A Development study of Drain Fluid Gastrografin as a Biomarker of Anastomotic Leak

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Signed patient information consent forms (PICF) are held for all participants.
No material from other sources were included so no permission to reproduce is needed.
Trial Registration with ANZCTR: ACTRN12619001687189
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Abstract

Purpose

Anastomotic leakage is the anathema of colorectal surgery. Its occurrence leads to increased morbidity and mortality and a prolonged hospital stay. Much work has gone into studying various biomarkers in drain fluid to facilitate early detection of anastomotic leak (AL). This 2a development study aims to assess the safety and feasibility of reliably detecting the iodine in Gastrografin (GG) in drain fluid and stool samples by Dual Energy Computed Tomography (DECT).

Methods

This is a prospective, observational, controlled, consecutive cohort study establishing the safety and feasibility of the detection of GG in surgical drain fluid and stool as a biomarker of anastomotic leak when patients with a low pelvic colorectal anastomosis undergo luminal flushing of the rectal tube with GG.

Results

Ten consecutive patients were allocated to the saline flush group and the following ten to the GG flush group. Three patients in the saline flush group developed an AL. One patient in the GG flush group developed an AL. An elevation in the drain fluid GG was detected using DECT on the day of clinical deterioration. None of the patients in the control group were found to have a positive result on DECT.

Conclusions

This observational 2a development study demonstrates the safety of a novel approach to the early detection of AL from extraperitoneal colorectal anastomoses. The technique requires validation in a larger cohort and a multi-centre study is planned to investigate the efficacy of GG rectal tube flushes as an early biomarker of AL in low pelvic anastomoses.

What does this paper add to the literature?

This paper assesses rectally administered Gastrografin (GG) as a novel drain fluid biomarker of anastomotic leak (AL). Few studies have investigated the extravasation of intraluminal substances. Intuitively, these are more likely to lead to the diagnosis of AL verses those that simply predict the possibility of a leak.

Keywords: Gastrografin, biomarker, anastomotic leak, Dual energy CT, drain fluid
Introduction

Anastomotic leakage (AL) is the anathema of colorectal surgery. Its occurrence leads to increased morbidity and mortality and a prolonged hospital stay. [1] Much work has been devoted to the identification of patients at high risk of AL, but nonetheless low risk patients still suffer AL and a delay in diagnosis has a measurable effect on mortality. [2]

Current approaches to the early detection of AL are nonspecific and insensitive. Systemic biomarkers have poor positive predictive value but better negative predictive value and are more useful in indicating which patients are at lower risk of AL, and thus are considered safe for discharge following surgery [3]. Drain fluid biomarkers have the potential to sample the environment around the anastomosis and have shown promise but suffer from a lack of validated specificity and cut off values. As a consequence, AL is often diagnosed at an advanced stage with the presentation of clinical symptoms and often secondary complications.

Gastrografin®, Bayer, Australia, is a water soluble, contrast solution commonly used for abdominal computer tomography (CT). In clinical practice, when administered orally or as an enema, Gastrografin (GG) acts as a radiological contrast for the detection of anastomotic leaks by CT.

The iodine in Gastrografin can be measured directly and quantitated with Dual Energy CT (DECT) and the protocol has been established and validated in a prior
study (Imaging protocol in Appendix 1)[4]. In patients who routinely have a rectal tube inserted, after a low colorectal anastomosis, this tube may be flushed with GG QID. Measuring the iodine in a sample of the drain fluid may serve as a biomarker (BM) of extravasation of the GG and thus a BM of AL of luminal contents.

The IDEAL collaboration has set out recommendations for the introduction of new surgical techniques. [5-7] Stage 2a Development studies report prospective outcomes from a single centre with a small and selected sample size. They require ethics approval [8], registration of the protocol and description of any modification to the technique as the study progresses. Outcomes reported are primarily safety but also technical and procedural success. [9]

This (Phase 1) 2a Development study aimed to assess the safety of GG rectal tube flushes and the feasibility of detecting Gastrografin in post-surgical samples by Dual Energy CT (DECT).

Methods

This is a prospective, observational, controlled, consecutive cohort, 2a Development study establishing the safety of GG rectal tube flushes. The study also assesses the feasibility of a new methodology for the detection of Gastrografin in surgical drain fluid and stool, following a low colorectal anastomosis and without a diverting loop ileostomy. This study was based at a
single private tertiary institution and with 3 participating surgeons. Patients were enrolled between November 2018 and September 2019.

Safety was assessed by recording the post-operative mortality and complications as graded by the Clavien-Dindo classification [10]. This study was prospective, signed patient consent was obtained and ethics approval was granted by the St Vincent’s Health and Aged Care (SVHAC) Human Research Ethics Committee (HREC): HREC 19/01. The trial was registered with the Australian New Zealand Clinical Trials Registry: ACTRN12619001687189.

Participants

Patients with the following inclusion and exclusion criteria were invited to participate.

**Inclusion criteria:**
1. Patients undergoing a rectal resection with an extraperitoneal anastomosis (ie. within 10cm of the anal verge), and without a diverting loop ileostomy;
2. Placement of a pelvic drain and rectal tube at surgery.

**Exclusion criteria:**
1. Allergy to iodine

A sample size of 20 was chosen. This is in line with the IDEAL recommendations for a 2a Development study. We recruited 20 consecutive patients undergoing a rectal resection with an extra-peritoneal anastomosis and without a diverting loop ileostomy. The first 10 patients underwent flushing of the rectal tube with
Normal Saline (30mls QID) and served as a control group. **This is the usual clinical practice in our institution, to prevent blockage of the rectal tube.** The subsequent 10 patients, who were eligible for participation, had their rectal tubes flushed with GG (30mls QID). The morning flush was administered at 6.00am. The drain fluid was collected at 6.30am daily by the night shift nurses. A sample of rectal tube fluid was also collected 15 to 30 mins after administration of the Saline or GG flush to assess the baseline intra-luminal iodine level.

**Gastrografin safety and applications**

Gastrografin (Diatrizoate, also known as amidotrizoate meglumine and sodium amidotrizoate, Bayer Australia, registered trademark of the Bayer group, Germany) is a hyperosmolar water-soluble iodinated radiological contrast media. GG is recorded as ARTG ID: 10684 on the Australian Register of Therapeutic Goods maintained by the Therapeutic Goods Administration (TGA). [11]

GG may be administered per orally or as an enema, and is used routinely in clinical practice. Its safety has been established in a number of randomised trials. [12] A meta-analysis of contrast enemas found that procedures involving GG were safe with only 1 reported complication of the 1169 procedures studied. GG and Urografin were the most common agents used in these radiological procedures. [13]
Aside from its routine use in radiology, GG has been safely employed orally in patients with small bowel obstruction and assessed as a prokinetic in prolonged post-operative ileus. [14, 15]

**Gastrografin Measurement by DECT**

Dual emission CT scanning (DECT) is a new technology that allows acquisition of 2 datasets from the same anatomical region at different voltages. [16] In contrast to single-spectrum imaging, which depicts the organs based on spatial distribution of the object attenuation, DECT is sensitive to the chemical composition. Hence, DECT is capable of differentiating materials with different atomic numbers. The organically bound iodine in Gastrografin is able to be simply quantitated using a DECT protocol described in appendix 1. The lower limit of detection of a solution containing GG was at a concentration of 0.097%, which correlated to an iodine density of > 1 mg/ml. The upper limit of the true negative levels of GG measured in drain fluid by DECT was derived by calculating the mean (+ 3 SD) iodine density (mg/ml) in GG negative samples. We have established measurements of iodine density above 1.2 mg/ml to represent a positive result for the presence of GG in a solution measured by DECT. [4]

**Pelvic Drains**

Participating surgeons in this study routinely place a pelvic drain after a low anterior resection with an extra-peritoneal anastomosis. It is possible that drains may be malpositioned or dislodged and this may lead to a false negative result in
the setting of AL detection. Whilst it is not intended that all patients should undergo routine imaging to confirm position, the majority of those experiencing a clinical AL will undergo CT imaging as part of usual clinical practice, presenting an opportunity for the assessment of the drain position in the pelvis.

**Rectal Tubes**

In our institution, it is routine practice to place a 28G Foley catheter per-anally and across the sphincter in all patients undergoing a rectal resection with an extra-peritoneal anastomosis. Balloons are inflated with 12mls of water or sutured in place with the balloon deflated. The purpose of the rectal tube is to decompress the rectum across the sphincter and often it will sit across the anastomosis. Rectal tubes are routinely flushed with 30mls saline QID following surgery to prevent blockage.

**Sample collection**

**Measurement of Drain fluid Gastrografin** levels (Day 1 to 5 or longer if drain remains in situ after 5 days)

Drain fluid samples were collected from Bellovac drains 15 to 30 mins after administration of the morning rectal tube flush. The drainage bag (A) is removed and the bellows emptied to a sterile plastic pot. A 10 mL sample was collected at Day 1, 2, 3, 4, 5 or until drain removed for the measurement of GG by DECT.
A single sample of rectal tube fluid was collected on the first post-operative day to measure the intra-luminal GG level.

The daily collection of drain or rectal fluid samples presents no risk, discomfort or inconvenience to the patient.

**Figure 1.** Drain fluid measurement

**Data Collection**

Consecutive, eligible patients provided written consent for inclusion in the study. Patient demographics, surgery details and post-surgical information was obtained. All patient information was de-identified by the assignment of a unique numeric code, known only to the investigators listed on this project.

Patient vital signs (pulse rate, blood pressure and temperature) were collected and recorded daily. All adverse events were assessed for any temporal relation to the administered GG flushes. AL is defined and graded by the criteria set out in the International Study Group document. [17]

**Blinding of samples for DECT**

The drain fluid samples were blinded to the reporting radiologist and scanned as a batch. The results were not available to the treating surgeon.


**Analysis and statistics**

All iodine measurements by DECT and statistical analysis was performed using de-identified data. Due to the small sample size in each arm of the study, descriptive statistics were computed for all measurements and displayed graphically using GraphPad Prism 8, GraphPad, San Diego, California, USA.

**Results**

Twenty-one patients were assessed for inclusion. One patient was ineligible due to an iodine allergy. The first ten consecutive patients were allocated to receive QID rectal tube flushes with 30 mls saline, as per standard care. Three of these patients experienced an AL (30%), as diagnosed by CT imaging and clinical course. The following consecutive ten patients were allocated to receive QID rectal tube flushes with 30mls GG. One patient in this group experienced an AL (10%). There was no mortality in either group. All patients underwent a stapled endoluminal anastomosis.

Three patients in the saline flushes group, who experienced an AL, were returned to the operating theatre (OT) where they underwent a laparoscopic abdominal lavage and a defunctioning ileostomy was fashioned. The one patient in the GG flushes group was also returned to OT for a laparoscopic abdominal lavage and defunctioning ileostomy. All ALs were graded as Grade C. [17]All 4
patients, who experienced an AL during the study, underwent a standard, single source CT scan on the day of clinical deterioration and the drains were all confirmed to be in the correct pelvic position. Patients who did not experience an AL did not undergo a CT scan.

The basic observations (temperature, pulse rate and blood pressure) were monitored and recorded for each patient during the study. No patient was found to have an adverse reaction to the GG during administration. The GG flushes were well tolerated by the patients. There was no prolongation of length of stay (LOS), or increase in AL rate observed in the GG flushes group.

The characteristics of the participants are summarised in Table 1.

**Table 1.** Characteristics of the two study groups; Saline and Gastrografin flushed rectal tubes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (N=20)</th>
<th>Saline flushes (n=10)</th>
<th>Gastrografin Flashes (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>65.5 (54.0 to 76.3)</td>
<td>67.0 (58.0 to 72.5)</td>
<td>64.0 (46.5 to 82.5)</td>
<td>0.999</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (40%)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td>0.650</td>
</tr>
<tr>
<td>Male</td>
<td>12 (60%)</td>
<td>5 (50%)</td>
<td>7 (70%)</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.5 (65.0 to 98.8)</td>
<td>70.0 (64.0 to 84.0)</td>
<td>94.5 (74.3 to 100.0)</td>
<td>0.112</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172.5 (163.3 to 179.5)</td>
<td>169.5 (160.8 to 177.8)</td>
<td>174.0 (169.0 to 180.0)</td>
<td>0.344</td>
</tr>
<tr>
<td>BMI, Kg/m^2</td>
<td>25.3 (23.9 to 29.6)</td>
<td>25.2 (22.3 to 26.6)</td>
<td>27.0 (24.6 to 32.7)</td>
<td>0.173</td>
</tr>
<tr>
<td>LOS, days</td>
<td>5.5 (3.0 to 10.0)</td>
<td>8.5 (3.8 to 10.3)</td>
<td>5.0 (3.0 to 6.3)</td>
<td>0.247</td>
</tr>
</tbody>
</table>
In this study, all drain fluid specimens following rectal tubes flushed with saline (Saline Flushes group) resulted in iodine density levels below the detection threshold of 1.23 mg/ml (Figure 2).

**Figure 2: Iodine density (mg/ml) in drain fluid specimens following colorectal surgery in patients not administered Gastrografin.** The dotted line represents the derived detection threshold of 1.230 mg/ml (mean + 3SD).

All rectal tube specimens (intra-luminal) from the saline flush group also returned results below the detection threshold (1.23 mg/ml iodine), as expected.
(Figure 3). All rectal tube specimens, from the GG flush group, returned measurable iodine levels. Thus, high levels of GG were present in the neo-rectal lumen of this group (Figure 3). Two samples collected from patients administered GG flushes resulted in iodine levels above the levels measurable by DECT (>100 mg/ml). This was expected due to beam hardening artefact in the setting of undiluted GG rectal tube flushes. There were no false positives or false negatives.

**Figure 3: Measurement of Gastrografin by Dual Energy CT, in stool of patients administered rectal tube flushes with Saline or Gastrografin**

Drain fluid measurements of GG in the saline flush group showed no increase in iodine in the three AL patients. (Figure 4).

**Figure 4. Log iodine density (mg/ml) measurements by DECT in drain fluid of patients administered saline rectal tube flushes.** Patients experiencing AL are shown in red, dotted line represents cut off at 1.23 mg/ml iodine density.
**Figure 5.** Log iodine density (mg/ml) measurements in the drain fluid of patients administered Gastrografin rectal tube flushes. One patient experienced AL is shown in red, dotted line represents detection cut off at 1.23 mg/ml iodine density.

In this series there were 3 false positive readings observed at day 2 and day 3 in the drain fluid of patients who did not experience an AL. (Figure 5) These 3 unexpected results were re-evaluated with Induction Coupled Mass Spectroscopy (ICPMS) and iodine measurements concur that the levels measured by DECT were positive for Iodine. The results measured by ICPMS confirm the accuracy of measurement by DECT, but don’t explain the unexpected results. Two of these abnormal results returned to levels below the cut-off threshold in patients with subsequent samples and the other patient had already had their drain removed and been discharged so no further samples were available for analysis. As all three patients were clinically well and their vital observations did not indicate any concerns, they did not undergo any cross-sectional imaging.

A CT scan was performed to investigate the one patient with a suspected AL in the GG Flushes group (Figure 6). This was performed without any routine enteric radiological contrast for the investigation but the extravasated GG flush was
readily apparent in the axial slices, highlighting and confirming the diagnosis of AL.

**Figure 6.** Axial CT scan to investigate suspected anastomotic leak in the one patient in the GG flushes group who suffered an AL.

At long term postsurgical follow up, all patients who experienced AL during the study had their ileostomy closed and intestinal continuity restored. There were no delayed ALs experienced by study participants.

**Discussion**

This study investigates the proof of concept of a novel biomarker of AL in patients with a low pelvic extraperitoneal colorectal anastomosis and without a diverting loop ileostomy. The flushing of rectal tubes with GG is safe and may be an early BM of AL. The measurement of BMs using DECT of ex vivo drain fluid avoids any exposure to ionizing radiation to the patients.

Many studies and systematic reviews have been published investigating drain fluid biomarkers. [2, 18, 19] The inflammatory cytokine biomarkers are difficult to interpret as they are measuring the environment around the anastomosis and tissue trauma, repair and remodelling would be expected processes occurring at
the operative site in the pelvis. Whilst they are poor biomarkers of AL, they indicate that non-specific inflammatory processes are occurring early in the post-operative period. A re-analysis of prior trial data by Sammour et al [20], suggested that peritoneal cytokines can predict clinically important AL on day 1 after surgery.

Pelvic drain use remains controversial [1]. A 2017 review assessed 7 systematic reviews, one randomised controlled trial and 7 cohort studies, and concluded that that routine drainage has no significant impact on the rate of colorectal AL but may have a selective utility when the operative field is not dry [21]. The included GRECCAR 5 randomised controlled trial reported no difference in the drained and undrained groups [22]. Earlier reviews have reported a lower AL rate and lower intervention rates in drained patients [23].

The use of rectal tubes is supported by a randomised controlled trial [24] and a subsequent meta-analysis [25]. The authors report that transanal tubes are safe and effective in decreasing the rate of clinically significant anastomotic leaks and mitigating the clinical consequences of leakage. The meta-analysis included 909 patients, with one RCT, one prospective study, and two retrospective studies. A 2018 retrospective study employing propensity score analysis, came to similar conclusions [26]. This represents Level 1 evidence following the Oxford Centre of Evidence Based Medicine [27].

In 2010, the International Study Group of Rectal Cancer, proposed a definition and grading system for anastomotic leak [17]. The authors recognised that
numerous definitions of AL were found in the literature, and that reported AL rates varied considerably from 3% to 23%. The agreed definition was: “a defect of the intestinal wall at the anastomotic site leading to a communication between the intra- and extraluminal compartments”. The thesis underpinning this study is that measurement of an exclusively luminal substance will be detectable in the extraluminal compartment, via a pelvic drain, as an AL occurs. The hope is that early detection of AL may allow investigation and intervention in the window before serious clinical deterioration occurs.

In this (Phase 1) 2a Development study, the DECT was able to detect and quantitate the iodine present in the rectum after regular GG flushes of the rectal tube in all ten patients administered GG. Furthermore, all values of rectal tube fluid and drain fluid in the ten patients administered saline flushes were below the cut-off threshold for iodine detection. The one patient, who suffered an AL in the GG flush group, showed an elevated GG level on the day of AL and was returned to OT for management. However, 3 other drain fluid specimens returned values above the cut-off threshold from patients who did experience a confirmed AL. Those patients recovered uneventfully and were well at follow up. Perhaps these patients did suffer a minor AL, detectable in the drain fluid only by DECT, but with no clinically significant effect on the patient or their vital signs. Sample collection error is another possibility. A larger study is required to further examine these findings but may prove to be valuable if minor ALs can be identified early in the post-operative course. This may lead to a heightened level of vigilance with more frequent clinical review and lower the threshold for cross-sectional imaging.
In an earlier pilot study [28], from this institution, extravasation of the enzyme Amylase was measured in the drain fluid of patients undergoing restorative surgery with an ileal J pouch for ulcerative colitis and without a diverting loop ileostomy. We found very high levels of the enzyme within the lumen of the pouch (over 1000 times the serum reference range) but levels in the drain fluid, of those patients who did not experience an AL, approximating the serum levels. We concluded that this extravasated intraluminal enzyme rose significantly in drain fluid samples of the patients who did experience an AL. We postulated that drain fluid analysis of extravasated intra-luminal substances may allow for the detection of AL before clinical deterioration of the patient.

In the IDEAL recommendations [5–7], 2a Development studies allow for protocol modifications to be made during a study and reported. No major protocol alterations occurred during this study, but ongoing nursing instruction and education was found to be important to ensure accurate sample collection and consistency.

A limitation of this study is the small sample size. The study was not powered to allow comparison of the control and intervention groups. The aim of safety assessment was delivered by the relatively blunt tool of recording graded complications and mortality. As all eligible patients did not have a defunctioning loop ileostomy, they represent a cohort of patients at a lower risk of AL. The risk factors, considered by the surgeons to arrive at the decision not to defunction the colorectal anastomoses, were not assessed.
The aim of this controlled development study was to demonstrate safety of the proposed study methodology and the feasibility of the DECT measurements of iodine in drain fluid and stool. The clinical application is to employ DECT measurements of iodine in drain fluid as an early BM of AL in colorectal surgery with an extraperitoneal anastomosis. Our preliminary findings achieve this objective and show promise of this novel approach but due to a small sample size in this 2a Development study, further evaluation is required.

**Conclusion**

This observational 2a Development study demonstrates the safety of a novel approach to the early detection of AL in low pelvic colorectal anastomoses and would appear to have merit. The technical protocol is feasible and reliable but requires validation in a larger cohort and a multi-centre study is planned to investigate the efficacy of GG rectal tube flushes as an early biomarker of AL in low pelvic anastomoses.

**Declarations**

**Funding**

No funding was received for this study. All investigations were performed ‘in kind’.

**Conflict of interest**
The investigators declare that there are no conflicts of interest.

**Author contributions**

All authors contributed to the study design, manuscript review and agree to be accountable for the final manuscript.

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References


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