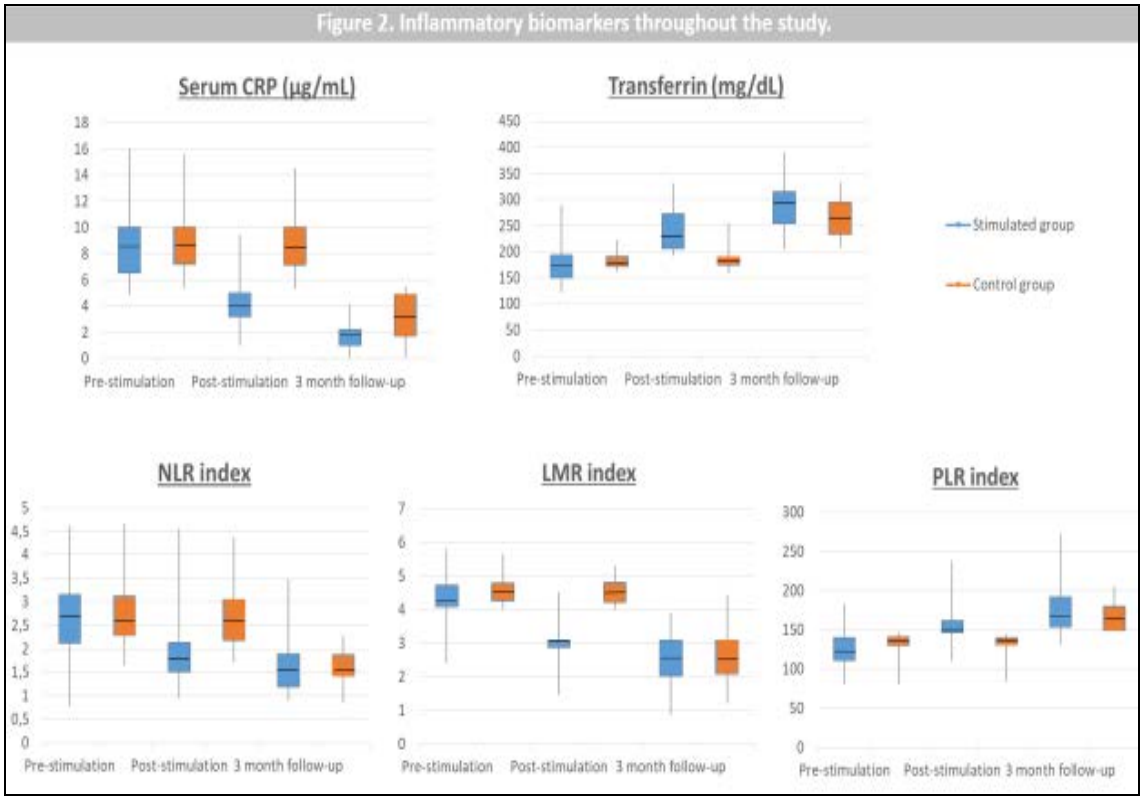


Figure 2. Inflammatory biomarkers throughout the study.

A significant decrease in the CRP value was observed in the SG after completing the stimulation phase (pre-stimulation CRP = 7.82 µg/mL and post-stimulation CRP = 4.09 µg/mL) compared to the CG that maintains high CRP values (pre-stimulation CRP = 8.42 µg/mL and post-stimulation CRP = 8.28 µg/mL), $p < 0.001$. At the three-month follow-up, the CRP values normalized, with CRP levels of 2.9 µg/mL in the CG and 1.58 µg/mL in the SG. NLR count figures significantly decreased in the SG from pre-stimulation (NLR = 2.58) to post-stimulation (NLR = 1.72) p < 0.001, while the figures of NLR in CG remained high (pre-stimulation NLR = 2.74 and post-stimulation NLR = 2.75), $p < 0.001$. At the three-month follow-up, the NLR ratio normalised in both groups, with an NLR value of 1.46 in the CG and of 1.57 in the SG. Regarding the LMR count a significant reduction was found in the SG after completing the stimulation phase (pre-stimulation LMR = 4.22 and post-stimulation LMR = 3.24), p < 0.001. The CG remained high LMR ratio (pre-stimulation LMR = 4.62 and post-stimulation LMR = 4.42). At the three-month follow-up, the LMR ratio normalized in both groups, with LMR value of 2.40in the CG and 2.66 in the SG. Result showed a significant increase in the PLR count in the SG after completing the stimulation phase from pre-stimulation PLR = 126.82 to post-stimulation PLR = 150.04, p < 0.001. Regarding the CG, the PLR score remained low (pre-stimulation PLR = 138.18 and post-stimulation PLR = 138.74). At the three- month follow-up, the PLR ratio normalized in both groups, with a PLR value of 161.54 in the CG and 167.67 in the SG.

Regarding transferrin values, they increased from 184 mg/dL to 238 mg/dL in the SG after completing the stimulation phase, but the CG maintained low transferrin values (pre-stimulation 181 mg/dL and post-stimulation 185 mg/dL), p < 0.001. At the three- month follow-up, transferrin values normalized, with 265 mg/dL in the CG and 289 mg/dL in the SG. The m-GPS was calculated in both groups without finding statistically significant differences in the baseline determination of both groups. The m-GPS in the CG was 1 in 65.7% of the patients (n = 23) and 2 in 34.3% (n = 12 patients), which figures that remained stable after stimulation. The m-GPS in the SG

was 1 in 64.7% of the patients (n = 22) and 2 in 35.3% (n = 12 patients). After stimulation, the m-GPS in SG was 0 in 91.2% of the patients (n = 31) and 1 in 8.8% (n = 3 patients), finding a significant decrease compared to the CG, $p < 0.001$.

Endoscopic/Histological Severity and Serological Biomarkers

When comparing the mean values of the different serological determinations by histological and endoscopic severity in DC, we found that patients with high severity index had higher mean values in the determination of CRP, NLR, and LMR, and lower mean values in the determination of PLR and transferrin. Nonetheless, these differences were not statistically significant (Table 2). There were no differences regarding baseline determinations between SG and CG.

After the stimulation phase, the increase in CRP, NLR, and LMR values was maintained in CG, being higher in the most severe cases of DC, without finding statistically significant differences. Likewise, no significant differences were found when comparing PLR and transferrin associated with the most severe index of DC (Table 2).After the stimulation phase, a decrease in the mean values of CRP, NLR, and LMR was observed in the SG with moderate and mild severity index and absence of histological and endoscopic DC. Although these differences were not statistically significant when compared with each other, when they were compared with baseline levels, a $p < 0.001$ was obtained. Likewise, there was no statistically significant increase in the mean values of PLR and transferrin in the group of moderate and mild severity index and absence of histological and endoscopic DC, but statistically significant differences were found when compared with baseline levels, with a $p < 0.001$ (Table2).

Table 2. Inflammatory markers according to endoscopic + histological severity index before and after stimulation.

Severity Index		Serum CRP					
		Stimulated Group (n = 34)			Control Group (n = 35)		
		n *	Me	RI	n	Me	RI
Pre-stimulation	Severe	9	11.03	±4.9	9	12.16	±3.14
	Moderate	21	10.51	±3.37	23	10.1	±1.96
	Mild	4	5.27	±0.37	3	7.83	±1.53
Post-stimulation	Severe	-		-	9	11.96	±2.91
	Moderate	3	7.35	±3.06	23	9.95	±2.09
	Mild	19	3.8	±1.72	3	7.67	±1.33
	Absent	12	4.2	±1.03	-		-
		NLR ratio					
Pre-stimulation	Severe	9	3.17	±1.53	9	2.92	±0.65
	Moderate	21	3.01	±1.1	23	2.87	±0.32
	Mild	4	2.58	±0.94	3	2.62	±0.47
Post-stimulation	Severe	-		-	3	2.98	±0.73
	Moderate	3	1.97	±0.85	23	2.83	±0.30
	Mild	19	1.7	±0.46	3	2.54	±0.45
	Absent	12	2.39	±1.28	-		-
		LMR ratio					
Pre-stimulation	Severe	9	4.82	±0.63	9	4.99	±0.31
	Moderate	21	4.24	±0.31	23	4.62	±1.47
	Mild	4	3.96	±1.17	3	4.44	±0.30
Post-stimulation	Severe	-		-	9	4.99	±0.57
	Moderate	3	3.75	±0.36	23	4.31	±0.27
	Mild	19	3.11	±0.71	3	4.36	±0.27
	Absent	12	3.72	±1.06	-		-
		PLR ratio					
Pre-stimulation	Severe	9	114.74	±19.79	9	130.37	±19.27
	Moderate	21	121.87	±20.83	23	134.82	±9.31
	Mild	4	131.76	±3.56	3	142.08	±1.95
Post-stimulation	Severe	-		-	9	132.66	±18.08
	Moderate	3	154.50	±26.07	23	134.57	±8.16
	Mild	19	158.37	±3.56	3	140.99	±1.84
	Absent	12	153.61	±31.31	-		-
		Serum Transferrin					
Pre-stimulation	Severe	9	174.66	±43.14	9	174.55	±10.51
	Moderate	21	184.96	±32.01	23	184.34	±10.09
	Mild	4	196.60	±17.53	3	211.66	±21.50
Post-stimulation	Severe	-		-	9	174.11	±9.08
	Moderate	3	248.66	±68.82	23	182.39	±11.05
	Mild	19	252.01	±42.32	3	217.33	±38.07
	Absent	12	232.25	±35.53	-		-

To describe the quantitative variables, the median (Me) and interquartile range (RI) were used. CRP: C-reactive protein. NLR (neutrophil-to-lymphocyte ratio). LMR (lymphocyte-to-monocyte ratio). PLR (platelet-to-lymphocyte ratio). n * of the stimulated group changes because the stimulation with probiotics produces a decrease in the endoscopic and histological severity of diversion colitis.

Safety and Adverse Effects

No serious complications were reported following the consumption of the probiotics in participants throughout the stimulation. Abdominal pain was the only symptom observed during stimulation, which was present in 20.5% of patients SG (n = 7) compared to 14,3% CG (n = 5) (no statistically significant differences). Abdominal pain was evaluated using the visual analog scale (VAS). Pain was moderated in SG, only present in the first stimulation sessions and disappearing afterwards. Pain was mild-moderate in CG in all stimulation sessions and disappeared after their completion.

4.4.5. Discussion

This is the first published randomized, double-blind study linking DC with alterations in inflammatory biomarkers. In this study, it was observed that serological pro- inflammatory biomarkers improved after stimulation with probiotics in SG compared to GC. The stimulation of the efferent loop with probiotics also improved the inflammation of the excluded colon mucosa, decreasing the inflammatory severity index and the inflammatory serum biomarkers. The differences for the studied biomarkers were statistically significant, $p < 0.001$. We observed a relationship between alterations in inflammatory biomarkers and endoscopic and histological severity index, although these findings were not statistically significant.

Several studies have been published about an alternative treatment to surgery, both in experimental models and phase III studies. The alternative to surgery consists in stimulation with different substances such as nutritional solutions, fecal transplantation, short-chain fatty acid (SCFA) enemas such as butyrate, acid 5 aminosalicylate (5-ASA), glucocorticoids, sucralfate and N-acetylcysteine among others, with different results [1,9,20-32]. Most of them showed inconsistent results, although there seems to be a decrease in intestinal mucosa inflammation, epithelial

lesions and neutrophil infiltration. Nowadays the most successful treatment is the closure of the protective ileostomy. However, there are no studies that mention whether these patients or experimental animals concomitantly present increase of inflammatory biomarkers. These serological alterations have been described and studied in pathologies such as inflammatory bowel disease, seeking the correction of these inflammatory parameters associated with dysbiosis, also with variable results [15-17]. For this reason, our results are encouraging, since they demonstrate a reduction in inflammatory parameters prior to the reconstruction of the transit, thus being a possible alternative for those patients who are not candidates for surgical treatment. The only side-effect was the appearance of colicky abdominal pain in 20.5% of the patients (n = 7), valued as moderate pain using the visual analog scale (VAS) and only associated with the first stimulation sessions, and disappearing afterwards. This effect has already been described in studies with stimulation of the efferent loop with short chain fatty acids [9,11,19].

The relationship between inflammatory biomarkers and probiotics has been studied in other pathologies such as acute diarrhea in adults, inflammatory bowel disease, active pouchitis and irritable bowel syndrome, among others [13,15,32-38]. The most studied probiotic bacteria are Bifidobacterium and Lactobacillus spp. These two bacteria produce a decrease of pro-inflammatory molecules and an increase of molecules that inhibit inflammation, also protecting against oxidative stress in humans and demonstrating an important role in intestinal dysbiosis [23,39-40]. Currently, the evidence showed that probiotics have a positive effect on systemic and oxidative inflammation, especially at the gastrointestinal level. L. acidophilus, one of the probiotics administered during our stimulation, has demonstrated its role in inflammatory regulation in multiple experimental studies [27,28,37-45]. A randomized controlled clinical trial conducted by Jafarnejad et al. [46] described the effect of supplementation with probiotics for 8 weeks, in this case, with VSL3 (a probiotic similar to the one administered in our study), on glycemic status and inflammatory

markers among pregnant women. The probiotic supplements contained eight strains of lactic acid bacteria (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L. acidophilus, L. plantarum, L. paracasei, and L. delbrueckii subsp. Bulgaricus). They found a statistically significant decrease in TNF alpha and CRP levels in the probiotic group compared to placebo [45]. Another clinical trial explained the efficacy of VSL3 probiotics administered in 24 patients with irritable Bowel Syndrome for 8 weeks, finding a significant improvement in symptoms, but without significant differences between the groups [47].

Finally, chronic inflammatory processes such as DC, can raise CRP, NLR, LMR values (inflammatory markers) and decrease levels of albumin, transferrin and another inflammatory marker such as the PLR index [48-49]. In our study the probiotics anti-inflamatory effect seemed to be demonstrated after the stimulation phase, where we observed a decrease of serological inflammatory parameters and normalization of CRP, PLR and transferrin values in the SG and a statistically significant decrease in NLR and LMR values, yet without normalization, with a $p < 0.001$. The normalization of the CRP, transferrin, NLR, LMR and PLR values was only achieved after reconstructive surgery and 3 months after surgery. These results could be explained by the ability of probiotics to interact with the intestinal mucosa, decreasing the molecular production of pro-inflammatory substances, and thereby decreasing the capacity for migration of inflammatory cells to the lamina propria, such as lymphocytes, eosinophils and plasma cells [31-33]. In addition, a correlation between high CRP values and an alteration in the percentage of lymphocytes in peripheral blood determinations has also been observed in previous studies [50]. Lymphocytes are the main components that infiltrate the mucosa and can range from mild to severe depending on the inflammation of the excluded colon segment in humans. However, in our study we did not find statistically significant differences between the increase of serum biomarkers and the severity index of DC.

Supporting our study results, it is worth noticing that patients had high adherence to treatment in both groups, only excluding patients with postoperative complications, without registering other losses during follow-up. In addition, our study population included only patients who met endoscopic and histological diagnostic criteria for DC, excluding patients with a diagnosis of nonspecific colitis despite presenting a protective ileostomy, since we consider that they could represent a misleading factor and could lead to error.

4.4.6. Conclusions

It could be concluded that endoscopic and histological severity index of diversion colitis is related to a greater alteration of inflammatory biomarkers in the blood and that stimulation with probiotics prior to reconstructive surgery produces an earlier normalization of these parameters, making it an option for patients who are not eligible for surgical treatment.

4.4.7. References

1. Szczepkowski M, Banasiewicz T, Kobus A. Diversion colitis 25 years later: the phenomenon of the disease. Int J Colorectal Dis. 2017; 32:1191–1196.
2. Glotzer DJ, Glick ME, Goldman H. Proctitis and colitis following diversion of the fecal stream. Gastroenterology. 1981; 80:438– 441.
3. Costa MD, Vieira de Melo CY, Amorim AC, Cipriano Torres De O, Dos Santos AC. Association Between Nutritional Status, Inflammatory Condition, and Prognostic Indexes with Postoperative Complications and Clinical Outcome of Patients with Gastrointestinal Neoplasia. Nutr. Cancer. 2016; 68, 1108–1114.

4. Delcea, C. et al. FRI0581 utility of the Glasgow Prognostic Score in systemic lupus erythematosus, in a Single Center Cohort of 130 Patients. Ann. Rheum. Dis.(Suppl 2). 2015; 638.2–638.
5. Zhao C, Ding C, Xie T, et al. Validation and optimization of the Systemic Inflammation-Based modified Glasgow Prognostic Score in predicting postoperative outcome of inflammatory bowel disease. Scientific Reports. 2018; 8:747.
6. Namiuchi, S. et al. The systemic inflammation-based Glasgow Prognostic Score as a prognostic factor in patients with acute heart failure. J. Cardiovasc. Med. 2015; 16, 409–415.
7. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: A decade of experience in patients with cancer. Cancer Treatment Reviews. 2013; 534–540.
8. Hirahara N, Fujii Y, Yamamoto, et al. Validation of a novel prognostic scoring system using inflammatory response biomarkers in patients undergoing curative thoracoscopic esophagectomy for esophageal squamous cell carcinoma. OncoTargets and Therapy 2017:10.
9. Kabir SI, Kabir SA, Richards R, Ahmed J, MacFie J. Pathophysiology, clinical presentation and management of diversion colitis: a review of in current literature. International Journal of Surgery. 2014: 1088-1092.
10. Keane C, Park J , Öberg S , Wedin A, Bock D , Grady G , et al. Functional outcomes from a randomized trial of early closure of temporary ileostomy after rectal excision for cancer. Br J Surg. 2019 Apr; 106 (5):645-652.
11. Rombey T, Panagiotopoulou IG, Hind D, Fearnhead NS, Preoperative bowel stimulation prior to ileostomy closure to restore bowel function more quickly and improve postoperative outcomes: a systematic review. Colorectal Dis. 2019; 21 (9): 994-1003.

12. Garfinkle R, Trabulsi N, Morin N, et al. Study protocol evaluating the use of bowel stimulation before loop ileostomy closure to reduce postoperative ileus: a multicenter randomized controlled trial. Colorectal Disease 2017; 19: 1024–1029.
13. Biagioli M, Capobianco D, Carino A, Marchianò S, et al. Divergent Effectiveness of Multispecies Probiotic Preparations on Intestinal Microbiota Structure Depends on Metabolic Properties. Nutrients. 2019 Feb; 11(2): 325.
14. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 2014; 11:506–514.
15. Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017; 152:327–339.
16. Cohen LJ, Cho JH, Gevers D, et al. Genetic factors and the intestinal microbiome guide development of microbe-based therapies for inflammatory bowel diseases. Gastroenterology. 2019; 156:2174–2189.
17. Basso PJ, Câmara NOS, Sales-Campos H. Microbial-based therapies in the treatment of inflammatory bowel disease—an overview of human studies. Front Pharmacol. 2018; 9:1571.
18. Shen Z, Zhu C, Quan Y, Yang Z, Wu S, Luo W, Tan B, Wang X. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J Gastroenterol 2018 January 7; 24(1): 5-14.
19. Harig JM, Soergel KH, Komorowsky RA, Wood CM. Treatment of diversion colitis with short-chain-fatty acid irrigation. N Eng J Med 1989; 320 (1): 23-28.

20. Alvarenga V Jr, Rodrigo GP, Esposito CC, Buongusto F, Castelo-Branco MT, Madi K, et al. Ascidian (chordate-tunicate) and mammalian heparin enemas attenuate experimental diversion colitis. Surgery. 2014;155(2):217 –27
21. Pacheco RG, Esposito CC, Muller LC, Castero-Branco MT, Quintella LP, Chagas VL, et al. Use of butyrate or glutamine in enema solution reduces inflammation and fibrosis in experimental diversion colitis. World J Gastoenterol. 2012;18:4278 –87.
22. Williams L, Armstrong MJ, Finan P, Sagar P, Burke D. The effect of faecal diversion on human ileum. Gut 2007; 56: 796 -801.
23. Beamish EL, Johnson J, Shaw EJ, Scott NA, Bhowmick A, Rigby RJ. Loop ileostomy -mediated fecal stream diversion is associated with microbial dysbiosis. Gut Microbes 2017; 8: 467 -78.
24. Schauber J, Bark T, Jaramillo E, Katouli M, Sandstedt B, Svenberg T. Local short-chain fatty acids supplementation without beneficial effect on inflammation in excluded rectum. Scand J Gastoenterol. 2000;35:184 –9.
25. Guillemot F, Colombel JF, Neut C, Verplanck N, Lecomte M, Romond C, et al. Treatment of diversion colitis by short-chain fatty acids. Prospective and double-blind study. Dis Colon Rectum. 1991;34:861864.
26. Martinez CA, de Almeida MG, da Silva CM, Ribeiro ML, da Cunha FL, Rodrigues MR, Sato DT, Pereira JA. Enemas with N-acetylcysteine can reduce the level of oxidative damage in cells of the colonic mucosa diverted from the faecal stream. Dig Dis Sci. 2013 Dec;58(12):3452-9.
27. Buanaim RP et al. Effects of anti-TNF-α in experimental diversion colitis. Acta Cir Bras. 2019;34(10).
28. Biagioli M., Laghi L., Carino A., Cipriani S., Distrutti E., Marchianò S., Parolin C., Scarpelli P., Vitali B., Fiorucci S. Metabolic Variability of a Multispecies Probiotic Preparation Impacts on the Anti-inflammatory Activity. Front. Pharmacol. 2017; 8:505.

29. Sanders M.E., Klaenhammer T.R., Ouwehand A.C., Pot B., Johansen E. Heimbach J.T., Marco M.L., Tennilä J., Ross R.P., Franz C., et al. Effects of genetic, processing, or product formulation changes on efficacy and safety of probiotics. Ann. N. Y. Acad. Sci. 2014; 1309:1–18.
30. Corridoni D, Pastorelli L, Mattioli B, Locovei S, Ishikawa D, Arseneau KO, et al. Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. PLoS One. 2012; 7(7):e42067
31. Guarner, F.; Khan, A.G.; Garisch, J.; Eliakim, R.; Gangl, A.; Thomson, A.; Krabshuis, J.; Lemair, T. Probiotics and Prebiotics; World Gastroenterology Organisation Global Guidelines; World Gastroenterology Organisation 2017; Volume 46, 468–481.
32. Sanders ME, Merenstein DJ, Ouwehand AC, Reid G, Salminen S, Cabana MD, et al. Probiotic use in at-risk populations. J Am Pharm Assoc JAPhA. 2016 Dec;56(6):680–6.
33. Lopes RCSO, Balbino KP, Jorge MP, Ribeiro AQ, Martino HSD, Alfenas RCG. Modulation of intestinal microbiota, control of nitrogen products and inflammation by pre/probiotics in chronic kidney disease: a systematic review. Nutr Hosp. 2018 Apr 27;35(3):722-730.
34. Grossi E, Buresta R, Abbiati R, Cerutti R, Pro-DIA study group. Clinical trial on the efficacy of a new symbiotic formulation, Flortec, in patients with acute diarrhea: a multicenter, randomized study in primary care. J Clin Gastroenterol. 2010 Sep;44 Suppl 1:S35–41.
35. Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C, et al. High-dose probiotics for the treatment of active pouchitis. Dis Colon Rectum. 2007 Dec;50(12):2075-2082.
36. Barra M, Danino T, Garrido D. Engineered Probiotics for Detection and Treatment of Inflammatory Intestinal Diseases. Front Bioeng Biotechnol. 2020 Mar 31;8:265.

37. Hod K, Sperber AD, Ron Y, Boaz M, Dickman R, Berliner S, Halpern Z, Maharshak N, Dekel R. A double-blind, placebo-controlled study to assess the effect of a probiotic mixture on symptoms and inflammatory markers in women with diarrhea-predominant IBS. Neurogastroenterol Motil. 2017 Jul;29(7).
38. Tenorio-Jiménez C, Martínez-Ramírez MJ, Del Castillo-Codes I, Arraiza-Irigoyen C, Tercero-Lozano M, Camacho J, Chueca N, García F, Olza J, Plaza-Díaz J, Fontana L, Olivares M, Gil Á, Gómez-Llorente C. Lactobacillus reuteri V3401 Reduces Inflammatory Biomarkers and Modifies the Gastrointestinal Microbiome in Adults with Metabolic Syndrome: The PROSIR Study. Nutrients. 2019 Jul 31;11(8):176.
39. Morgan DM, Cao Y, Miller K, McGoldrick J, Bellavance D, Chin SM, Halvorsen S, Maxner B, Richter JM, Sassi S, Burke KE, Yarze JC, Ludvigsson JF, Staller K, Chung DC, Khalili H, Microscopic Colitis is Characterized by Intestinal Dysbiosis. Clin Gastroenterol Hepatol. 2020 Apr;18(4):984-986.
40. Rossi M, Amaretti A. Bifidobacteria: genomics and molecular aspects. In: Mayo B, van Sinderen D, editors. Probiotic properties of bifidobacteria. UK: Horizon Scientific Press. 2010; 97-123.
41. Yang YJ, Chuang CC, Yang HB, Lu CC, Sheu BS. Lactobacillus acidophilus ameliorates H. pylori-induced gastric inflammation by inactivating the Smad7 and NFkappaB pathways. BMC Microbiology. 2012;12:38. 7.
42. Borthakur A, Bhattacharyya S, Kumar A, Anbazhagan AN, Tobacman JK, Dudeja PK. Lactobacillus acidophilus alleviates platelet-activating factor-induced inflammatory responses in human intestinal epithelial cells. PLoS One. 2013;8:e75664.
43. Li H, Zhang L, Chen L, Zhu Q, Wang W, Qiao J. Lactobacillus acidophilus alleviates the inflammatory response to enterotoxigenic Escherichia coli K88 via inhibition of the NF-kappaB and p38 mitogen-activated protein kinase signaling pathways in piglets. BMC Microbiol. 2016;16:273.

44. Stenman LK, Waget A, Garret C, Klopp P, Burcelin R, Lahtinen S. Potential probiotic Bifidobacterium animalis ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. Benef Microbes. 2014;5:437-45.
45. Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Abbasi MM, Dolatkhah N. Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial. Asia Pac J Clin Nutr. 2018; 27(3):581-591.
46. Jafarnejad S, Saremi S, Jafarnejad F, Arab A. Effects of a multispecies probiotic mixture on glycemic control and inflammatory status in women with gestational diabetes: a randomized controlled clinical trial. J Nutr Metab. 2016; 2016:5190846.
47. Michail S, Kenche H. Gut microbiota is not modified by randomized, double-blind, placebo-controlled trial of VSL#3 in diarrheapredominant irritable bowel syndrome. Probiotics Antimicrob Proteins. 2011;3:1–7.
48. Afsar et al. Role of Mean Platelet Volume As An Adjunct in Evaluation of Acute Inflammation. Annals of Pathology and Laboratory Medicine, Vol. 4, Issue 4, 2017.
49. Korniluk A, Koper-Lenkiewicz OM, Kaminska J, Kemona H, Dymicka-Piekarska V.Mean Platelet Volume (MPV): New Perspectives for an Old Marker in the Course and Prognosis of Inflammatory Conditions. Mediators of Inflammation 2019, Article ID 9213074
50. Zhao C, Ding C, Xie T, Zhang T, Dai X, Wei Y, Li Y, Gong J, Zhu W. Validation and optimization of the Systemic Inflammation-Based modified Glasgow Prognostic Score in predicting postoperative outcome of inflammatory bowel disease: preliminary data. Sci Rep. 2018 Jan 15;8(1):747.

Discusión

5.1. Principales hallazgos.

La estimulación con probióticos del asa eferente de la ileostomía de protección de pacientes intervenidos de carcinoma colorrectal es un método seguro y eficaz (artículo 2 y 3). Tras la estimulación se objetivó una disminución del grado de severidad en el grupo estimulado frente al grupo control tanto a nivel endoscópico como histológico. Se consiguió una reducción del grado de colitis por diversión en el 100% de los pacientes del GE, con la desaparición de la colitis por diversión macroscópica en el 35,3%. Se demostró en ambos casos la asociación entre la estimulación con probióticos y la reducción de la colitis por diversión con una relación intensa, con la aparición de un único efecto secundario reconocido, dolor abdominal tipo cólico, en un 20% de los pacientes, valorado como dolor moderado mediante la escala visual analógica (EVA) y sólo asociado a las primeras sesiones de estimulación, desapareciendo posteriormente. Este efecto secundario ya fue descrito en estudios con estimulación del asa eferente con ácidos grasos de cadena corta.

La estimulación con probióticos del asa eferente de la ileostomía de protección de pacientes intervenidos de carcinoma colorrectal disminuye la clínica de CD tras la cirugía en el 75% de los pacientes y en el 100% a corto plazo tras la reconstrucción del tránsito intestinal (artículo 2 y 3). Se observó asociación entre la ausencia de clínica y la estimulación con un coeficiente de correlación intensa, frente al GC, en el que presentaron clínica el 100% de los pacientes. Este hecho se mantiene en el seguimiento al primer mes del restablecimiento del tránsito, con un 94,3% de pacientes sintomáticos en el GC frente al 2,9% en el GE. Sin embargo en el seguimiento a corto plazo las cifras tienden a igualarse, consiguiendo el 100% de asintomáticos en el GE y llegando al 71,4% en el GC, desapareciendo la relación directa entre el tercer y el sexto mes, cuando la colitis por diversión endoscópica y microscópica se hace prácticamente inexistente. Este hecho coincide con el efecto modulador transitorio y

limitado que ejercen los probióticos sobre la mucosa intestinal y con la curva de recuperación de la función intestinal publicada en otros estudios.

Precisamente este efecto modulador, nos hace pensar que la estimulación con probióticos del asa eferente de la ileostomía de protección de pacientes intervenidos de carcinoma colorrectal, puede ser una alternativa para pacientes que no son candidatos a cirugía de reconstrucción del tránsito (artículos 1, 2, 3 y 4). Actualmente para estos pacientes sólo existe tratamiento sintomático para paliar la clínica asociada a la colitis por diversión. Buscando un tratamiento alternativo a la cirugía se han publicado varios estudios, tanto en modelos experimentales como estudios fase III, mediante la estimulación con diversas sustancias como soluciones nutricionales, trasplante fecal, enemas de ácidos grasos de cadena corta (SCFA) como el butirato, ácido 5 aminosalicilato (5-ASA), glucocorticoides, sucralfato y N-acetilcisteína entre otros con diferentes resultados (Harig 1989; Guillemot F, 1991; Schauber J, 2000; Williams L, 2007; Pacheco RG, 2012; Martinez CA, 2013; Alvarenga V, 2014; Kabir SI, 2014; Szczepkowski M, 2017; Beamish EL, 2017; Kleinwort A, 2017; Buanaim RP, 2019). En su mayoría mostraron resultados inconsistentes, aunque parece existir una disminución de la inflamación de la mucosa intestinal, reduciendo las lesiones epiteliales y la infiltración de neutrófilos de forma generalizada. El tratamiento más exitoso continúa siendo a día de hoy el cierre de la ileostomía de protección.

El grado de severidad endoscópica e histológica de la colitis por diversión se relaciona con una mayor alteración de los biomarcadores inflamatorios en sangre (artículo 4). La estimulación del asa eferente con probióticos mejoró la inflamación de la mucosa de colon excluida, reduciendo el grado inflamatorio y disminuyendo los biomarcadores séricos inflamatorios. También se observó una tendencia entre valores más alterados y grado de severidad endoscópica e histológica.

La estimulación con probióticos previa a la reconstrucción del tránsito produce una normalización de estos biomarcadores serológicos de forma precoz (artículo 4). Como

hemos explicado previamente, la colitis por diversión es una inflamación crónica del intestino excluido tras la creación de una ileostomía de protección. Esta inflamación crónica produce una alteración de los biomarcadores serológicos tal y como sucede en otras enfermedades inflamatorias. Por otro lado, ya ha quedado demostrado el efecto antiinflamatorio de los probióticos (Biagioli M, 2019). El ácido láctico producido por las cepas de Lactobacilos, y en menor medida por Bifidobacterias, es un regulador negativo de mediadores proinflamatorios tales como metaloproteasas, TNF alfa y monocitos, a la vez que inducen la expresión de antinflamatorios como la IL-17, IL-22, IL-23 y EGR 2, aumentando las células CD4 y macrófagos en la lámina propia del colon (Corridoni D, 2012; Biagiol M, 2019). Este efecto antiinflamatorio parece demostrarse en nuestro estudio tras la fase de estimulación al objetivar una disminución en el GE de los parámetros inflamatorios serológicos preoperatorios, con normalización de los valores de PCR, PLR y transferrina y una disminución estadísticamente significativa de los valores de NLR y LMR sin llegar a normalizarse. Estos resultados se explican por la capacidad de los probióticos de interactuar con la mucosa intestinal disminuyendo la producción molecular de sustancias proinflamatorias, y con ello disminuyendo la capacidad de migración de células inflamatorias a la lámina propia, tales como linfocitos, eosinófilos y células plasmáticas.

La estimulación con probióticos es independiente del inicio de tolerancia oral y restablecimiento del tránsito intestinal (artículo 1 y 2). En cuanto al efecto sobre la función intestinal tras la reconstrucción del tránsito, no se han observado diferencias entre ambos grupos, con un tiempo de restablecimiento del tránsito y tolerancia oral no estadísticamente significativos. Aunque durante la fase de estimulación se ha observado un aumento de las deposiciones y emisión de gas durante en el GE frente al GC, hecho que relacionamos de forma directa con una disminución o desaparición de la colitis por diversión tras la estimulación con probióticos con una relación moderada/intensa. Este inicio del tránsito intestinal previo a la reconstrucción permite

identificar obstrucciones funcionales distales a la ileostomía que puedan originar íleo mecánico tras la cirugía y que pueden resolverse mediante dilataciones endoscópicas o por el contrario identificar cierto grado de incontinencia asociada al síndrome de resección anterior, lo que permite realizar una educación de esfínteres precoz.

La estimulación con probióticos es independiente de la aparición de complicaciones postoperatorias y la estancia hospitalaria (artículos 1 y 2). No hubo diferencias en la aparición de complicaciones intraoperatorias ni postoperatorias. Sí se observó una disminución notable del tiempo quirúrgico en GE que asociamos al engrosamiento del cabo distal secundario a la estimulación y que facilita la realización de la anastomosis. La diferencia en cuanto a la estancia hospitalaria entre el GE y el CG, con una mediana de 4 días en GE y 5 días en GC, asociándolo a las 24 horas de diferencia en la tolerancia oral entre GE, en el que un 58% de los pacientes toleran dieta líquida en las primeras 24-48 horas, frente al GC que tarda 48-72 horas.

La aparición de íleo paralítico secundario al cierre de la ileostomía de protección es independiente de la estimulación del asa eferente con probióticos (artículo 1). No se ha demostrado diferencias en cuanto a la aparición de íleo postoperatorio, apareciendo en 29% de los pacientes del GE y en el 31% de los pacientes del GC. Estos hallazgos discrepan con las revisiones sistemáticas publicadas por Rombey y Garfinkle (Rombey T, 2019; Garfinkle R, 2019) y otros estudios publicados sobre el íleo postoperatorio que parece mejorar tras estimulación del asa eferente previa al cierre de la ileostomía (Menéndez P, 2013; Abrisqueta J, 2014; Rombey T, 2019). Aunque ambos concluyen en la falta de evidencia para recomendar la estimulación del asa eferente de forma rutinaria y hacen hincapié en la necesidad de realizar estudios multicéntricos con mayor calidad científica que permitan comprobar la validez externa de los mismos.

Parece existir relación entre la aparición de íleo paralítico postoperatorio tras la reconstrucción del tránsito y la existencia previa en el postoperatorio de la cirugía del cáncer colorrectal. Se objetivó que la incidencia de IPP era exactamente igual a la presentada en la cirugía colorrectal, y que esto se debía a que todos los pacientes que presentaron íleo tras la reconstrucción del tránsito también lo habían presentado en la cirugía colorrectal previa. Este hecho es importante, dado que el cierre de la ileostomía de protección no comparte los factores de riesgo tradicionales de la cirugía colorrectal como son el tiempo quirúrgico, aumento de líquidos y la pérdida de sangre entre otros.

5.2. Explicación de los principales hallazgos

Actualmente existen varios artículos publicados sobre la estimulación del asa eferente (Menéndez P, 2013; Abrisqueta J, 2014; Garfinkle R, 2017; Fernández López F, 2019). Todos concluyen, al igual que nuestro estudio que es un método, seguro, eficaz y reproductible. La primera revisión sistemática fue publicada por Rombey y col. en 2019 (Rombey, 2019), ésta incluyó 8 estudios con un total de 267 pacientes. En ella, a pesar de los prometedores resultados iniciales, no existen pruebas de calidad suficiente como para recomendar la implementación rutinaria de la estimulación intestinal preoperatoria en la práctica clínica. Esto se debe a la heterogeneidad de los estudios. En su mayoría son estudios con bajo poder estadístico y con resultados difíciles de comparar entre sí, ya que algunos comparan pacientes sometidos a proctocolectomía con anastomosis ileoanal en J y otros pacientes con resección anterior baja, sólo uno fue aleatorizado, tres eran casos clínicos únicos y había diversidad en cuanto a la sustancia usadas durante la estimulación y duración de la misma.

Además, respecto a la seguridad, la estimulación del asa eferente es un método con escasa morbilidad. Se demostró en ambos casos la asociación entre la estimulación

con probióticos y la reducción de la colitis por diversión con una relación intensa, con la aparición de un único efecto secundario reconocido, dolor abdominal tipo cólico, en un 20% de los pacientes, valorado como dolor moderado mediante la escala visual analógica (EVA) y sólo asociado a las primeras sesiones de estimulación, desapareciendo posteriormente. Este efecto secundario ya fue descrito en estudios con estimulación del asa eferente con ácidos grasos de cadena corta (Harig JM, 1989; Kabir SI, 2014; Rombey T, 2019).

Por otro lado, nuestro estudio es el primero que aplica la estimulación del asa eferente en la colitis por diversión, objetivando una disminución del grado de severidad en el grupo estimulado frente al grupo control tanto a nivel endoscópico como histológico. Esta reducción preoperatoria de la CD tras la estimulación con probióticos es relativamente importante, ya que no existe abundante bibliografía sobre la colitis por diversión y su relación con la alteración microbiota. En los dos últimos años se han publicados dos estudios sobre el microbioma intestinal, Mishima (Mishima Y, 2020) y Knox (Knox NC,2019); a los que hay que sumar, por un lado la última revisión sistemática publicada sobre la colitis por diversión por Kabir y col. en 2014 (Kabir SI, 2014), que revisa un total de 3305 artículos, incluyendo finalmente 35 estudios, que analiza la fisiopatología, presentación clínica y tratamiento de la colitis por diversión que concluye que hay una gran variabilidad de formas de presentación y un único tratamiento definitivo que es la reconstrucción del tránsito.

Precisamente esta disminución del grado de severidad histológico y endoscópico, nos hace pensar que la estimulación con probióticos del asa eferente de la ileostomía de protección de pacientes intervenidos de carcinoma colorrectal, puede ser una alternativa para pacientes que no son candidatos a cirugía de reconstrucción del tránsito. Actualmente para estos pacientes sólo existe tratamiento sintomático para paliar la clínica asociada a la colitis por diversión. Buscando un tratamiento alternativo a la cirugía se han publicado varios estudios, tanto en modelos experimentales como

estudios fase III, mediante la estimulación con diversas sustancias como soluciones nutricionales, trasplante fecal, enemas de ácidos grasos de cadena corta (SCFA) como el butirato, ácido 5 aminosalicilato (5-ASA), glucocorticoides, sucralfato y N-acetilcisteína entre otros con diferentes resultados(Harig 1989; Guillemot F, 1991; Schauber J, 2000; Williams L, 2007; Pacheco RG, 2012; Martinez CA, 2013; Alvarenga V, 2014; Kabir SI, 2014; Szczepkowski M, 2017; Beamish EL, 2017; Kleinwort A, 2017; Buanaim RP, 2019). En su mayoría mostraron resultados inconsistentes, aunque parece existir una disminución de la inflamación de la mucosa intestinal, reduciendo las lesiones epiteliales y la infiltración de neutrófilos de forma generalizada.

Así mismo, en el estudio macroscópico y microscópico, se aprecian alteraciones asociadas a la CD, tanto en el GC como en el GE sin objetivar relación estadísticamente significativa entre severidad, clínica ni tiempo desde la creación del estoma de protección, hallazgos similares a los publicados en estudios previos (Son DN, 2013; Baek SJ, 2014; Kabir SI, 2014; Szczepkowski M, 2017). A nivel macroscópico o endoscópico, se ha demostrado una disminución significativa de todos los hallazgos endoscópicos en el GE frente al GC, con una correlación intensa entre la estimulación del asa eferente con probióticos y la disminución de la colitis por diversión macroscópica. Cabe destacar una notable disminución del grado de severidad del edema y el eritema mucoso, convirtiéndose de grado severo o moderado a formas leves o simplemente desapareciendo, hecho que relacionamos con el efecto antinflamatorio de los probióticos; junto con la desaparición por completo de las úlceras en el GE tras la fase de estimulación también con una correlación intensa. Las úlceras asociadas a CD en humanos son un hallazgo inconstante. Parece existir asociación con cambios estructurales tardíos del epitelio superficial (Kabir SI, 2014; Szczepkowski M, 2017). En nuestro estudio, los pacientes que presentaban úlceras mucosas son los que llevaban más tiempo desde la creación del estoma. Estos

hallazgos se ajustan a las observaciones previas realizadas en humanos y en modelos animales (Pacheco RG, 2012; Alvarenga V, 2014).

A nivel microscópico o histológico, en estudios morfológicos de biopsias colónicas en pacientes con ileostomía de protección han demostrado una inflamación crónica en más del 50% de las muestras, con un incremento del tejido linfoide en el 100% de las biopsias anatomopatológicas (Szczepkowski M, 2017; Buanaim RP, 2019). La alteración histológica más característica de la CD es la hiperplasia folicular linfoide, presente en modelos experimentales y biopsias de pacientes a partir de los 60 días posteriores a la creación del estoma de protección (Kleinwort A, 2017), con una infiltración de la mucosa por linfocitos que puede ser desde leve a severa en función de la inflamación del segmento de colon excluido. Pacheco et al. describió la hiperplasia folicular linfoide en el 100% de los sujetos de estudio en su modelo animal sobre colitis por diversión y una atrofia de las criptas en aproximadamente 24% de los sujetos a las seis semanas de la creación del estoma y en el 42% tras 17 semanas (Pacheco RG, 2012; Alvarenga V, 2014). En nuestro estudio, los cambios histopatológicos se caracterizaron al igual que en estudios previos principalmente por la hiperplasia folicular linfoide y la infiltración de la lámina propia por linfocitos, apareciendo desestructuración de la arquitectura de las criptas y criptitis en aquellos pacientes con mayor tiempo desde la creación del estoma. Se aprecia al igual que ocurría con los hallazgos macroscópicos una reducción significativa de las alteraciones microscópicas en el GE frente al GC, con una correlación intensa entre la estimulación del asa eferente con probióticos y la disminución o desaparición de las alteraciones histológicas. Estos hallazgos se explican por la capacidad de los probióticos de interactuar con la mucosa intestinal disminuyendo la producción molécular de sustancias proinflamatorias (Biagioli M, 2019), y con ello disminuyendo la capacidad de migración de células inflamatorias a la lámina propia, tales como linfocitos, eosinófilos y células plasmáticas.

En cuanto a la clínica asociada a la CD, la estimulación con probióticos del asa eferente de la ileostomía de protección de pacientes intervenidos de carcinoma colorrectal disminuye la clínica de CD tras la cirugía en el 75% de los pacientes y en el 100% a corto plazo tras la reconstrucción del tránsito intestinal. Se mantiene en el seguimiento al primer mes del restablecimiento del tránsito. Sin embargo en el seguimiento a corto plazo las cifras tienden a igualarse, desapareciendo la relación directa entre el tercer y el sexto mes, cuando la colitis por diversión endoscópica y microscópica se hace prácticamente inexistente. Este hecho coincide con el efecto modulador antiinflamatorio, transitorio y limitado que ejercen los probióticos sobre la mucosa intestinal (Kang SI, 2016; Sartor RB, 2017; Basso PJ, 2018; Cohen LJ, 2019; Amoroso C, 2020) y con la curva de recuperación de la función intestinal publicada en otros estudios (Beamish EL, 2017; Keane C, 2019).

Este efecto modulador antiinflamatorio es el que buscamos para conseguir una disminución de los biomarcadores serológicos alterados. Nuestro estudio es el primer estudio aleatorizado, doble ciego, publicado, que relaciona la colitis por diversión con las alteraciones de los estos biomarcadores inflamatorios. Si bien, ya conocemos que en la colitis por diversión se produce una inflamación inespecífica en la mucosa del segmento de colon excluido tras la creación de un estoma de protección (Szczepkowski M, 2017). A día de hoy no existen trabajos que relacionen ésta inflamación con la elevación de los parámetros serológicos. Por el contrario, la relación entre biomarcadores inflamatorios y probióticos sí ha sido estudiada en otras patologías como diarrea aguda en el adulto, enfermedad inflamatoria intestinal, pouchitis activa y síndorme de intestino irritable entre otras (Gionchetti P, 2007; Grossi E, 2010; Sartor RB, 2017; Hod K, 2019; Biagioli M, 2019; Tenorio-Jiménez C, 2019; Barra M, 2020).

Las bacterias probióticas mas estudiadas pertenecen al género Bifidobacterium y Lactobacillus spp. Éstas disminuyen moléculas proinflamatorias y aumentan moléculas que inhiben la inflamación, protegiendo además contra el estrés oxidativo en humanos

y demostrando un papel importante sobre la disbiosis intestinal (Rossi M, 2010; Beamish EL, 2017; Morgan DM, 2020). Actualmente la evidencia de la que disponemos nos demuestra que los probióticos tienen un efecto positivo sobre sobre la inflamación sistémica y oxidativa, sobre todo a nivel gastrointestinal. L. acidophilus, uno de los probióticos administrados durante nuestra estimulación, he demostrado su papel en la regulación inflamatoria en múltiples estudios experimentales (Rossi M, 2010; Jafarnejad S, 2016; Biagioli M, 2017; Hod K, 2017; Hajifaraji M, 2018; Tenorio-Jiménez C, 2019, Buanaim RP, 2019; Morgan DM, 2020). Un ensayo clínico controlado aleatorizado realizado por Jafarnejad et al. (Jafarnejad S, 2016). Describe el efecto de la suplementación de con probióticos, en este caso, con VSL3, compuesto probiótico similar al administrado en nuestro estudio, durante 8 semanas, sobre el estado glucémico y los marcadores inflamatorios entre mujeres embarazadas. Los suplementos probióticos contenían ocho cepas de bacterias del ácido láctico (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L.acidophilus, L.plantarum, L.paracasei y L. delbrueckii subsp. Bulgaricus). Encontraron una disminución estadísticamente significativa en los niveles de TNF alpha y PCR en el grupo de los probióticos en comparación con el placebo. Resultados superponibles a otros estudios (Hajifaraji M, 2018). Otro ensayo clínico donde se evalúa la eficacia de los probióticos VSL3 administrados durante 8 semanas en 24 pacientes con Síndrome de Intestino Irritable, encontrando una mejora significativa de la sintomatología, pero sin diferencias significativas entre los grupos (Michail S, 2011).

En nuestro estudio, para llevar a cabo la fase de estimulación, utilizamos un preparado con probióticos formado por la combinación de 8 cepas (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L.acidophilus, L.plantarum, L.paracasei y L. delbrueckii subsp. Bulgaricus), demostrando el efecto antiinflamatorio tras la fase de estimulación al objetivar una disminución en el GE de los parámetros inflamatorios serológicos, con normalización

de los valores de PCR, PLR y transferrina y una disminución estadísticamente significativa de los valores de NLR y LMR sin llegar a normalizarse. La normalización de los valores de PCR, transferrina, NLR, LMR y PLR sólo se consigue tras la reconstrucción del tránsito, en el seguimiento a 3 meses de la cirugía. Al igual que ocurría con las alteraciones histológicas, estos hallazgos se explican por la capacidad de los probióticos de interactuar con la mucosa intestinal disminuyendo la producción molécular de sustancias proinflamatorias (Biagioli M, 2019), y con ello disminuyendo la capacidad de migración de células inflamatorias a la lámina propia, tales como linfocitos, eosinófilos y células plasmáticas.

En cuanto a la aparición de íleo paralítico secundario al cierre de la ileostomía de protección y la estimulación del asa eferente con probióticos, no hemos encontrado relación. Estos hallazgos discrepan con las revisiones sistemáticas publicadas por Rombey (Rombey T, 2019) y Garfinkle (Garfinkle R, 2019). En 2019 Garfinkle y col. publican la primera revisión sistemática que sintetiza la literatura existente hasta el momento. Reúne 9.528 pacientes en 67 estudios, la mayoría retrospectivos. Los autores señalan que su incidencia depende de la definición que utilicemos de íleo, y que estas variaciones provocan gran heterogeneidad en los estudios incluidos, por lo que necesitaremos continuar investigando y definirlo con criterios homogéneos. De hecho, más de la mitad de los estudios de este metaanálisis (39/67) no tenían criterios definidos de íleo postoperatorio (estudios con menor incidencia), y entre los que lo tenían, no coincidían en la definición. Ambos concluyen en la falta de evidencia para recomendar la estimulación del asa eferente de forma rutinaria y hacen hincapié en la necesidad de realizar estudios multicéntricos con mayor calidad científica que permitan comprobar la validez externa de los mismos. Esta falta de consenso en la definición creemos que explicaría nuestros hallazgos. Ante esta necesidad de encontrar un denominador común para poder comparar los diversos estudios nos basamos en el estudio publicado por el grupo de trabajo de la ASER (American Society

of Enhanced Recovery) y POQI (Perioperative Quality Initiative) (Hedrick TL, 2018), que establece definiciones basadas en la puntuación de la sintomatología para la disfunción gastrointestinal postoperatoria (englobando términos como íleo, íleo paralítico, íleo postoperatorio o íleo prolongado) que incluyen la tolerancia oral, aparición de náuseas, vómitos, distensión abdominal con timpanismo y duración de estos síntomas. Este sistema de puntuación validado llamado I-FEED (Alsharqawi N, 2020, Anexo 8) es el que hemos utilizado en nuestro estudio, ya que nos parece la forma más objetiva y extrapolable de determinar la aparición de íleo postoperatorio, definido como disfunción gastrointestinal postoperatoria, diferenciarlo así de la intolerancia gastrointestinal postoperatoria que se resuelve en 24-48 horas, y poder compararlo con otros estudios en un futuro.

Basándonos en revisiones sistemáticas sobre el uso de probióticos en el tratamiento de otras patologías gastrointestinales (Redman MG, 2014; Zhang Y, 2016; Kang SI, 2016), consideramos que esta estimulación conseguiría la repoblación del segmento excluido, activando los mecanismos celulares de absorción, aumentando la motilidad y disminuyendo la atrofia del asa eferente, para que, tras el cierre de la ileostomía de protección, el retorno a la normalidad sea más rápido, con un menor tiempo en el reestablecimiento del tránsito y tolerancia oral, disminuyendo la aparición de íleo postoperatorio y con ello disminuyendo la estancia hospitalaria. En nuestro estudio, tras la inclusión y aleatorización de 73 pacientes no se detectaron diferencias estadísticamente significativas en cuanto a las variables sociodemográficas, clínicas ni quirúrgicas. Sí se objetivó una notable disminución del tiempo quirúrgico en GE que asociamos al engrosamiento del cabo distal secundario a la estimulación y que facilita la realización de la anastomosis. La diferencia en cuanto a la estancia hospitalaria fue de 1 día, asociándose a las 24 horas de diferencia en la tolerancia oral entre GE, en el que más del 50% de los pacientes toleran dieta líquida en las primeras 24-48 horas, frente al GC que tarda 48-72 horas. Estos resultados son similares a los

reportados por otros estudios (Menéndez P, 2013; Abrisqueta J, 2014; Rombey T, 2019). Además se objetivó que la incidencia de íleo postoperatorio era exactamente igual a la presentada en la cirugía colorrectal, y que esto se debía a que todos los pacientes que presentaron íleo tras la reconstrucción del tránsito también lo habían presentado en la cirugía colorrectal previa. Este hecho es importante, dado que el cierre de la ileostomía de protección no comparte los factores de riesgo tradicionales de la cirugía colorrectal como son el tiempo quirúrgico, aumento de líquidos y la pérdida de sangre entre otros (Wolthuis AM, 2016; Sugawara K, 2018; Rybakov EG, 2018; Garfinkle R, 2019).

Finalmente, hay que destacar el aumento de las deposiciones y emisión de gas durante en el GE frente al GC durante la fase de estimulación, hecho que relacionamos de forma directa con una disminución o desaparición de la colitis por diversión tras la estimulación con probióticos. Este inicio del tránsito intestinal previo a la reconstrucción permite identificar obstrucciones funcionales distales a la ileostomía que puedan originar íleo mecánico tras la cirugía y que pueden resolverse mediante dilataciones endoscópicas o por el contrario identificar cierto grado de incontinencia asociada al síndrome de resección anterior, lo que permite realizar una educación de esfínteres precoz previo al cierre definitivo del estoma, aumentando notablemente la calidad de vida de los pacientes (Kim KH, 2011; Abrisqueta J, 2014).

5.3. Fortalezas y limitaciones

La realización del presente estudio fue motivada por la ausencia de evidencia científica clara sobre el beneficio de la estimulación del asa eferente. Con un diseño doble ciego mantenido hasta la finalización del mismo, una definición estandarizada de disfunción gastrointestinal postoperatoria no utilizada hasta el momento. Los autores señalan que la incidencia de IPP depende de la definición que utilicemos de íleo, y que

estas variaciones provocan gran heterogeneidad en los estudios incluidos, por lo que necesitaremos continuar investigando y definirlo con criterios homogéneos. Ante esta necesidad de encontrar un denominador común, nos basamos en el sistema de puntuación validado llamado I-FEED (Hendrick TL, 2018; Alsharqawu N, 2020), ya que nos parece la forma más objetiva y extrapolable de determinar la aparición de íleo postoperatorio, definido como disfunción gastrointestinal postoperatoria, diferenciarlo así de la intolerancia gastrointestinal postoperatoria que se resuelve en 24-48 horas, y poder compararlo con otros estudios en un futuro.

Además, nuestro estudio es el primer estudio aleatorizado, doble ciego, publicado, que relaciona la colitis por diversión con las alteraciones de los biomarcadores serológicos inflamatorios. Si bien, ya conocemos que en la colitis por diversión se produce una inflamación inespecífica en la mucosa del segmento de colon excluido tras la creación de un estoma de protección. A día de hoy no existen trabajos que relacionen ésta inflamación con la elevación de los parámetros serológicos.

Como hemos comentado anteriormente en el capítulo de metodología, la estimulación del asa eferente de la IP fue realizada en todos los casos por el mismo cirujano, ajeno al procedimiento quirúrgico y al seguimiento postoperatorio del paciente, del mismo modo, el equipo quirúrgico que intervino al paciente y lo evaluó durante el postoperatorio no era sabedor del grupo al que pertenecía cada caso. Valoramos este aspecto del trabajo con suma importancia, ya que consideramos clave, el valor estadístico que aporta al estudio que sean los mismos cirujanos colorrectales los que operen todos los pacientes y que estos sean ajenos al grupo que ha sido asignado a cada paciente. Con esto se evita el sesgo que se produce cuando están implicados varios cirujanos, con la consecuente diferencia de indicaciones, opiniones y gestos quirúrgicos que existen entre ellos.

También es importante destacar que, en nuestro estudio, para llevar a cabo la fase de estimulación, utilizamos un preparado con probióticos formado por la

combinación de 8 cepas (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L.acidophilus, L.plantarum, L.paracasei y L. delbrueckii subsp. Bulgaricus), todos del mismo lote, para minimizar así las posibles diferencias que se habían observado en otros estudios, en los cuales diferentes lotes del mismo producto fabricado en diferentes sitios, ejercen diferentes propiedades in vitro e in vivo (Sanders ME, 2014; Biagioli M, 2017). Se componen por tanto de Lactobacilos, Bifidobacterias y Estreptococos.

A favor de nuestro estudio hay que destacar, una alta adherencia al tratamiento en ambos grupos, siendo excluidos solamente pacientes con complicaciones postoperatorias, sin registrar otras pérdidas durante el seguimiento. Además nuestra población de estudio incluyó sólo pacientes que cumplían criterios diagnósticos endoscópicos e histológicos de colitis por diversión, excluyendo pacientes con diagnóstico de colitis inespecífica a pesar de presentar ileostomía de protección, ya que consideramos que podrían representar un factor de confusión e inducir a error.

Finalmente, aunque para la realización de nuestro estudio se calculó el tamaño muestral basado en la literatura existente, siendo necesario 30 paciente por grupo y hemos reclutado más, creemos que el tamaño muestral puede representar la limitación fundamental de nuestro estudio, por lo que estos resultados deben ser considerados con cautela y necesitaremos ampliar el “n” en estos futuros trabajos sobre la función intestinal y la estimulación preoperatoria previo al cierre de la IP.

5.4. Implicaciones de los resultados del estudio

A lo largo de este estudio hemos presentado esta modificación de una técnica ya descrita con anterioridad en la literatura (Menéndez P, 2013; Abrisqueta J, 2014; Garfinkle R, 2017; Vázquez-Melero A, 2018; Fernández López F, 2019; Rombey T, 2019), confirmando su seguridad, fiabilidad y reproducibilidad. A partir de esto y

basándonos en la sencillez, se podrían **establecer protocolos** para que cada paciente pudiese realizar diariamente y en su domicilio la estimulación con probióticos previa la cirugía de reconstrucción del tránsito. Hemos de destacar, que durante la realización de este estudio, los pacientes de ambos grupos han tenido que acudir al hospital en días alternos durante los 20 días previos a la cirugía, en ocasiones suponiendo un desplazamiento de más de una hora desde su domicilio. Con la realización de un protocolo para la aplicación de esta técnica, los pacientes acudirían al hospital para una primera consulta, dónde se les explicaría el fundamento de la técnica así como el modo de realizarla y los materiales necesarios, asegurándoles siempre un soporte sanitario (teléfono, consultas externas, consultas por urgencias…) ante cualquier incidencia que pudiese surgir durante la estimulación previa al cierre de la IP. En resumen, el objetivo en el futuro será, que una técnica que se ha mostrado segura y reproducible en el ámbito hospitalario pueda ser aplicada en régimen domiciliario previamente a la cirugía.

Por otro lado, al desarrollar uno de los objetivos específicos de esta tesis nos encontramos múltiples estudios que valoran e intentar dar solución al IPP meter estudios IPP, y una gran variabilidad en la definición del mismo. Ante esta necesidad de encontrar un **denominador común para poder comparar los diversos estudios** nos basamos en el estudio publicado por el grupo de trabajo de la ASER (American Society of Enhanced Recovery) y POQI (Perioperative Quality Initiative) (Hendrick TL, 2018), que establece definiciones basadas en la puntuación de la sintomatología para la disfunción gastrointestinal postoperatoria (englobando términos como íleo, íleo paralítico, íleo postoperatorio o íleo prolongado) que incluyen la tolerancia oral, aparición de náuseas, vómitos, distensión abdominal con timpanismo y duración de estos síntomas. Este sistema de puntuación validado llamado I-FEED (Alsharqawu N, 2020) es el que hemos utilizado en nuestro estudio, ya que nos parece la forma más objetiva y extrapolable de determinar la aparición de íleo postoperatorio, definido

como disfunción gastrointestinal postoperatoria, diferenciarlo así de la intolerancia gastrointestinal postoperatoria que se resuelve en 24-48 horas, y poder compararlo con otros estudios en un futuro.

También, hay que destacar, el inicio preoperatorio de emisión de gas y heces durante la fase de estimulación del estudio. Paralelo a la estimulación se ha observado un aumento de las deposiciones y emisión de gas durante esta fase en GE frente al GC. Este inicio del tránsito intestinal previo a la reconstrucción permite **identificar obstrucciones funcionales distales** a la ileostomía que puedan originar íleo mecánico tras la cirugía y que pueden resolverse mediante dilataciones endoscópicas o por el contrario identificar cierto grado de incontinencia asociada al síndrome de resección anterior, lo que permite realizar una educación de esfínteres precoz (Kim KH, 2011; Abrisqueta J, 2014).

Finalmente, dado los hallazgos aportados por este estudio, en el que se aprecia la disminución de la CD tras estimulación con probióticos en el 75% de los pacientes y la desaparición endoscópica e histológica en el 25%. Así como una disminución de la clínica de CD, la estimulación del asa eferente con probióticos puede ser una **alternativa para aquellos pacientes que no son candidatos a la cirugía** de reconstrucción del tránsito por comorbilidad.

5.5. Futuras líneas de investigación

Los resultados de este estudio nos han ayudado a confirmar y refutar las hipótesis que inicialmente teníamos cuando comenzamos. Esto nos ha llevado por un lado a las conclusiones del presente estudio y por otro nos ha abierto nuevas líneas de investigación que en lo sucesivo iremos desarrollando.

Por un lado, creemos que la estimulación del asa eferente con probióticos previa al cierre de la IP no sólo supone una mejoría clínica de la CD, sino que repercute

notablemente sobre la calidad de vida del paciente sometido a una cirugía de reconstrucción del tránsito (Ayaz-Alkaya S. 2019), por ello se abre una nueva línea de investigación en la que junto al proceso de estimulación, se le administrará al paciente a lo largo del proceso varios cuestionarios de calidad de vida repetidos en el tiempo. Los cuestionarios a utilizar serán Short Form SF-36, Estoma-QoL, EuroQoL-5D-5L y el Test Holschneider. Todos ellos miden la calidad de vida en relación a diferentes aspectos del sujeto por lo que se complementan entre sí.

Por otro lado, hemos basado nuestro estudio en la influencia de los cambios fisiopatológicos intestinales causados en parte por la pérdida de flora intestinal en el segmento de colon excluido (Williams L, 2007; Beamish EL, 2017; Keane C, 2019, Rombey T, 2019) estudio comparativos y metaanálisis; y en la mejoría de la CD tras la manipulación microbiana mediante la estimulación de probióticos previa al cierre de la ileostomía de protección, lo que nos permitiría normalizar la función autoinmune alterada, restaurar la homeostasis y disminuir la inflamación de la mucosa y con ella normalizar los biomarcadores inflamatorios serológicos. Si bien esta notable mejoría tras la administración de probióticos no hemos podido correlacionarla con el aumento en el segmento de colon excluido de las bacterias vivas no patógenas administradas a través del asa eferente. Por ello una nueva línea de investigación es la determinación mediante técnica de PCR (reacción en cadena de la polimerasa) de las muestras obtenidas mediante endoscopia y criopreservadas en el laboratorio de Microbiología comparando pre-estimulación y post-estimulación, tanto de los pacientes participantes en el actual estudio como de los que seguimos reclutando en el momento actual.

En cuanto a la aparición de IPP, es cierto que nuestro estudio difiere de otros publicados (Rombey T, 2019; Garfinkle R, 2017), aunque los resultados podrían correlacionarse con las últimas revisiones sistemáticas publicadas, que concluyen que se necesitan más estudios multicéntricos con mayor calidad científica. Si bien, hemos observado una relación directa entre la aparición de IPP tras la reconstrucción del

tránsito en los pacientes en los que previamente ya se había presentado tras la cirugía del carcinoma colorrectal. Se objetivó que la incidencia de íleo postoperatorio era exactamente igual a la presentada en la cirugía colorrectal, y que esto se debía a que todos los pacientes que presentaron íleo tras la reconstrucción del tránsito también lo habían presentado en la cirugía colorrectal previa. Este hecho es importante, dado que el cierre de la ileostomía de protección no comparte los factores de riesgo tradicionales de la cirugía colorrectal como son el tiempo quirúrgico, aumento de líquidos y la pérdida de sangre entre otros (Wolthuis AM, 2016; Sugawara K, 2018; Rybakov EG; 2018; Garfinkle R, 2019). Este hallazgo ha supuesto una nueva línea de investigación sobre la que actualmente estamos trabajando, y que es objeto de un nuevo Proyecto I+i becado por la Junta de Andalucía.

Conclusiones

1. La estimulación del asa eferente con probióticos es un método seguro y eficaz.
2. Provoca una disminución o desaparición del grado de colitis por diversión macroscópica y microscópica en el 100% de los pacientes estimulados, con una disminución en el 75% y una desaparición en el 25%.
3. Disminuye la clínica de colitis por diversión tras la cirugía en el 75% de los pacientes y en el 100% a corto plazo tras la reconstrucción del tránsito intestinal.
4. Puede ser una alternativa para pacientes que no son candidatos a cirugía de reconstrucción del tránsito.
5. El grado de severidad endoscópica e histológica de la colitis por diversión se relaciona con una mayor alteración de los biomarcadores inflamatorios en sangre.
6. La estimulación con probióticos previa a la reconstrucción del tránsito produce una normalización de estos parámetros de forma precoz.
7. La estimulación con probióticos es independiente del inicio de tolerancia oral y restablecimiento del tránsito intestinal.
8. La estimulación con probióticos es independiente de la aparición de complicaciones postoperatorias y la estancia hospitalaria.
9. La aparición de íleo paralítico secundario al cierre de la ileostomía de protección es independiente de la estimulación del asa eferente con probióticos.
10. Parece existir relación entre la aparición de íleo tras la reconstrucción y la existencia previa en el postoperatorio de la cirugía del cáncer colorrectal.

Referencias

Aaron R, Sasson and Elin R, Sigurdson. Surgery of rectal cancer. Colorrectal cancer, multimodality management. 2020. Cap 18. 337-340.

Abrisqueta J, Abellan I, Lujan J, Hernandez Q, Parrilla P. Stimulation of the efferent limb before ileostomy closure: a randomized clinical trial. Dis Colon Rectum 2014; 57: 1391–6.

Afsar et al. Role of Mean Platelet Volume As An Adjunct in Evaluation of Acute Inflammation. Annals of Pathology and Laboratory Medicine, Vol. 4, Issue 4, 2017.

Alvarenga V., Pacheco RG, Esposito CC et al., "Ascidian (chordate-tunicate) and mammalian heparin enemas attenuate experimental diversion colitis," Surgery (United States), 2014: 155:2, 217–227.

Alsharqawi N, Alhashemi M, Kaneva P, et al. Validity of the I-FEED score for postoperative gastrointestinal function in patients undergoing colorectal surgery. Surg Endosc. 2020 May;34(5):2219-2226.

Amoroso C, Perillo F, Strati F, Fantini M, Caprioli F, Facciotti F. The Role of Gut Microbiota Biomodulators on Mucosal Immunity and Intestinal Inflammation. Cells. 2020 May 16; 9(5).

Arredondo J, Pastor E, Simó V, Beltrán M, Castañón C, Magdaleno MC, Matanza I, Notarnicola M, Ielpo B. Neoadjuvant chemotherapy in locally advanced colon cancer: a systematic review. Tech Coloproctol. 2020 Oct; 24(10):1001-1015. Doi: 10.1007/s10151-020-02289-4. Epub 2020 Jul 14. PMID: 32666362.

Ayaz-Alkaya S. Overview of psychosocial problems in individuals with stoma: a review of literature. Int Wound J. 2019 Feb; 16 (1):243-249.

Baek SJ, Kim SH, Lee CK, Roh KH, Keum B, Kim CH, Kim J (2014) Relationship between the severity of diversion colitis and the composition of colonic bacteria: a prospective study. Gut Liver 8(2):170–176.

Baldassarre ME, Di Mauro A, Tafuri S, Rizzo V et al. Effectiveness and Safety of a Probiotic-Mixture for the Treatment of Infantile Colic: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial with Fecal Real-Time PCR and NMR-Based Metabolomics Analysis. Nutrients. 2018 Feb; 10(2): 195.

Barra M, Danino T, Garrido D. Engineered Probiotics for Detection and Treatment of Inflammatory Intestinal Diseases. Front Bioeng Biotechnol. 2020 Mar 31; 8 : 265.

Basso PJ, Câmara NOS, Sales-Campos H. Microbial-based therapies in the treatment of inflammatory bowel disease—an overview of human studies. Front Pharmacol. 2018; 9:1571.

Beamish EL, Johnson J, Shaw EJ, Scott NA, Bhowmick A, Rigby RJ. Loop ileostomy - mediated fecal stream diversion is associated with microbial dysbiosis. Gut Microbes 2017; 8: 467 -78.

Beart RW, Steele GD Jr, Menck HR, et al. Management and survival of patients with adenocarcinoma of the colon and rectum: a national survey of the commission on cancer. J Am Coll Surg 1995; 181:225–236. Ver si hay alguno más reciente con sintomatología.

Bhalla A, Peacock O, Tierney GM, Tou S, Hurst NG, Speake WJ et al. Day-case closure of ileostomy: feasible, safe and efficient. Colorectal Dis 2015; 17: 820‑823.

Biagioli M, Capobianco D, Carino A, Marchianò S, et al. Divergent Effectiveness of Multispecies Probiotic Preparations on Intestinal Microbiota Structure Depends on Metabolic Properties. Nutrients. 2019 Feb; 11(2): 325.

Biagioli M., Laghi L., Carino A., Cipriani S., Distrutti E., Marchianò S., Parolin C., Scarpelli P., Vitali B., Fiorucci S. Metabolic Variability of a Multispecies Probiotic Preparation Impacts on the Anti-inflammatory Activity. Front. Pharmacol. 2017; 8:505.

Borthakur A, Bhattacharyya S, Kumar A, Anbazhagan AN, Tobacman JK, Dudeja PK. Lactobacillus acidophilus alleviates platelet-activating factor-induced inflammatory responses in human intestinal epithelial cells. PLoS One. 2013; 8 : e75664.

Breen RE, Garnjobst W. Surgical procedures for carcinoma of the rectum. A historial review. Dis Colon and rectum 1983; 26: 680-685.

Buanaim RP et al. Effects of anti-TNF-α in experimental diversion colitis. Acta Cir Bras. 2019; 34(10).

Callaway LK, McIntyre HD, Barrett HL, Foxcroft K, Tremellen A, Lingwood BE, Tobin JM, Wilkinson S, Kothari A, Morrison M, et al. Probiotics for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Women Diabetes Care. 2019 Mar; 42(3):364-371.

Chen J, Wang DR, Yu HF, et al. Defunctioning Stoma in Low Anterior Resection for Rectal Cancer: A Meta-Analysis of Five Recent Studies. Hepato-gastroenterol 2012; 59:1828-1831.

Chenyan Zhao, Chao Ding, Tingbin Xie, et al. Validation and optimization of the Systemic Infammation-Based modifed Glasgow Prognostic Score in predicting postoperative outcome of infammatory bowel disease. Scientific Reports (2018); 8:747.

Cohen LJ, Cho JH, Gevers D, et al. Genetic factors and the intestinal microbiome guide development of microbe-based therapies for inflammatory bowel diseases. Gastroenterology. 2019; 156:2174–2189.

Corridoni D, Pastorelli L, Mattioli B, Locovei S, Ishikawa D, Arseneau KO, et al. Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. PLoS One. 2012; 7(7):e42067.

Danielsen AK, Park J, Jansen JE, Bock D, Skullman S, Wedin A, et al. Early Closure of a Temporary Ileostomy in Patients With Rectal Cancer: A Multicenter Randomized Controlled Trial. Ann Surg 2017; 265: 284 -90

Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019 Oct 19; 394(10207):1467-1480. Doi: 10.1016/S0140-6736(19)32319-0. PMID: 31631858.

Farag S, Rehman S, Sains P, Baig MK, Sajid MS. Early vs delayed closure of loop defunctioning ileostomy in patients undergoing distal colorectal resections: an integrated systematic review and meta-analysis of published randomized controlled trials. Colorectal Dis 2017; 19: 1050–1057.

Fernández López F, González López J, Paz Novo M, Ladra González MJ, Paredes Cotoré J. Estimulación preoperatoria del asa eferente de la ileostomía con ácidos grasos de cadena corta. Cir Esp. 2019; 97: 54-6.

Fisberg, M.; Machado, R. History of yogurt and current patterns of consumption. Nutr. Rev. 2015, 73, 4–7.

Ford AC, Moayyedi P, Lacy BE, et al.; Task Force on the Management of Functional Bowel Disorders. American College of Gastroenterology monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. Am J Gastroenterol. 2014; 109(suppl 1): S2-S26. 22.

Gao R, Gao Z, Huang L, Qin H. Gut microbiota and colorectal cancer. Eur J Clin Microbiol Infect Dis. 2017 May; 36(5):757-769. Doi: 10.1007/s10096-016-2881-8. Epub 2017 Jan 7. PMID: 28063002; PMCID: PMC5395603.

Garfinkle R, Trabulsi N, Morin N, et al. Study protocol evaluating the use of bowel stimulation before loop ileostomy closure to reduce postoperative ileus: a multicenter randomized controlled trial. Colorectal Disease 2017; 19: 1024–1029.

Garfinkle R, Savage P, Boutros M, Landry P, Reynier P, Morin N et al. Incidence and predictors of postoperative ileus after loop ileostomy closure: a systematic review and meta-analysis. Surg Endosc 2019; 33(8):2430-2443.

Gasbarrini, G.; Bonvicini, F.; Gramenzi, A. Probiotics History. J. Clin. Gastroenterol. 2016, 50, S116–S119.

Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C, et al. High-dose probiotics for the treatment of active pouchitis. Dis Colon Rectum. 2007 Dec; 50(12):2075-2082.

Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A and Arnold D. ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017; 28(Suppl 4): vi22–40.

Glotzer DJ, Glick ME, Goldman H (1981) Proctitis and colitis following diversion of the fecal stream. Gastroenterology 80:438‑ 441.

Graney MI, Graney CM. Colorectal surgery from antiquity to the modern era. Dis Colon and rectum 1980; 23: 432-441.

Grossi E, Buresta R, Abbiati R, Cerutti R, Pro-DIA study group. Clinical trial on the efficacy of a new symbiotic formulation, Flortec, in patients with acute diarrhea: a multicenter, randomized study in primary care. J Clin Gastroenterol. 2010 Sep; 44 Suppl 1:S35–41.

Guillemot F, Colombel JF, Neut C, Verplanck N, Lecomte M, Romond C, et al. Treatment of diversion colitis by short-chain fatty acids. Prospective and double-blind study. Dis Colon Rectum. 1991; 34: 861864.

Guarner, F.; Khan, A.G.; Garisch, J.; Eliakim, R.; Gangl, A.; Thomson, A.; Krabshuis, J.; Lemair, T. Probiotics and Prebiotics; World Gastroenterology Organisation Global Guidelines; World Gastroenterology Organisation 2017; Volume 46, pp. 468–481.

Guo, X.; Long, R.; Kreuzer, M.; Ding, L.; Shang, Z.; Zhang, Y.; Yang, Y.; Cui, G. Importance of functional ingredients in yak milk-derived food on health of Tibetan nomads living under high-altitude stress: A review. Crit. Rev. Food Sci. Nutr. 2014, 54, 292–302.

Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Abbasi MM, Dolatkhah N. Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial. Asia Pac J Clin Nutr. 2018; 27(3):581-591.

Hardy KJ. Surgical history. Evolution of the stoma. Aust N Z J Surg 1989; 59:71-77.

Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Abbasi MM, Dolatkhah N. Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial. Asia Pac J Clin Nutr. 2018; 27(3):581-591.

Harig JM, Soergel KH, Komorowsky RA, Wood CM (1989 Jan 5) Treatment of diversión colitis with short-chain-fatty acid irrigation. N Eng J Med 320 (1): 23-28.

Heald RJ, Husband EM, Ryal RD. The mesorectum in rectal cancer surgery-the clue to pelvic recurrence?. Br J surg 1982; 69: 613-616.

Heald RJ. “The holly plane” of rectal surgery. J. R. Soc med 1988; 81; 503-508.

Heald RJ, Santiago I, Pares O, Carvalho C, Figueiredo N. The Perfect Total Mesorectal Excision Obviates the Need for Anything Else in the Management of Most Rectal Cancers. Clin Colon Rectal Surg 2017; 30:324–332.

Hedrick TL, McEvoy MD, Mythen MMG, et al. American Society for Enhanced Recovery and Perioperative Quality Initiative Joint Consensus Statement on Postoperative Gastrointestinal Dysfunction Within an Enhanced Recovery Pathway for Elective Colorectal Surgery. Anesth Analg 2018; 126: 1896–907.7.

Hill C., Guarner F., Reid G., Gibson G.R., Merenstein D.J., Pot B., Morelli L., Canani R.B., Flint H.J., Salminen S., et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 2014; 11:506–514.

Hirahara N, Fujii Y, Yamamoto, et al. Validation of anovel prognostic scoring system using inflammatory response biomarkers in patients undergoing curative thoracoscopic esophagectomy for esophageal squamous cell carcinoma. OncoTargets and Therapy 2017:10.

Hod K, Sperber AD, Ron Y, Boaz M, Dickman R, Berliner S, Halpern Z, Maharshak N, Dekel R. A double-blind, placebo-controlled study to assess the effect of a probiotic mixture on symptoms and inflammatory markers in women with diarrhea-predominant IBS. Neurogastroenterol Motil. 2017 Jul; 29(7).

Huser N, Michalski CW, Erkan M, et al. Systematic review and meta-analysis of the role of defunctioning stoma in low rectal cancer surgery. Ann Surg 2008; 248: 52-60.

Jacobs M, Verdeja JG, Goldstein HS. Minimally invasive colon resection (laparoscopic colectomy). Surg laparosc endosc 1991; 1: 144-150.

Jafarnejad S, Saremi S, Jafarnejad F, Arab A. Effects of a multispecies probiotic mixture on glycemic control and inflammatory status in women with gestational diabetes: a randomized controlled clinical trial. J Nutr Metab. 2016; 2016:5190846.

Kabir SI, Kabir SA, Richards R, Ahmed J, MacFie J. Pathophysiology, clinical presentation and management of diversión colitis: a review of current literatura.International Journal of Surgery 12 (2014): 1088-1092.

Kang SI, Oh HK, Kim MJ, Son IT, Kim DW, Kang SB. The effect of probiotics (CJLP243) on bowel function restoration after ileostomy closure in patients with rectal cancer: A pilot randomised controlled trial. Colorectal dis 2016; 18 (Supplement 1): 127.

Keane C, Park J , Öberg S , Wedin A, Bock D , Grady G , et al. Functional outcomes from a randomized trial of early closure of temporary ileostomy after rectal excision for cancer. Br J Surg. 2019 Apr; 106 (5):645-652.

Keller DS, Berho M, Perez RO, Wexner SD, Chand M. The multidisciplinary management of rectal cancer. Nat Rev Gastroenterol Hepatol. 2020 Jul; 17(7):414-429. Doi: 10.1038/s41575-020-0275-y. Epub 2020 Mar 12. PMID: 32203400.

Kim KH, Yu CS, Yoon YS, Yoon SN, Lim SB, Kim JC. Effectiveness of biofeedback therapy in the treatment of anterior resection syndrome after rectal cancer surgery. Dis Colon Rectum. 2011; 54: 1007–13.

Kleinwort A, Döring P, Hackbarth C, Heidecke CD, Schulze T. Deviation of the Fecal Stream in Colonic Bowel Segments Results in Increased Numbers of Isolated Lymphoid Follicles in the Submucosal Compartment in a Novel Murine Model of Diversion Colitis. Biomed Res Int. 2017; 2017: 5265969. Doi: 10.1155/2017/5265969

Knox NC, Forbes JD, Van Domselaar G, et al. The gut microbiome as a target for ibd treatment: are we there yet? Curr Treat Options Gastroenterol. 2019; 17:115–126.

Komorowski RA. Histologic spectrum of diversion colitis. Am J Surg Pathol. 1990; 14:548 –54.

Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemona H, Dymicka-Piekarska V. Mean Platelet Volume (MPV): New Perspectives for an Old Marker in the Course and Prognosis of Inflammatory Conditions. Mediators Inflamm. 2019 Apr 17; 2019:9213074. Doi: 10.1155/2019/9213074. PMID: 31148950; PMCID: PMC6501263.

Kumar M, Hemalatha R, Nagpal R, Singh B, et al. Probiotic approaches for targeting inflammatory bowel disease: an update on advances and opportunities in managing the disease. Int J Probiotics Prebiotics. 2016; 11(3-4): 99–116.

Lazorthes F, Fages P, Chiotasso P, et al. Reseccion of the rectum with construction of a colonic reservoir and coloanal anastomósis for carcinoma of the rectum. Br J Surg 1986; 73: 136-138.

Leong AF. Selective total mesorectal excision for rectal cancer. Dis Colon and rectum 2000; 43: 1237-1240.

Li H, Zhang L, Chen L, Zhu Q, Wang W, Qiao J. Lactobacillus acidophilus alleviates the inflammatory response to enterotoxigenic Escherichia coli K88 via inhibition of the NF-kappaB and p38 mitogen-activated protein kinase signaling pathways in piglets. BMC Microbiol. 2016; 16: 273.

Liu Y, Alookaran JJ, Rhoads JM. Probiotics in Autoimmune and Inflammatory Disorders. Nutrients. 2018 Oct 18; 10(10):1537. Doi: 10.3390/nu10101537. PMID: 30340338; PMCID: PMC6213508.

Lloyd-Price J, Arze C, Ananthakrishnan AN, et al. Multi-omics of the gut microbial ecosystem in bowel diseases. Nature. 2019; 569:655–662.

Lopes RCSO, Balbino KP, Jorge MP, Ribeiro AQ, Martino HSD, Alfenas RCG. Modulation of intestinal microbiota, control of nitrogen products and inflammation by pre/probiotics in chronic kidney disease: a systematic review. Nutr Hosp. 2018 Apr 27; 35(3):722-730.

Louis, P.; Flint, H.J. Formation of propionate and butyrate by the human colonic microbiota. Environ. Microbiol. 2017, 19, 29–41.

Lucas C, Barnich N, Nguyen HTT. Microbiota, Inflammation and Colorectal Cancer. Int J Mol Sci. 2017 Jun 20; 18 (6):1310. Doi: 10.3390/ijms18061310. PMID: 28632155; PMCID: PMC5486131.

Luján J, Valero G, Hernández Q, et al. Randomized clinical trial comparing laparoscopic and open surgery in patients with rectal cáncer. Br J surg 2009; 96: 982-9.

Martinez CA, de Almeida MG, da Silva CM, Ribeiro ML, da Cunha FL, Rodrigues MR, Sato DT, Pereira JA. Enemas with N-acetylcysteine can reduce the level of oxidative damage in cells of the colonic mucosa diverted from the faecal stream. Dig Dis Sci. 2013 Dec; 58 (12): 3452-9.

Marusch F, Koch A, Schmidt U, et al. Value of a protective stoma in low anterior resections for rectal cancer. Dis Colon Rectum 2002; 45: 1164-1171.

Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 2017; 246: 207 -14.

Marteau P., Shanahan F. Basic aspects and pharmacology of probiotics: An overview of pharmacokinetics, mechanisms of action and side-effects. Best. Pract. Res. Clin. Gastroenterol. 2003; 17:725–740.

McFarland, L.V. From yaks to yogurt: The history, development, and current use of probiotics. Clin. Infect. Dis. 2015, 60, S85–S90.

Menéndez P, Garcia A, Lozano E, Pelaez R. Effectiveness of afferent loop stimulation prior to ileostomy closure. Cir Esp 2013; 91: 547-8.

Michail S, Kenche H. Gut microbiota is not modified by randomized, double-blind, placebo-controlled trial of VSL#3 in diarrheapredominant irritable bowel syndrome. Probiotics Antimicrob Proteins. 2011; 3: 1–7.

Michima Y, Sartor RB. Manipulating resident microbiota to enhance regulatory immune function to treat inflammatory bowel diseases. J Gastroenterol. 2020; 55(1): 4–14.

Montminy EM, Jang A, Conner M, Karlitz JJ. Screening for Colorectal Cancer. Med Clin North Am. 2020 Nov; 104 (6):1023-1036. doi: 10.1016/j.mcna.2020.08.004. PMID: 33099448.

Morgan DM, Cao Y, Miller K, McGoldrick J, Bellavance D, Chin SM, Halvorsen S, Maxner B, Richter JM, Sassi S, Burke KE, Yarze JC, Ludvigsson JF, Staller K, Chung DC, Khalili H, Microscopic Colitis is Characterized by Intestinal Dysbiosis. Clin Gastroenterol Hepatol. 2020 Apr; 18(4):984-986.

Morze J, Danielewicz A, Przybyłowicz K, Zeng H, Hoffmann G, Schwingshackl L. An updated systematic review and meta-analysis on adherence to mediterranean diet and risk of cancer. Eur J Nutr. 2021 Apr; 60(3):1561-1586. doi: 10.1007/s00394-020-02346-6. Epub 2020 Aug 8. PMID: 32770356; PMCID: PMC7987633.

Pacheco RG, Esposito CC, Müller LC, Castelo-Branco MT, Quintella LP, Chagas VL, de Souza HS, Schanaider A (2012) Use of butyrate or glutamine in enema solution reduces inflammation and fibrosis in experimental diversion colitis. World J Gastroenterol 2012:18(32):4278–4287.

Parc R, Tiret E, Frileux P, et al. Reseccion and coloanal anastomósis with colonic reservoir for rectal cancer. Br J Surg 1986; 73: 139-141.

Qiu XY, Li YH, Lin GL, Zhou JL, Xiao Y, Wu B, Qiu HZ. [Protective colostomy and protective ileostomy for the prevention of anastomotic leak in patients with rectal cancer after neoadjuvant chemoradiotherapy and radical surgery]. Zhonghua Wei Chang Wai Ke Za Zhi. 2021 Jun 25; 24 (6): 523-529. Chinese. doi: 10.3760/cma.j.cn.441530-20210304-00100. PMID: 34148317.

Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory

markers, and gut colonization in overweight adults: a randomized, controlled trial. Mediators Inflamm. 2014; 2014: 348959.

Redman MG, Ward EJ, Phillips RS. The efficacy and safety of probiotics in people with cancer: a systematic review. Ann Oncol. 2014; 25 (10): 1919-1929.

Rombey T, Panagiotopoulou IG, Hind D, Fearnhead NS, Preoperative bowel stimulation prior to ileostomy closure to restore bowel function more quickly and improve postoperative outcomes: a systematic review. Colorectal Dis. 2019; 21 (9): 994-1003.

Rossi M, Amaretti A. Bifidobacteria: genomics and molecular aspects. In: Mayo B, van Sinderen D, editors. Probiotic properties of bifidobacteria. UK: Horizon Scientific Press; 2010. pp. 97-123.

Rybakov EG, Shelygin YA, Khomyakov EA, Zarodniuk IV. Risk factors for postoperative ileus after colorectal cancer surgery. Colorectal Dis 2018; 20: 189–194.

Sanders ME, Merenstein DJ, Ouwehand AC, Reid G, Salminen S, Cabana MD, et al. Probiotic use in at-risk populations. J Am Pharm Assoc JAPhA. 2016 Dec; 56 (6): 680–6.

Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017; 152:327–339.

Schauber J, Bark T, Jaramillo E, Katouli M, Sandstedt B, Svenberg T. Local short-chain fatty acids supplementation without beneficial effect on inflammation in excluded rectum. Scand J Gastoenterol. 2000; 35: 184 –9.

Sharma A, Deeb AP, Rickles AS, Iannuzzi JC, Monson JRT, Fleming FJ. Closure of defunctioning loop ileostomy is associated with considerable morbidity. Colorectal Dis 2013; 15: 458 -62.

Son DN, Choi DJ, Woo SU, Kim J, Keom BR, Kim CH, Baek SJ, Kim SH. Relationship between diversion colitis and quality of life in rectal cancer. World J Gastroenterol. 2013; 19(4):542–549.

Stenman LK, Waget A, Garret C, Klopp P, Burcelin R, Lahtinen S. Potential probiotic Bifidobacterium animalis ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. Benef Microbes. 2014; 5:437-45.

Sugawara K, Kawaguchi Y, Nomura Y, Suka Y, Kawasaki K, Uemura Y et al. Perioperative factors predicting prolonged postoperative ileus after major abdominal surgery. J Gastrointest Surg 2018; 22: 508–515.

Szczepkowski M, Banasiewicz T, Kobus A. Diversion colitis 25 years later: the phenomenon of the disease. Int J Colorectal Dis (2017) 32:1191‑1196.

Tan WS, Tang CL, Shi L, et al. Meta-analysis of defunctioning stomas in low anterior resection for rectal cancer. Br J Surg 2019; 96: 462-472.

Tenorio-Jiménez C, Martínez-Ramírez MJ, Del Castillo-Codes I, Arraiza-Irigoyen C, Tercero-Lozano M, Camacho J, Chueca N, García F, Olza J, Plaza-Díaz J, Fontana L, Olivares M, Gil Á, Gómez-Llorente C. Lactobacillus reuteri V3401 Reduces Inflammatory Biomarkers and Modifies the Gastrointestinal Microbiome in Adults with Metabolic Syndrome: The PROSIR Study. Nutrients. 2019 Jul 31; 11 (8): 176.

Thanikachalam K, Khan G. Colorectal Cancer and Nutrition. Nutrients. 2019 Jan 14; 11(1): 164. doi: 10.3390/nu11010164. PMID: 30646512; PMCID: PMC6357054.

Tong T, Guan Y, Xiong H, Wang L, Pang J. A Meta-Analysis of Glasgow Prognostic Score and Modified Glasgow Prognostic Score as Biomarkers for Predicting Survival Outcome Carcinoma. Front Oncol. 2020; 10: 1541.

Vázquez-Melero A, Loizate Totoricaguena A, García Alonso P. ClinicalTrials.gov Identifier: NCT03424447. Stimulation of the Efferent Loop Before Loop Ileostomy Closure. Vol. 2018. U.S. National Library of Medicine, Bethesda (Maryland), 2018.

Warschkow R, Steffen T, Thierbach J, et al. Risk factors for anastomotic leakage after rectal cancer resection and reconstruction with colorestostomy. A prospective study with bootstrap analysis. Ann Surg Oncol 2011; 18: 2772-2782.

Wilkins T, Sequoia J. Probiotics for Gastrointestinal Conditions: A Summary of the Evidence. Am Fam Physician. 2017 Aug 1; 96 (3): 170-178. PMID: 28762696.

Williams L, Armstrong MJ, Finan P, Sagar P, Burke D. The effect of faecal diversion on human ileum. Gut 2007; 56: 796 -801.

Wolthuis AM, Bislenghi G, Fieuws S, de Buck van Overstraeten A, Boeckxstaens G, D'Hoore A. Incidence of prolonged postoperative ileus after colorectal surgery: a systematic review and meta-analysis. Colorectal Dis 2016; 18: O1–9.

Yang YJ, Chuang CC, Yang HB, Lu CC, Sheu BS. Lactobacillus acidophilus ameliorates H. pylori-induced gastric inflammation by inactivating the Smad7 and NFkappaB pathways. BMC Microbiology. 2012; 12: 38. 7.

Zhang Y, Li L, Guo C, et al. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. BMC Gastroenterol. 2016; 16 (1): 62.

Zhao-hua Shen, Chang-xin Zhu, Yong-sheng Quan, Zhen-yu Yang, Shuai Wu, Wei-wei Luo, Bei Tan, Xiao-yan Wang. Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. World J Gastroenterol 2018 January 7; 24(1): 5-14.

Zhao C, Ding C, Xie T, Zhang T, Dai X, Wei Y, Li Y, Gong J, Zhu W. Validation and optimization of the Systemic Inflammation-Based modified Glasgow Prognostic Score in predicting postoperative outcome of inflammatory bowel disease: preliminary data. Sci Rep. 2018 Jan 15;8(1):747.

Zielińska A, Włodarczyk M, Makaro A, Sałaga M, Fichna J. Management of pain in colorectal cancer patients. Crit Rev Oncol Hematol. 2021 Jan; 157: 103122. doi: 10.1016/j.critrevonc.2020.103122. Epub 2020 Oct 19. PMID: 33171427.

Anexos

ANEXO 1

Autorización del Comité de Ética de la Investigación de la provincial de Huelva

Servicio Andaluz de Salud
CONSEJERÍA DE IGUALDAD, SALUD Y POLÍTICAS SOCIALES

Dª Mª VICTORIA ALONSO MARTÍNEZ, Secretaria del Comité de Ética de la Investigación de la provincia de Huelva,

CERTIFICA

Que este Comité ha evaluado Favorablemente, a propuestas de la investigadora ***Dª. Ángela Rodríguez Padilla***, para que se realice el ***Proyecto de Investigación*** titulado:

Efectos sobre la colitis por diversión tras la estimulación del asa eferente con probióticos previo al cierre de ileostomía en pacientes intervenidos de carcinoma colorrectal.
*Código: **PI 010/17***

La capacidad de los investigadores y los medios disponibles en el ***Complejo Hospitalario Universitario de Huelva***, son apropiados para llevar a cabo el estudio.

Y que este Comité considera que dicho estudio cumple los requisitos éticos y legales para ser realizado en dicho centro por ***Dª. Ángela Rodríguez Padilla***.

Lo que firmo, en Huelva a **29 de marzo de 2017**

Firmado:

CONSEJERÍA DE SALUD

Dª. Mª Victoria Alonso Martínez

ANEXO 2

CONSENTIMIENTO INFORMADO

D./DÑA……..……,
(NOMBRE Y APELLIDOS DEL REPRESENTANTE LEGAL) CON D.N.I……., EN CALIDAD DE REPRESENTANTE LEGAL DE ……DECLARO:
(NOMBRE Y APELLIDOS DEL PACIENTE)

Que el Dr. ……..…., me ha solicitado verbalmente y por escrito mi participación para colaborar con el trabajo de investigación:

"Efectos sobre la colitis por diversión tras estimulación del asa eferente con probióticos previo al cierre de ileostomía de protección en pacientes intervenidos de carcinoma colorrectal"

El objetivo del estudio es estudiar la incidencia colitis por diversión tras cierre de ileostomía de protección en pacientes intervenidos de neoplasia de recto; este aspecto no ha sido evaluado a nivel nacional y es de enorme trascendencia socioeconómica y sobre la calidad de vida de los pacientes que son intervenidos.

Me han explicado que mi participación consiste en colaborar, tras ser instruido en la estimulación del asa eferente intestinal, durante el mes previo a la intervención quirúrgica, así como realización de colonoscopia con toma de biopsias previa y del control tras la reconstrucción del tránsito; facilitando el acceso a las pruebas complementarias recogidas en mí historial clínico. En algún caso se me podrá solicitar la confirmación de algún dato que faltara en dicho historial.

También se me ha explicado que mi participación es voluntaria y que puedo retirarme del estudio en el momento en que así lo desee, sin que esto repercuta en la atención médica que recibo; igualmente los datos suministrados serán manejados de manera

confidencial y se garantizará que no se hará difusión que permita identificar al paciente ni se harán públicos los resultados con información que permita llegar a la persona que dio la información.

He comprendido las explicaciones, que se me han facilitado en un lenguaje claro y sencillo; y el médico que me ha atendido me ha permitido realizar todas las observaciones y me ha aclarado todas las dudas que le he planteado.

También comprendo que, en cualquier momento y sin necesidad de dar ninguna explicación, puedo revocar el consentimiento que ahora presto.

Por ello, manifiesto que estoy satisfecho con la información recibida y que comprendo el alcance del estudio.

Y en tales condiciones CONSIENTO participar en el estudio.

Firma del paciente (o repr. Legal)	Firma del médico

En Huelva, a……..de……de 20……

REVOCACIÓN

Revoco el consentimiento prestado en fecha ……., y no deseo proseguir con mi participación en el estudio, que doy con ésta por finalizado.

_____ _____

Firma del paciente (o repr. Legal) Firma del médico

En Huelva, a……..de……de 20……

ANEXO 3

HOJA DE INFORMACIÓN A LOS PACIENTES

Si usted decide participar en este estudio, como paciente tiene derecho a ser informado acerca de los beneficios y riesgos derivados del cierre de la ileostomía de protección y de la reconstrucción del tránsito digestivo.

El propósito de esta información no es preocuparle, simplemente representa un esfuerzo para que usted conozca mejor los hechos y pueda tomar la decisión libre y voluntaria, de autorizar o rechazar dicho procedimiento.

Estudio:

Este estudio trata de comprobar la eficacia de la estimulación probiótica en la colitis por diversión.

¿Qué le vamos a hacer y por qué?:

Se le va a realizar un cierre de la ileostomía de protección mediante anastomosis (unión de ambos cabos intestino), por este procedimiento se restaura la continuidad del intestino, cerrando el ano artificial.

¿Cómo se realiza?

Durante los 15 días previos a la reconstrucción acudirá a Consultas Externas para la estimulación de uno de los cabos de su estoma. Posteriormente, en la intervención se practicará una incisión alrededor del estoma y, según los casos o dificultad de la técnica, una incisión abdominal; ello permite la unión de los dos extremos intestinales mediante una sutura. A veces es necesario la colocación de material protésico para reparar la pared del abdomen o el orificio donde estaba el estoma.

Cabe la posibilidad de que durante la cirugía haya que realizar modificaciones del procedimiento por los hallazgos intraoperatorios para proporcionar el tratamiento más adecuado. También es posible que, a pesar de realizar la intervención, no sea posible conseguir el cierre del estoma.

¿Qué beneficios y alternativas puede tener?

El cierre del estoma permitirá restablecer la continuidad del tubo digestivo. Podrá realizar la defecación normal, a través del ano. No existe otro método para realizar el cierre del ano artificial.

¿Qué riesgos existen?

Cualquier actuación médica tiene riesgos. La mayor parte de las veces estos riesgos no se materializan, y la intervención no produce daños o efectos secundarios indeseables. Pero a veces no es así. Por eso es importante que usted conozca los riesgos que pueden aparecer en este proceso o intervención.

Los más frecuentes:

- Infección o sangrado de la herida quirúrgica, flebitis, retención aguda de orina, diarrea, alteraciones de la continencia fecal, que habitualmente ceden tras un periodo de adaptación, retraso en la recuperación de la motilidad intestinal y dolor prolongado en la zona de la operación.

Los más graves:

- Infección intraabdominal, fístula intestinal por fallo de cicatrización de la sutura intestinal, hemorragia y obstrucción itestinal.
- Estas complicaciones habitualmente se resuelven con tratamiento médico pero pueden llegar a requerir una reintervención, generalmente de urgencia,

para su tratamiento, siendo en ocasiones necesario volver a realizar la ostomía. Existe un riesgo mínimo de mortalidad.

Los derivados de sus problemas de salud:

- Las patologías concurrentes en cada paciente (diabetes, obesidad, inmunodepresión, hipertensión, anemia, edad avanzada…) pueden aumentar la frecuencia o la gravedad de riesgos o complicaciones por lo que, en estos casos, el riesgo quirúrgico es mayor.

<u>También debe saber:</u>

- A veces, durante la intervención, se producen hallazgos imprevistos. Pueden obligar a tener que modificar la forma de hacer la intervención y utilizar varienates de la misma no contempladas inicialmente.

- A veces es necesario tomar muestras biológicas para estudiar mejor su caso. Pueden ser conservadas y utilizadas posteriormente para realizar investigaciones relacionadas con la enfermedad que usted padece. No se usarán directamente para fines comerciales. Si fueran a ser utilizadas para otros fines distintos se le pediría posteriormente el consentimiento expreso oara ello. Si no da su consentimiento para ser utilizadas en investigación, las muestras se destruirán una vez dejen de ser útiles para documentar su caso, según las normas del centro. En cualquier caso, se protegerá adecuadamente la confidencialidad en todo momento.

- También puede hacer falta tomar imágenes, como fotos o vídeos. Sirven para documentar mejor el caso. También pueden usarse para fines docentes de difusión del conocimiento científico. En cualquier caso serán usadas si usted da su autorización. Su identidad siempre será preservada de forma confidencialidad.

ANEXO 4

CUESTIONARIO ENDOSCOPIA DIGESTIVA BAJA

NHC _____ Endoscopista _____

Fecha Endoscopia _____

A cumplimentar por el endoscopista que analiza la biopsia:

Friabilidad mucosa

Presente ☐ Ausente ☐

Edema mucosa

Leve ☐ Moderado ☐ Severo ☐

Úlceras

Presente ☐ Ausente ☐

Eritema

Leve ☐ Moderado ☐ Severo ☐

Pólipos inflamatorios

Presente ☐ Ausente ☐

Estenosis

Leve ☐ Moderado ☐ Severo ☐

¿Colitis por Diversión? Si ☐ No ☐

A cumplimentar por el Investigador Principal o Investigador Asociado tras la realización de la colonoscopia:

Colonoscopia

Control ☐ Tras estimulación ☐

Puntuación final

	Leve	Moderado	Severo
Grado Colitis por Diversión	☐	☐	☐

ANEXO 5

CUESTIONARIO BIOPSIA ANATOMOPATOLÓGICA

NHC _____ Patólogo _____

Fecha Biopsia _____

A cumplimentar por el patólogo que analiza la biopsia:

Hiperplasia folicular linfoide
Presente ☐ Ausente ☐

Infiltración lámina propia linfocitos
Leve ☐ Moderado ☐ Severo ☐

Eosinófilos
Presente ☐ Ausente ☐

Infiltración lámina cél. plasmáticas
Leve ☐ Moderado ☐ Severo ☐

Abscesos criptas
Presente ☐ Ausente ☐

Distorsión arquitectura criptas
Leve ☐ Moderado ☐ Severo ☐

¿Colitis por Diversión? Si ☐ No ☐

A cumplimentar por el Investigador Principal o Investigador Asociado tras análisis anatomopatológico:

Biopsia
Control ☐ Tras estimulación ☐

Puntuación final

	Leve	Moderado	Severo
Grado Colitis por Diversión	☐	☐	☐

ANEXO 6

CUESTIONARIO PACIENTES TRAS ESTIMULACIÓN

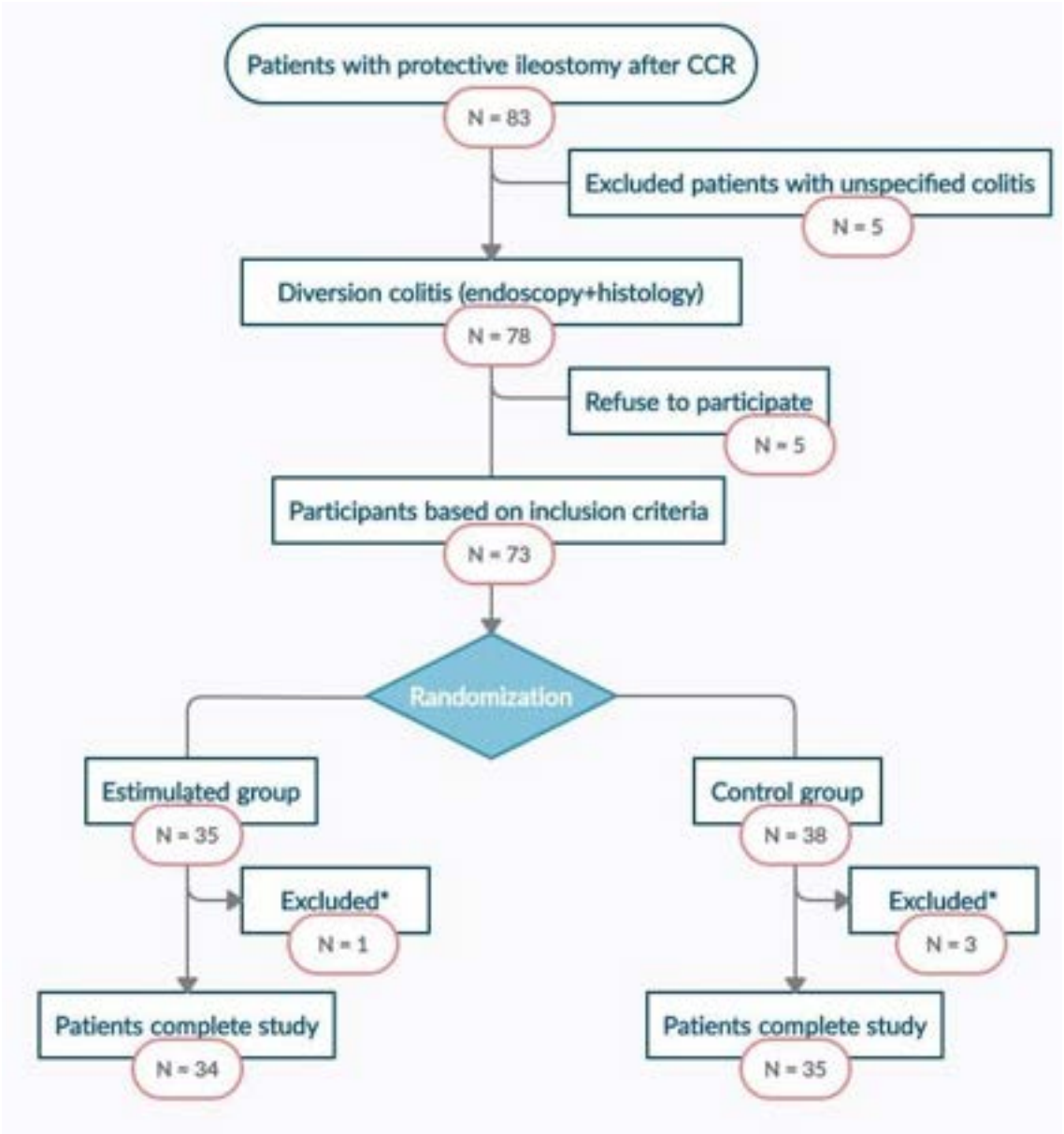
ID PACIENTE

Fecha INICIO / /20 . Fecha INICIO / /20 .

Día	Dolor abdominal	Emisión gas por el ano	Emisión heces por el ano	Nºde deposiciones	Control esfinteriano	Incidencias	Medicación
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

ANEXO 7

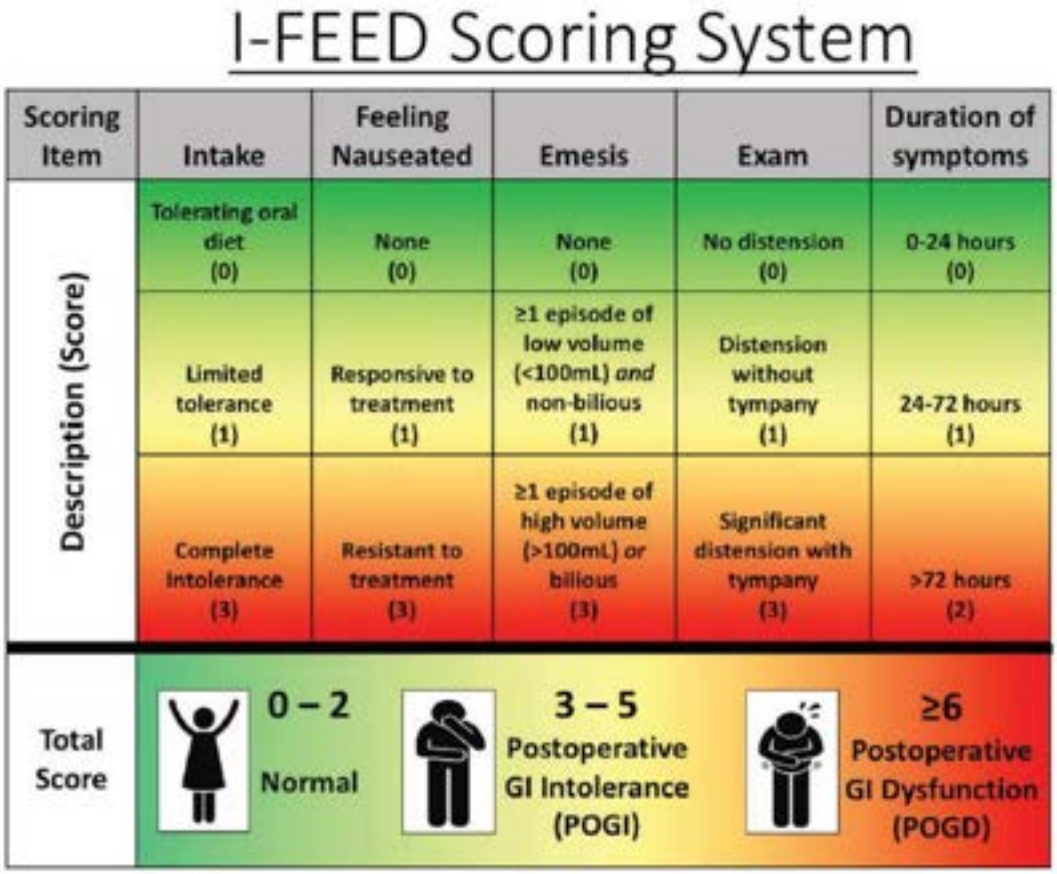
DIAGRAMA DE FLUJO DE SELECCIÓN DE PACIENTES



CCR: cáncer colorrectal.

ANEXO 8

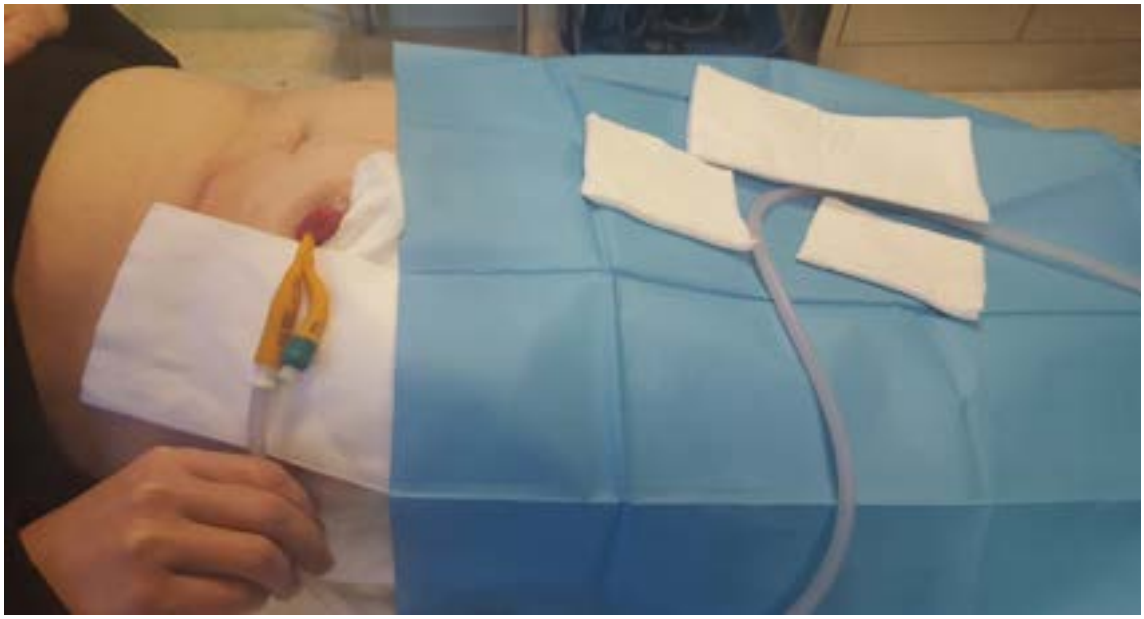
ESCALA I-FEED

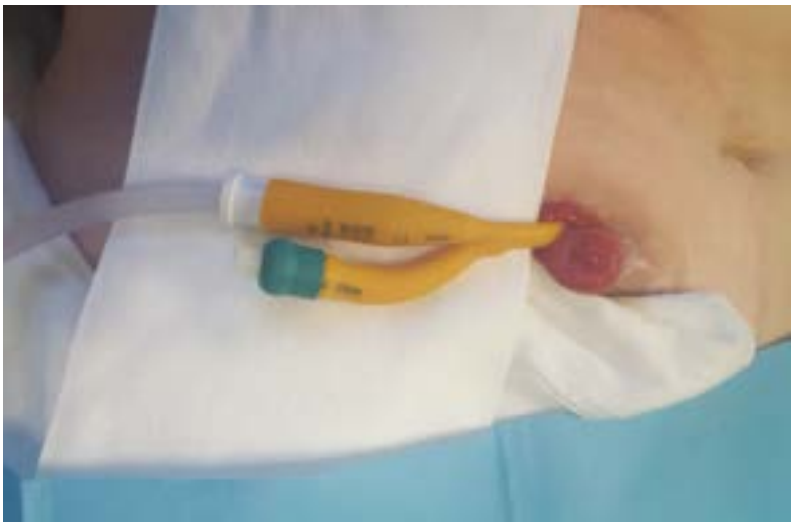


The I-FEED scoring system was created out of the need for a consistent objective definition of impaired postoperative GI function. The scoring system attributes 0–2 points for each of the 5 components based on the clinical presentation of the patient and categorizes patients into normal (0–2), postoperative GI intolerance (3–5), and postoperative GI dysfunction (≥6). GI indicates gastrointestinal; I-FEED, Intake, Feeling nauseated, Emesis, physical Exam, and Duration of symptoms; POGD, postoperative gastrointestinal dysfunction; POGI, postoperative gastrointestinal intolerance.

ANEXO 9

FOTOS PROCEDIMIENTO ESTIMULACIÓN Y MATERIALES





Estimulación preoperatoria del asa eferente con probióticos. Se introdujo a través del cabo desfuncionalizado una sonda estéril de Foley de 14 Ch conectado a un sistema de suero para permitir la infusión lenta, durante 20-30 minutos, de una solución con 4.5mg de probióticos diluidos en 250ml de suero fisiológico 0.9%.

ANEXO 10
INDICIOS DE CALIDAD

Artículo 1:

Autores/as: Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez-Salgado J, Balongo-García R, Ruiz-Frutos C.

Título: Postoperative Ileus after Stimulation with Probiotics before Ileostomy Closure

Publicado en: Nutrients. 2021 Feb 15;13(2):626. doi: 10.3390/nu13020626

Año de publicación: 2021

Indicios de calidad: Journal Citation Report (2020):

- Impact Factor: 5.717

- Nutrition and dietetics-SCIE: 17/89 (Q1).

- SJR 1.42.

Artículo 2:

Autores/as: Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Rada-Morgades R, Gómez-Salgado J, Ruiz-Frutos C.

Título: Diversion Colitis and Probiotic Stimulation: Effects of Bowel Stimulation Prior to Ileostomy Closure.

Publicado en: Frontiers in Medicine 2021 Jun 8:654573. doi: 10.3389/fmed.2021.654573

Año de publicación: 2021

Indicios de calidad: Journal Citation Report (2020):

- Impact Factor: 5.091

- JCI: Medicine, General & Internal: 44/313 (Q1).

- SJR 1.39.

Artículo 3:

Autores/as: Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez-Salgado J, Rada-Morgades R, Ruiz-Frutos C.

Título: Macro and Microscopic Findings after Probiotics Stimulation

Publicado en: Biology (Basel). 2021 Apr 6;10(4):303. doi: 10.3390/biology10040303.

Año de publicación: 2021

Indicios de calidad: Journal Citation Report (2021):

- Impact Factor: 5.079

- Biology -SCIE: 16/93 (Q1).

- SJR 1.73

Artículo 4:

Autores/as: Rodríguez-Padilla Á, Morales-Martín G, Pérez-Quintero R, Gómez-Salgado J, Ruiz-Frutos C.

Título: Serological Biomarkers and Diversion Colitis: Changes after Stimulation with Probiotics

Publicado en: Biomolecules. 2021 May 2;11(5):684. doi: 10.3390/biom11050684

Año de publicación: 2021

Indicios de calidad: Journal Citation Report (2021):

- Impact Factor: 4.879

- Biochemistry & Molecular Biology -SCIE: 96/298 (Q2).

- SJR 1.13.