

The value of biomarkers in early diagnosis of anastomotic leak following colorectal tumor resection: a review of the literature between 2012 and 2017

Wei Ge¹ and Gang Chen¹

¹Department of General Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu Province, P. R. China

Correspondence to: Gang Chen, **email:** 331812195@qq.com

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ABSTRACT

Anastomotic leakage (AL) is one of the most serious complications of colorectal resection, causing sepsis, reducing overall in-hospital survival and increasing the risk of local and distant recurrences. So, early diagnosis of AL is very important. To improve the early diagnosis of AL following colorectal resection, a number of studies have investigated potential biomarkers to predict AL. In this review, we summarized the biomarkers, which had been shown to be useful in early diagnosis of AL. The results showed that c-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), cytokines, and so on were hot study biomarkers, which were proved to be reliable predictors of AL. Besides, drainage indexes such as microbiological study, potential of hydrogen (PH), carcinoembryonic antigen (CEA), and so on also helped to indicate early AL. Although there have been many studies on this field, rigorous homogeneous and prospective trials are demanded to explore ideal biomarkers to early diagnose AL.

INTRODUCTION

At present, surgery is the main treatment of colorectal tumor. Enhanced recovery after surgery (ERAS) has gained momentum in the management of the colorectal cancer patient, which reduces morbidity and shortens the hospital stay [1]. Anastomotic leakage (AL) is one of the most serious complications of colorectal resection. Studies showed that AL occurs mainly around the 5th–7th postoperative day (POD) [2]. Early discharge according to ERAS carries a potential risk of developing AL when a patient is already out of the hospital. AL is a devastating complication, causing sepsis, reducing overall in-hospital survival and increasing the risk of local and distant recurrences [3, 4]. So, early diagnosis of AL is very important.

To improve the early diagnosis of AL following colorectal resection, a number of studies have investigated potential biomarkers to predict AL. In this review, we summarized the biomarkers, which had been shown to be useful in early diagnosis of AL.

C-reactive protein

C-reactive protein (CRP) is a serum acute-phase reactant synthesized almost exclusively in the liver, released in response to stimulation by proinflammatory cytokines. Its production is part of a nonspecific acute-phase response to most forms of tissue damage, infection, inflammation, malignant neoplasia and so on. The median concentration of CRP is 0.8mg/L in adults, however, it may increase owing to acute-phase stimulus, stimulated by IL-6, α -TNF and IL-1 β originating at the site of inflammation [5]. The half-life of CRP is very approximately 19h. All these characteristics make CRP a valuable biomarker for inflammatory response, early detection postoperative complication and so on [5]. Measuring CRP postoperatively to monitor the development of inflammatory and inflectional complication is considered standard care in many hospitals.

AL after colorectal resection may result in abdominal infection and also improve the CRP level, so elevated CRP may indirectly indicate AL theoretically. CRP has been

reported in many studies to be raised significantly days before the diagnosis of AL. Almeida et al. performed a study to demonstrate the value of postoperative serum CRP in the identification of increased risk of AL after colorectal surgery. According to the result, the diagnosis of AL was mainly between the 4th and 11th postoperative day (POD, mean 7th POD). The cut-off value of 140 mg/L on POD3 maximized the sensitivity (78%) and specificity (86%) of serum CRP in assessing the risk of leakage [6]. Serum CRP has been evaluated in other 11 studies [7–17]. In general, the CRP level was raised significantly days before the diagnosis of AL. The reported cut-off values for CRP ranged from 130 to 245 mg/l for POD 3–5. Also, some results showed that CRP was a good negative predictive value for AL. The details were summarized in Table 1, Table 2, and Table 3. There are also studies to determine whether CRP in drain fluid can serve as screening tool for AL [18, 19]. However, the results showed that examination CRP in drain fluid could not help to detect AL.

Procalcitonin

Procalcitonin (PCT) is the prohormone of calcitonin, synthesized by the parafollicular C-cells of the thyroid gland. PCT is consist of 116 amino acids, whose normal level in the circulation is very low (<0.05 ng/ml). Bacterial infections induce a generalized release of PCT from all differentiated cell types all over the body. Therefore, the level of PCT could rise significantly in the serum of patients. According to the literature, PCT would increase quickly after 2 to 3 hours following infection and rise to 700 ng/ml in severe sepsis [20]. According to the characteristic of PCT, a value of which less than 0.5 ng / mL represents low risk of bacterial infection, while, a value of greater than 2 ng / mL shows a high risk of bacterial infection. PCT has an important characteristic that its level does not increase following inflammation of non-infectious origin, which is different from CRP. Besides, PCT has another interesting characteristic that its level increases before clinical signs present. All these characteristics make PCT as an ideal tool for early diagnosis of AL after colorectal surgery.

There are a series of studies showed that PCT help to early diagnose AL following colorectal surgery. Urszula et al. performed a study to assess the usefulness of the assessment of PCT in the diagnosis of AL. 157 patients underwent major elective colorectal surgery were involved in this study. They demonstrated the high accuracy of the PCT concentrations measured on day 1 following colorectal resection in the early prediction of AL. Low PCT value at this time point can be especially helpful in excluding AL from the differential diagnosis [21]. Another study by Marek showed that PCT measurement on POD 3 following colorectal cancer resection can positively identify patients at low risk of anastomotic leakage. The cut-off for PCT on POD 3 was 3.83 ng/ml, with sensitivity of 75% and specificity of 100% for AL [12]. A study performed in

Spain also concluded that PCT is reliable predictor of major anastomotic leak after colorectal resection. Raised PCT serum concentration on postoperative days 3 to 5 renders necessary a careful evaluation of the patient before discharge [9]. Similar conclusions had been got in other researches [7, 10, 14, 18]. There are also studies to determine whether PCT in drain fluid can serve as screening tool for AL [19]. Unfortunately, the results showed that examination of PCT in drain fluid could not help to detect AL.

White blood cell count

White blood cell count (WBC) plays an important role in elimination of microorganisms and wound healing. If inflammation is present in the body due to any reason (infection, trauma or tumoral differentiation), WBC presence or increases at inflammation site are common. So the level of WBC could reflect the degree of inflammation. AL would cause inflammatory reaction as the intestinal contents flowed into the abdominal cavity. Therefore, WBC may act as an indicator to predict AL. According to this deduction, a series of studies had been tried to verify this hypothesis. Torben et al. performed a study to determine whether the WBC has a role as early predictor of post-operative septic complications including AL in patients operated laparoscopically in a fast-track regimen. The result showed that the best cut-off value for WBC as a predictor of septic complications was observed on POD2, where $WBC > 12 \times 10^9$ had a sensitivity of 90% and a specificity of 62%. So they concluded that WBC maybe a weak diagnostic marker in prediction of post-operative septic complications, including AL [8]. Stephen et al. also showed that WBC maybe a potential marker of AL. following colorectal surgery [16]. However, some other studies got the opposite conclusion. There was another study aimed to demonstrate the value of WBC in the identification of increased risk of AL after colorectal surgery. Comparison of postoperative serum WBC values did not show any significant differences between AL group and non-AL group until the POD6 [6]. Another two studied got the similar conclusion that WBC was not an ideal biomarker for predicting AL [10, 14]. Therefore, further studies should be performed to explore the value of WBC in the early diagnosis of AL.

Cytokines

Some cytokines such as IL-6, IL-8, IL-10 and TNF α are produced in response to various inflammation mediators. In the group of patients with tumors and inflammatory disease of bowel, the levels of these cytokines may be related to underlying disease to a great extent. Base on these characteristics, cytokines maybe the potential biomarker for AL following colorectal surgery. Serum and peritoneal cytokines have been tried to predict AL. Kostan et al. performed a study to verify whether serum IL-6 could be a hallmark of AL. The result showed

Table 1: The general characteristics of the studies included in this review investigating potential biomarkers to predict AL

First author, year, reference	Study design	Open approach	Operation for cancer	Num	AL rate
Almeida, 2012, [6]	Prospective	82%	74.60%	173	13.90%
Lagoutte, 2012, [7]	Prospective	65%	52%	100	13%
Torben, 2012, [8]	Retrospective	0%	—	129	18%
Alvaro, 2013, [9]	Prospective	79%	73.20%	205	8.30%
Giaccaglia, 2014, [10]	Prospective	89.90%	93.90%	99	7.10%
Kostan, 2014, [11]	Prospective	48%	100%	84	10%
Marek, 2015, [12]	Prospective	47.30%	100%	55	9.10%
Waterland, 2016, [13]	Prospective	36%	—	727	7.90%
Valentina, 2016, [14]	Prospective	25%	100%	504	5.60%
Michal, 2017, [15]	Prospective	—	100%	724	4.60%
Stephen, 2017, [16]	Prospective	—	100%	197	5.60%
Burke, 2017, [17]	Prospective	77.70%	100%	211	12.80%
Ismail, 2017, [18]	Prospective	92%	100%	50	14%
Komen, 2014, [19]	Prospective	—	—	243	8%
Urszula, 2016, [21]	Prospective	21.70%	60.50%	157	10.30%
Tarik, 2016, [22]	Prospective	—	64.60%	206	8.30%
Elyamani, 2011, [23]	Prospective	100%	100%	56	14.30%
Käser, 2014, [25]	Retrospective	—	—	1106	7.30%
Liu, 2013, [26]	Prospective	97.50%	100%	753	7.60%
Liron, 2016, [27]	Prospective	30.50%	63.80%	105	9.50%

that IL-6 levels were not different between patients with and without AL at any time point. So, IL-6 was not a prefer biomarker for AL [11]. Similar conclusion had been got by Urszula. Their results showed that patients who developed AL had higher concentrations of IL-6 on POD1, but the difference was not significant. So they thought that IL-6 is not a good early marker for developing AL [21]. There were also studies to confirm the value of peritoneal cytokines in detecting AL. Accumulating evidence suggests that peritoneal cytokine concentrations may predict AL after colorectal surgery. Tarik et al. showed that concentration levels of peritoneal IL-6 and IL-10 on day 1 after colorectal surgery were predictive of AL (area under receiver operating characteristic curve, 0.72 and 0.74; $p = 0.006$ and 0.004). These results suggest that peritoneal levels of IL-6 and IL-10 are predictive of AL [22, 23]. Whereas, another study showed that peritoneal IL-6 was not a good index to predict AL and increasing levels of TNF α is predictive [18]. So, further studies should be made to explore to the value of cytokines in the use of early diagnosis of AL.

Microbiological study in drainage

When AL attacks in colorectal surgery, intestinal bacteria may flow into the abdominal cavity. Ruiter et

al. reported that the composition of the microbial flora present in the abdominal fluid of patients critically ill with abdominal sepsis varies depending on location of the perforation [24]. In lower gastrointestinal perforation, the most frequently isolated aerobic organisms were *E. coli*, *Klebsiella*, and *Pseudomonas* species. The predominant anaerobes were *Bacteroides* [24]. So that testing the concentration of these concentration bacteria in drainage may in turn predict AL. Based on this assumption, Elyamani et al. performed a bacteriological study to predict AL. Their result showed that *Escherichia coli*, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism were significantly more in AL group in first, third, fifth days postoperatively. They concluded that intraperitoneal bacterial colonization might be an additional diagnostic tool that can support the decision making of surgeons for early detection of AL in colorectal surgery [23]. Another study by Ismail et al. got similar conclusion that bacterial proliferation in drain fluid is predictive for assessing of AL [18].

Some uncommon markers in serum

There are some uncommon markers in serum had also been tried to diagnose AL early. These markers are mainly divided into inflammatory markers and

Table 2: The sample source, testing biomarkers and testing time of the studies included in this review

First author, year, reference	Sample source	Biomarkers included	Time interval
Almeida, 2012, [6]	Serum	CRP, WBC	Daily (between POD1 and POD9)
Lagoutte, 2012, [7]	Serum	CRP, PCT	Daily (between POD1 and POD4)
Torben, 2012, [8]	Serum	CRP, WBC	Daily until discharge
Alvaro, 2013, [9]	Serum	PCT, CRP	Daily (between POD1 and POD5)
Giaccaglia, 2014, [10]	Serum	WBC, CRP, PCT	POD1, POD3 and POD5
Kostan, 2014, [11]	Serum	CRP, Calprotectin, IL-6	Daily during hospital
Marek, 2015, [12]	Serum	CRP, PCT	POD 1 and POD 3
Waterland, 2016, [13]	Serum	CRP	Daily (between POD1 and POD7)
Valentina, 2016, [14]	Serum	PCT, CRP, WBC	POD3 and POD5
Michal, 2017, [15]	Serum	CRP, NLR	POD0 and POD4
Stephen, 2017, [16]	Serum	CRP, PCT, WCC, GGT	Daily (between POD1 and POD5)
Burke, 2017, [17]	Serum	CRP	Daily (between POD1 and POD7)
Ismail, 2017, [18]	Drainage and serum	Serum (CRP, PCT); Drainage (bacterial proliferation, IL-6, TNFa)	Serum (POD1 and POD3), Drainage (POD1, POD3, and POD5)
Komen, 2014, [19]	Drainage	CRP, LBP, PCT	Daily (between POD1 and POD5)
Urszula, 2016, [21]	Serum	PCT, IL-6	POD0 and POD1
Tarik, 2016, [22]	Drainage and serum	IL-6, IL-8, IL-10, and TNFa	POD1
Elyamani, 2011, [23]	Drainage	Microbiological study, IL-6, IL-10, TNF	POD 1, POD 3, and POD5
Käser, 2014, [25]	Serum	Sodium level, WBC	As close as possible to POD5
Liu, 2013, [26]	Drainage	PH	Daily (between POD1 and POD12)
Liron, 2016, [27]	Drainage	CEA	POD1, POD2, and POD3

CRP: C-reactive protein; WBC: White blood cell count, PCT: Procalcitonin, NLR: Neutrophil to lymphocyte ratio, LBP: Lipopolysaccharide-binding protein, PH: Potential of hydrogen, CEA: Carcinoembryonic antigen, POD: Postoperative day.

intestinal damage markers. Inflammatory markers such as calprotectin, gamma-glutamyl transferase, neutrophil to lymphocyte ratio (NLR) had been studied. Kostan et al. tried to test calprotectin as suitable biomarker for AL. They found that calprotectin had the best diagnostic accuracy to detect AL postoperatively. Highest diagnostic accuracy was obtained when CRP and calprotectin were combined at POD3, yielding sensitivity of 100%, specificity of 89%, positive likelihood ratio = 9.09, and negative likelihood ratio = 0.00 [11]. So calprotectin may be a potential biomarker for detecting AL worth further study. Gamma-glutamyl transferase was assessed Stephen et al. for predictive utility in diagnosing AL. They got the result that there was no association of gamma-glutamyl transferase with AL [16]. NLR is another hot inflammation marker and it is also been assessed the prognostic value in the development of AL. The accuracy of NLR in the detection of AL using area under curve was 0.68 with the optimal cut-off value of 6.5, sensitivity 69%, specificity 78%, PPV 49% and NPV 88% [15]. According to this

result, NLR on POD4 possesses the ability to predict the development of AL.

Markers of gut damage might be suitable for detecting AL early, as these are hallmarks of AL. Kostan et al. tested intestinal damage markers such as intestinal fatty acid binding protein, liver fatty acid binding protein, and ileal bile acid binding protein to predict AL. They found that preoperative intestinal fatty acid binding protein levels predicted anastomotic leakage at a cutoff level of 882 pg/mL with sensitivity of 50%, specificity of 100%, positive likelihood ratio = infinite and negative likelihood ratio = 0.50 [11]. So that preoperative intestinal fatty acid binding protein measurement can be used for AL risk assessment. Besides, some markers of internal environment were used to act as biomarker of AL. There was a study to define the significance of hyponatremia as a marker of AL after colorectal surgery. They found that hyponatremia could be a specific and relevant marker of AL after colorectal surgery with a specificity of 93% and a sensitivity of 23% [25]. However, such studies are few and need to be confirmed further.

Table 3: The effective biomarkers, and diagnostic efficiency of the studies included in this review

First author, year, reference	Effective biomarkers	Diagnostic efficiency
Almeida, 2012, [6]	CRP (POD3, cutoff value:140 mg/L)	Sensitivity 78%, specificity 86%
Lagoutte, 2012, [7]	CRP (POD3, cutoff value: 13.0 mg/dL), PCT(POD3, cutoff value: 0.068 mg/dL)	CRP (sensitivity 70%, specificity 70%), PCT (sensitivity 70%, specificity 70%)
Torben, 2012, [8]	CRP (POD3, cutoff value:200 mg/L), WBC (POD2, cutoff value:12*10^9)	CRP (sensitivity 68%, specificity 74%); WBC (sensitivity 90%, specificity 62%)
alvaro, 2013, [9]	PCT (POD5, cutoff value:0.31 ng/ml)	Sensitivity 72%, specificity 100%
Giaccaglia, 2014, [10]	PCT (POD3 and POD5)	Negative predictive value (96.7% and 96.7% respectively)
Kostan, 2014, [11]	CRP: POD4, cutoff value: 99 mg/L,	Sensitivity 100%, specificity 64%
Marek, 2015, [12]	CRP (POD3, cutoff value: 245.64 mg/l), PCT (POD3, cutoff value:3.83 ng/ml)	CRP (sensitivity100%, specificity 98%), PCT (sensitivity 75%, specificity 100%)
Waterland, 2016, [13]	CRP (POD3 and POD4 with cut-off value of 209 and 123.5 mg/ml in laparotomy); CRP (POD2 with cut-off value of 146.5 mg/ml in laparoscopy)	CRP (sensitivity 80%, specificity 80% on POD3 and sensitivity 94%, specificity 60% on POD4 in laparotomy); CRP (sensitivity 75%, specificity 70% on POD2 in laparoscopy)
Valentina, 2016, [14]	PCT, CRP	Negative predictive value
Michal, 2017, [15]	CRP (POD4, cutoff value:180 mg/L), NLR (POD4, cutoff value:6.5)	CRP (sensitivity 75%, specificity 91%), NLR (sensitivity 69%, specificity 78%)
Stephen, 2017, [16]	CRP, cutoff value: 53 units	Sensitivity 90.9%, specificity 95.7%. a daily rise of around 50 units being most predictive
Burke, 2017, [17]	CRP (POD5, cutoff value:132 mg/l),	CRP (sensitivity 70%, specificity 76.6%),
Ismail, 2017, [18]	Serum CRP and PCT (POD3), Drain TNF-a and bacterial proliferation (POD5)	
Komen, 2014, [19]	LBP	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.
Urszula, 2016, [21]	PCT (POD1, cutoff value:1.09 ng/mL)	Sensitivity 87%, specificity 87%
Tarik, 2016, [22]	Peritoneal IL-6 (POD1, cutoff value:3091 pg/ml); peritoneal IL-10 (POD1, cutoff value:504 pg/ml)	Peritoneal IL-6 (sensitivity 86%, specificity 52%), peritoneal IL-10 (sensitivity 86%, specificity 62%)
Elyamani, 2011, [23]	Cytokines (IL-6, IL-10, TNF), microbiological study (Escherichia coli, Klebsiella, Pseudomonas species, and bacteroid micro-organism)	
Käser, 2014, [25]	Hyponatremia	Sensitivity 23%, specificity 93%
Liu, 2013, [26]	PH: POD3, cutoff value: 6.978	Sensitivity 98.7%, specificity 94.7%
Liron, 2016, [27]	CEA (1000 ng/mL)	

Some uncommon markers in drainage

Testing peritoneal biomarkers to detect AL seems to be more direct and accurate. So expect for CRP, PCT, WBC, and microbiological study, there were some other peritoneal markers had been tried to predict AL following

colorectal surgery. Liu et al. try to demonstrate the value of potential of hydrogen (PH) of pelvic drainage in the identification of increased risk of AL after anterior resection for rectal cancer with a double stapling technique. They got positive result and concluded that an early and persistent decline of PH value of pelvic

drainage fluid after rectal surgery with anastomosis, is a marker of AL. A cut-off value of 6.978 determined at 25°C on POD3 maximizes the sensitivity and specificity [26]. Evidences showed that carcinoembryonic antigen (CEA) elevation were associated with the inflammation process that accompanies AL [19]. So the idea that measuring CEA of drain as an indicator of AL was put forward. According to this idea, Liron et al. evaluated CEA levels in abdominal drains after colorectal resection to assess its potential as an early marker to predict AL. The result showed that early anastomotic dehiscence was associated with a significant elevation of CEA in drain fluids. So they concluded that significant elevation of CEA levels in abdominal drains in the early postoperative period may indicate early anastomotic dehiscence [27]. Besides, there were also researchers aimed to determine if lipopolysaccharide-binding protein (LBP) can serve as screening tool for colorectal AL. They got that increased concentrations of LBP in drain fluid are significantly associated to a higher chance of AL and could contribute in a future prognostic model for AL [19]. Peritoneal biomarkers for detecting AL is very promising and worth further attempt.

Prospect

With the advance of ERAS, patients underwent colorectal resection discharged before AL appears. So, early detection of AL is important and significant. This paper above had summarized the studies about biomarkers for early diagnosing of AL. Some results were valuable and got some biomarkers to detect AL such as CRP, CPT, Microbiological study and so on. However, owing to the effect of sample size and research design, these studies did not get the unified conclusion. Besides, according the results, these indexes are more suitable for negatively predicting AL. So new ideal biomarkers for early diagnosing AL are need to be discovered. In my opinion, biomarkers in drain may be potential to detect AL early and accurately. As when AL happens, exception occurs firstly in anastomoses, so testing the drain around the anastomoses may get the sign early. Further, when AL happens, the intestinal juice may fly into the abdominal cavity. The intestinal juice contains a lot of bacteria and is acidic, so testing the PH of drain the marking microbiological study may contribute to detecting AL. Although there have been studies on this field, these studies were so few and the simple sizes were not large. Rigorous homogeneous and prospective trials are demanded to further confirm the role of drain in the early diagnosis of AL following colorectal resection.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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