NSAID Administration Post Colorectal Surgery Increases Anastomotic Leak Rate – Systematic Review/Meta-Analysis

by © Aryan Modasi MD, BHSc (Thesis) submitted

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Abstract

Background

Current enhanced recovery guidelines suggest that opioid sparing medications should be used for analgesia whenever possible following colorectal surgery. The present study aims to assess whether postoperative NSAID use is associated with an increased anastomotic leak rate.

Methods

A systematic review was performed for studies investigating anastomotic leak rate following NSAID use versus control after colonic or rectal anastomosis. Meta-analysis was performed to assess for overall risk of anastomotic leak with NSAID use, as well as sub-group analysis to compare selective vs non-selective NSAIDs and drug-specific NSAID safety profiles.

Results

Seven studies were included in the final review. Use of an NSAID post-operatively was associated with an overall increased risk of anastomotic leakage [OR 1.58 (1.23, 2.03), $P = 0.0003$]. Non-selective NSAIDs were associated with an increased risk [OR 1.79 (1.47, 2.18), $P < 0.00001$], but selective NSAIDs were not. The non-selective NSAID diclofenac was associated with an increased leak rate [OR 2.79 (1.96, 3.96), $P < 0.00001$], but ketorolac was not [OR 1.36 (0.89, 2.06), $P = 0.16$].

Conclusions

Great caution must be taken when prescribing NSAIDs following colonic or rectal anastomotic creation. The risk and safety profile varies within the NSAID class and further research is needed to clarify which NSAIDs are safe for use and which are not.

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List of Abbreviations

ASA……. American Society of Anesthesiologists (Physical Status Classification System)

COX…….…… Cyclooxygenase (enzyme)

ERAS…….…… Early Recovery After Surgery

M-H…….…… Mantel-Haenszel Test

NSAID…….…..... Non-Steroidal Anti-Inflammatory Drug

OR…….……Odds Ratio

PRISMA…….. Preferred Reporting Items for Systematic Reviews and Meta-Analyses

REV-MAN…….. Review Manager (software)

ROBINS-I…… Risk of Bias in Non-Randomized Studies (risk of bias assessment tool)

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Introduction

It can be argued that no domain within general surgery has undergone as much scrutiny and refinement within the last decade as the post-operative management of colorectal patients[18]. With increasing acceptance and implementation of Early Recovery after Surgery (ERAS) protocols, much focus has been placed on decreasing the length of hospital stay post-operatively without compromising patient safety. Given the myriad of complications associated with opioid and narcotic use, a multi-modality approach using non-steroidal anti-inflammatories (NSAIDs) for pain control has increasingly become an integral component of patient care in many centers.

NSAIDs, which act through inhibition of the cyclooxygenase (COX) pathways, have been shown to significantly reduce the amount of opioids required post-operatively, while simultaneously decreasing pain, shortening duration of ileus and length of hospital stay, and improving patient satisfaction[47]. However, NSAIDS may also have an impact on wound healing, which in animal studies has been shown to have an impact on anastomotic leak rates[1]. Anastomotic leaks, a significant and potentially life-threatening complication, lead to peritonitis/sepsis, reoperation, and increased mortality. The implications of a clinically significant association between NSAIDs and post-operative anastomotic leaks would be far-reaching, requiring significant reassessment of our current post-operative management regime.

This study aims to determine if NSAID administration given post-operatively following colorectal anastomosis increases the incidence of anastomotic leakage. A positive association between NSAID use and anastomotic leak rates would indicate that their routine use post-operatively is not safe and alternatives will need to be found. Furthermore, if a positive

association is found we aim to explore this further to determine if this is a class-specific, or even drug-specific effect within the broader NSAID class, or if it's generalizable to all NSAIDs as a whole. On the contrary, if it can be proven definitively that there is no association between NSAIDs and anastomotic leakage, then more definitive recommendations can be made with respect to the use of NSAIDs (such as by the ERAS society), and many institutes/surgeons who have been reluctant to implement an opioid sparing approach can confidently and safely alter their practice habits.

Co-authorship Statement

Completion of this thesis would not be possible without the great support of my supervisors/co-authors. Contributions were made in the following domains:

Design and Identification of Research Protocol:

Dr. Chris Smith – Proposal of the initial research question (*Are NSAIDs associated with an increased anastomotic leak rate?)*

Dr. Aryan Modasi - Design of the initial research protocol/methodology, review of prior literature within the proposed realm of our study/creation of formal literature review

Drs. Bryan Curtis, David Pace, Marshall Godwin – Review of proposed research protocol/methodology, with suggestions for improvement

Practical Aspects of Research:

Dr. Aryan Modasi – Determination of eligibility criteria, database review, study selection and data collection (with independent verification from a second physician (Dr. Sara Afraz) and Dr. David Pace in the event of disagreement). Risk of bias assessments for included studies

Data Analysis:

Dr. Aryan Modasi – Determination of summary measures, synthesis of results, creation of forest plots and interpretation of data

Dr. Marshall Godwin – Review of final results and guidance with regards to summary measures used and interpretation of data

Manuscript Preparation:

Dr. Aryan Modasi – Write up of manuscript. Review and amendment of manuscript based on feedback from co-authors.

Drs. Bryan Curtis, Marshall Godwin, David Pace, Chris Smith – Review of manuscript with feedback to improve the formatting, presentation, grammar, figures (Dr. Marshall Godwin in particular), and final interpretation of data

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Chapter 1 – Background

1.1 – Colorectal Anastomosis

A colorectal anastomosis is the restoration of intestinal continuity following the removal of a portion of the colon or rectum. Following resection of a portion of the colon, whether for malignant or benign causes, two formerly distant portions of the intestine are attached together to form an anastomosis.

Anatomically the intestinal tract can be categorized into two distinct segments, the large intestine (colon) and small intestine. The small intestine is the most proximal segment of the two, and as the name implies, has the smaller diameter of the two. It functions to absorb the products of digestion (carbohydrates, proteins, lipids, and vitamins) into the bloodstream. The large intestine, also called the colon, lies distal to the small intestine and its main function is to absorb water. Beyond the colon at the very distal end of the gastrointestinal tract lies the rectum and anus. The rectum functions act as a temporary storage site for feces.

Whenever a portion of the colon or rectum is removed, and intestinal continuity is restored by reattaching two distant portions of the intestinal tract, a colonic or rectal anastomosis is formed. For example, when the proximal half of the colon is resected for a cecal cancer (the cecum is the most proximal portion of the large intestine), an anastomosis is made between the distal small bowel and the transverse colon (mid-colon). This type of anastomosis is called an ileocolic anastomosis. The method by which these two segments of intestine are reattached varies based on the surgeon's preference. Most surgeons will use a stapling device to create the anastomosis, however performing a hand-sewn anastomosis, where the two segments of bowel

are sutured together, is also accepted. Current research indicates that both techniques give equal results with regards to post-operative leak rates[13].

1.2 – Anastomotic Leakage

The post-operative anastomotic leak is one of the most feared complications following any colorectal anastomotic procedure. As the name entails, it arises when there is leakage of intestinal contents from the connection where the two segments of bowel are attached. Anastomotic leaks are associated not only with significant morbidity (increased length of stay in hospital, need for re-operation, need for stoma following reoperation, etc.), but a significantly increased mortality rate (from 2.5% up to 15.8%)[14]. Furthermore, research now suggests that following surgery for rectal and colon cancer, anastomotic leakage is associated with an increased risk for local cancer recurrence[15].

The overall incidence of anastomotic leakage is generally recorded between 2 to 7%, with the lowest incidence found in patients undergoing an ileocolic anastomosis (1 – 3%), and the highest incidence found following colo-anal anastomosis (10 – 20%)[16]. Most leaks become clinically apparent (pain, fever, tachycardia, peritonitis, etc.) between post-operative days 5 and 7, however up to 12% can occur after post-op day 30 with subtle symptoms (low-grade fever, prolonged ileus, etc.)[17].

Well documented risk factors for anastomotic leakage include poor pre-operative patient health (quantified using the American Society of Anesthesia score – Appendix 1), emergency surgery[18], prolonged operative time (likely secondary to more difficult dissection and

anastomosis)[18], obesity[19], and anastomotic ischemia (measured using laser Doppler flowmetry)[20]. One area of controversy within the general surgical community is the risk of leakage associated with post-operative NSAID usage. This study aims to address this controversial topic and provide some clarity into the safety of non-steroidal anti-inflammatory drugs following colorectal surgery.

1.3 – Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of medication used worldwide for their analgesic (pain-relieving), antipyretic (fever-reducing), and anti-inflammatory effects. Within the NSAID class there are more than 20 different drugs. The primary mechanism of action of these medications is the prevention of prostaglandin synthesis. Prostaglandins are a group of hormone-like compounds produced in the body. There are various types of prostaglandins producing an array of effects on the human body, but central to their role in the inflammatory process is their ability to function as powerful vasodilators. Prostaglandins are produced following the oxidation of arachidonic acid by the cyclooxygenase (COX) enzymes.

NSAIDs work by inhibiting one or both of the two important isoforms of the COX enzyme: COX-1 and COX-2. COX-1 is expressed in most tissues and is responsible for baseline levels of most prostaglandins, while COX-2 is generally undetectable in most tissues and its expression is increased during states of inflammation. Reduction in COX enzyme activity leads to reduced prostaglandin production, which in-turn leads to impaired tissue inflammatory activity. This anti-inflammatory effect has been shown to impair collagen cross-linkage in

healing intestinal tissue, which has been shown to decrease the tissue's mechanical strength[25]. As a result, there has been increasing concern regarding the safety of NSAIDs during colonic and rectal anastomotic healing. Similar questions have come up in other surgical fields as well, such as within the orthopedic surgery community, where it has been demonstrated that NSAIDs impair bone and fracture healing[49].

NSAIDs can be further sub-classified depending on which COX enzyme is affected. Non-selective NSAIDs are those that target both the COX-1 and COX-2 enzymes. Due to the widespread nature of COX-1, and its multitude of effects, non-selective NSAIDs can lead to a variety of side-effects. Chief amongst these is the risk of gastric ulceration secondary to the reduction in gastric protection that is normally provided by prostaglandins. As a result, selective NSAIDs were created to target just the COX-2 enzyme. These medications produce analgesic and anti-inflammatory effects similar to non-selective NSAIDs, but with fewer gastric and duodenal ulcers. Despite the dichotomous classification, there are many different drugs within the the NSAID class with differing affinities for the COX-1 and COX-2 enzymes. Certain non-selective NSAIDs, such as diclofenac, have an inhibitory profile that favors the COX-2 enzyme to such an extent that they in-effect act very similar to selective NSAIDs[39]. Furthermore, different medications within the NSAID class have differing analgesic properties irrespective of their sub-classification. Non-selective NSAIDs such as ibuprofen, ketorolac, and diclofenac have been shown to be superior to other non-selective NSAIDs, such as paracetomol, when it comes to pain control[50]. While these drugs are all grouped under one class, they vary significantly in their risk and efficacy profiles.

1.4 – Early Recovery After Surgery (ERAS)

To understand the importance of NSAID safety following colorectal surgery, one must understand the importance and clinical impact the ERAS movement has had on current surgical practice. The underlying principle of ERAS (Early Recovery After Surgery) is to provide evidence-based protocols to reduce hospital length of stay, and expedite return to baseline health and functional status. The ERAS guidelines provide strategies for optimal preoperative, intraoperative, and postoperative strategies (See Appendix 2)[21].

Data from hospitals that have implemented the ERAS protocol have shown reduced hospital length of stay, earlier return of bowel function, and earlier ambulation[22]. Taken all together, this implies that patients return to their baseline health quicker and returning to work in a shorter period of time, hospitals are discharging patients sooner and saving significant amounts of money, and most important of all, patients are experiencing significantly fewer healthcare-associated lung, urinary tract, and surgical site infections[23].

Looking at the current ERAS guidelines for colorectal surgery (Appendix 2), one can see the recommendation for opioid-sparing analgesia in the postoperative period. Opioids are known to cause sedation, postoperative nausea and vomiting, urinary retention, ileus, and respiratory depression[24], all of which can delay discharge. The primary pharmacologic alternative to opioids are NSAIDs (nonsteroidal anti-inflammatory drugs). However, much debate has lingered in the surgical community regarding the safety of NSAIDs following colorectal surgery, and whether or not there is an increased risk of anastomotic leakage associated with their usage (See

Literature Review). With this systematic review we aim to find an answer to the question – "Are NSAIDs associated with an increased risk of anastomotic leakage following colorectal surgery?".

Chapter 2 – Literature Review

2.1 - Animal Studies

Introduction

Any clinical question must be founded on an underlying pathophysiological principle. Assessment of tissue and anastomotic healing in human patients can only measure the clinical end point of anastomotic leakage. In-vivo assessment of tissue healing on a cellular level cannot be done with human patients in the post-operative period. In contrast, assessment of tissue and anastomotic healing in animal studies allows for experimental analyses that look at the underlying cellular processes that occur during the healing process and how they are affected by NSAIDs. When quantifying anastomotic integrity/strength experimentally, two measures that are commonly used are anastomotic breaking strength and anastomotic bursting pressure.

Anastomotic breaking (tensile) strength is measured using an instrument called a Lloyd's tensiometer. The two ends of the anastomosis are placed in two separate clamps which are pulled apart at a constant speed. The machine produces a load-strain curve which is quantified into a score produced by the accompanying software. The anastomotic bursting pressure is measured by inserting an oxygen insufflator into the bowel lumen and attaching it to a manometer. The intraluminal pressure is increased by gas insufflation in increments of 10 mmHg, and held at each pressure for 10 seconds. The bursting pressure is measured as the pressure at which gas leakage occurred. Many experimental animal studies use one or both of these measures.

Literature Review

Cahill RA et al., 2004[25]

Randomized trial on 40 adult Sprague–Dawley rats who underwent laparotomy, descending colonic transection and hand-sewn re-anastomosis. The rats were randomized to receive either a selective COX-2 inhibitor (rofecoxib, 10 mg/kg) or an equal volume of water via an orogastric tube (tube placed down the throat and into the stomach) before operation and then daily after surgery. Animals were euthanized after 3 or 7 days, and their anastomosis was assessed using bursting pressure and tensile strength measurement. Haematoxylin and eosin-stained intestinal sections were also examined and scored by a blinded independent observer.

Upon final analysis, the researchers found a correlation between NSAID use and anastomotic leakage. Five animals in the group receiving rofecoxib developed colonic anastomotic complications, compared with none in the control group ($P = 0.048$). Two had developed colonic perianastomotic abscesses by day 3, and three had frank colonic anastomotic dehiscence by day 7. When comparing the two groups, the researchers found that the rats that had received rofecoxib had markedly lower bursting pressures at both post-op day 3 and 7 (both $P = 0.02$), and similarly the tensile strength was weaker in this group at both time points ($P = 0.04$ at day 3). Histologically however, there were no significant differences in the cellular (inflammatory cell or fibroblast influx) or structural (blood vessel formation or collagen deposition) composition around the anastomoses between the groups.

The conclusion reached by the team was that COX-2 inhibitors may in-fact significantly decrease the wound strength of post-operative colonic anastomosis. The differences in

mechanical wound strength, despite the presence of similar degrees of fibroblast influx and collagen formation, suggested, according to the authors, that there was an impairment in collagen cross-linking in the rats receiving COX-2 inhibitors.

De Hingh IH et al., 2006[26]

Randomized trial whereby male Wistar rats were operated on and received both an ileal and a colonic anastomosis, at 15 cm proximal to the cecum and 3 cm proximal to the peritoneal reflection. The rats were randomized to receive either celecoxib (a selective COX-2 inhibitor), in doses of 15, 50 or 200 mg/kg/day, or normal saline (placebo) daily starting the day before the operation. The rats were then euthanized on post-op day 3 and anastomotic strength was assessed by measuring the bursting pressure and breaking strength. The team then went on to perform histologic and biochemical analysis on the intestinal tissue.

Administration of celecoxib, at all doses tested, resulted in a significantly higher ileal leak rate than in control rats ($P = 0.002$), but there was no difference in the colonic anastomosis leak rates. Ileal bursting pressures were significantly lower in those treated with celecoxib 200 mg/kg/day than in the control group ($P = 0.004$), however the colonic bursting pressures were not affected by celecoxib. There were no differences in breaking strength in either group for ileal nor colonic anastomoses. Finally, and very interestingly, on histologic intestinal analysis of rats that had not undergone surgery, COX-2 was undetectable, while in those who had just undergone a resection and anastomosis COX-2 was widely expressed in both the ileum and colon.

What the team concluded was that the COX-2 inhibitor impaired the healing of the ileal anastomosis, but not the colonic anastomosis. These findings (regarding the effect of COX-2 inhibitors on colonic anastomoses) are contradictory to those found by Cahill et al. in 2004. The team led by de Hingh postulated that this may be secondary to pharmacokinetic difference between the different COX-2 inhibitors used in the two studies, however this hypothesis has yet to be proven. Never-the-less, the recommendation was made to exercise caution as COX-2 inhibitors may impair anastomotic healing, in particular with ileal anastomoses.

Klein M et al., 2011[27]

Randomized trial on 32 Wistar rats who underwent a descending colon resection with primary colonic anastomosis. The rats were randomized to receive either diclofenac (a non-selective NSAID) at 4mg/kg/24 hours or saline intramuscular injection twice daily. On post-operative day 3 the rats were euthanized and the breaking strength and COX-2 levels of the anastomoses were measured.

The researchers found a significantly reduced level of COX-2 enzyme in the tissues of the rats who received diclofenac, however there were no differences between the two groups in anastomotic breaking strength. Furthermore, there was no correlation between COX-2 levels and breaking strength. The conclusion of the research team was that while diclofenac decreases the COX-2 enzyme levels in the intestinal tissue, this does not pose an increased risk of anastomotic leakage, and diclofenac appears to be safe in the early healing of colonic anastomoses.

Klein M et al., 2012[28]

Randomized trial on 60 Wistar rats who underwent a descending colectomy with colonic anastomosis. A separate expanded polytetrafluoroethylene (ePTFE) tube was placed subcutaneously into the rats back as well. The tube was to be taken out and analyzed for hydroxyproline (an essential component of tissue healing) content on post-op day 7. The rats were randomized to receive diclofenac 4mg/kg/day or saline (placebo) post-operatively. The rats were then euthanized on post-op day 7 and both the ePTFE tube was removed as well as the colonic anastomosis.

On final analysis, no difference in anastomotic breaking strength was seen between the two groups. There was however a 38% decreased collagen deposition level (as estimated by the hydroxyproline level) in the ePTFE tubes of the diclofenac group rats ($P = 0.03$). Collagen is a critical component of the tissue healing cascade, however since the anastomotic breaking strength was equal in both groups, this decrease was not clinically relevant. Therefore, the research team concluded that while diclofenac may cause a histologically significant decrease in collage deposition, there is no clinically significant increased risk of anastomotic breakdown associated with this.

Van der Vijver RJ et al., 2013[29]

Two experiments performed on 40 Wistar rats. In the first experiment, 20 rats were given a colonic anastomosis 3 cm from the peritoneal reflection (descending colon) as well as a second anastomosis in the distal ileum 15cm proximal to the cecum. The rats were then randomized into two groups. Group 1 received buprenorphine (an opioid) for post-op analgesia, while group 2

received carprofen (a COX-2 inhibitor). For the second experiment, 20 rats were randomized to receive either an ileal anastomosis only or a colonic anastomosis only. All the rats in experiment 2 then went on to receive carprofen for post-op analgesia.

The rats were euthanized on post-operative day 3 and the anastomoses were examined. In experiment one, carprofen was associated with a significantly elevated ileal anastomotic leakage rate ($P = 0.01$), but no change in the colonic leakage rate. In the second experiment, where the rats only had 1 anastomosis, the use of carprofen led to an ileal leakage rate of 80%, whereas all the colonic anastomoses remained intact ($P = .0007$).

In assessing the mean bursting pressure, the ileal anastomoses had a significantly lower bursting pressure in the carprofen group in experiment 1 than in the control group ($P = .0005$), but there was no significant difference in the breaking strength. The bursting pressure did not change for the colonic anastomosis. Finally, the collagen activity level was assessed on all the specimens (by measuring the hydroxyproline levels) and no differences were found at the ileal or colonic anastomoses across groups.

The conclusion derived from this study is that the COX-2 inhibitor carprofen impairs anastomotic healing in the ileum, but not the colon. These results were similar to those found by de Hingh et al, in 2006[26]. The authors cite a 2005 study which describes COX-2 expression as being highest in the ileum[30], and hypothesize that the elevated expression in the ileum may explain why COX-2 inhibition affects ileal anastomoses to a far greater extent than colonic anastomoses. The findings from this study suggest caution when using NSAIDs, particularly COX-2 inhibitors, following anastomotic creation.

Yauw S et al., 2014[31]

Study performed on rats aimed at assessing if diclofenac affects anastomoses differently depending on the location in the intestine. Ninety-five Wistar rats were randomized to one of 6 groups. The groups differed based on the location of the anastomosis the rats were given (ileum vs proximal colon vs distal colon), and the timing of diclofenac administration (no diclofenac vs diclofenac starting post-op day 0 vs post-op day 1 vs post-op day 2). The rats were euthanized 3 days after starting diclofenac (or post-op day 3 for the group that didn't receive diclofenac) and the leak rate and anastomotic strength was assessed.

The investigators found that the leak rate did not differ between the ileum and proximal colon, however both had significantly higher leak rates than the distal colon ($P = 0.001$). Delaying the administration of diclofenac to post-operative day 1 or 2 resulted in a gradual reduction in the leak rates. In assessing the bursting pressure and breaking strength, the only significant change that was found was a significantly lower bursting pressure in the ileum compared to the distal colon when diclofenac was administered.

The findings in this study correlate very interestingly with previous rat model findings. Diclofenac caused proximal colon leakage rates similar to that observed with ileal anastomoses, but had no effect on the distal colon. These results show that in the large bowel, diclofenac only affects the healing of the proximal colon anastomosis. Furthermore, the incidence of leakage in the proximal colon was significantly lower when administration of diclofenac was delayed one to two days. These findings suggest an early detrimental effect from diclofenac on ileal and

proximal colon anastomoses, and that caution should be taken when prescribing diclofenac as an early postoperative painkiller following gastrointestinal surgery.

Drakopoulou S et al., 2016[32]

Study designed to assess the affect of lornoxicam (non-selective NSAID) on proximal colonic anastomoses. Twenty-eight Wistar rats were randomly assigned to two groups. All rats underwent ascending colonic transection followed by a hand-sewn anastomosis. Group 1 received intraperitoneal lornoxicam before and daily after surgery. Group 2 received an equal intraperitoneal volume of placebo. Half of the rats in each group were euthanized on post-op day 3 and the remaining on post-op day 7. Macro and microscopic indicators of anastomotic healing were then assessed.

The rats in the lornoxicam group did have higher rates of anastomotic leakage, but this did not reach statistical significance. On histologic exam, the colonic anastomosis in the lornoxicam group was found to have significantly lower fibroblast infiltration levels by post-operative day 3, and significantly less granulation tissue by post-operative day 7. Fibroblasts are critical in wound healing and lay the structural framework for new tissue growth. Similarly, granulation tissue is the newly formed tissue bed upon which connective tissue and blood vessels form to regenerate tissue. Both these factors are critical to wound healing, and anastamotic tissue repair in particular. In keeping with all this, the rate of histologic necrosis was found to be higher in the lornoxicam group, an indicator of microvascular ischemia and delayed tissue healing.

The authors conclude that while lornoxicam was not found to significantly increase anastomotic dehiscence in the rat model, histological factors associated with the healing process of a newly reconstructed colonic anastomosis were shown to be impaired. Taken all together, this indicates that there may in-fact be a true detrimental effect attributable to NSAIDs that would be borne out in a larger study. Caution is recommended with the use of NSAIDs following colonic anastomosis.

Summary

While there is conflicting animal data regarding the potential association with NSAID use and anastomotic leakage, there appear to be a general trend seen in the literature. There appears to be a location dependent effect of NSAIDs on anastomotic healing. Three of the studies found an association between NSAID use and ileal anastomotic leakage, but when descending colon anastomoses were examined that association was no longer present[26, 29, 31]. Given the increased expression of COX-2 in the small bowel compared to the distal colon[30], it's hypothesized that COX-2 inhibition may decrease tissue healing with the ileum being affected to a greater extent than the distal colon[29]. This location dependent effect is not simply a matter of small bowel vs large bowel anastomosis however. As explained in the study by van der Vijver et al. in 2013[29], proximal colonic anastomoses and ileal anastamoses seem to be affected to a similar degree by NSAIDs, yet distal colonic anastomoses show no detrimental effects[29]. Furthermore, looking at the two studies which found no increased risk of leakage following NSAID administration, both examined distal colonic anastomoses exclusively[27, 28]. Their negative findings fall in line with the location dependent findings of other studies, and cannot be generalized to state that there is no association between NSAID use and anastomotic leakage elsewhere in the gastrointestinal tract.

The pathophysiological mechanism by which NSAIDs may impair anastomotic healing is still up for debate. While studies have shown COX-2 expression to be greatest in the ileum[30], and it has been shown that COX-2 expression is decreased following NSAID use, this has not been found to correlate with a lower anastomotic mechanical strength[27]. Furthermore, the effect that NSAIDs have on collagen deposition and cross-linking remains unclear. In 2005 Cahill et al.[25] performed a study where they found no decrease in collagen deposition in intestinal tissue following NSAID use, despite a decrease in their mechanical strength. They hypothesized that what occurs is an impairment in cross-linkage rather than a change in the amount of collagen present. Similarly, in 2013 van der Vijver et al.[29] found no change in collagen present in ileal or colonic tissues following NSAID usage, despite an increased ileal leak rate. In contrast, in 2012 Klein et al. [28] found a statistically significant reduction in collagen present in colonic tissue following NSAID administration, but no change in the breaking strength of the tissues.

What can be taken away is that there does seem to be a true, location dependent effect that NSAIDs have on the gastrointestinal tract, and histological factors associated with the healing process certainly seem to be affected. NSAIDs appears to decrease fibroblast infiltration, decrease granulation tissue formation, decrease re-epithelialization, and increase microscopic necrosis[32]. Taken all together this can certainly lead to impaired wound healing and tissue regeneration. Finally, while the results and findings across studies are not always consistent, one must remember that a variety of NSAIDs are used in these studies, each with their own unique pharmacodynamic profile. Not only is there a distinction between selective and non-selective

NSAIDs, but within these classes each drug has a different affinity for the COX enzymes, resulting in differing physiologic responses.

2.2 – NSAIDs Post Colorectal Anastomosis (Previous Meta-Analyses)

Introduction

Animal studies suggest that NSAIDs may impair anastomotic healing, but the question remains: Does this risk translate to real-life, clinical practice? With the current push towards non-opioid analgesia in the post-operative period, concrete clinical evidence is needed to convince clinicians that the most effective tool in the non-opioid armamentarium, NSAIDs, are not safe for routine use.

The literature regarding NSAID safety following colorectal surgery was, for the most part, quite sparse prior to the turn of the century. As more attention was placed on decreasing patient length of stay and limiting narcotic use, NSAID use has increased and many have questioned if it is safe to do so. In the last 15 years many attempts have been made to tackle this question. Some have found an increased risk of leakage with NSAID use, some have not. As the number of studies has increased, researchers have begun to combine these results, making an effort to increase the statistical power, improve the size effect estimates, and ultimately resolve the uncertainty that remains. That is of course the goal of any systematic review and meta-analysis.

Literature Review

Rushfeldt CF et al., 2011[33]

Systematic review from 2011 involving three observational retrospective cohort studies (887 patients). Two of the studies assessed post-operative diclofenac use while the other looked at celecoxib. All three studies found a significantly increased risk of anastomotic leakage following administration of the the NSAID in question. No mention was made in the article regarding other confounding factors for anastomotic leakage, or the distribution of such factors amongst the three articles. For example, there was no mention of the type of anastomosis in each article, the use of a protective ostomy, average length of surgery, pre-existing patient health, etc. There were also no secondary outcomes assessed besides anastomotic leakage rate.

As the first systematic review looking at this topic the warning flag was raised that there may be a real risk associated with NSAID use. But as an isolated review there were several limiting factors present. The small number of studies, lack of bias assessment, lack of confounder assessment, and lack of a final overall quantitative effect estimate all limit interpretation of the authors results. The take-away message from this study is that NSAIDs may increase the risk of anastomotic leakage, but more evidence would be needed before a definitive conclusion could be made.

Burton TP et al., 2013[34]

Systematic review and meta-analysis comprised of six randomized control trials (480 patients). All six studies were designed and powered to assess the effect of NSAID usage on post-operative ileus. As a secondary outcome three of these studies quantified the post-operative

anastomotic leak rate amongst the NSAID and non-NSAID groups. The other three articles mentioned anastomotic leakage in the context of patient exclusion within their study. The six included trials involved NSAID usage of any kind, within 48 hours of an anastomosis of the small bowel, colon, or rectum. Risk of bias was assessed using the Jadad score[35]. The primary outcome measured in the meta-analysis was anastomotic dehiscence. Secondary outcomes measured included pain scores, morphine equivalent doses, and time to return of flatus and stools.

In four of the six trials the incidence of anastomotic dehiscence was higher in the NSAID group than in control group. Synthesized, the overall rate of anastomotic leakage was 5.1% in the NSAID group vs 2.4% in the control group. Despite an odds ratio of 2.16, this difference did not reach statistical significance ($P = 0.1$).

Most studies showed a moderate beneficial effect from NSAIDs for all secondary end points. There was no difference between groups in movement-evoked pain scores on POD 0 or POD 1, but they were significantly lower on POD 2. Pain scores at rest were significantly lower in the NSAID group on POD 0, 1, and 2. Opioid usage during the first 48 hours was significantly lower in the NSAID group, with an average time to return of bowel function 0.43 days earlier in the NSAID group in than in the control group ($P < 0.00001$).

This meta-analysis illustrates the dilemma that surrounds the NSAID conundrum. On the one hand there seems to be an indication that the risk of leak following NSAID usage is increased. But this difference does not reach statistical significance. Whether because of an

under-powered meta-analysis that failed to demonstrate a true effect, or a difference between groups that came about by pure chance, no definitive answer to the clinical question at hand can be had. Looking at the articles included within this meta-analysis, one can see that none of the six articles assessed anastomotic leakage as a primary, or even pre-determined secondary, outcome. As such these studies were not sufficiently powered to give a significant result with regards to NSAIDs and anastomotic leakage. In-fact, of the six included studies, three of them only mention anastomotic leakage in the context of excluding patients from their study because they leaked. This is because all six of these studies were actually designed and powered to assess the affect of NSAIDs on post-operative ileus and resumption of bowel function, not anastomotic leakage. In this regard, the studies demonstrated a small but significant beneficial effect from NSAIDs with regards to improved post-op pain scores, quicker return to bowel function, and decreased reliance on opioids. All of which are important clinical measures. Beneficial effects like these make NSAIDs an enticing analgesic option for many surgeons, and the lack of definitive proof demonstrating increased harm makes it difficult to abandon them.

Bhangu A et al., 2014[36]

Systematic review and meta-analysis involving 8 studies (4,464 patients) aimed to assess whether postoperative NSAID use increased the risk of anastomotic leakage. Articles that involved either a small bowel or colon anastomosis were included. Assessment of bias was performed using the Newcastle Ottawa Scale[37].

Overall use of NSAIDs was significantly associated with anastomotic leak ($P < 0.001$). On sub-group analysis, an adverse effect was seen with non-selective NSAIDs ($P < 0.001$), but

not with selective NSAIDs ($P = 0.2$). When looking at individual drugs within the NSAID class, the significant effect remained when only studies using diclofenac were included ($P < 0.001$). Considering only patients receiving ketorolac, the effect was no longer significant ($P = 0.1$). The effect with celecoxib also remained non-significant ($P = 0.2$).

This latest review, demonstrating a significant association between NSAID usage and anastomotic leakage, involved the largest total number of patients to date. This was the first review to look at not only selective vs. non-selective NSAIDs, but to analyze individual drugs within each class. If there truly is an increased risk of anastomotic leakage it may not be uniform across all NSAIDs. Not only were the non-selective NSAIDs the only class found to harbor a significant association, but within this class, diclofenac (a non-selective NSAID) was found to pose an increased risk while Ketorolac (also a non-selective NSAID) did not. Whether or not this is a true medication effect, or secondary to unseen confounding factors (i.e. other differences in patient factors or patient care between groups) remains unknown at this time. The question regarding NSAID safety following gastrointestinal surgery may be more complicated than first perceived. It may be that based on differing pharmacodynamics and bioavailability certain NSAIDs may pose an increased risk to patients, while others do not.

Summary

Systematic reviews and meta-analyses provide a standardized approach to examining the medical literature. The goal is to provide a definitive answer to guide clinical practice. The current literature however does not provide uniform agreement on the impact of NSAIDs post-operatively. The first review (performed without meta-analyses) from 2011 describes a potential

association between NSAIDs and leaks[33], but significant methodological flaws and low study/patient numbers limit the applicability of such findings. Two meta-analyses completed in 2013 and 2014 then went on to find contradictory evidence regarding this topic. The first study found no significant relationship between NSAIDs and increased leak risk, yet the second one published just one year later did find a significant risk[34, 36].

Currently no consensus exists regarding the safety of NSAIDs post-operatively. NSAIDs are a class of medication with many benefits over opioid medications, and the risk profile has yet to be clearly elucidated. Contradictory evidence exists not only regarding the safety of these medications, but regarding the risk of individual medications within the NSAID class. Furthermore, when looking at the research that has been done on rats (See Section on Animal Studies), one can see that there is a clear location dependent effect that NSAIDs have on the gastrointestinal tract. The small bowel appears to be affected to a far greater extent by post-operative NSAIDs than the distal colon, and any study looking to elucidate the effect of NSAIDs following colorectal surgery must take this into account. The three previous reviews done on this topic have included both small and large bowel anastomoses, and tabulated this as an overall effect on anastomotic healing. Any systematic review that aims to assess the risk of NSAIDs specifically on colorectal anastomoses must isolate only those studies that involve the colon and exclude those that involved small bowel to small bowel anastomoses.

2.3 – Drug Specific Risk of Anastomotic Leak

Introduction

As a greater emphasis is being placed on elucidating the safety profile of NSAIDs we are beginning to gain a greater appreciation for the variability that exists within the NSAID class. Differing inhibitory and bioavailability profiles lead to different local and systemic effects.

As discussed in the Background section, NSAIDs can be classified as COX-2 selective or non-selective inhibitors. However, this classification is an oversimplification of a very complex pharmacodynamic picture. Non-selective NSAIDs are thought to indiscriminately inhibit both COX-1 and COX-2 enzymes. However, different non-selective NSAIDs have differing affinities for the COX-1 and COX-2 enzymes[38]. Diclofenac, which is categorized as a nonselective NSAID, preferentially inhibits COX-2 and behaves very much like a selective NSAID[39]. On the other hand, ketorolac, another nonselective NSAID, has significant preferential inhibition of COX-1 over COX-2 and displays a very different risk and safety profile from that of diclofenac[39].

Often times different drugs within a class are viewed as being essentially interchangeable, with a physician's preference often relating more-so to their previous experience and comfort with the medication, rather than evidence-based justification of one drug over another. However, analysis of different NSAIDs (diclofenac and ketorolac being the most commonly studied), may reveal that broad generalization of safety between drugs even within the same sub-class is inappropriate, and the real question that should be asked is not "Do NSAIDs pose an increased risk for anastomotic leakage?", but rather "Are there specific classes or medications within the broader NSAID group that are associated with an increased risk of anastomotic leakage?".

Literature Review

Klein M et al., 2012[7]

Cohort study looking at a prospectively collected Danish database of 2,756 patients who underwent surgery for colorectal cancer, and received a colonic or rectal anastomosis. Treatment group consisted of 885 patients who received an NSAID for at least two days' duration within the first seven days after surgery. Ibuprofen was administered to 655 (74%), diclofenac to 226 (26%). The remaining 1,871 patients who did not receive an NSAID, or received an NSAID for less than two days' duration, served as controls.

The proportion of patients with anastomotic leakage was significantly higher in the NSAID groups than in the control group. Anastomotic leakage occurred in 12.8% of patients treated with diclofenac, 8.2% treated with ibuprofen, and in 5.1% of controls ($P<0.001$ for diclofenac vs. controls; $P=0.004$ for ibuprofen vs. controls). After unadjusted analyses when compared with controls, the absolute risk of anastomotic leakage was increased by 7.8% after diclofenac treatment and 3.2% after ibuprofen treatment. After the final multivariate logistic regression was performed however, only a significant association with diclofenac remained ($P<0.001$), and ibuprofen was no longer associated with an increased risk of post-operative anastomotic leak ($P = 0.2$). There was no significant difference in 30-day postoperative mortality between the three groups.

Within the control group there were 231 patients who received an NSAID for less than two days' duration. When this subgroup was compared to the remaining controls who did not receive an NSAID at all, no difference was found in the proportion of anastomotic leakage between the two groups.

The take-away message was that following colorectal cancer resection, there exists an increased risk of anastomotic leakage with postoperative diclofenac treatment but not ibuprofen treatment. The authors hypothesize that COX-2 inhibition causes micro-thromboses or micro-emboli that then impairs the anastomotic blood supply, leading to anastomotic leakage. This hypothesis is based on research done on patients following coronary artery bypass grafting, where the incidence of postoperative thrombotic cardiovascular events increased greatly with COX-2 inhibitor treatment[40]. Also, it has been shown that there exists a lower risk of thromboembolic events with ibuprofen and other non-selective drugs[41], and this could explain why only diclofenac (a non-selective NSAID with very high COX-2 inhibition) and not ibuprofen (primarily COX-1 inhibition) increased the risk of anastomotic leakage in this study.

Saleh F et al., 2014[11]

Retrospective review performed on patients who underwent elective colorectal surgery. Aimed at assessing the association between leak rate and ketorolac use within the first 5 post-operative days. Total of 731 patients identified as having a resection with primary anastomosis included in the study. Control group comprised of 376 patients who did not receive an NSAID.

Treatment group included a total of 355 patients who received ketorolac within 5 days after surgery. The primary outcome of interest was leakage after primary anastomosis. A secondary outcome of interest was whether the total ketorolac dose received was associated with an increase in anastomotic leak rate.

There were a total of 24 leaks (3.3 %) in the study population, with 12 leaks in both the no ketorolac (3.2 %) and ketorolac (3.4 %) groups ($P = 0.886$). Subgroup analysis was performed using patients in the ketorolac group to assess if a dose-dependent relationship existed between ketorolac use and anastomotic leakage. This time a significant association between dose and leak rate was found, indicating that higher doses of ketorolac post-operatively may be associated with higher leak rates.

This study suggests that ketorolac appears to be safe when given post-operatively after colorectal surgery. However, in subgroup analysis it was suggested that leakage may be more likely with higher total doses of ketorolac. As discussed in this paper, the authors cite differences in ketorolac's inhibitory profile as a likely explanation for why no association was found between ketorolac and anastomotic leakage. Ketorolac has been found to be up to 1,000 times more selective for the COX-1 enzyme compared to COX-2[42], and this may explain why unlike other NSAIDs that inhibit COX-2 to a greater extent ketorolac appears to be safe following colorectal surgery. The significant dose-dependent association discussed in this study however suggests that at higher doses ketorolac causes COX-2 inhibition at a level sufficient to pose a significant risk to patients. Further research would be needed to prove this hypothesis.

Subendran J et al., 2014[6]

Matched nested case-control study using a prospectively collected database. Cases were defined as those who had an anastomotic leak postoperatively. Controls were chosen using 1:1 matching with cases based on underlying disease, type of surgery, age (within 5 years), sex, and year of surgery (within 5 years). The primary and secondary exposure variables were, respectively, use of any NSAID and use of ketorolac specifically. A total of 270 patients (135 case-control pairs) were included in the study. Four pairs were excluded because the anastomotic leak occurred more than 12 months after the initial surgery.

In adjusted analysis, use of any NSAID was associated with a non-significant increase in leaks ($P = 0.06$), while use of ketorolac was associated with a significantly higher risk of anastomotic leaks ($P = 0.02$). There was no significant association between cumulative ketorolac dose and anastomotic leakage ($P = 0.7$). There was no significant association between anastomotic leakage and the number of days of NSAID ($P = 0.2$) or ketorolac use ($P = 0.97$).

This study did not find a significant increase in anastomotic leakage with the use of postoperative NSAIDs overall, but there was a significant increase in anastomotic leaks with the use of ketorolac specifically. These findings are contradictory to those found by Saleh et al. in 2014[11], which found ketorolac not to be associated with an increased risk of anastomotic leakage. The aforementioned study did however find a dose-dependent relationship between ketorolac and post-operative leakage, which this study, despite finding an overall increased risk of leak with ketorolac, did not find. Furthermore, both studies looked at data that occurred around the same time, from the same city – Toronto (albeit in different hospitals). These conflicting findings

underlie the confusion regarding the safety of NSAIDs as a whole post-operatively. They do however underlie the principle that there may very well be a drug dependent effect within the NSAID class, and further research is needed to clarify this effect.

Bakker N et al., 2016[8]

Retrospective database review of 856 patients who underwent an elective colon or rectal resection with a primary anastomosis. There were 732 patients with colon cancer, 282 (38.5%) of whom did not receive an NSAID, 288 (39.3%) received diclofenac, 93 (12.7%) received nabumetone, and 69 patients (9.4%) received ibuprofen. There were also 124 patients with rectal cancer, 40 (32%) of whom did not receive an NSAID and 84 (68%) who received diclofenac. The primary outcome measured was the incidence of anastomotic leakage.

Overall, patients receiving NSAIDs had a higher anastomotic leak rate compared to patients who did not ($P = 0.038$). In the colon cancer group, there was a significantly higher leak rate in the diclofenac group (11.8%) compared to the group receiving no NSAIDs (6.0%), the nabumetone group (1.1%), and the ibuprofen group (4.3%) ($P = 0.002$). In the rectal cancer group there were no leaks in the no NSAIDs group, and 11 patients (13.1%) with an anastomotic leak in the diclofenac group ($p = 0.017$). There was no significant relationship between the post-operative day that diclofenac was started and the leak rate. There was also no significant relationship between the duration of diclofenac use within the first five postoperative days and the leak rate.

This study found that diclofenac was an independent risk factor for anastomotic leakage, while the other NSAIDs that were used, nabumetone and ibuprofen, did not increase the risk. Of note, nabumetone is an NSAID with preferential COX-2 inhibition, similar to diclofenac, yet unlike diclofenac it was not associated with an increased leak rate. The theory that COX-2 inhibition on its own is what causes leakage is an obvious over-simplification. There does however seem to be an increasingly large pool of data indicating that diclofenac is not safe for use following colorectal surgery.

Summary

To answer the ultimate question regarding NSAID safety, it appears that one must be aware that there is no single answer. Up until now all attention has been placed on answering the question, "Do NSAIDs impair anastomotic healing?", but the results of such an inquiry have been conflicting to say the least. Looking critically through the available literature, there seems to be a real indication that some NSAIDs pose a real risk after surgery, but others do not. Diclofenac in particular has been found repeatedly to be associated with an increased post-operative anastomotic leak rate, while the COX-1 preferential inhibitor, ibuprofen, has not.

But the issue is not simply one of COX-1 vs COX-2 inhibition, otherwise one would expect all COX-2 selective inhibitors to be associated with an increased leak risk. For example, in 2016 Bakker et al.[8] looked at both diclofenac and nabumetone, two drugs with very high COX-2 inhibitory profiles. If the issue was simply one of COX-2 inhibition one would expect both drugs to be associated with an increased leak rate, however this was not the case. Only diclofenac demonstrated an increased risk when given post-operatively, nabumetone did not.

Furthermore, in 2014 Subendran et al.[6] looked at just ketorolac, an NSAID with a much higher COX-1 inhibitory profile than COX-2, and assessed its association with post-operative anastomotic leakage. Their findings, that ketorolac is significantly associated with an increased leak risk, would go against any hypothesis that this is simply a COX-2 inhibitory effect.

The take away message is that the question regarding NSAID safety is much more complicated than once believed. At this point, where no conclusive recommendation exists regarding post-operative NSAID use, if it's believed a risk exists it may be best to recommend against their use as an entire class. It will take much longer to identify which drugs within the class are proven safe and which are proven dangerous, and given the push towards non-opioid analgesia from initiatives such as the ERAS movement, a safe first step may be to caution against their use as a whole. In the long-run though there needs to be an appreciation that this may in-fact be a drug-specific effect, and as research into individual drugs becomes available, the recommendations regarding their use can be further refined.

Chapter 3 – Methods

3.1 – PRISMA Statement

The following systematic review and meta-analysis was completed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[43]. These guidelines were developed in 2005 following a three-day meeting designed to expand upon the existing QUOROM (Quality of Reporting of Meta-Analysis) Statement. The rationale behind the PRISMA statement has been to ensure the transparency and completeness in the reporting of systematic reviews and meta-analyses, and as such those guidelines have been used in the design of this study. An up-to-date checklist using the current PRISMA guidelines can be found in Appendix 3.

3.2 – Protocol

Prior to study commencement a research protocol was created as part of an initial research abstract outlining the process by which data sources would be obtained, the outcomes of interest to be assessed, and the means by which data would be compiled and analyzed. This protocol was agreed upon by all members of the research team and forwarded along to the Memorial University Clinical Epidemiology program for approval. The methodology outlined in that initial protocol was followed throughout study completion and will be outlined in the following sections.

3.3 – PICO

Patients: Patients undergoing colorectal resection and primary anastomosis

Intervention: Administration of an NSAID post-operatively (within the first 7 days)

Control: Patients who haven't received an NSAID post-operatively (first 7 days)

Outcomes: Primary outcome - Post-operative anastomotic leak rate

Sub-group analysis - Selective vs non-selective NSAIDs, ketorolac vs diclofenac

3.4 – Eligibility Criteria

Randomized control trials, cohort studies, and case-control studies performed on humans undergoing a colonic or rectal resection with a primary anastomosis were eligible. As there is currently very limited research available looking at this topic, a decision was made to use both randomized control trials and non-randomized observational studies. Given that the pooling of crude events from non-randomized studies may be misleading as they do not take into account confounding factors, a decision was made to use multivariate adjusted risk estimates from the observational data whenever possible.

Colonic anastomoses were defined as any anastomosis in which at least one limb involved colonic tissue (i.e. ileocolic anastomoses involving small bowel and colon were classified as a colonic anastomosis). Similarly, rectal anastomoses were defined as an anastomosis whereby at least one limb included rectal tissue (i.e. colorectal anastomosis following proximal rectal resection). No restriction was placed on publication date or language. Included trials were required to involve patients who received a primary colonic or rectal anastomosis and post-operative NSAID analgesia within 7 days of surgery, as well as a control

group of patients undergoing a similar surgery who did not receive NSAIDs (could have received a placebo or any other form of non-NSAID analgesia). Studies were required to have assessed anastomotic leakage as a primary outcome.

Studies were excluded if they were animal based, did not involve a colonic or rectal anastomosis, were editorials or case reports, did not look at anastomotic leak rates as a primary outcome, or involved an additional intervention in addition to NSAID analgesia in the treatment arm (i.e. NSAIDs plus steroids vs placebo). Studies that included small-bowel anastomoses were not included. This decision was made primarily based on animal studies that have demonstrated a location-dependant effect of NSAIDs on the gastrointestinal tract (See Literature Review). It's been shown that the small bowel is affected by non-steroidal anti-inflammatories to a greater extent than the distal colon[26, 29, 31], therefore it was decided that to accurately assess the effect of NSAIDs on colonic and rectal anastomoses studies that included small bowel anastomoses needed to be excluded.

3.5 – Information Sources

Studies were identified by searching electronic databases and scanning reference lists of articles. No limits were placed on language. It was decided that if foreign papers were identified they would be translated and included in the article review. The databases searched included MEDLINE, EMBASE, and CINAHL. The final search was completed on November 24, 2016.

The MEDLINE database was selected as it is generally regarded to be one of the most comprehensive sources of health care related research in the world. The EMBASE database

covers much of the same subject matter as the MEDLINE database, but provides an additional focus on drugs and pharmacology. Given the focus of this study on NSAID medications the EMBASE database was deemed to be an important source of research information. Lastly, to ensure that we performed a well rounded and complete literature search we also included the CINAHL database in our search. CINAHL is generally regarded as a very clinically oriented database with a focus on nursing and allied health disciplines.

3.6 – Search

Given the narrow scope of this research question and the limited number of studies available, a broad search strategy was employed to ensure that any and all relevant articles were assessed. The following two search terms were used with all 3 databases: "anastomosis" and "NSAIDs". Given that the search term "NSAIDs" is synonymous with the term "non-steroidal anti-inflammatory" and the singular form – "NSAID", these three terms were grouped together using the "OR" function. As an example, our MEDLINE database search consisted of the following inquiry: "Anastomosis AND (NSAID OR NSAIDs OR non-steroidal anti-inflammatory)". The same strategy was employed for the EMBASE and CINAHL database searches.

3.7 – Study Selection

Study eligibility was evaluated in an un-blinded standardized manner by two independent physicians affiliated with Memorial University. Any disagreements between reviewers was to be resolved by consensus. Initial screening was performed by analysis of article

titles/abstracts. Articles were then read in full to assess for final inclusion/exclusion into the systematic review and meta-analysis.

3.8 – Data Collection Process

Data was extracted independently by one author, with independent verification by a second physician/researcher. Data was input manually into the Review Manager (Rev-Man) 5.3 software system. Discrepancies were to be resolved with a discussion between the two independent researchers. If consensus could not be reached a third independent researcher would decide.

3.9 – Data Items

Extracted data from each study included: (1) study design and study inclusion/exclusion criteria; (2) type of intervention (including type of NSAID, duration, and frequency of use); (3) type of outcome measured, including the definition used for diagnosing an anastomotic leak; and (4) individual anastomotic leak rates from the intervention and control groups in each study.

3.10 – Risk of Bias in Individual Studies

Due to the nature of the question being examined, all studies were of a non-randomized, retrospective nature. As per Cochrane guidelines a risk of bias assessment was performed using the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I) tool[2]. Studies were graded on risk of bias to due confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes,

and selection of the reported units. A final overall risk of bias was then determined. Overall risk of bias was placed into one of five categories[3]:

1: *Low Risk of Bias* – the study is comparable to a well performed RCT

2: *Medium Risk of Bias* – the study provides sound evidence for an RCT, but cannot be considered comparable to a well performed RCT

3: *Serious Risk of Bias* – the study has some important problems

4: *Critical Risk of Bias* – the study is too problematic to provide any useful evidence and should not be included in any synthesis

5: *No Information* on which to base a judgment about risk

3.11 – Summary Measures

Meta-analysis was to be performed by computing the odds ratio (OR) from the original data using the Cochran-Mantel–Haenszel method (with a 95% confidence interval). An odds ratio greater than 1 was indicative of an increased risk of anastomotic leakage in the group administered NSAIDs. A *P*-value of 0.05 was considered significant for all analyses. The primary outcome measure was the incidence of anastomotic leakage following surgery in each group.

The Cochran-Mantel–Haenszel test (also known as the Mantel-Haenszel test) is used to

assess the association between a binary intervention/exposure (such as NSAID exposure and no NSAID exposure), and a binary/dichotomous outcome (such as the presence of an anastomotic leak and the absence of a leak). The Mantel-Haenszel test provides an estimate of the common odds ratio, and tests whether the overall degree of association between the exposure and the outcome is significant.

Although it is theorized by some that the relative risk is an easier outcome to interpret clinically, and that misinterpretation of the odds ratio can lead to over/under estimation of the risk associated with an exposure[45], our decision to include case-control studies as part of the analysis precludes the use of relative risk as the principle summary measure. Both odds ratio and relative risk are summary measures that can be used for dichotomous outcomes.

3.12 – Synthesis of Results

Synthesis and graphical representation of the meta-analysis was to be performed with the Review Manager (Rev-Man) 5.3 software, using a random-effects model. A forest plot would be created to display the results of each study as well as the overall result. Inter-study heterogeneity would then be assessed using the I^2 statistic.

The I^2 statistic describes the percentage of variation across studies that is due to heterogeneity rather than by chance alone[44]. In comparison to the chi-squared test (another test of heterogeneity), the I^2 statistic is far more reliable in meta-analyses involving smaller numbers of studies. When the number of studies is low, the chi-squared value becomes underpowered making interpretation of a non-significant value very difficult. Given the focused nature of our

clinical question, and presumed low number of available studies, it was believed that the I^2 statistic would be a more reliable measure of heterogeneity.

Along with the I^2 statistic, it was also determined that a random-effects model would be used instead of a fixed-effects model. The fixed-effects model works under the assumption that the true effect size for all studies in a meta-analysis are identical, and that any variation seen is due to sampling error. Meanwhile the random-effects model assumes that the true effect size varies from one study to another, and that the selected studies represent a random sample of possible effect sizes that could have been observed. As such, in the fixed-effects model smaller studies are given a very small weight, while in the random-effects model smaller studies are given a comparatively larger weight as it is believed that each study provides unique information about a different effect size.

In our study we chose a random-effects model from the start. Based on our review of the existing literature (see Literature Review), we believed that the effect size for each study would vary based on the intrinsic differences observed in each study. Across studies there were significant differences ranging from which NSAIDs were being administered, their duration and timing, the type of anastomosis created, to the diagnostic criteria used to identify post-operative leaks. Because of these differences, it was believed that each study would have its own unique true effect size and that a fixed-effects model would be inappropriate.

3.13 – Risk of Bias Across Studies

Publication bias was assessed via a graphical representation of precision vs effect

estimate (i.e. a funnel plot). This graphical representation plots the effect estimates from individual studies (in the form of an odds ratio), against the inverse standard error of the aforementioned studies. The underlying principle of such as plot being that the effect estimates from less precise studies (higher standard error) will scatter more widely at the bottom of the graph, with the spread narrowing among the more precise (lower standard error) studies. In the absence of bias, the plot should resemble a symmetrical inverted funnel.

Heterogeneity, reporting bias, and chance may all lead to asymmetry or other shapes in funnel plots[46]. Reporting bias arises when the publication of research findings is influenced by the nature of the results. Statistically significant "positive" results are more likely to be published, published rapidly, published in English, published more than once, published in high impact journals, and cited by others. Data that would lead to negative results may be filtered, manipulated, or presented in such a way that they become positive. These biases would cause an asymmetry if, for example, studies that indicate that NSAIDs increase the leak rate are more likely to published than studies that show no effect, or vice-versa.

3.14 – Additional Analysis

Further sub-group analysis was planned based on the findings from our literature review. Given the hypothesis that there may be a drug specific effect that NSAIDs have on the healing of colorectal anastomoses (see Literature Review), further sub-group analysis was planned to stratify patients based on the type of NSAID they received (selective vs non-selective). We then aimed to further investigate this drug specific effect by looking at different medications within a single class. Ketorolac and diclofenac are two medications that have been studied in great detail,

and both belong to the non-selective NSAID class. We aimed to look at the drug specific effect of each medication versus placebo and see if there were significant differences. In doing so, we planned to answer the following questions:

A) Are NSAIDs as a whole associated with an increased risk of anastomotic leakage?

B) If so, is this effect isolated to one class of NSAIDs?

C) And if so, is this effect uniform across different medications within the same class?

Chapter 4 – Results

4.1 – Study Selection

A search of MEDLINE, EMBASE, and CINHAL yielded a total of 432 articles. After adjusting for duplicates we were left with a total of 373 non-duplicate articles (see Figure 4.1 for PRISMA diagram). These 373 articles were then screened and we were then left with 31 articles remaining for full-text review. Of these remaining articles 24 were excluded because they did not meet our inclusion/exclusion criteria. Seven articles were experimental rat studies, 6 were review papers or author replies, 6 looked at comparison groups that did not meet inclusion/exclusion criteria (i.e. one NSAID vs. another, or an NSAID plus another intervention vs placebo) or primary outcomes that did not meet inclusion/exclusion criteria (i.e. length of hospital stay), 1 was a poster abstract that did not meet inclusion criteria, 1 was a project proposal, 1 included bariatric surgery patients in their patient pool, and 2 were previously completed meta-analyses. Seven articles remained which met our inclusion/exclusion criteria and were included in the systematic review. We then went on to check the reference lists of relevant papers that were both included and not included in our analysis for articles we may have missed in our initial screening. No articles that we had not previously screened were found.

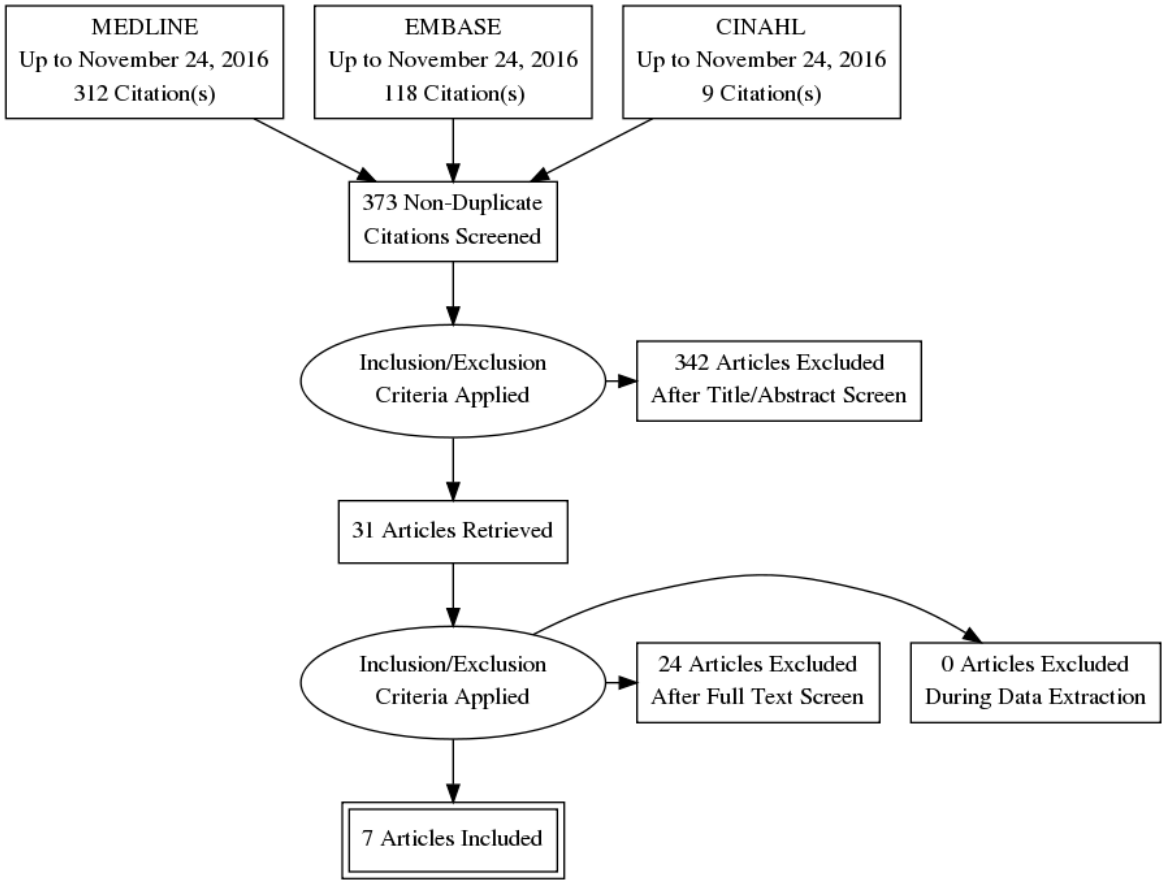

Figure 4.1: PRISMA Diagram

4.2 – Study Characteristics

In total 9,835 participants were involved in the 7 selected studies. All studies were published in English with 3 originating from North America (Canada and the United States), and 4 from Europe (Denmark and the Netherlands). Three were single-centered studies and 4 were multi-centered. With respect to their study methodology, 6 of the selected studies were of a retrospective cohort design, while one (Subendran 2014) was a nested, matched case-control study (See Tables 1 – 7 for full study details).

The main inclusion criteria in all studies involved adults undergoing colonic or rectal resection, who received a primary anastomosis and then an NSAID within 7 days following surgery. No other gastro-intestinal anastomosis was included in any of the 7 studies. The NSAIDs given in 5 of the studies were only non-selective NSAIDs, 1 study looked at both selective and non-selective NSAIDs, and 1 did not specify which type of NSAID was used. Of the 6 studies that specified the exact NSAID given, 4 involved diclofenac and 2 involved ketorolac.

The primary outcome of interest in all 7 studies was post-operative anastomotic leak. In 5 studies an anastomotic leak was confirmed whenever signs of leakage were present on imaging or subsequent surgery. In the other 3 studies a leak was considered present only when it was confirmed at re-operation. Six of the 7 studies included secondary outcome analysis in their paper, however the secondary outcomes were quite varied across studies.

4.3 – Risk of Bias Within Studies

The risk of bias assessment was completed using the ROBINS-I tool (see Appendix 4 – 10). All studies were graded using the official ROBINS-I scoring guidelines[3]. Four studies were deemed Moderate Risk based on baseline differences in potential confounding variables between the control and intervention groups. Interestingly, in three of those four studies (Bakker et al. 2006[8], Klein et al. 2009[10], and Saleh et al. 2014[11]) patients in the control group began with poorer baseline health, while in the end it was the NSAID group that was found to have a significantly higher leak rate.

The other 3 studies were deemed Serious Risk. Paulasir et al., 2015[5] was deemed Serious Risk as the authors did not classify and account for rectal vs colonic anastomosis – a potential confounding factor given previous literature indicating a location specific effect that NSAIDs exhibit throughout the gastrointestinal tract (see Literature Review). Subendran et al., 2014[6] was classified as Serious Risk because the authors did not take into account pre-existing co-morbidities in their analysis. Pre-existing comorbidities play a major role in risk stratification of patients for post-operative anastomotic leak, and without documentation of what if any differences existed between groups, one cannot be sure if the results found within this study were secondary to the intervention in question (NSAIDs), or to unknown differences in baseline health amongst patients. Similarly, Klein et al., 2012[7] was classified as being at Serious Risk of bias because of a failure to assess whether or not patients received a protective stoma in their analysis. Patients with a protective stoma are at lower risk of clinically apparent anastomotic leakage, and as such differences between groups in protective stoma formation must be taken into account.

4.4 – Results of Individual Studies/Synthesis of Results

Overall NSAID Use and Anastomotic Leak Rate

The anastomotic leak rate in each study for both intervention and control groups is listed in Figure 4.2. NSAIDs were associated with a significantly higher anastomotic leak rate in 4 of the 7 studies, with an overall leak rate of 300/3555 (8.44%) compared to 302/6280 (4.81%). When synthesized using our pre-ordained parameters (M-H analysis, OR with random-effects model) the overall anastomotic leak rate was found to be significantly higher in the NSAID

group [OR 1.58 (1.23, 2.03), $P = 0.0003$]. Heterogeneity as defined by our I^2 statistics was classified as moderate (38%).

Comparison: NSAIDs versus No NSAIDs post colorectal surgery
Outcome: Anastomotic leak

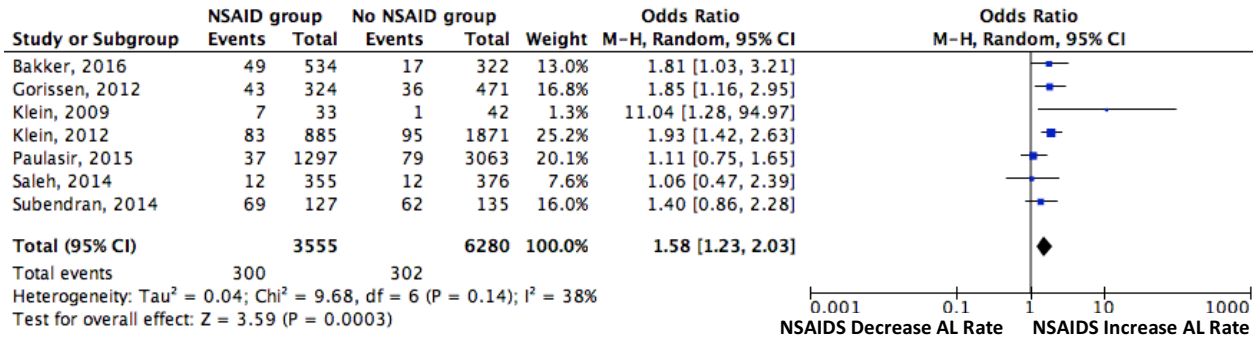

Figure 4.2: NSAIDs post colorectal surgery and anastomotic leak rate

Class Specific Anastomotic Leak Rate

Of the 7 included studies, documentation of NSAID type was provided by 6 (the exception being Paulasir et al., 2015[5]). All 6 studies included non-selective NSAIDs in their intervention group, while 1 (Gorrisen et al., 2012[9]) included both selective (celecoxib and meloxicam) and non-selective NSAIDs.

When looked at in isolation, Gorrisen et al., 2012[9] found an increased anastomotic leak rate with non-selective NSAIDs compared to selective NSAIDs [OR 2.13 (1.24, 3.65); $P = 0.006$]. Selective COX-2 inhibitors were not associated with an increased risk of anastomotic leak when compared to controls [OR 1.17 (0.50, 2.74), $P = 0.7$].

We then isolated patients from the 6 studies who took only non-selective NSAIDs and compared them to the controls in each study. Of this patient group, a significantly elevated anastomotic leak rate was found in 4 of the 6 studies (Figure 4.3). The overall leak rate in the non-selective NSAID group was found to be 248/2310 (11.64%), compared to 240/3499 (6.86%) in the control group (OR 1.77 [1.43, 2.20], P < 0.00001). Heterogeneity between groups was low (I^2 = 8%).

Comparison: Non-selective NSAIDs versus No NSAIDs post colorectal surgery
Outcome: Anastomotic leak

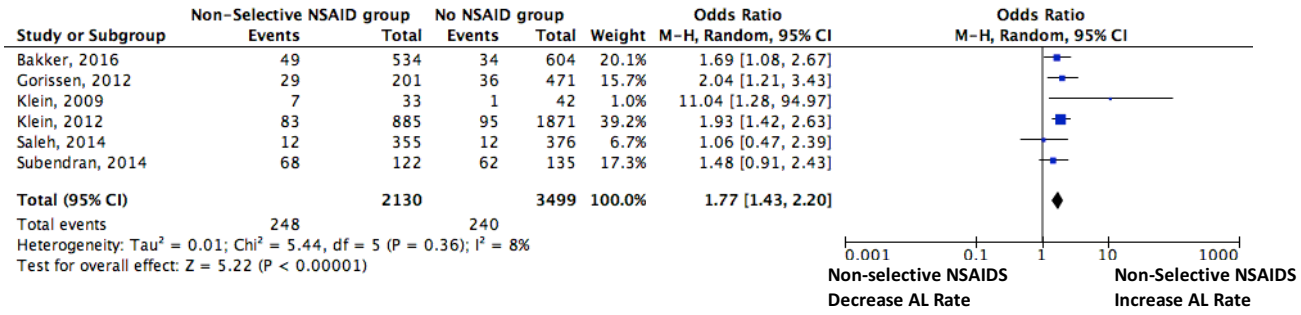

Figure 4.3: *Non-selective NSAIDs post colorectal surgery and anastomotic leak rate*

Drug Specific Anastomotic Leak Rate

Used by patients in 4 of the 6 studies with drug-specific documentation, diclofenac was the most commonly used medication across studies. The second most commonly used medication was ketorolac (present in 2 of 6 studies). Both medications are classified as non-selective NSAIDs, and a drug-specific analysis was done to assess for heterogeneity within the non-selective class with regards to risk profiles.

Of the 4 studies in which diclofenac was said to have been used, 3 provided isolated

anastomotic leak rates for patients taking diclofenac (Figure 4.4). In all 3 studies, diclofenac was associated with a statistically significant increased anastomotic leak rate [OR 2.74 (1.94, 3.88), $P < 0.00001$]. The overall leak rate in patients taking diclofenac across these studies was 81/631 (12.84%) compared to 113/2235 (5.06%) in controls. The one study that did not include specific numbers for diclofenac (Gorrisen et al., 2012[9]), did however indicate that diclofenac was the most commonly used non-selective NSAID amongst their study population, and non-selective NSAIDs as a class were found to be associated with a significantly increased post-operative leak rate [14.5% leak rate; OR 2.13 (1.24, 3.65); $P = 0.006$]. Ketorolac specific leak rates were documented in two studies (Figure 4.5), with neither finding ketorolac to be associated with a significant change in the post-operative leak rate [OR 1.36 (0.89, 2.06), $P = 0.16$]. Heterogeneity was absent ($I^2 = 0\%$) in both the diclofenac and ketorolac sub-group analyses.

Comparison: Diclofenac versus No NSAIDs post colorectal surgery
Outcome: Anastomotic leak

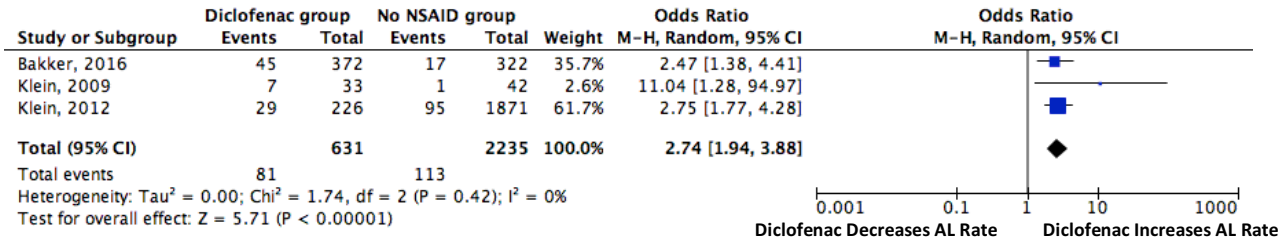

Figure 4.4: Diclofenac post colorectal surgery and anastomotic leak rate

Comparison: Ketorolac versus No NSAIDs post colorectal surgery
Outcome: Anastomotic leak

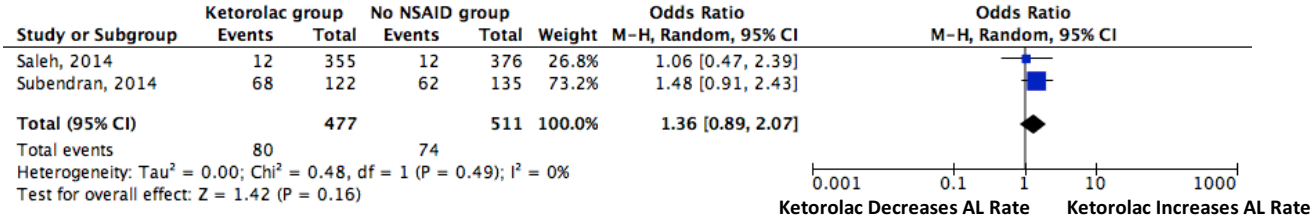

Figure 4.5: Ketorolac post colorectal surgery and anastomotic leak rate

4.5 – Risk of Bias Across Studies

Moderate heterogeneity was found in the overall NSAID vs control group analysis (I^2 = 38%). This heterogeneity was further assessed using a funnel plot (Figure 4.6). Given the relatively low number of studies included in this analysis the general shape of our funnel plot remained fairly symmetrical. When looking at the forest plot one study stands out, that being the least precise study at the base of the pyramid (Klein et al., 2009[10]). As expected, the least precise study demonstrates a size effect farthest from the mean. The studies with the highest precision all clustered very closely to the mean at the top of our plot, and the study with the lowest precision was positioned farthest from the mean at the base of our plot. While the left side of the base remains bare in this plot (which would represent studies that showed a protective effect with NSAIDs), this is likely to be secondary to the low number of studies included, rather than an inherent bias. As such there is no indication from our funnel plot that there is a significant risk of bias present across studies

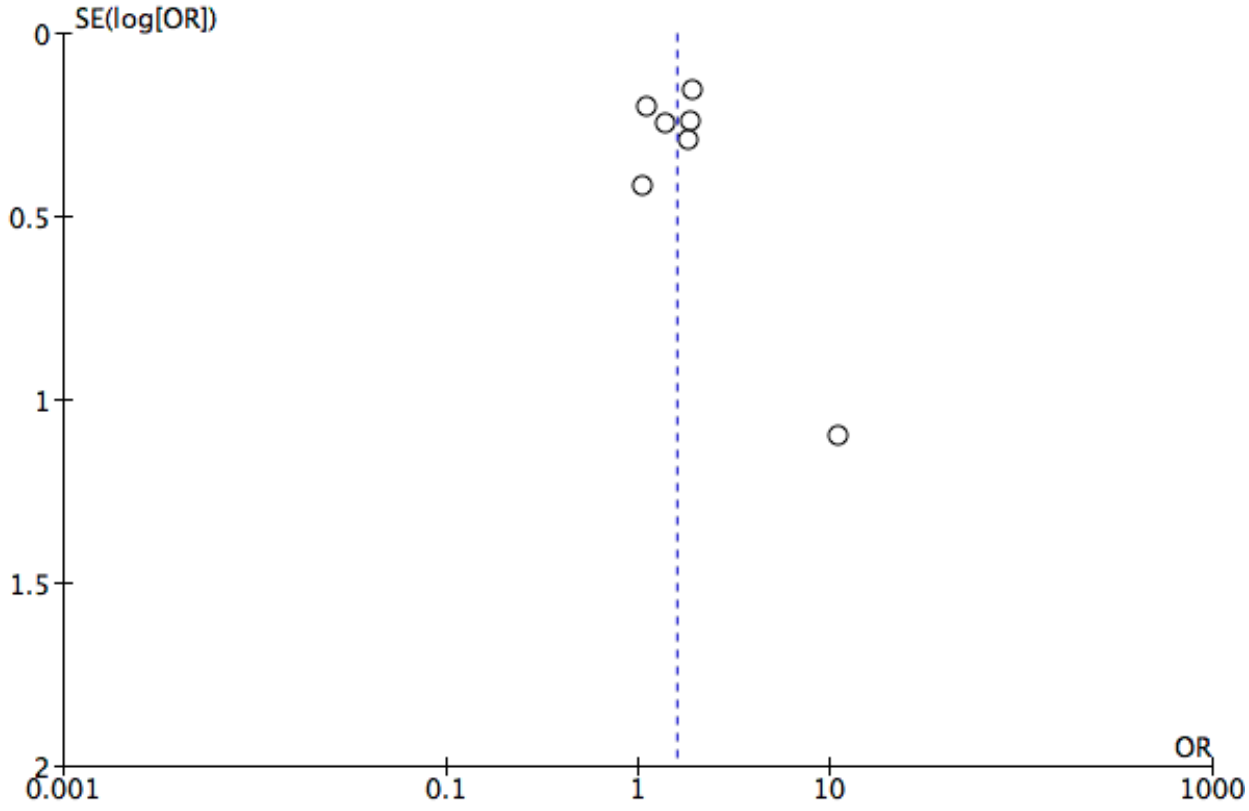

Figure 4.6: Funnel plot (NSAID use vs No NSAID use)

Chapter 5 – Discussion

Overall, the evidence in this study indicates that non-selective NSAIDs are associated with an increased risk of anastomotic leakage following colorectal surgery. When grouped together as an entire class, NSAIDs in our study were found to significantly increase the leak rate of colonic and rectal anastomoses. With further sub-group analysis however this effect was not found to be consistent across all NSAIDs, with medications within the same class demonstrated conflicting risk profiles.

This meta-analysis is the first to look at isolated colonic and rectal anastomotic leak rates, as well as the first to perform drug-specific analysis on the effect of certain NSAIDs on anastomotic healing. As demonstrated in several animal studies, the effect that NSAIDs have on anastomotic healing varies based on the location within the gastrointestinal tract[26, 29, 31]. Small bowel anastomoses have been shown to be affected by post-operative NSAID use to a greater extent than distal colonic anastomoses. Previous meta-analysis performed to assess the impact of NSAIDs on anastomotic healing have failed to take into account this potential location dependent effect that NSAIDs have on the gastrointestinal tract[33, 34, 36].

While this study has taken a first step towards stratifying the impact that NSAIDs have based on anastomotic location, there are still significant limitations present. In our study we grouped all colonic and rectal anastomoses as one category. Research in 2013 from van der Vijver et al.[29] however indicated that the different segments of the colon may have be a variable response to NSAIDs, and that proximal colonic anastomoses may demonstrate a risk profile analogous to that of small bowel anastomoses, while distal colonic anastomoses are affected to a

far lesser degree and do not demonstrate the same risk profile.

In our study we did not analyze patients based on proximal or distal colonic anastomoses, nor did we stratify them based on colonic versus rectal anastomoses. This was in large part due to fact that the studies that have been published looking at colorectal anastomoses and NSAID use do not differentiate between the proximal and distal colon in their analysis. As such we could not obtain the data needed to perform a sub-group analysis such as this. Six of the seven studies we included in our analysis did include anastomotic location within their demographic information, which was used to assess for baseline differences in confounding variables between groups. None of those studies went on to use that information to perform sub-group data analysis based on anastomotic type though (i.e. ileocolic anastomotic leak rate, recto-sigmoid anastomotic leak rate, etc.). Given the current literature indicating that NSAIDs affect not only the gastrointestinal tract to different degrees based on location, but also different segments of the colon based on location, future research should look to either isolate their patients to only one type of anastomosis (i.e. ileocolic anastomoses following right hemi-colectomy), or to provide stratified data based on anastomotic location.

As to why NSAIDs impair anastomotic healing and why different segments of the gastrointestinal tract are affected differently, the pathophysiological mechanism still remains up for debate. Histological factors associated with the healing process certainly seem to be affected following NSAID use. These medications appear to decrease fibroblast infiltration, decrease granulation tissue formation, decrease re-epithelialization, and increase microscopic necrosis[32]. Taken all together this can certainly lead to impaired wound healing and tissue regeneration.

Furthermore, NSAIDs appear to have an effect on collagen deposition and cross-linking, although this effect is not yet clear. In 2004 Cahill et al.[25] hypothesized that NSAIDs impair cross-linkage rather than collagen production, a finding echoed in 2013 by van der Vijver et al.[29] who found no change in collagen concentration within ileal or colonic tissues after NSAID use. In a 2012 study by Klein et al.[28] however, there was in-fact find a statistically significant reduction in collagen in colonic tissue following NSAID administration, contradicting the findings from the previous two studies.

Although it's unclear what the overall effect is on intestinal collagen structure/function, studies have shown that COX-2 expression is greatest in the ileum and that this expression decreases after NSAID use[29, 30]. This has led to theories that the increased leak rate associated with NSAIDs may in-fact be a COX-2 specific inhibitory effect. When tested clinically however this has not been proven to be the case. In 2014 Bhangu et al.[36] performed sub-group analysis in their meta-analysis to stratify selective vs. non-selective NSAIDs. They found that only the non-selective NSAID group was associated with an increased anastomotic leak rate post-operatively, while the selective COX-2 inhibitor group did not demonstrate any significant change in leak rate. These findings are in-line with our own results, in which we found that when patient data was analyzed based on class of NSAID received, only the non-selective NSAID group demonstrated an increased risk of anastomotic leakage.

We then went on to take this analysis one step further and performed an additional sub-group analysis on individual drugs within the non-selective NSAID class. In our study we found the association between post-operative leak rate and medication use inconsistent when two non-

selective NSAIDs, ketorolac and diclofenac, were compared to controls. While both are categorized within the same class of medication, only diclofenac was associated with an increased leak rate. This would indicate that the question regarding NSAID safety is much more complicated than once believed, and that in the long-run there needs to be an appreciation that this may in-fact be a drug-specific effect.

Nevertheless, there are limitations that must be taken into account when interpreting this data. Because we narrowed down our research question to a very focused topic, colorectal anastomotic healing following post-operative NSAID use, the number of studies present within this meta-analysis is relatively low. This is especially important when considering the sub-group analysis that was performed. Only 1 study included selective NSAID medications in their published data, and when performing our medication-specific analysis only 3 studies were included in the diclofenac group, and 2 in the ketorolac group. The individual results from these sub-group analyses (that diclofenac, but not ketorolac or selective NSAIDs were significantly associated with an increased leak rate) must be taken with a grain of salt, as further research is needed to truly clarify these class and drug-specific risk profiles. The real take-away message is that the question regarding NSAID safety is a very complex one that cannot be answered with a broad study that generalizes all NSAIDs as one medication with the same risk and safety profile.

Furthermore, another inherent limitation of a review such as this is the reliance on non-randomized data. Prospective, randomized clinical trials provide the strongest evidence upon which recommendations can be made. Within this paper nearly half of the studies examined posed potential bias secondary to confounding factors that were unaccounted for by the study

authors. Of the remaining studies that measured these confounding factors, to some degree or another they all contained baseline differences between intervention and control groups that made final size effect interpretations difficult. As with any study reliant on observational data, it is difficult to differentiate association with causation. While some studies did show an increased leak rate with NSAID usage, there also remains the possibility that those patients with a post-operative leak required greater pain control in the immediate post-operative setting, necessitating the addition of an NSAID to their management plan. Potential confounders such as these highlight why a true answer to the question regarding NSAIDs and their safety can't be made until high-quality randomized studies are performed.

Within the context of our study, alternative analytic approaches to decrease the confounding inherent in observational studies include instrumental variable analysis and propensity score analysis. Instrumental variable analysis, which has primarily been used in the context of economic research in the past, relies on the use of a third variable, an "instrumental variable", which correlates with treatment selection (i.e. NSAID use in this study) but not directly with the outcome variable (i.e. anastomotic leakage). The instrumental variable creates variance to estimate the effect of the treatment on the outcome. The inherent difficulty in using this approach is in finding an instrumental variable that correlates strongly with the treatment variable while also not having a direct effect on the outcome, other than indirectly through the treatment. This often times relies on expert opinion. The availability of such data in the context of many of these observational studies is often times limited as well. Such an analysis is best performed when large data sets are available, as is the case in many large scale economic analyses.

Going forward significant efforts will need to be made to confidently identify which individual NSAIDs increase the risk of anastomotic leakage and which don't. Given the increase in non-opioid analgesia in many centers these days, there are now opportunities to develop large, prospectively randomized control trials comparing individual NSAIDs to both placebo and each other. These studies would minimize many of the inherent biases present in the retrospective reviews available today, and allow for individualized assessment of NSAIDs rather than the class based studies we currently have. Large prospective studies, such as a theoretical one that randomized patients to a diclofenac group, a ketorolac group, and a placebo/traditional opioid analgesia group, would ultimately lead to the production of definitive guidelines regarding drug safety during anastomotic healing.

5.1 – Conclusion

Anastomotic leakage results in significant morbidity and mortality for patients. While current ERAS guidelines recommend non-opioid analgesia post-operatively for colorectal patients, our findings indicate that the use of NSAIDs in the immediate post-operative period (within 7 days) in these patients may increase the risk of a post-operative anastomotic leak.

Where no conclusive recommendation currently exists regarding post-operative NSAID use, we advise great caution when prescribing NSAIDs following colonic or rectal anastomotic creation. While our findings indicate that there may be certain NSAIDs that are safe for post-operative analgesia, given the large amount of work still needed to properly clarify the individual risk profiles of these medications, a safe first step may be to caution against their use entirely. As

more data regarding individual NSAIDs becomes available, further refinement of the guidelines can be implemented to ensure that these medications are administered only when proven safe.

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Tables

Table 1 – Study Characteristic Table (Bakker et al. 2006)

Bakker et al. 2006

Risk of Bias	**Moderate** Groups dissimilar with respect to baseline confounding variables
Study Design	**Retrospective Cohort Study**
Population	**856 Patients** Netherlands Single-Center Inclusion Criteria: Patients undergoing an elective colon or rectal resection with primary anastomosis because of malignancy and treated within an enhanced recovery program Exclusion Criteria: Benign disease, acute operation, colostomy closure, and any form of stoma except for a deviating ileostomy in rectal resection with primary anastomosis after preoperative chemo/radiation
Intervention	**N = 534** NSAID started on the second postoperative day and continued until oral analgesia could be reduced. Relevant dose of NSAID defined as a prescription of more than one consecutive day. Between 2006 and 2009, ibuprofen was prescribed for patients who were 70 years of age and younger and nabumetone was prescribed for patients older than 70 years of age. Between 2010 and 2013, the protocol was changed and diclofenac was the NSAID prescribed for all patients
Control	**N = 322** No NSAIDs given post-operatively
Outcome	**Anastomotic Leak Rate** No time limit given from surgery Included abscess formation in the quadrant of the anastomosis requiring drainage as an anastomotic leak Secondary Outcomes: Surgical site infection, fascial dehiscence, ileus, pneumonia, urinary tract infection, in-hospital mortality
Results	Intervention: 9.2% Control: 5.3% **p<0.05** **Odds Ratio: 1.81 [1.03, 3.21]**

Table 2 – Study Characteristic Table (Gorissen et al. 2012)

Gorrissen et al. 2012	
Risk of Bias	**Moderate** Groups dissimilar with respect to baseline confounding variables
Study Design	**Retrospective Cohort Study**
Population	**795 Patients** Netherlands Multi-Center Inclusion criteria: Patients undergoing colonic or rectal resection with primary anastomosis between Jan. 2008 and Dec. 2010 Exclusion criteria: none given
Intervention	**N = 324** Any NSAID use within the first 5 days post-operatively
Control	**N = 471** No NSAIDs given post-operatively
Outcome	**Anastomotic Leak Rate** Defined as clinical and radiological signs of anastomotic leakage as confirmed by re-operation or occurrence of an enterocutaneous fistula Secondary outcome: Complications according to Dindo-Clavien classification system
Results	Intervention: 13.2% Control: 7.6% **p<0.05** **Odds Ratio:** **1.85 [1.16, 2.95]**

Table 3 – Study Characteristic Table (Klein et al. 2009)

Klein et al. 2009	
Risk of Bias	**Moderate** Groups dissimilar with respect to baseline confounding variables that were measured
Study Design	**Retrospective Cohort Study**
Population	**75 Patients** Denmark Single-Center Inclusion criteria: Patients undergoing laparoscopic colorectal resection with primary anastomosis between October 2004 and June 2007. All cases done by the same operating team. Exclusion criteria: none given
Intervention	**N = 33** Oral diclofenac (150mg/day) starting POD #1
Control	**N = 42** No diclofenac given
Outcome	**Anastomotic Leak Rate** Defined as clinically significant leakage where re-operation was needed Secondary outcomes: None
Results	Intervention: 21.2% Control: 2.4% **p<0.05** **Odds Ratio: 11.04 [1.28, 94.97]**

Table 4 – Study Characteristic Table (Klein et al. 2012)

Klein et al. 2012	
Risk of Bias	**Serious** Not all potential confounding variables measured Groups dissimilar with respect to baseline confounding variables that were measured 4 patients excluded from analysis following commencement of intervention (because took NSAID other than ibuprofen/diclofenac) Participants excluded due to missing data
Study Design	**Retrospective Cohort Study**
Population	**2,756 Patients** Denmark Multi-Center Inclusion criteria: Patients with available electronic medical records who had undergone an elective operation for colorectal cancer between January 1, 2006 and December 31, 2009 with either colonic or rectal resection, and received a primary anastomosis. Exclusion criteria: none given
Intervention	**N = 885** NSAID taken post-operatively (at least two days' treatment) within the first 7 days, with a relevant daily dose of at least 50 mg for diclofenac and at least 800 mg for ibuprofen
Control	**N = 1,871** No NSAID taken post-operatively
Outcome	**Anastomotic Leak Rate** Defined as as clinical leakage requiring acute surgical intervention such as re-laparoscopy or re-laparotomy Radiological or endoscopic drainage was not considered surgical intervention Secondary Outcome: Mortality within 30 days
Results	Intervention: 9.4% Control: 5.1% **p<0.05** **Odds Ratio: 1.93 [1.42, 2.63]**

Table 5 – Study Characteristic Table (Paulasir et al. 2015)

Paulasir et al. 2015	
Risk of Bias	**Serious** Not all potential confounding variables measured. Groups dissimilar with respect to baseline confounding variables that were measured.
Study Design	**Retrospective Cohort Study**
Population	**4,360 Patients** United States Multi-Center Inclusion criteria: Non-pregnant patients over the age of 18 who underwent colon and rectal surgery with bowel anastomosis between July 2012 through February 2014 Exclusion criteria: Age under 18, current pregnancy, American Society of Anesthesiologists (ASA) class 5 and 6, the presence of preoperative open wounds with or without infection
Intervention	**N = 1,297** NSAID (not specified) taken on post-op day 1
Control	**N = 3,063** No NSAID taken post-operatively
Outcome	**Anastomotic Leak Rate** Defined as as a clinically diagnosed leak at the site of the intestinal anastomosis requiring one or more of the following interventions: antibiotic treatment, percutaneous drainage, re-operation with new anastomosis, re-operation with proximal diversion, or re-operation with end stoma Secondary outcomes: any surgical site infection, sepsis, death within 30 days of surgery
Results	Intervention: 2.9% Control: 2.6% **p>0.05** **Odds Ratio: 1.11 [0.75, 1.65]**

Table 6 – Study Characteristic Table (Saleh et al. 2015)

	Saleh et al. 2015
Risk of Bias	**Moderate** Groups dissimilar with respect to baseline confounding variables that were measured
Study Design	**Retrospective Cohort Study**
Population	**731 Patients** Canada Multi-Center Inclusion criteria: Patients who underwent elective colorectal surgery with primary anastomosis between March 2004 and December 2011 Exclusion criteria: Patients who took any NSAID other than ketorolac within 5 days of surgery
Intervention	**N = 355** Intraoperative and/or postoperative ketorolac administered within 5 days of surgery
Control	**N = 376** No ketorolac received within the first 5 post-operative days
Outcome	**Anastomotic Leak Rate** Defined as a documented leak at the time of re-operation and/or radiologically confirmed based on contrast leakage or abscess at the site of the anastomosis with or without percutaneous drainage Secondary outcome: whether the total ketorolac dose received was associated with an increase in anastomotic leak rate.
Results	Intervention: 3.4% Control: 3.2% **p>0.05** **Odds Ratio: 1.06 [0.47, 2.39]**

Table 7 – Study Characteristic Table (Subendran et al. 2014)

Saleh et al. 2015	
Risk of Bias	**Serious** Not all potential confounding variables measured.
Study Design	**Nested matched case-control study**
Population	**262 Patients** Canada Single-Center Inclusion criteria: All patients who had elective colorectal surgery at Mount Sinai Hospital in Toronto between January 2001 and June 2012 Exclusion criteria: None given
Cases	**N = 131** Anastomotic leak postoperatively (within 12 months) as identified by radiologic investigations and/or direct confirmation at the time of re-operation
Control	**N = 131** 1:1 matching with cases based on underlying disease, type of surgery, age (within 5 years), sex, and year of surgery (within 5 years)
Exposure	**Primary Exposure:** Any post-operative NSAID use within the first 5 days post-surgery Secondary Exposure: Use of ketorolac
Results	Cases: 52.7% exposed to NSAIDs Control: 44.3% exposed to NSAIDs **p>0.05** **Odds Ratio: 1.40 [0.86, 2.28]**

Appendix

Appendix 1 – American Society of Anesthesiologists (ASA) Physical Status Classification System[48]

ASA 1	A normal healthy patient
ASA 2	A patient with mild systemic disease
ASA 3	A patient with severe systemic disease
ASA 4	A patient with severe systemic disease that is a constant threat to life
ASA 5	A moribund patient who is not expected to survive without the operation
ASA 6	A declared brain-dead patient whose organs are being removed for donor purposes

Appendix 2 – ERAS guidelines post colorectal surgery[21]

Preoperative Period
Education
Counseling
Optimization of medical comorbidities
Mechanical bowel prep and oral antibiotics
Fasting from fried or fatty foods or meat for eight hours
Fasting from light meals and unclear liquids (eg, tea and toast, juice with pulp, milk) for six hours
Fasting from clear liquids (excludes alcoholic beverages, beverages with milk, juice with pulp) for two hours
No premedication
Carbohydrate drink two hours prior to the procedure

Intraoperative Period
Thromboprophylaxis
Antibiotic prophylaxis
High-concentration inspired oxygenation
Thoracic epidural analgesia
Normothermia
Fluid optimization
Minimally invasive surgical approach
No nasogastric tubes
No intra-abdominal or perineal drains (except in settings such as colonic spillage or purulent drainage)

Postoperative Period
Enteral nutrition beginning on day 1
High-calorie supplements twice daily
Opioid-sparing analgesia
Multimodal antiemetic regimen
Removal of urinary catheter, typically on postoperative day 1
Mobilization using a structured program, typically on the evening of the procedure

Appendix 3 - PRISMA Checklist[43]

Section/Topic	#	Checklist Item
TITLE		
Title	1	Identify the report as a systematic review, meta-analysis, or both.
ABSTRACT		
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of what is already known.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).
METHODS		
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.
RESULTS		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
DISCUSSION		
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.
FUNDING		
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

Appendix 4 – Risk of Bias Assessment (ROBINS-I Tool) - Bakker et al. 2016

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – Confounding variables taken into account in analysis, however groups dissimilar in baseline characteristics with respect to confounding variables The patients in the colon group receiving no NSAIDs were older, had a higher ASA score, more often had cardiac comorbidity, pulmonary comorbidity or diabetes, and more often used prednisone compared to patients receiving diclofenac or ibuprofen. For rectal cancer patients, baseline characteristics showed no differences between the group receiving no NSAIDs and the diclofenac group. Patients in the colon group receiving no NSAIDs more often had an open resection, more often had a hand-sewn anastomosis and had more perioperative blood loss compared to patients receiving diclofenac. In the rectum group, patients receiving no NSAIDs more often had an open resection and less often had a deviating ileostomy compared to patients receiving diclofenac.	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI

1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes – Logistic regression analysis used	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Favours NO NSAID group having more leaks (older patients, higher ASA score, more pre-existing comorbidities, and more often were on prednisone at baseline in NO NSAID group) – (Opposite of actual result)	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?	NA	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – No co-interventions present	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No – Multiple subgroups were analysed, however they were all discussed in the results	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	MODERATE	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	The overall direction was towards finding a higher anastomotic leak rate in the NO NSAID group, which was in-fact the opposite of the effect found (there was a higher leak rate in the YES NSAID group).	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 5 – Risk of Bias Assessment (ROBINS-I Tool) - Gorissen et al. 2012

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – Confounding variables taken into account in analysis, however groups dissimilar in baseline characteristics with respect to confounding variables. There was a significantly higher frequency of pre-existing pulmonary disease in the group treated with combined NSAIDs/Selective Cox-2 Inhibitors. There was also a significantly greater use of laparoscopic techniques in the combine NSAIDs/ Selective Cox-2 Inhibitors group.	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes – Logistic regression analysis used	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Unpredictable	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?	NA	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA– There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	MODERATE	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 6 – Risk of Bias Assessment (ROBINS-I Tool) - Klein et al. 2009

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – All confounding variables taken into account in analysis, however groups dissimilar in baseline characteristics with respect to confounding variables. The two groups were not homogenous with respect to gender, ASA group and operation type. The No diclofenac group was significantly older, had a significantly higher ASA classification, and had sig. more rectal anastomoses.	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes – Logistic regression analysis used	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	The difference in the baseline characteristics that were measured favours a higher leak rate in the NO Diclofenac group (the opposite of the actual result)	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	The difference in the baseline characteristics that were measured would favour a higher leak rate in the NO Diclofenac group, which was the opposite of the results found in this study.	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 7 – Risk of Bias Assessment (ROBINS-I Tool) - Klein et al. 2012

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – Not all confounding variables measured (presence of diverting ostomy not measured), and there were significantly more laparoscopic cases in the NO NSAID group	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes – Logistic regression analysis used	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Serious - Confounder not measured (presence of diverting ostomy), and disproportionate number of laparoscopic cases in the NO NSAID group	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Unpredictable	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	Yes – 4 patients in the NSAID group (out of 885) were not used in the analysis because the used an NSAID other than ibuprofen or diclofenac	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Yes – Administration of another NSAID besides ibuprofen/diclofenac would be related to other analgesia (NSAID or otherwise) received	NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Yes – The administration of other NSAIDs (besides ibuprofen/diclofenac) could be associated with the outcome (anastamotic leakage)	NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Serious	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Yes – 10 patients of 2766 excluded because of missing data about presence or absence of post-op leakage	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?	NI	NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?	NI	NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study), and researchers responsible for compiling data were blinded to outcomes for patients	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	Serious	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 8 – Risk of Bias Assessment (ROBINS-I Tool) - Paulasir et al. 2015

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – Not all confounding variables measured (rectal vs colonic anastomosis not differentiated), and there were significantly differences in baseline confounders between the two groups (age, ASA class, co-morbidities, surgical priority, surgical approach)	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes –logistic regression analysis	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Serious – Potential confounder not measured (colonic vs rectal anastomosis), and disproportionate confounding characteristics between the two groups	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Unpredictable	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	Serious	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 9 – Risk of Bias Assessment (ROBINS-I Tool) - Saleh et al. 2014

Signalling questions	Description	Response options
Bias due to confounding		
1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – All confounding variables measured, but there were differences between the two groups with respect to the confounding variables. The NSAID group was younger, had a lower ASA classification, and had fewer comorbidities.	Y / PY / PN / N
If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes –logistic regression analysis	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate –Disproportionate confounding characteristics between the two groups	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Favours the NSAID group having fewer leaks	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Potentially favours less leaks in the NSAID group, as they were younger, with a lower ASA classification at baseline	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info

Appendix 10 – Risk of Bias Assessment (ROBINS-I Tool) - Subendran et al. 2014

	Signalling questions	Description	Response options
Bias due to confounding			
	1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	Yes – Did not measure all important confounding variables (pre-existing comorbidities not take into account in analysis)	Y / PY / PN / N
	If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, go to question 1.3.	No	NA / Y / PY / PN / N / NI
	1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)		NA / Y / PY / PN / N / NI

Questions relating to baseline confounding only		
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes –logistic regression analysis	NA / Y / PY / PN / N / NI
1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No	NA / Y / PY / PN / N / NI
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA	NA / Y / PY / PN / N / NI
1.8. **If Y/PY to 1.7**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Serious – Baseline patient comorbidities not measured	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to confounding?	Unpredictable	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4	No	Y / PY / PN / N / NI
2.2. **If Y/PY to 2.1**: Were the post-intervention variables that influenced selection likely to be associated with intervention?		NA / Y / PY / PN / N / NI
2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes	Y / PY / PN / N / NI
2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	Yes	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to classification of interventions?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?		Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	NA – There were no important co-interventions	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Yes	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Yes	Y / PY / PN / N / NI
4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?		

Bias due to missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Yes	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	No	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No	Y / PY / PN / N / NI
5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	No	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	No –Retrospective review (outcome assessors were unaware of future study)	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes	Y / PY / PN / N / NI
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	No	Y / PY / PN / N / NI
Risk of bias judgement	Low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Bias in selection of the reported result		
Is the reported effect estimate likely to be selected, on the basis of the results, from...		
7.1. ... multiple outcome *measurements* within the outcome domain?	No	Y / PY / PN / N / NI
7.2 ... multiple *analyses* of the intervention-outcome relationship?	No	Y / PY / PN / N / NI
7.3 ... different *subgroups*?	No	Y / PY / PN / N / NI
Risk of bias judgement	Moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall bias		
Risk of bias judgement	Serious	Low / Moderate / Serious / Critical / NI
Optional: What is the overall predicted direction of bias for this outcome?	Unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

For detailed information regarding scoring guidelines please refer to:

Sterne JAC, Higgins JPT, Elbers RG, Reeves BC and the development group for ROBINS-I. Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I): detailed guidance, updated 12 October 2016. Available from http://www.riskofbias.info