Clinical Science

Acute phase proteins in drain fluid: a new screening tool for colorectal anastomotic leakage? The APPEAL study: analysis of parameters predictive for evident anastomotic leakage

Niels Komen, M.D.^{a,*}, Juliette Slieker, M.D.^a, Paul Willemsen, M.D.^b, Guido Mannaerts, M.D., Ph.D.^c, Piet Pattyn, M.D., Ph.D.^d, Tom Karsten, M.D., Ph.D.^e, Hans de Wilt, M.D., Ph.D.^{a,f}, Erwin van der Harst, M.D., Ph.D.^g, Yolanda B. de Rijke, Ph.D.^h, Magdalena Murawska, Ph.D.ⁱ, Johannes Jeekel, M.D., Ph.D.^a, Johan F. Lange, M.D., Ph.D.^a, The APPEAL Study Group

^aDepartment of Surgery, Erasmus University Medical Center, Rotterdam, The Netherlands; ^bDepartment of Surgery, ZNA Middelheim, Antwerp, Belgium; ^cDepartment of Surgery, St. Franciscus Gasthuis, Rotterdam, The Netherlands; ^dDepartment of Surgery, University Hospital Ghent, Ghent, Belgium; ^eDepartment of Surgery, Reinier de Graaf Groep, Delft, The Netherlands; ^fDepartment of Surgery, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; ^gDepartment of Surgery, Maasstad Ziekenhuis, Rotterdam, The Netherlands; ^hDepartment of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁱDepartment of Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands

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Abstract

BACKGROUND: We aim to determine if C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), and procalcitonin (PCT) in drain fluid can serve as screening tools for colorectal anastomotic leakage (CAL).

METHODS: Patients included in this multicenter prospective observational study underwent left hemicolectomy, sigmoid resection, high anterior resection, low anterior resection, or subtotal colectomy. During the first 5 postoperative days, CRP, LBP, and PCT were determined on drain fluid.

RESULTS: In total 243 patients were included, of whom 19 (8%) developed CAL. CRP levels were higher in patients with leakage on day 3 and day 5, levels of LBP were higher on days 2, 3, and 4, and PCT levels were higher on day 5. Multivariate analysis showed LBP to be significantly related to CAL. An increase in the average initial value at the first postoperative day with 1 standard deviation increased the risk of leakage by 1.6 times.

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* Corresponding author. Tel.: +32-485-638784; fax: +32-382-51308.

E-mail address: nielskomen@hotmail.com

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CONCLUSION: Increased concentrations of LBP in drain fluid are significantly associated to a higher chance of CAL and could contribute in a future prognostic model for CAL.
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Despite the vast body of evidence, colorectal anastomotic leakage (CAL) remains a poorly understood complication of colorectal surgery. The reported incidence of CAL is estimated between 2.4% and 19%,^{1–3} and mortality rates because of sepsis and multiorgan failure are around 15%.⁴ With current screening and diagnostic methods, the interval between construction of the colorectal anastomosis and diagnosis of leakage varies between 6 and 13 days.^{5–7} Several studies have suggested that delay of diagnosis of CAL is associated with higher mortality rates and that only early management improves clinical outcome.^{8–10} Therefore, new screening methods allowing detection of CAL in the early postoperative phase are needed. A biomarker reflecting the local inflammation, ie around the anastomosis, could be an objective screening tool for CAL in an early phase.

C-reactive protein (CRP) is an acute-phase protein displaying rapid and pronounced rise of its serum concentration in response to infection or inflammation. It is mainly produced by hepatocytes; however, Kupffer cells, blood monocytes, and alveolar macrophages have been shown to produce CRP as well.¹¹

Lipopolysaccharide-binding protein (LBP) is a glycoprotein produced mainly in the liver, but is also produced in the skin, lung, intestine, and at local sites of injury and infection. Particularly LBP produced at local sites of infection is considered to contribute to bacterial clearing.¹²

Procalcitonin (PCT) is a potential marker of acute inflammation that is released from different parenchymal tissues and differentiated cell types throughout the body, mostly from the liver and adipose tissue, in response to interleukin levels increased in case of bacterial infections.^{13–16}

The aim of this study is to determine if CRP, LBP, and PCT in drain fluid can serve as noninvasive screening tools for CAL in the early postoperative phase after colorectal surgery.

Methods

Patients included in the Analysis of Parameters Predictive of Evident Anastomotic Leakage (APPEAL) study received left-sided colorectal resection with the construction of an anastomosis and were given an intra-abdominal drain. Seven medical centers in The Netherlands and Belgium participated in this study. The study, registered in the Dutch Trial Register (<http://www.trialregister.nl>, study number NTR 1258), was approved by the medical ethical committee of all the participating centers, in accordance with the ethical standards of the Helsinki Declaration of 1975, and all patients gave informed consent. Participating centers included patients consecutively between January 2007 and December 2009.

Inclusion and exclusion criteria

Patients undergoing left hemicolectomy, sigmoid resection, high anterior resection (with partial mesorectal excision), low anterior resection (with total mesorectal excision), and subtotal colectomy with ileorectal anastomosis were included. Oncologic resections as well as resections for benign diseases were included. Emergency operations were excluded because of the high probability of coexisting tissue damage and logistical difficulties. Reversals of colostomy were also excluded since the primary disease was already treated. Furthermore, patients under 18 years of age, patients who refused to participate, and patients who did not receive a drain were excluded.

Surgical procedure

The surgical procedure was left to the surgeon's discretion. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain. Guidelines concerning bowel preparation in the participating centers were respected. Patients were operated by laparotomy or laparoscopy and the anastomosis was stapled or hand-sutured. A diverting stoma was constructed according to the surgeon's preference. To obtain drain fluid, a drain was placed at the anastomotic site and was left in place during the first 5 postoperative days. The type of drain used was left to the surgeon's discretion.

Determination of C-reactive protein, lipopolysaccharide-binding protein, and procalcitonin

Drain fluid reservoirs were emptied 2 times a day with 12-hour intervals, respecting the rules of sterility. The evening collection was disposed of. The morning collection was centrifuged for 10 min at 2,800g and 4°C. The supernatant was brought into different cryotubes that were frozen at –80°C to allow analysis in batch.

LBP was determined by a 2-sited chemiluminescent immunometric enzyme assay (IMMULITE 1000, Siemens, Los Angeles, CA). For CRP determination, the Tina-quant assay on the Hitachi 912 (Roche Diagnostics, Penzberg, Germany) was used. PCT was determined with an immunoluminometric assay (LUMI-test PCT, BRAHMS Diagnostika, Berlin, Germany).

Definitions

The endpoint of the APPEAL study was symptomatic CAL. This was defined as a clinically manifest

insufficiency of the anastomosis leading to a clinical state requiring intervention, confirmed by radiological studies, reoperation, or fecal discharge from the drain. Radiologic confirmation of CAL was defined as extravasation of endoluminally administered water-soluble contrast and/or significant perianastomotic air on computed tomography or X-ray. Radiological studies were not routinely performed, only in case of clinical suspicion of CAL. Interventions to treat CAL consisted of therapeutic drainage (prolonged stay of drain), use of therapeutic antibiotics, or a surgical intervention, ie construction of a diverting stoma, disconnection of the anastomosis, and construction of a new anastomosis or a colostomy. All postoperative fistulas communicating with the surgical anastomosis were classified as a leak. Postoperative abscesses were classified as anastomotic leakage if there was extravasation of enteric contrast on radiological studies, if there was significant perianastomotic air, or if communication with the anastomosis was noted after radiologic drainage. Postoperative mortality was defined as patients who died within 30 days of operation, in hospital and after discharge.

Data collection

Patients were followed from their preoperative admission on the ward until the first postoperative follow-up at the outpatient clinic. Demographic data of the patients, operative details, postoperative events, and follow-up data were obtained through a standardized case record form and entered into a database. In case of CAL, the postoperative day of diagnosis was noted along with the manifestation of CAL, the diagnostic tool for detection of the leak, and the treatment.

Statistics

Categorical data are presented as numbers with percentages, numerical data are presented as means $\pm$ standard deviation of the mean (normally distributed), or medians with interquartile ranges (not normally distributed). Univariate analysis was performed using a chi-square test in case of categorical data and an unpaired *t* test or one-way analysis of variance (normally distributed) and Mann–Whitney *U* test (not normally distributed) in case of numerical data. To determine whether the individual CRP, PCT, and LBP fluctuations in time were related to the risk of CAL, we fitted mixed effects model with random cubic splines for the normalized responses of CRP, LBP, and PCT to account for nonlinear character of the responses. Each model contained the following fixed effects: age, American Society of Anesthesiologists classification, sex, approach (laparoscopic or open), use of corticosteroids, time effects of the order up to 3 or less (depending on the response), and the interaction terms between time effects and factors American Society of Anesthesiologists classification, sex, approach (laparoscopic or open), and use of corticosteroids. Each model contained as random effects a random intercept and random effects of time.

From each univariate model random effects were estimated using empirical Bayes estimates and plugged into the logistic model for CAL and the survival model for time to CAL. To correct for uncertainty of the plug-in covariates, we sampled random effects from the fitted polynomial mixed regression models and fitted the logistic/survival models for each sample. As the final estimate, we took an average of the obtained estimates. Final standard errors were calculated taking into account within and between samples variability.

Results

A total of 243 patients were included. The mean age was 64 ± 12 years; 135 patients (56%) were men and 108 (44%) were women. Thirty-three patients (14%) were treated for inflammatory diseases, 206 patients (84%) were treated for malignancy, and 4 patients (2%) had ischemic colitis. Fifty-six patients (23%) underwent preoperative radiotherapy, and in 59 patients (25%) a defunctioning stoma was constructed. A total of 92 (38%) patients underwent a laparoscopic procedure and 151 patients (62%) were operated through laparotomy.

Nineteen patients (8%) developed clinical CAL. In 9 patients it became manifest as sepsis, in 7 patients as peritonitis, 2 patients developed a presacral abscess, and 1 patient developed an intra-abdominal abscess. In 8 patients the diagnosis was made by computed tomography, in 7 patients by relaparotomy, and in 4 patients fecal discharge from the drain occurred. Median interval between operation and confirmation of CAL was 6 days (range 2 to 26 days). Two patients (.8%) developed an infection at the drain insertion site, both in the group without CAL. Average hospital stay of patients with CAL was significantly longer (28 ± 22 days vs 13 ± 13 days; $P < .0001$). In the group of patients with CAL 3 died (16%), whereas 6 patients (3%) died in the group without CAL ($P = .002$).

Table 1 shows the baseline characteristics of the patients with CAL compared to the patients without CAL. The incidence of pulmonary comorbidity and the number of patients using corticosteroids were significantly higher in the leakage group.

Drain fluid production was compared between the groups with and without leakage. No significant difference between the 2 groups was found.

Univariate analysis shows that CRP levels are significantly higher in patients with leakage on day 3 and day 5 postoperatively (Fig. 1). Levels of LBP are significantly higher in patients with leakage on days 2, 3, and 4 after the operation (Fig. 2). The PCT concentration is significantly higher on day 5 in case of leakage (Fig. 3). In the multivariate analysis for LBP, we found a significant effect of the random intercept after correcting by sampling method (Table 2). This suggests that the higher the initial value of LBP the higher the risk of leakage. Both LBP and the random effects were normalized. Therefore, this means that an increase in the average initial value of LBP

Table 1 Univariate analysis of baseline characteristics of the total APPEAL population: patients without CAL versus the patients with CAL

Variable	No CAL (<i>n</i> = 224)	CAL (<i>n</i> = 19)	<i>P</i> value
Age (y)	64 ± 12	65 ± 13	.765
Sex			
Male	125 (93%)	10 (7%)	.789
Female	99 (92%)	9 (8%)	
BMI (kg/m ²)			
<25	80 (91%)	9 (9%)	.155
25–30	109 (98%)	5 (2%)	
>30	35 (88%)	5 (12%)	
Smoking			
Yes	50 (94%)	3 (6%)	.750
No	168 (92%)	15 (8%)	
Steroids			
Yes (chronic)	2 (40%)	3 (60.0%)	.000
Yes (perioperatively)	16 (80%)	4 (20%)	
No	205 (94%)	12 (6%)	
ASA score			
1–2	173 (93%)	13 (7%)	.532
3–4	50 (89%)	6 (11%)	
Neoadjuvant radiotherapy			
Yes	51 (91%)	5 (9%)	.945
No	173 (93%)	14 (7%)	
Type of resection			
TME/LAR	76 (92%)	7 (8%)	.727
PME/HAR	55 (95%)	3 (5%)	
Left hemicolectomy	21 (96%)	1 (4%)	
Sigmoid resection	68 (90%)	8 (10%)	
Subtotal colectomy	4 (100%)	0 (0%)	
Height anastomosis (cm)			
>7	149 (94%)	10 (6%)	.211
<7	74 (89%)	9 (11%)	
Construction anastomosis			
Stapled	178 (93%)	14 (7%)	.735
Handsewn	45 (90%)	5 (10%)	
Configuration anastomosis			
End-to-end	57 (86%)	9 (14%)	.259
End-to-side	16 (94%)	1 (6%)	
Side-to-end	122 (94%)	8 (6%)	
Side-to-side	23 (96%)	1 (4%)	
Protective ileostomy			
Yes	55 (93%)	4 (7%)	.933
No	167 (92%)	15 (8%)	

ASA = American Society of Anesthesiologists; CAL = colorectal anastomotic leakage; HAR = high anterior resection; LAR = low anterior resection; PME = partial mesorectal excision; TME = total mesorectal excision.

at the first postoperative day with 1 standard deviation increases the risk of leakage by $\exp(.5) = 1.6$ times. In addition, we found the use of steroids to be independently associated with CAL as well. No significant results were obtained in the multivariate analysis of PCT and CRP.

Univariate analysis of the acute phase protein (APP) data on each postoperative day was also performed with outcome variables other than CAL. Comparison of means between patients undergoing laparoscopic versus open surgery resulted in significantly higher concentrations in the open surgery group of CRP on day 1 ($P = .014$) and 2 ($P = .018$) and of PCT on day 2 ($P = .026$). No significantly different

concentrations for LBP were found. Similar analysis comparing patients with a diverting stoma to patients without a stoma showed no significant results for neither of the 3 analyzed APPs ($P > .120$). Indication for operation (malignancy, inflammatory, and other) as outcome variable also did not reveal significant results ($P > .087$).

Comments

APPs are produced in the liver, extrahepatically and at the site of injury in case of LBP. Therefore levels at the

Figure 1 Differences in CRP concentrations (mg/L) between patients with and without CAL. *Postoperative day on which differences are significant (day 3, $P = .003$; day 5, $P = .013$).

local milieu could be a more specific indicator for local infection, like CAL.

CRP plays a role in recognizing pathogens and activating the complement system and phagocytic cells. Furthermore, it contributes to restoration of normal structure and function of injured tissues.¹⁷ CRP in plasma has been used for a long time to objectify disease activity in infective or noninfective inflammatory states.¹⁸ For CRP in drain fluid, univariate analysis shows a significant difference between patients with CAL and patients without CAL on days 3 and 5 postoperatively. Multivariate analysis renders no significant results. The differences between laparoscopic versus open surgery may be explained by the greater tissue damage resulting from open surgery, leading to a greater inflammatory reaction.

LBP enhances the inflammatory response to gram-negative bacteria by transferring LPS (Lipopolysaccharide) to a host membrane protein (m)CD14, causing the release of proinflammatory cytokines that recruit neutrophils as an early, innate immune response.¹⁹ High serum LBP levels have been shown to correlate with the onset of bacteremia and sepsis.²⁰ Since gram-negative bacteria are abundantly present in the colon, significantly higher LBP concentrations in patients with CAL on days 2, 3, and 4 postoperatively could be expected. Moreover, multivariate analysis

Figure 2 Differences in LBP concentrations (µg/mL) between patients with and without CAL. *Postoperative day on which differences are significant (day 2, $P = .040$; day 3, $P = .039$; day 4, $P = .003$).

has shown that an increase of LBP levels in drain fluids is associated with a significantly higher risk for CAL.

In a nonseptic condition, PCT is produced primarily in neuroendocrine C cells of the thyroid.¹⁵ However, bacterial infection has been shown to induce a release of PCT from different parenchymal tissues and differentiated cell types throughout the body. The serum level of PCT has been suggested to be a sensitive indicator of ongoing abdominal sepsis and could help in deciding whether to perform a re-laparotomy.²¹ In drain fluid, a significant difference between patients with CAL and patients without CAL was found only on day 5 postoperatively. However, multivariate analysis renders no significant results. Levels of PCT were significantly higher in the open surgery group on day 2. Perhaps this could be because of a larger intraoperative spill in the open group. However, on day 1 the difference is not significant contradicting this hypothesis.

This is the first study concerning acute phase proteins in drain fluid and was conducted to study if APPs have diagnostic value when determined in the perianastomotic environment. Several studies in search of a biomarker for CAL in drain fluid have been performed. The majority of studies concerns cytokines like interleukin-1b and tumor necrosis factor- α .²² Unfortunately, no thresholds could be defined because of the great variability in results, rendering these parameters as not yet suited for clinical use.²³ The same problem is encountered with LBP and, in analogy to cytokine data, consequently one can only conclude that a correlation exists between LBP levels in drain fluid and CAL. Probably a combination of biomarkers allows for a more specific screening of CAL and further studies should aim to find such a biomarker profile.

The number of samples used for each analysis does not add up to the number of included patients. This is because of variable and insufficient production of drain fluid in most cases. Peritoneal lavage could be a solution; however, this may interfere with the concentrations of the APPs. Few samples are missing because of the accidental removal of the drain by the patient, early intervention for CAL, and accidental loss of drain fluid either at the ward or at the processing laboratory.

Corticosteroids are known to impair wound healing, however, their influence on the healing of colorectal anastomoses is unclear and studies have reported conflicting results.^{24–26} This study shows the use of corticosteroids to be correlated to CAL. It confirms the results published by Sliker et al, who used the same database.²⁷

To date, the use of prophylactic drainage remains controversial. Several level 1 studies have shown that prophylactic drainage does not have a beneficial or a detrimental effect on the incidence of CAL and on the morbidity afterwards.^{28–30} A prospective study concerning pelvic anastomosis showed a higher leakage rate after routine irrigation-suction drainage in elective anterior resection.³¹ A retrospective study showed drainage and the use of a defunctioning stoma to be beneficial in terms of reoperation rates as a result of anastomotic leakage after

Figure 3 Differences in PCT concentrations (ng/mL) between patients with and without CAL. *Postoperative day on which differences are significant (day 5, $P = .013$).

total mesorectal excision.³² Despite this controversy surrounding the necessity to drain, prophylactic drainage remains a common practice in many hospitals, particularly after rectal surgery.³³ In addition, the outcome measures used in these studies consist of leakage rates, hospital stay, radiological anastomotic leakage, infectious complications, and patient comfort. In this study, we have shown diagnostic potential of the drain. Therefore, considering the low complication rate of prophylactic drainage, it should be placed routinely during surgery to allow collection of drain fluid in the early postoperative period.

The statistical methods used in this article allow analysis of longitudinal data on an individual level, ie analysis of individual profiles of the acute phase proteins. We found that an increase in LBP levels with 1 standard deviation leads to a 1.6 times increased risk of CAL in the individual patient. This means that LBP and CAL are related. However, since it is not possible to calculate a standard deviation on one measurement, this finding by itself cannot be used in clinical practice. In addition, because of the vast variability of the obtained values between patients and between samples of the same patient, it is not possible to define general cutoff values. A personalized, “tailor made” approach is mandatory. In analogy with cardiovascular research concerning risk scores (Framingham score), a more personalized approach can be achieved by the development of a prognostic model, determining whether repeated measurements of a specific biomarker, in this study LBP can ultimately provide a better understanding of disease

progression. The most common are dynamic predictions of survival probabilities using the recorded longitudinal information, namely, landmarking³⁴ and joint modeling.³⁵ Because the subject-specific longitudinal trajectories can be quite complex (eg nonlinear, plateaus), different features of these trajectories may be more predictive for the event of interest. In our study, we do not encounter strictly joint-modeling setting, meaning that the recorded longitudinal information influences the survival submodel but not the other way around. Therefore using the 2-stage model is sufficient. Based on that model we can perform a dynamic prediction of the patient-specific hazard for a new patient by updating the patient-specific random effect related to LBP intercept as more longitudinal data for this patient are available. For this purpose, we can apply the similar procedure as in the typical joint modeling situation by using Metropolis–Hastings sampling.³⁶ To simplify matters, a statistical black box should be visualized that allows calculation of the chance of CAL after putting in a diagnostic factor like the concentration of LBP on postoperative days 1 and 3 for example. Other postoperative signs and symptoms like fever, serum CRP, and white blood cell count could be factored in as well.

Conclusion

Increased concentrations of LBP in drain fluid are significantly associated to a higher chance of CAL and could contribute in a future prognostic model for CAL.

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Table 2 Final logistic regression model for CAL after correcting for standard error using sampling method

Coefficient	log HR	Standard error	P value
Intercept	−2.63	.47	1.8×10^{-8}
B.s [.,3]	.30	.19	.12
int.s LBP	.50	.22	.024
Use of steroids at home (Yes vs No)	4.14	1.32	.002
Use of steroids intraoperatively (Yes vs No)	1.56	.71	.027
Approach (Open vs Laparoscopy)	−.83	.57	.14

The intercept estimate reflects the reference value of the log hazard ratio for the average person in a population.

B.s [.,3] = b-spline basis function of order 3; HR = hazard ratio; Int.s LBP = random intercept for LBP; LBP = lipopolysaccharide-binding protein.

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