Serial estimation of serum C-reactive protein and procalcitonin for early detection of anastomotic leak after elective intestinal surgeries-prospective cohort study

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ABSTRACT

Objective: Anastomotic leak can adversely affect the outcome of surgery especially if detected late. The present study was carried out to detect anastomotic leak early in the postoperative period using serial estimation of procalcitonin (PCT) and C-reactive protein (CRP).

Material and Methods: A single centre prospective cohort study was done on patients undergoing elective gastrointestinal surgery with anastomosis. Serial estimation of serum procalcitonin and C-reactive protein was done on the first five postoperative days. Other parameters such as hemoglobin, total protein, albumin and WBC counts were noted perioperatively. Patients were followed up to 60. postoperative day to assess for anastomotic leak, wound infection and other septic foci.

Results: Eighty-four patients were included in the study. Anastomotic leak rate was 26.19% (22/84) and 3/22 patients died in anastomotic leak group. Wound infection rate was 23.81%.The cut off value of CRP on third postoperative day in detecting anastomotic leak was 44.322 mg/dl with sensitivity of 72.73%, specificity of 66.13% and accuracy of 59.52%. The cut off value for WBC count measured perioperatively in detecting anastomotic leak was 9470 cell/mm^3 with sensitivity of 72.73%, specificity of 56.45% and accuracy of 59.74%. Serum procalcitonin, haemoglobin, total protein and albumin measured were not sensitive enough to detect the anastomotic leak early.

Conclusion: Measuring CRP on the third postoperative day can predict anastomotic leak with a cut off value of 44.32mg/dl. Patients with raised CRP need careful evaluation to rule out anastomotic leak before deciding on early discharge.

Keywords: C-reactive protein, procalcitonin, anastomotic leak

INTRODUCTION

Anastomotic leak (AL) is the most dreadful complication, which can occur following all intestinal anastomotic surgeries. The incidence of AL following intestinal surgeries is 2-20% (1). The incidence is high in esophagectomy and colorectal surgeries and was reported as 10% and 2-19% respectively (2,3). Mortality following AL after colorectal and esophageal surgeries are 30% and 30-60% respectively (4,5) 70 patients undergoing colorectal surgery were prospectively analyzed in a single-center tertiary teaching hospital. Demographic and surgical data were obtained. Serum procalcitonin was taken before surgery and at day three (72 hours). AL can present early or late. It is usually diagnosed between 7 to 12 days postoperatively. Late AL presents after one-month postoperatively (6). It is always the priority of any surgeon to identify these ALs at the earliest possible so as to avoid mortality and morbidity.

AL will lead to bacterial contamination of the peritoneal cavity, which leads to rise in inflammatory mediators such as C-reactive protein (CRP) and procalcitonin (PCT) before it presents clinically. AL can be detected early in elective cases, where wound infection and peritoneal contamination are less due to preoperative bowel preparation and prophylactic preoperative antibiotics. Some studies have reported the role of PCT and CRP for early detection of AL in a group of specific surgery

like colorectal and oesophageal surgery (3,4,7-10) it is clinically valuable to detect anastomotic leak early after esophagectomy in esophageal cancer. The purpose of this study is to investigate the associations between routine postoperative laboratory findings and anastomotic leak and to analyze the laboratory findings to find out an independent predictive marker for anastomotic leak. In addition, this study compares cases treated with neoadjuvant therapy (NT. However, there are not many reports documenting the efficacy of these parameters in overall alimentary tract surgery.

This study was carried out to determine the role of PCT and CRP plasmatic concentration as an early detector of AL following elective gastrointestinal surgery.

MATERIAL and METHODS

This study was a prospective cohort study carried out in a tertiary center in India. Institutional ethics committee approval was obtained (JIP/IEC/201/1040). Written Informed consent was taken from all the participants in this study and patients were given full freedom to withdraw at any point of time during the study.

Patients and Data Collection

All patients aged more than or equal to 18 years, who had undergone elective gastrointestinal surgery with an anastomosis, were included in the study. Patients with fever or focus of sepsis preoperatively, patients who had received intraperitoneal chemotherapy and in patients in whom, covering stoma was done with anastomosis were excluded from the study.

All patients, who had fulfilled the inclusion and exclusion criteria, were included in the study after taking informed written consent. Following surgery on the first five postoperative days blood was collected daily at 8 a.m. for PCT and CRP measurement.

The CRP levels were estimated using ELISA kit manufactured by Calbiotech USA® (11). The PCT levels were estimated using ELISA kit manufactured by Raybiotech® (12). The normal reference value of PCT as per the kit was less than 0.15 ng/ml and the normal reference value of CRP was less than 3 mg/l.

The patient's data, such as age, sex, telephone number, diagnosis, indication for surgery, previous surgical history, perioperative hemoglobin, total leucocyte counts (TLC), total protein and albumin were recorded. Hemoglobin and TLC were measured using Sysmex Xt-2000i which works on Coulter principle (13). Albumin was measured in Beckman-Coulter AU5800 using spectophotometry method and bromocresol green was used as dye. Total protein was measured in Beckman-Coulter AU5800 using photometric biuret end point method (14). Confounding variables such as surgical site infection and any other postoperative septic foci were also noted. Patients were followed up postoperatively for any signs and symptoms of leak and any other postoperative complications.

AL was defined as any clinical signs of leakage, confirmed by radiological examination, endoscopy, clinical examination of the anastomosis (i.e, palpation of the anastomosis), or reoperation (15). The patient was labeled as having AL if he/she had clinical evidence of leak like peritoneal signs, bile or faecal content in the drain or if ultrasound guided aspiration of the free fluid or localized collection at the anastomotic site reveals bile or faecal matter or if water soluble contrast leak is seen on fluoroscopy or computed tomography.

Serum PCT, CRP, haemoglobin, TLC, total protein and albumin were analysed for the predictability of AL. The incidence of leak following elective intestinal anastomosis in this hospital, the risk factors associated with leak in the population studied, the sensitivity and specificity of serial estimation of serum CRP and PCT to detect leak early were calculated. The patients were followed up on post-operative day (POD) 60 over telephonic interview and development of enterocutaneous fistula or late post-operative leak were recorded.

Sample Size

Sample size was calculated using the formula, $N = Z^2 \times P (1-P) / [d^2 \times (1-\text{prevalence})]$ when p was specificity. The sensitivity of PCT measured for five post-operative days to predict AL was 100% and specificity was 72%. The specificity of CRP measured for five post-operative days to predict AL was 83% (8). With expected specificity of 72% and expected prevalence of AL as 9.4%, the sample size was calculated as 84 with 95% confidence interval, 10% relative precision. The specificity of PCT was lower than that of CRP. Hence sample size was calculated using the specificity of PCT. Power of the study was kept at 80%.

Statistical Analysis

All statistical analysis was done using SPSS version 20. Categorical variables such as presence of AL were expressed as proportions. Continuous variables such as PCT, CRP, haemoglobin, total protein, albumin and TLC levels were expressed as mean (SD) or median (IQR) depending upon the normality of distribution. The total protein, albumin, haemoglobin and TLC showed normal distribution. CRP and PCT levels measured on all five days, did not follow normal distribution. Receiver Operating Characteristic (ROC) curve was plotted between day specific PCT, CRP, perioperative haemoglobin, total protein, albumin, TLC and the presence of AL, to determine optimum cut-off value for early detection of AL, using sensitivity and specificity. The differences in haemoglobin, total protein, albumin and TLC between the AL and NAL patients were analysed using student t test. PCT and CRP was analysed using Mann-Whitney U test. p value less than 0.05 was taken as significant.

RESULTS

This study was carried out from January 2017 to December 2018. A total of 84 patients were included in the study. Surgical

Table 1. Demographic and clinical parameters studied in AL and NAL groups

Patient characteristics	AL group (N= 22)	NAL group (N= 62)	p*
Sex			0.138
Female (n= 31)	11 (35.48%)	20 (64.52%)	
Male (n= 53)	11 (20.75%)	42 (79.25%)	
Age groups			0.074
18-45 years (n= 27)	11 (40.74%)	16 (59.26%)	
45-60 years (n= 27)	7 (25.93%)	20 (74.07%)	
More than 60 years (n= 29)	4 (13.79%)	25 (86.21%)	
Mortality	3 (13.64%)	2 (3.22%)	0.076
Wound infection	6 (27.27%)	14 (22.58%)	0.657

AL: Anastomotic leak, Non AL group: Non-anastomotic leak group.
*Chi-square test.

Table 2. Comparison of procalcitonin and CRP between AL and NAL groups

Test	POD	Non-AL group Median (IQR)	AL group Median (IQR)	p*	AUC
Procalcitonin	1	210.98 (32.43-657.05)	22.06 (11.54-206.44)	0.009	0.312
Procalcitonin	2	16.27 (49.60-652.02)	26.2 (15.98-212.99)	0.013	0.322
Procalcitonin	3	176.72 (39.17-582.03)	7.55 (7.4-198.45)	0.002	0.274
Procalcitonin	4	216.35 (51.82-625.56)	20.456 (7.25-261.48)	0.017	0.328
Procalcitonin	5	162.64 (33.03-450.16)	15.63 (7.23-352.36)	0.009	0.315
CRP	1	25.04 (16.24-35.14)	34.89 (29.15-54.89)	0.041	0.647
CRP	2	27.82 (16.54-41.61)	34.39 (27.16-38.08)	0.137	0.607
C RP	3	27.09 (16.34-53.98)	44.32 (35.28-61.37)	0.033	0.654
CRP	4	34.90 (16.30-58.07)	51.23 (30.19-51.23)	0.148	0.604
CRP	5	29.44 (16.23-48.98)	45.68 (32.57-48.51)	0.104	0.617

POD: Post operative day, on AL group-non anastomotic leak group, AL group-anastomotic leak group, AUC: Area under the curve, IQR: Interquartile range.
*Mann-Whitney U test.

procedures done for the patients were gastric (42), colonic (13), pancreaticobiliary (20), esophageal (3), stoma closure (4) and small bowel surgeries (2). AL developed among 22/84 patients (26.19%). The distribution of anastomotic leak among various procedures were 12, 3, 6, and 1 in gastric, colonic, pancreaticobiliary and oesophageal anastomosis respectively. AL did not show any statistical difference between the sex and different age groups (Table 1). Overall mortality in the study was 5.95% (5/84). The mortality in the AL group (3/22; 13.64%) when compared to the mortality in the NAL group (2/62; 3.22%) although higher, the difference was not significant (p= 0.076). The overall incidence of postoperative surgical site infection was 20/84 (23.81%). The incidence of wound infection in the AL group was 27.27% and for the NAL group was 22.58%. The difference in distribution of wound infection was not statistically significant (p= 0.657). The clinical demographic parameters studied in AL and NAL groups were shown in Table 1.

The median and interquartile range for serum CRP and procalcitonin for all five postoperative days, were calculated. The area under the curve calculated for CRP on all five postoperative days was above 0.500 (Table 2).

The mean and standard deviation of haemoglobin, TLC, total protein and albumin were calculated. The area under the curve of TLC was found to be significant (Table 3).

The AUC for serum CRP was 0.654 on day three reaching the maximum, than other postoperative days. The cut off value was taken as more than 44.32 mg/dl with sensitivity of 72.73% and specificity of 66.13% (Figure 1) (Table 4). When the cut off value of the perioperative TLC count was taken as more than 9470cells/mm^3, the sensitivity was 72.73% and the specificity was 56.45%, (Figure 2) (Table 4).The AUC was found to be 0.641.

Table 3. Comparison of hemoglobin, total protein, albumin and TLC between AL and NAL groups

Test	Non AL group Mean (SD)	AL group Mean (SD)	p*	AUC
Hemoglobin	10.27 (1.81)	11 (1.76)	0.108	0.355
Total protein	5.6 (1.11)	5.62 (0.92)	0.945	0.452
Albumin	2.96 (0.59)	3.06 (0.63)	0.541	0.417
TLC	10131.94 (4468.81)	12203.64 (4472.41)	0.009	0.641

Non AL group: Non anastomotic leak group, AL group: Anastomotic leak group, AUC: Area under the curve, SD: Standard deviation, TLC: Total leucocyte counts. *Student t-test.
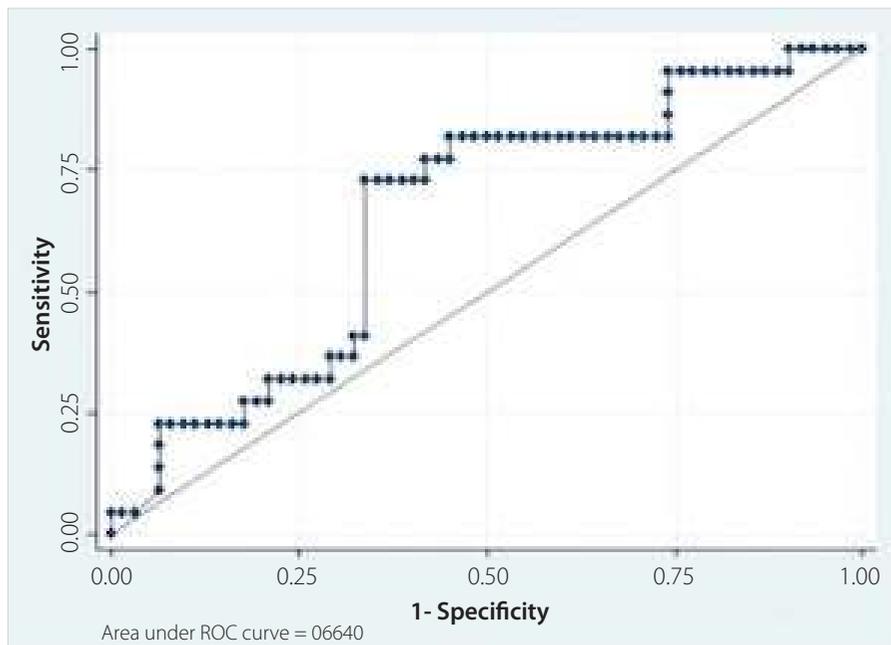
Figure 1. Receiver operating characteristic curve of serum C-reactive protein on postoperative day three.

Table 4. Best cut off value of CRP and TLC along with AUC, sensitivity and specificity for detecting anastomotic leak

| Test	POD	AUC	Cut off	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
CRP	3	0.654	44.32 mg/dl	72.73%	66.13%	30%	75.93%	59.52%
TLC		0.641	9470 cells/mm^3	72.73%	56.45%	28.95%	89.74%	59.74%

CRP: C-reactive protein, POD: Post operative day, AUC: Area under the curve, TLC: Total leucocyte counts.

DISCUSSION

AL is a serious life-threatening complication that can occur after a gastrointestinal anastomosis. It is associated with high mortality and morbidity due to the sepsis it causes. Also, overall survival reduces, as the chance of recurrence of malignancy increases due to delay in adjuvant therapy. Early diagnosis of AL can reduce the mortality and morbidity significantly. CRP is an acute phase reactant produced by liver, in response to infection, ischemia and tissue damage (10). It starts to rise two hours following insult

and peaks at 48 hours (3). In normal circumstances, C cells of the thyroid gland produce PCT. In sepsis, white blood cells, pancreas, spleen, kidney, colon, adipocytes and the brain produce PCT. It starts rising at 3-4 hours and peaks at 8 to 24 hours (4). In the present study, the aim was to determine whether serological analysis could detect AL, prior to its clinical presentation.

The present study showed that the plasmatic concentration of CRP on third POD with the cut off value of more than 44.32 mg/dl was significantly associated with AL. The perioperative TLC,
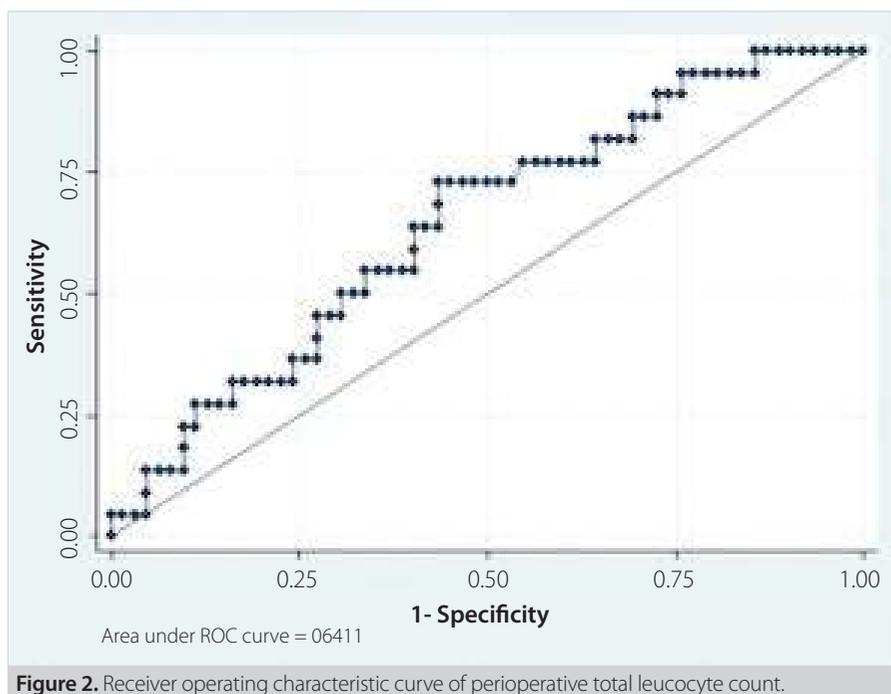
Figure 2. Receiver operating characteristic curve of perioperative total leucocyte count.

more than 9470 cells/mm^3 had predicted AL early. Postoperative serum PCT was not an early predictor of AL. The total protein, albumin and hemoglobin level measured perioperatively had no association with AL.

Garcia-Granero et al. have reported about early prediction of AL after colorectal resection using PCT and CRP (8). The study have shown that CRP and PCT were reliable predictors from third to fifth postoperative days with AUC more than 0.800. The best predictor was PCT on day five with the cutoff of 0.31 ng/ml, with sensitivity of 100%, specificity of 72%, and negative predictive value of 100% and positive predictive value of 17%. Aiolfi et al. have reported a systematic analysis and Bayesian meta-analysis on five studies including 850 patients on early prediction of esophageal AL using CRP (9). This study have demonstrated that CRP values on POD three and five had very good diagnostic accuracy with the AUC of 0.800. The cut off values derived for POD three and five were 17.6 mg/dl and 13.2 mg/dl respectively. Hayati et al. have reported on early prediction of colorectal AL using serum PCT on POD 3 (4). The study have shown that PCT cut off value was 5.29 ng/ml with sensitivity of 100%, specificity of 85%, the positive predictive value of 23% and the negative predictive value of 100%. The early predictor of AL associated with pancreaticoduodenectomy surgery was analyzed only in very few studies (16,17). The studies mentioned above showed that the sensitivity and negative predictive value of CRP and PCT in predicting AL of various surgeries was 100%. So, these serological tests can be used to rule out AL, postoperatively. The present study showed that the plasmatic concentration of CRP on third POD was significantly increased in patients with AL and the se-

rial estimation of serum PCT postoperatively was not associated with AL. This may be because of raised postoperative wound infection rate, which is an important confounding factor.

A meta-analysis showed that the overall incidence of AL was 9% (18). In the present study, it was found that the incidence of AL was 26.19%, which was high as many of the patients in present study had hypoalbuminemia, though it is not showing any statistical significance. The mean value of albumin in the present study was 2.96 mg/dl and 3.05 mg/dl in NAL and AL groups respectively.

Zarnescu et al. have reported the risk factors related to AL in colorectal surgery (19). The general factors which were associated with increased chance of AL in colorectal surgery were male sex, malnutrition, serum total protein less than 6 g/dl and albumin less than 3.5 g/dl, hemoglobin less than 9.9g%, blood transfusions, American Society of Anesthesiologists (ASA) score more than or equal to three, prolonged operating time and chronic steroid therapy. Local factors, associated with increased chance of AL, are low rectal anastomosis, less than 6 cm from anal verge, neoadjuvant radiotherapy, intraperitoneal chemotherapy, Hyperthermic intraperitoneal chemotherapy and bevacizumab. In the present study, it was found that risk factors, which reflect the general nutritional status of the patient such as hemoglobin, total protein, albumin had no association with the AL. Other risk factors such as neoadjuvant radiotherapy or chemotherapy, surgical techniques, duration of surgery, chronic kidney disease were not studied in our study.

The present study showed that the serum CRP value above 44.32 mg/dl on POD three can detect AL with sensitivity of

72.73%, specificity of 66.13%, positive predictive value of 30%, negative predictive value of 75.93% and accuracy of 59.52%. Since the negative predictive value of CRP was more (75.93%), it can be used as a tool to rule out AL. The present study also showed that raised peri-operative TLC is also associated with AL.

The merits of this study are the risk factors associated with AL such as hemoglobin, total protein and albumin were also analyzed. Other causes of raised CRP and PCT such as wound infection was also considered and analyzed. Duration of follow up was also long i.e, 60 days to include delayed postoperative complications.

The limitation of this study is the high wound infection rate in the study population, which may be because of poor nutritional status of the patients and the malignant nature of the disease.

CONCLUSION

The plasmatic concentration of more than 44.32 mg/dl of CRP on POD three, and >9470 cells/mm^3 of postoperative TLC were found to detect the AL early with a high negative predictive value. So, this can be utilized for discharging patients early after elective gastrointestinal surgeries. However, serum procalcitonin was not found to be a predictor of anastomotic leak. Risk factors such as low serum total protein, albumin and hemoglobin were not associated with anastomotic leak.

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Elektif bağırsak ameliyatlarından sonra anastomoz kaçağının erken tespiti için serum c-reaktif protein ve prokalsitonin seri tahmini prospektif kohort çalışması

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ÖZET

Giriş ve Amaç: Özellikle geç fark edildiğinde anastomoz kaçağı cerrahi sonucunu kötü bir şekilde etkileyebilir. Bu çalışma, prokalsitonin (PCT) ve C-reaktif protein (CRP) sıralı tahmini kullanılarak anastomoz kaçağının postoperative dönemde erken tespit edilmesi için yürütüldü.

Gereç ve Yöntem: Anastomozlu elektif gastrointestinal cerrahi operasyonu olan hastalarda tek merkezli prospektif kohort bir çalışma yürütüldü. İlk beş postoperative günde, serum prokalsitonin (PCT) ve C-reaktif protein (CRP) sıralı tahmini uygulandı. Hemoglobin, total protein, albümin ve akyuvar sayısı gibi diğer parametreler perioperatif olarak kaydedildi. Hastalar; anastomoz kaçağı, yara enfeksiyonu ve diğer septik odaklar açısından postoperative 60. güne kadar takip edildi.

Bulgular: Çalışmaya 84 hasta dahile dildi. Anastomoz kaçağı oranı %26,19 idi ve anastomoz kaçağındaki 3/22 hasta kaybedildi. Yara enfeksiyon oranı %23,81 idi. Üçüncü postoperative günde anastomoz kaçağının tespiti için CRP eşik değeri 44,322 mg/dl olarak bulunurken duyarlık, özgüllük ve doğruluk oranları sırasıyla %72,73, %66,13 ve %59,52 idi. Anastomoz kaçağının tespiti için perioperative öçlülen akyuvar sayısının eşik değeri 9470 hücre/mm³ olarak bulunurken duyarlık, özgüllük ve doğruluk oranları sırasıyla %72,73, %56,45 ve %59,74 idi. Ölçülen serum prokalsitonin, hemoglobin, total protein ve albumin, anastomoz kaçağının erken dönemde tespiti açısından yeterince duyarlı değildi.

Sonuç: Postoperatif üçüncü günde CRP ölçümü, 44,32 mg/dl eşik değeri iel anastomoz kaçağına öngörebilir. CRP yüksek hastalar, erken taburculuk öncesi anastomoz kaçağı açısından değerlendirilmelidir.

Anahtar Kelimeler: C-reaktif protein, prokalsitonin, anastomoz kaçağı