Blood Galectin-3 Levels Predict Postoperative Complications after Colorectal Cancer Surgery

Akihisa Matsuda¹, Marina Yamada¹, Satoshi Matsumoto¹, Nobuyuki Sakurazawa¹, Youichi Kawano¹, Kumiko Sekiguchi¹, Takeshi Yamada², Takeshi Matsutani², Masao Miyashita³ and Hiroshi Yoshida³

¹Department of Surgery, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan
²Department of Surgery, Nippon Medical School, Tokyo, Japan

Background: Recent studies suggested that galectin-3 may act as a pro-inflammatory damage-associated molecular pattern. The aim of this study is to investigate the association between blood galectin-3 and postoperative complications (POC) after colorectal cancer (CRC) surgery.

Methods: Blood samples were taken from 35 patients with CRC before surgery, immediately after surgery, and on postoperative days (POD) 1, 3, 5, and 7. Blood galectin-3 and interleukin-6 levels were measured by commercially available ELISA. Patients were divided into those with (POC group) and without POC (no-POC group).

Results: Significantly higher galectin-3 levels were observed pre- and postoperatively in the POC group (n=10) compared with those of the no-POC group (n=25). Galectin-3 levels on POD1 showed the best predictive potential for POC (cut-off: 3.18 pg/mL, area under the curve: 0.868).

Conclusions: These results indicate that increased perioperative blood galectin-3 levels may be associated with POC after CRC surgery. (J Nippon Med Sch 2019; 86: 142–148)

Key words: colorectal cancer, galectin-3, postoperative complication, surgery

Introduction

The incidence of colorectal cancer (CRC) in Japan is increasing, and CRC was recognised as the commonest cancer in 2015¹. Although surgical resection has been a mainstay for the treatment of CRC, high rates of postoperative complications (POC) (about 30%) and mortality (about 4%) are reported, despite improvements in surgical techniques and perioperative management²,³. Recent studies demonstrated that POC were associated with poorer long-term outcomes after CRC surgery⁴–⁷, and prevention of POC is thus a critical issue for surgeons. But prevention is difficult, because multiple risk factors promote the occurrence of POC, such as comorbidities, surgical procedures, operation time, and transfusion of blood⁸–¹¹. Recent interest has focused on perioperative pro-inflammatory immunological host responses as distinct predictive biomarkers for POC after CRC surgery¹²–¹⁶.

Galectin-3 is a 31-kDa chimeric galectin characterized by a single C-terminal carbohydrate recognition domain (CRD) and an N-terminal aggregating domain that interacts with non-carbohydrate ligands to allow the formation of oligomers¹⁷–¹⁹. The functions of galectin-3 are diverse, including roles in cell migration, adhesion, apoptosis, and inflammatory and immunomodulatory activities. Studies have shown that galectin-3 is associated with several inflammatory diseases, such as sepsis and airway inflammation¹⁸–²⁰. Galectin-3 was recently shown to provoke the inflammatory responses in sepsis, and has emerged as a ‘damage-associated molecular pattern (DAMP)’²¹. However, the influence of galectin-3 on surgical outcomes following gastrointestinal surgery is unknown. We evaluated the association between blood galectin-3 levels and the development of POC after elec-
Galectin-3 and Postop Complications

Table 1 Clinical characteristics of patients with and without postoperative complications and results of univariate analyses

| Variables                  | No POC group (n=25) | POC group (n=10) | P value |
|----------------------------|---------------------|------------------|---------|
| Sex (male/female)          | 16/9                | 7/3              | 1.000   |
| Age (years)                | 67.2±1.6            | 68.4±2.7         | 0.711   |
| BMI (kg/m²)                | 22.6±0.6            | 22.4±1.0         | 0.800   |
| Comorbidity (yes/no)       | 15/10               | 7/3              | 0.709   |
| ASA score (1/2/3)          | 12/13/0             | 3/7/0            | 0.458   |
| Tumor location (Colon/Rectum) | 13/12            | 3/7              | 0.285   |
| Pathological stage (JSSCR) | 2/2/13/6/2          | 1/2/1/5/1        | 0.105   |
| Surgical approach (open/laparoscopic) | 1/24          | 2/8              | 0.190   |
| Resection of other organ (yes/no) | 1/24            | 1/9              | 0.496   |
| Surgical duration (min)    | 270±19              | 364±39           | 0.046   |
| Intraoperative blood loss (mL) | 64±19              | 289±102          | 0.059   |
| Preoperative blood exam     |                     |                  |         |
| White blood cells (counts/μL) | 6,356±309          | 6,375±820        | 0.983   |
| C-reactive protein (mg/dL) | 0.5±0.1             | 0.7±0.5          | 0.679   |
| IL-6 (ng/mL)               | undetectable        | undetectable     | -       |
| Albumin (g/dL)             | 3.9±0.1             | 3.7±0.1          | 0.246   |
| Galectin-3 (ng/mL)         | 2.8±0.4             | 4.4±0.7          | 0.045   |

Values are expressed as mean ± SE. POC; postoperative complications, BMI; body mass index, ASA; American Society of Anesthesiologists, JSSCR; Japan Society for Cancer of the Colon and Rectum

Table 2 Details of postoperative complications

| Clavien-Dindo grading | Postoperative complications | n   |
|-----------------------|-----------------------------|-----|
| I                     | Superficial SSI             | 2   |
| II                    | Minor anastomotic leakage   | 1   |
|                      | Intraabdominal abscess      | 1   |
| IIIa                  | Deep SSI                    | 1   |
|                      | Adhesional small bowel obstruction | 1 |
| IIIb                  | Major anastomotic leakage   | 2   |
|                      | Intraabdominal abscess      | 1   |

SSI: surgical site infection

Materials and Methods

Subjects

This single-institutional prospective study from January 2014 to December 2015 was conducted at the Department of Surgery of Nippon Medical School Chiba Hokusoh Hospital. Thirty-five patients with CRC with elective primary tumor resection were included in this study. Antibiotic prophylaxis was administered for all patients. Patients under eighteen years old, having ongoing infection, preoperative chemotherapy and/or radiation therapy, or severe organ dysfunction were excluded from this study. The approach of surgery, open or laparoscopic was determined by patient factors and the surgeons’ decision. The study was performed based on the standards of the Helsinki Declaration. This study protocol was approved by the Ethics Committee of the institution (approval no. 562).

Postoperative complications were categorised by the Clavien-Dindo grading system2. Complications of grade ≥2 occurring before the end of the individual follow-up period of 30 days postoperatively were defined as POC in this study. The included patients (n=35) were divided into either group based on the presence (POC group, n=10) or absence (no-POC group, n=25) of POC. Related clinical data were collected at chart review.

Blood Collection and Measurements

Blood samples were collected from the antecubital vein before (pre) and immediately after surgery (post), and on 1, 3, 5, and 7 postoperative days (POD) in the morning. White blood cell count, C-reactive protein (CRP), and albumin levels were routinely monitored at the institutional laboratory. The detection limit of CRP was 0.02 mg/dL. The serum was extracted by centrifugation at 1,000× for 10 min at 4°C and stored at −80°C. Blood galectin-3 and interleukin (IL)-6 levels were analysed by commercially available kits (DuoSet ELISA, R&D Systems, Minneapolis, MN, USA) at all the stated time points.
Perioperative Changes in Galectin-3

Patients in the POC group showed a significant increase of preoperative galectin-3 levels than the no-POC group (Table 1). Galectin-3 levels increased immediately after surgery in both groups and then recovered (Fig. 1). Galectin-3 levels immediately after surgery were significantly higher than preoperative levels in both groups, while levels in the POC group were significantly higher than the no-POC group throughout all perioperative periods.

Perioperative Inflammatory Responses

The perioperative changes in pro-inflammatory markers, including WBC, CRP, and IL-6, are summarized in Table 3. Preoperative WBC and CRP levels were equivalent in the POC and no-POC groups, but both parameters were significantly higher in the POC group on POD 5 and 7. However, no significant differences in IL-6 levels were observed throughout the entire perioperative period, because of the high degree of individual variation.

Evaluation of Galectin-3 as a Predictive Marker for POC

ROC curve analyses of blood galectin-3 levels pre, post, and on POD1 versus POC are shown in Figure 2. Levels at all three time points showed relatively high predictive potentials for POC (>-0.7 of the area under the ROC curve (AUC)). Among these, the POD1 value showed the best predictive performance (cut-off: 3.18 pg/mL, AUC: 0.868, 95% confidence interval [CI]: 0.745–0.991), with a sensitivity and specificity of 68.0% and 90.0%, respectively.
Galectin-3 and Postop Complications

Table 3. Perioperative inflammatory responses related to postoperative complications

| Variables | Pre | POD1 | POD3 | POD5 | POD7 |
|-----------|-----|------|------|------|------|
| White blood cells (counts/μL) | 6.35±0.39 | 6.27±0.82 | 7.18±0.50 | 9.07±1.29 | 10.09±1.29 |
| C-reactive protein (mg/dL) | 0.4±0.1 | 0.4±0.12 | 0.7±0.3 | 0.7±0.3 | 0.5±0.1 |
| IL-6 (ng/mL) | 12.1±2.31 | 25.7±7.7 | 133±33 | 187±37 | 98±33 |

Values are expressed as mean ± SE. ud: undetectable. *P<0.05 vs. No POC group.

Discussion

The results of this study demonstrate that high perioperative blood galectin-3 levels are associated with POC after CRC surgery. We also showed that surgical stress transiently increased blood galectin-3 levels immediately after surgery, with gradual recovery of levels thereafter.

Galectins comprise a group of evolutionarily conserved proteins present in vertebrates, invertebrates, and fungi. Fifteen mammalian galectins have been discovered to date, all including a characteristic CRD of about 130 amino acids, through which they can bind β-galactosides. Galectin-3 is classified in the chimera-type group, with a C-terminal CRD and a large N-terminal protein-binding domain. The distribution of galectin-3 is wide-range throughout the body, including in the digestive and urogenital tracts, lungs, and heart. Also, galectin-3 is abundantly expressed in myeloid cells (monocytes, macrophages, dendritic cells, neutrophils) and in epithelial and endothelial cells. It is located predominantly in the cytoplasm, and can be secreted extracellularly following stimulation with various agents such as lipopolysaccharide, under both physiological and pathophysiological conditions. Extracellular galectin-3 is involved in various functions, including immunity against pathogens and in both acute and chronic inflammation. Recent studies have demonstrated that galectin-3 can recognize microbial structures as a pathogen-recognition receptor, as well as having pro-inflammatory properties eliciting infiltration of neutrophils and other immune cells, and that it can also be released as a DAMP. Regarding its role as a DAMP, Mishra et al. recently reported that galectin-3 was released in the lungs of mice in a Francisella novicida lethal sepsis model, and elicited neutrophil infiltration, inflammatory cytokine release, vascular injury, and the release of various inflammatory mediators from neutrophils.

Blood galectin-3 level has been broadly applied as a biomarker in a number of inflammatory diseases, such as cancers, heart failure, stroke, rheumatoid arthritis, and sepsis. Mueller et al. reported an approximately 2.7-fold increase in blood galectin-3 levels in human sepsis than healthy controls. Oever et al. also reported that galectin-3 levels were higher in patients with infections (viral respiratory infections, bacterial sepsis, and candidaemia) than controls or patients with non-infectious inflammatory diseases (gout or autoimmune inflammatory syndrome). The ability of galectin-3 to segregate between an infection and non-infectious inflammation is superior to
CRP; but, galectin-3 could not segregate between bacterial and Candida sepsis. Interestingly, galectin-3 levels were 2-fold higher in patients with Gram-negative than Gram-positive infections, though the difference was not significant.23 The immunological characteristics of galectin-3 may explain the postoperative increase in blood levels in patients with POC in the current study. Notably, nine of the 10 patients with POC had infectious complications. Furthermore, Gram-negative enterobacteria, such as Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa, are known to be isolated predominantly from surgical site infections and could be responsible for causing inflammation.26

The early detection of POC would aid clinical decision-making and provide significant opportunities to achieve better patient outcomes. Pro-inflammatory and immunological blood biomarkers have recently received much attention in relation to their abilities to predict POC after CRC surgery.17-20. CRP is used routinely as a pro-inflammatory marker to monitor postoperative condition, and has been widely reported to predict POC after CRC surgery; however, its best predictive potential is based on levels measured on POD3 or 4, which is relatively late for effective interventions given the usual diagnostic period for POC (POD 5 in our study cohort). Retting et al. recently reported that higher IL-6 blood levels on POD1 provided the best predictive value for POC after major abdominal surgery compared with CRP, tumor necrosis factor-α, and leukocyte count. However, the predictive potential of IL-6 on POD1 in the current study was relatively low (AUC: 0.67) compared with that for galectin-3 (AUC: 0.868). Neither CRP nor IL-6 levels demonstrated any predictive value in our study cohort.

It should be noted that the significant difference of blood galectin-3 levels was observed preoperatively, which could imply an important and intrinsic pathophysiological difference between the two groups. The POC group had a slight increase of preoperative CRP levels (but not statistically significant) without any evidence of infection. This result suggested that patients in the POC group were preoperatively under “low-grade chronic inflammation condition”, which is associated with dormant pathological conditions, such as visceral obesity, arteriosclerosis, and diabetes mellitus. These disease conditions are reported to have higher blood levels of galectin-3 than control patients.30-31. The preoperative low-grade chronic inflammation condition could be a trigger for an exaggerated hyperinflammation induced by surgical stress and result in the occurrence of POCs.32,33

The results of our study offer several potential clinical benefits. In the case of patients with blood galectin-3 levels below the cut-off value, surgeons can be reassured...
that POC are unlikely to occur, thus allowing immediate patient discharge. In contrast, higher galectin-3 levels can alert surgeons to the possibility of POC at an early stage, allowing the patient to be reassessed and managed accordingly.

This observational study had certain limitations: 1) this was a single-institution study with a small cohort of patients, which may have affected the predictive potential of the marker to discriminate between the presence and absence of POC; 2) POD1 blood samples were collected at different times after surgery, because the starting time of surgery varied; 3) the degree of surgical stress may differ among different procedures.

In conclusion, surgical stress may increase blood galectin-3 levels and high perioperative blood galectin-3 levels may be associated with POC after CRC surgery. Future studies with larger sample sizes are warranted to clarify the predictive value of galectin-3, its role in the pathophysiology of POC, and its potential in terms of therapeutic interventions for POC.

Acknowledgements: This study was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (15K10037: Matsuda A). We thank Susan Furness, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of Interest: All authors have no conflict of interest to declare.

References
1. Service CI: Projected Cancer Statistics, 2016 (http://ganjo ho.jp/en/public/statistics/short_pred.html).
2. Alves A, Panis Y, Mathieu P, Mantion G, Kwiatkowski F, Slim K, Association Francaise de C: Postoperative mortality and morbidity in French patients undergoing colorectal surgery: results of a prospective multicenter study. Arch Surg 2005; 140: 278–283, discussion 284.
3. Sjo OH, Larsen S, Lunde OC, Næsbakken A: Short term outcome after emergency and elective surgery for colon cancer. Colorectal Dis 2009; 11: 733–739.
4. Artiniyan A, Orcutt ST, Anaya DA, Richardson P, Chen GJ, Berger DH: Infectious postoperative complications decrease long-term survival in patients undergoing curative surgery for colorectal cancer: a study of 12,075 patients. Ann Surg 2015; 261: 497–505.
5. Brown SR, Mathew R, Keding A, Marshall HC, Brown JM, Jayne DG: The impact of postoperative complications on long-term quality of life after curative colorectal cancer surgery. Ann Surg 2014; 259: 916–923.
6. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P: Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg 2011; 253: 890–899.
7. Richards CH, Platt JJ, Anderson JH, McKee RF, Horgan PG, McMillan DC: The impact of perioperative risk, tumor pathology and surgical complications on disease recurrence following potentially curative resection of colorectal cancer. Ann Surg 2011; 254: 83–89.
8. Song L, Mao J, Zhang J, Ibrahim MM, Li LH, Tang JW: Annexin A7 and its binding protein galectin-3 influence mouse hepatocellular carcinoma cell line in vitro. Biomed Pharmacother 2014; 68: 377–384.
9. Song L, Tang JW, Owusu L, Sun MZ, Wu J, Zhang J: Galectin-3 in cancer. Clin Chim Acta 2014; 431: 185–191.
10. Buljan M, Situm M, Tomas D, Milosevic M, Kruslin B: Prognostic value of galectin-3 in primary cutaneous melanoma. J Eur Acad Dermatol Venereol 2011; 25: 1174–1181.
11. Dumont P, Berton A, Nagy N, Sandras F, Tinton S, Demetter P, Mascarf F, Allouei A, Decaestecker C, Salmon I: Expression of galectin-3 in the tumor immune response in colon cancer. Lab Invest 2008; 88: 896–906.
12. McSorley ST, Khor BY, MacKay GJ, Horgan PG, McMillan DC: Examination of a CRP first approach for the detection of postoperative complications in patients undergoing surgery for colorectal cancer: A pragmatic study. Medicine (Baltimore) 2017; 96: e6133.
13. McSorley ST, Ramanathan ML, Horgan PG, McMillan DC: Postoperative C-reactive protein measurement predicts the severity of complications following surgery for colorectal cancer. Int J Colorectal Dis 2015; 30: 913–917.
14. Platt JJ, Ramanathan ML, Crosbie RA, Anderson JH, McKee RF, Horgan PG, McMillan DC: C-reactive protein as a predictor of postoperative infective complications after curative resection in patients with colorectal cancer. Ann Surg Oncol 2012; 19: 4168–4177.
15. Rettig TC, Verwijnenen L, Dijkstra IM, Boerma D, van de Garde EM, Noordzij PG: Postoperative Interleukin-6 Level and Early Detection of Complications After Elective Major Abdominal Surgery. Ann Surg 2016; 263: 1207–1212.
16. Watt DG, McSorley ST, Park JH, Horgan PG, McMillan DC: A Postoperative Systemic Inflammation Score Predicts Short- and Long-Term Outcomes in Patients Undergoing Surgery for Colorectal Cancer. Ann Surg Oncol 2017; 24: 1100–1109.
17. Abdelkrim MM, Hamama MA, Farergy SE, Farag AG, El Shafey EN, Farouk S, Elnawardany NF: Diagnostic and prognostic role of galectin 3 expression in cutaneous melanoma. Am J Dermatopathol 2010; 32: 809–814.
18. Dancer YJ, Truong LD, Zhai Q, Shen SS: Expression of Galectin-3 in renal neoplasms: a diagnostic, possible prognostic marker. Arch Pathol Lab Med 2010; 134: 90–94.
19. Issa SE, Christensen AF, Lindegaard HM, Hetland ML, Horslev-Petersen K, Stengard-Pedersen K, Eljbjerg BJ, Lottenburger T, Ellingsen T, Pedersen JK, Junker K, Svendsen A, Tarp U, Ostergaard M, Junker P: Galectin-3 is Persistently Increased in Early Rheumatoid Arthritis (RA) and Associates with Anti-CCP Seropositivity and MRI Bone Lesions, While Early Fibrosis Markers Correlate with Disease Activity. Scand J Immunol 2017; 86: 471–478.
20. Wang A, Zhong C, Zhu Z, Xu T, Peng Y, Xu T, Peng H, Chen CS, Wang J, Ju Z, Li Q, Geng D, Sun Y, Zhang J, Yuan X, Chen J, Zhang Y, He J: Serum Galectin-3 and Poor Outcomes Among Patients With Acute Ischemic Stroke. Stroke 2018; 49: 211–214.
21. Mishra BB, Li Q, Steichen AL, Binstock BJ, Metzger DW, Teale JM, Sharma J: Galectin-3 functions as an alarmin:
pathogenic role for sepsis development in murine respiratory tularemia. PLoS One 2013; 8: e59616. doi: 10.1371/journal.pone.0059616. Epub 2013 Mar 20.

22. Dindo D, Demartines N, Clavien PA: Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004; 240: 205–213.

23. ten Oever J, Giamarellos-Bourboulis EJ, van de Veerdonk FL, Stelma FF, Simon A, Janssen M, Johnson M, Pachot A, Kullberg BJ, Joosten MG: Circulating galectin-3 in infections and non-infectious inflammatory diseases. Eur J Clin Microbiol Infect Dis 2013; 32: 1605–1610.

24. Mueller T, Leitner I, Egger M, Hallmayer M, Dieplinger B: Association of the biomarkers soluble ST2, galectin-3 and growth-differentiation factor-15 with heart failure and other non-cardiac diseases. Clin Chim Acta 2015; 445: 155–160.

25. Tonouchi H, Ohmori Y, Tanaka K, Mohri Y, Kobayashi M, Kusunoki M: Postoperative weight loss during hospital stays in patients with gastric cancer undergoing surgical resection. Hepatogastroenterology 2008; 55: 803–806.

26. Kobayashi M, Mohri Y, Inoue Y, Okita Y, Miki C, Kusunoki M: Continuous follow-up of surgical site infections for 30 days after colorectal surgery. World J Surg 2008; 32: 1142–1146.

27. Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ: Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. Clin Res Cardiol 2010; 99: 323–328.

28. Korner H, Nielsen HJ, Soreide JA, Nedrebo BS, Soreide K, Knapp JC: Diagnostic accuracy of C-reactive protein for intraabdominal infections after colorectal resections. J Gastrointest Surg 2009; 13: 1599–1606.

29. Warschkow R, Steffen T, Beutner U, Muller SA, Schmied BM, Tarantino I: Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. Int J Colorectal Dis 2012; 27: 1377.

30. Yu L, Ruifrok WP, Meissner M, Bos EM, van Goor H, Sanjabi B, van der Harst P, Pitt B, Goldstein IJ, Koerts JA, van Veldhuisen DJ, Bank RA, van Gilst WH, Silljé HH, de Boer RA: Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. Circ Heart Fail 2013; 6: 107–117.

31. Weigert J, Neumeier M, Wanninger J, Bauer S, Farkas S, Scherer MN, Schnitzbauer A, Schäffler A, Aslanidis C, Schölmerich J, Buechler C: Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes. J Clin Endocrinol Metab 2010; 95: 1404–1411.

32. Sagawa M, Yoshimatsu K, Yokomizo H, Yano Y, Okayama S, Usui T, Yamaguchi K, Shiozawa S, Shimakawa T, Katsube T, Kato H, Nariyaka Y: Worse Preoperative Status Based on Inflammation and Host Immunity Is a Risk Factor for Surgical Site Infections in Colorectal Cancer Surgery. J Nippon Med Sch 2017; 84: 224–230.

33. Matsuda A, Jacob A, Wu R, Aziz M, Yang WL, Matsutani T, Suzuki H, Furukawa K, Uchida E, Wang P: Novel therapeutic targets for sepsis: regulation of exaggerated inflammatory responses. J Nippon Med Sch 2012; 79: 4–18.

(Received, July 8, 2018)

(Accepted, January 22, 2019)