

## Original Article

# IMP3 expression in biopsy specimens of colorectal cancer predicts lymph node metastasis and TNM stage

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**Abstract:** IMP3 is associated with lymph node metastasis and TNM stage and is a good independent prognostic biomarker for colorectal cancer (CRC). However, the expression status and clinical implication of IMP3 in biopsy specimens have not yet been studied. We aim to address whether the presence of IMP3 expression in preoperative biopsies of CRC could predict lymph node metastasis and TNM stage. In this study, we examined IMP3 expression in paired biopsy and resection specimens of 71 CRC and analyzed the correlation of IMP3 expression with clinicopathological parameters. In the biopsy specimens, IMP3 positive expression was observed in 56 of 71 cases (78.9%) whereas negative expression was observed in 15 of 71 cases (21.1%). In the resection specimens, IMP3 positive expression was detected in 83.1% cases (59/71) whereas negative expression was detected in 16.9% cases (12/71). The absolute concordance rate between biopsy and resection specimens was 90.1% (64/71). The Spearman correlation test documented the existence of a strong linear correlation between the percentage of IMP3-positive cells in the biopsy and resection specimen ( $r = 0.629$ ;  $P < 0.001$ ). IMP3 expression in resection specimens was significantly related to histological grade ( $P = 0.043$ ), T classification ( $P = 0.035$ ), lymph node metastasis ( $P = 0.023$ ), TNM stage ( $P = 0.007$ ), tumor border ( $P = 0.049$ ) and tumor budding ( $P = 0.012$ ). IMP3 expression in biopsy specimens was significantly related to lymph node metastasis ( $P = 0.004$ ), TNM stage ( $P = 0.005$ ) and tumor budding ( $P = 0.001$ ). In conclusion, IMP3 expression in biopsy specimens could be used to predict lymph node metastasis and TNM stage in CRC patients.

**Keywords:** Colorectal cancer, biopsies, IMP3, lymph node metastasis, TNM stage

## Introduction

Colorectal cancer (CRC) is the third most common cancers in the worldwide [1]. In 2012, more than 140,000 individuals in the United States were diagnosed with CRC, 36% of individuals will die of their disease [2]. To improve this result, the modern evolution of treatment of CRC towards a multidisciplinary management [3]. Advances in preoperative chemotherapy and radiation have reduced the disease recurrence and increased survival in high risk diseases [4]. In many patients with rectal cancer, it is now well accepted that preoperative radiation or chemoradiation therapy given to reduce the risks of cancer relapse [3, 4]. The preoperative chemotherapy for resectable liver metastases from CRC reduced the risk of events of progression-free survival by a quarter and was compatible with major surgery [5].

Study have shown that patients with locally advanced, but resectable, colon cancer can be appropriately selected for neoadjuvant chemotherapy with CT scanning and can safely undergo preoperative chemotherapy followed by colonic resectional surgery, without incurring significant perioperative morbidity [6]. Consequently, the use of preoperative chemotherapy and radiation may become routine in the management of CRC.

The American Joint Committee on Cancer (AJCC) TNM stage is the most important prognostic factor for patients with CRC. Accurate preoperative staging of CRC are essential for deciding on treatment strategies in the multidisciplinary management. Locally advanced cancers are normally treated with preoperative therapy before surgical resection. The most frequently used imaging modalities for preopera-

tive staging of CRC are ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT (PET/CT) [7, 8]. These imaging modalities are unreliable in identifying lymph node metastasis (accuracies of MRI are 39-95%, CT are 22-73%, US are 62-83%) [8]. Inaccurate radiological staging might result in inappropriate preoperative chemotherapy and radiotherapy. Therefore, the need for additional predictive marker for preoperative staging that would enable better categorization of the patients and eventually provide a guide for individualized therapy.

Preoperative chemotherapy and radiotherapy can lead to partial or complete pathological regression of CRC. Complete pathological response rates are described in 10% to 27% of patients with rectal cancer [9]. In the circumstance, the resection specimen can't be used to detect by immunohistochemistry for prognostic evaluation, and the pretherapeutic biopsy may be the only tissue suitable for analysis. The challenge for the pathologist will be to determine and validate new prognostic and predictive markers by using pretherapeutic biopsies.

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a member of the insulin-like growth factor (IGF) mRNA binding protein (IMP) family that consists of IMP1, IMP2, and IMP3 [10]. Large cohort and independent studies have demonstrated that IMP3 on resection specimens is associated with lymph node metastasis and TNM stage and is a good independent prognostic biomarker for CRC [11-14, 17]. To date, no systematic study has been performed to ascertain the concordance of IMP3 expression between paired endoscopic biopsy and resection specimens in CRC patients. To the best of our knowledge, the expression status and clinical implication of IMP3 have not yet been studied in biopsy specimens.

In this study, we examined IMP3 expression in paired biopsy and resection specimens of CRC and determined the correlation of IMP3 expression with clinicopathological parameters. We aim to address whether the presence of IMP3 expression in preoperative biopsies of CRC could predict lymph node metastasis and TNM stage.

## Materials and methods

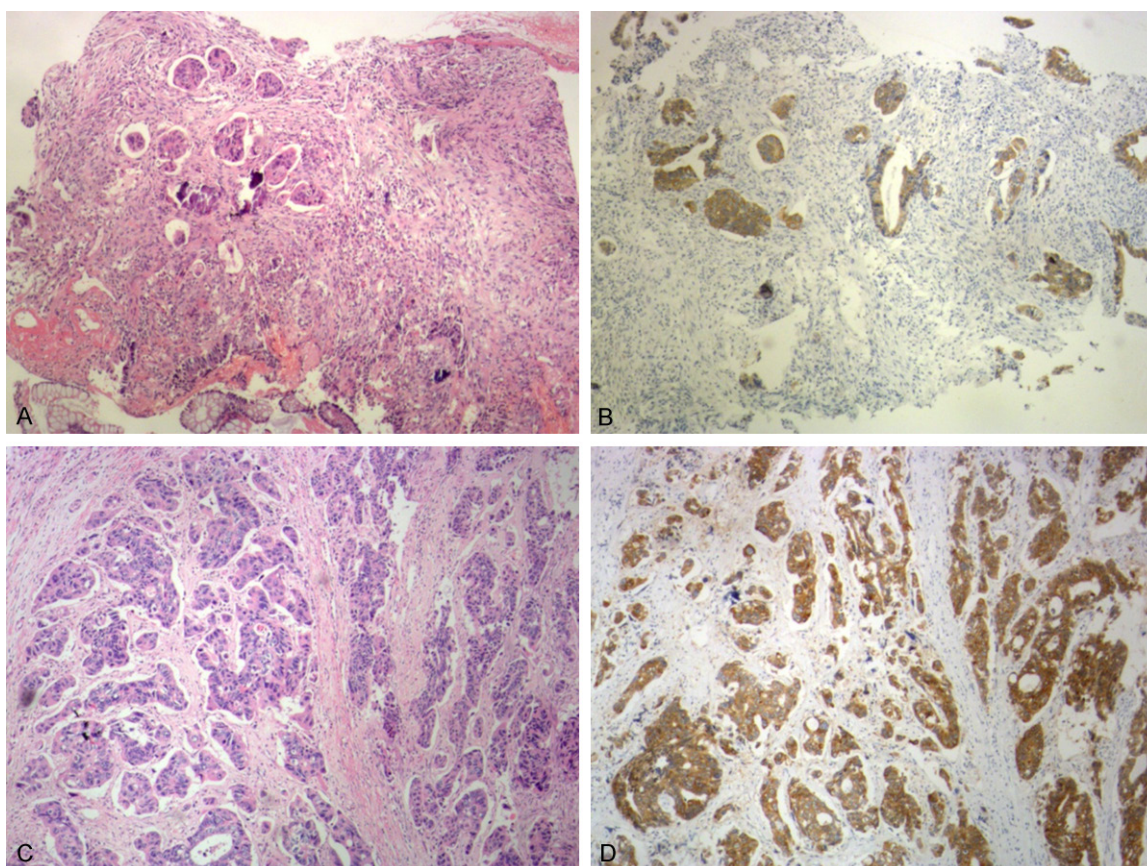
### *Patients*

The experimental protocol used in this study was approved by the Human Ethics Review Committee of the Third Affiliated Hospital. Patients who had both preoperative biopsy and subsequent resection specimens at our institution were included in the study. Seventy one patients were identified from the databases. Clinical data were obtained from patient records including age, gender, tumor location, tumor size, preoperative CT scan and information on preoperative therapy. There were 38 males and 33 females with an average age of 61.5 years (range, 25-88 years). No patient underwent preoperative radiotherapy or chemotherapy. All specimens were routinely fixed in 10% buffered formalin and embedded in paraffin, and 4  $\mu$ m sections were cut and stained with hematoxylin and eosin (H&E). All H&E slides of both the biopsy and resection specimens were reevaluated, and the diagnosis was confirmed by two experienced pathologists (W. QingZhu and Z. Tong). For biopsy specimens, the presence of invasive carcinoma was required for the study. All resection specimens were classified according to the TNM classification system. Histological subtype, presence of lymphovascular invasion, Lymph node metastasis, tumor border and tumor budding were assessed. The histological grade was assessed according to the WHO 2010 criteria [15].

Tumor budding was defined as dedifferentiated single cells or clusters of < 5 cells at the invasive tumor front. The extent of tumor budding was assessed on CDX2-stained whole tissue sections as described previously by Karamitopoulou et al [16]. The CDX2-stained whole tissue sections were first examined at low magnification (4  $\times$ ) and selected the area with the highest density of peritumoral budding. The average number of buds was counted in 10 HPFs (40  $\times$ ). Tumors were divided into two groups according to the average number of budding: low grade ( $\leq$  10 buds) and high grade (> 10 buds).

### *Immunohistochemical analysis*

Immunohistochemical staining was performed on 4  $\mu$ m sections of paraffin-embedded tissue. Briefly, the tissue sections were deparaffinized



**Figure 1.** Biopsy (A) and resection specimens (C) of colorectal cancer stained with hematoxylin and eosin. The tumor shows IMP3 positive expression in biopsy (B) and resection specimens (D) ( $\times 40$ ).

and rehydrated. Heat-induced antigen retrieval was carried out at 95°C for 15 min in citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide solution. Sections were immunostained using a monoclonal mouse anti-human antibody against IMP3 (clone 69.1; 1:100 dilution; Dako North America, Carpinteria, CA, USA) and CDX2 (clone EPR2764Y; 1:100 dilution; Gene Tech Company, Ltd., Shanghai, China). The GTVision™ Detection system (Gene Tech) and 3,3'-diamino-benzidine (Gene Tech) were used to detect antibody-conjugated peroxidase activity. Lastly, the sections were counterstained with haematoxylin, dehydrated, and mounted. Pancreatic carcinoma cases were used as positive controls. Negative control sections were prepared by substituting non-immune IgG for the primary antibody. Immunostaining was assessed by an experienced pathologist (W. QingZhu) who was blinded to the clinical data of the patients.

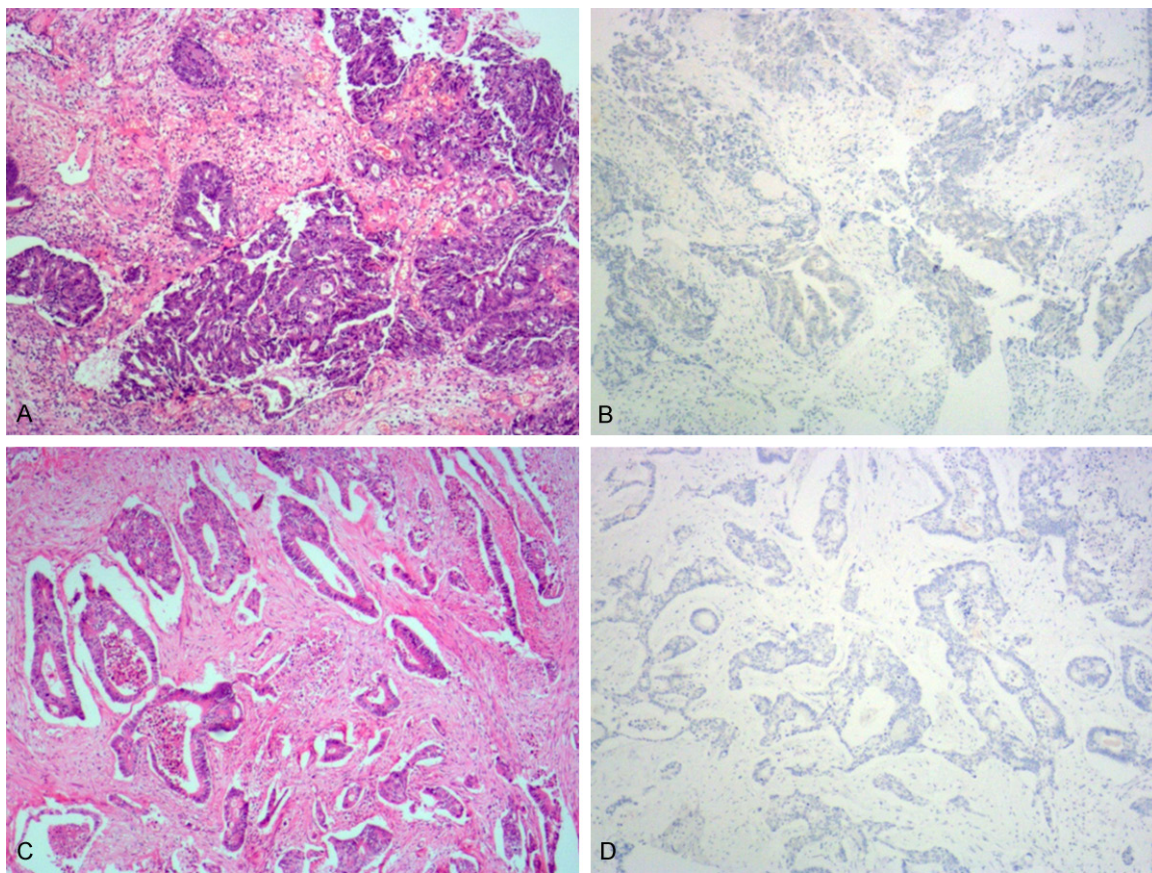
Samples with cytoplasmic IMP3 staining were scored as positive. Intensity was classified as

negative, weak, moderate, or strong. The percentage of positive cells was graded using the following five categories: 0 (1%-10% of cells stained), 1 (11%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (76%-100%). IMP3 staining of more than 10% of the tumor cells in biopsy and resection specimens was considered positive. Nuclear staining for CDX2 was considered positive in resection specimens.

## Statistical analysis

The Spearman correlation coefficient was used to analyze the correlation between percentage of IMP3-positive cells in the biopsy and resection specimens. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the biopsy and CT scan were calculated. The correlation of IMP3 expression with clinicopathological parameters was evaluated by Pearson chi-squared test. *P* values less than 0.05 were considered statistically significant. SPSS (Version 13.0; SPSS Inc. Chicago, IL, USA) were used to analyze the data.





**Figure 2.** Biopsy (A) and resection specimens (C) of colorectal cancer stained with hematoxylin and eosin. The tumor shows IMP3 negative expression in biopsy (B) and resection specimens (D) ( $\times 40$ ).

## Results

### *Correlation between IMP3 expression in the biopsy and resection specimens*

In the biopsy specimens, IMP3 positive expression was observed in 56 of 71 cases (78.9%) whereas negative expression was observed in 15 of 71 cases (21.1%). IMP3 staining intensity was weak, moderate and strong in 15 (26.8%), 17 (30.4%) and 24 (42.8%) specimens, respectively. In the resection specimens, IMP3 positive expression was detected in 83.1% cases (59/71) whereas negative expression was detected in 16.9% cases (12/71). IMP3 staining intensity was weak, moderate and strong in 13 (22.0%), 19 (32.2%) and 27 (45.8%) specimens, respectively. Of the 56 IMP3 positive expression in biopsy specimens, 54 (96.4%) were confirmed (**Figure 1**), whereas 2 (3.6%) were negative in the resection specimens (false positive). Of the 15 IMP3 negative expression in biopsy specimens, 10 (66.7%) were con-

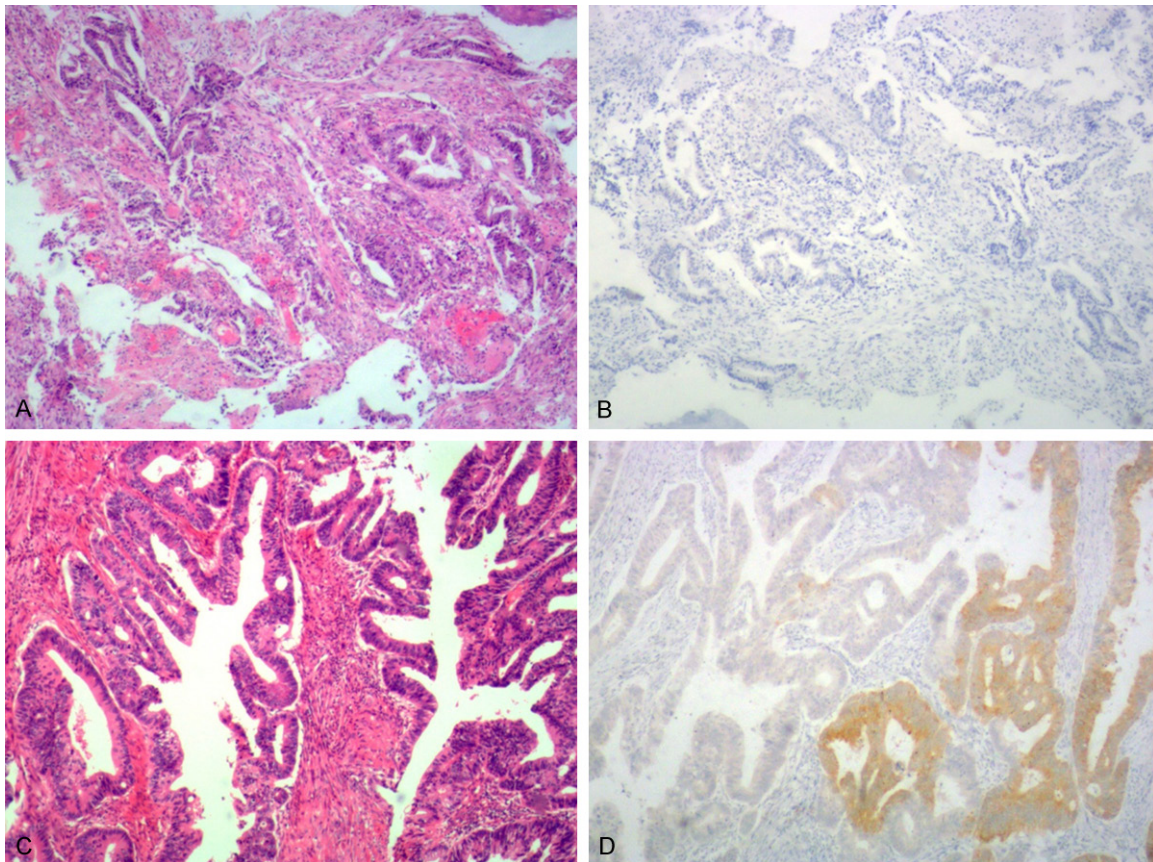
firmed (**Figure 2**), whereas 5 (33.3%) were positive in the resection specimens (false negative) (**Figure 3**). The absolute concordance rate between biopsy and resection specimens was 90.1% (64/71), whereas a discrepancy was found in 7 cases (9.9%). The sensitivity, specificity, PPV, and NPV for biopsy specimens were 91.5%, 83.3%, 96.4%, and 66.7%, respectively. The Spearman correlation test documented the existence of a strong linear correlation between the percentage of IMP3-positive cells in the biopsy and resection specimen ( $r = 0.629$ ;  $P < 0.001$ ) (**Table 1**).

### *Relationship between IMP3 expression in the biopsy and resection specimens and clinico-pathological characteristics*

IMP3 expression in resection specimens was significantly related to histological grade ( $P = 0.043$ ), T classification ( $P = 0.035$ ), lymph node metastasis ( $P = 0.023$ ), TNM stage ( $P = 0.007$ ), tumor border ( $P = 0.049$ ) and tumor budding ( $P$



## IMP3 expression in biopsy specimens



**Figure 3.** Biopsy (A) and resection specimens (C) of colorectal cancer stained with hematoxylin and eosin. The tumor shows IMP3 negative expression in biopsy specimens (B) and heterogeneous IMP3 expression (one area negative and the other positive) in resection specimens (D) ( $\times 40$ ).

**Table 1.** The concordance of IMP3 expression between biopsy and resection specimens

	Biopsy					Total	
	1%-10%	11%-25%	26%-50%	51%-75%	76%-100%		
Resection							P < 0.001
1%-10%	10	1	0	1	0	12	
11%-25%	2	1	4	0	3	10	
26%-50%	2	2	0	4	9	17	
51%-75%	0	0	0	1	3	4	
76%-100%	1	1	2	1	23	28	
Total	15	5	6	7	48	71	

= 0.012). In contrast, There was no association between IMP3 expression and age ( $P = 0.233$ ), gender ( $P = 0.788$ ), tumor location ( $P = 0.718$ ), tumor size ( $P = 0.291$ ), histological subtype ( $P = 0.846$ ) and lympho-vascular invasion ( $P = 0.311$ ) (**Table 2**).

IMP3 expression in biopsy specimens was significantly related to lymph node metastasis ( $P =$

0.004), TNM stage ( $P = 0.005$ ) and tumor budding ( $P = 0.001$ ). In contrast, There was no association between IMP3 expression and age ( $P = 0.458$ ), gender ( $P = 0.987$ ), tumor location ( $P = 0.708$ ), tumor size ( $P = 0.130$ ), histological grade ( $P = 0.640$ ), histological subtype ( $P = 0.257$ ), T classification ( $P = 0.115$ ), tumor border ( $P = 0.236$ ) and lympho-vascular invasion ( $P = 0.058$ ) (**Table 2**).

## IMP3 expression in biopsy specimens

**Table 2.** IMP3 expression in biopsy and resection specimens and clinicopathological characteristics

Characteristic	Biopsy				Resection			
	Total	positive (%)	negative (%)	P	positive (%)	negative (%)	P	
Colorectal cancer	71	56 (78.9)	15 (21.1)		59 (83.1)	12 (16.9)		
Age (years)				P = 0.458			P = 0.233	
≤ 45	10	7 (70.0)	3 (30.0)		7 (70.0)	3 (30.0)		
> 45	61	49 (80.3)	12 (19.7)		52 (85.2)	9 (14.8)		
Gender				P = 0.987			P = 0.788	
Female	33	26 (78.8)	7 (21.2)		27 (81.8)	6 (18.2)		
Male	38	30 (78.9)	8 (21.1)		32 (84.2)	6 (15.8)		
Tumor location				P = 0.708			P = 0.718	
Left sided	56	43 (76.8)	13 (23.2)		47 (83.9)	9 (16.1)		
Right sided	15	13 (86.7)	2 (13.3)		12 (80.0)	3 (20.0)		
Tumor size				P = 0.130			P = 0.291	
d ≤ 4 cm	26	18 (69.2)	8 (30.8)		20 (76.9)	6 (23.1)		
d > 4 cm	45	38 (84.4)	7 (15.6)		39 (86.7)	6 (13.3)		
Histological subtype				P = 0.257			P = 0.846	
Nonmucinous	64	50 (78.1)	14 (32.1.)		53 (82.8)	11 (17.2)		
Mucinous	7	6 (85.7)	1 (14.3)		6 (80.0)	1 (20.0)		
Histological grade				P = 0.640			P = 0.043	
Well and Moderate	59	44 (74.6)	15 (25.7)		47 (79.7)	12 (20.3)		
Poor	12	12 (100)	0 (0.0)		12 (100)	0 (0.0)		
T classification				P = 0.115			P = 0.035	
T1-T2	10	6 (60.0)	4 (40.0)		6 (60.0)	4 (40.0)		
T3-T4	61	50 (82.0)	11 (18.0)		53 (86.9)	8 (13.1)		
TNM stage				P = 0.005			P = 0.007	
I-II	34	22 (64.7)	12 (35.3)		24 (70.6)	10 (29.4)		
III-IV	37	34 (91.9)	3 (8.1)		35 (94.5)	2 (5.4)		
Lymph node metastasis				P = 0.004			P = 0.023	
Present	33	31 (93.9)	2 (6.1)		31 (93.9)	2 (6.1)		
Tumor border				P = 0.236			P = 0.049	
Infiltrating	47	39 (83.0)	8 (17.0)		42 (76.6)	5 (23.4)		
Pushing	24	17 (70.8)	7 (29.2)		17 (70.8)	7 (29.2)		
Tumor budding				P = 0.001			P = 0.012	
Low (≤ 10 buds)	35	22 (62.9)	13 (37.1)		25 (71.4)	10 (28.6)		
High (> 10 buds)	36	34 (94.4)	2 (5.6)		34 (94.4)	2 (5.6)		
Lympho-vascular invasion				P = 0.058			P = 0.311	
Absent	32	22 (68.8)	10 (31.2)		25 (78.1)	7 (21.9)		
Present	39	34 (87.2)	5 (12.8)		34 (87.2)	5 (12.8)		

### Association of IMP3 expression in the biopsy and CT scan with lymph node metastases

Sixty four patients who had information of CT scan in detecting lymph node metastases were identified from the databases (**Table 3**). For lymph node metastases, IMP3 expression in the biopsy had a sensitivity of 93.9%, a specificity of 34.2%, a PPV of 55.4%, and an NPV of 86.7%, whereas CT had a sensitivity of 45.2%,

a specificity of 72.7%, a PPV of 60.9%, and an NPV of 58.5%. IMP3 expression in the biopsy had a higher sensitivity and a lower specificity than CT scan for lymph nodal metastases.

### Discussion

IMP3 plays a pivotal role in the tumor cell proliferation, adhesion, invasion, and metastasis [10, 17]. Four large studies have shown that

**Table 3.** Association of CT scan with lymph node metastases

	Total	CT scan	
		negative (%)	positive (%)
Lymph node metastasis			
Absent	33	24 (72.7)	9 (27.3)
Present	31	17 (54.8)	14 (45.2)

IMP3 is an independent prognostic biomarker which predicts metastasis and poor prognosis in CRC. Lochhead et al [11] studied 671 CRCs, and demonstrated that IMP3 positivity was associated with poor differentiation ( $P = 0.0003$ ), stage III-IV disease ( $P = 0.0081$ ). Yuan et al [12] found diffuse IMP3 expression correlated with large tumor ( $P = 0.0452$ ), high-stage tumor ( $P = 0.0417$ ), lymph node metastasis ( $P = 0.0232$ ), high lymph node ratio ( $P = 0.0016$ ), and lower 5-year survival ( $P = 0.0012$ ). Li et al [13] also observed that overexpression of IMP3 was significantly associated with T classification ( $P = 0.001$ ), nodal involvement ( $P < 0.001$ ), and the AJCC stage ( $P < 0.001$ ). Lin et al [14] reported that high IMP3 expression was associated with differentiation, lymphoid metastasis, TNM stage, Ki-67 labeling index and a poor patient outcome ( $P < 0.05$ ). We demonstrated that IMP3 expression in resection specimens was significantly related to histological grade ( $P = 0.043$ ), T classification ( $P = 0.035$ ), lymph node metastasis ( $P = 0.023$ ), TNM stage ( $P = 0.007$ ), tumor border ( $P = 0.049$ ) and tumor budding ( $P = 0.012$ ). The presented data herein are consistent with previously published reports, which suggest that IMP3 expression in resection specimens can be used to predict lymph node metastasis and TNM stage. In addition, our results showed a significant correlation between IMP3 expression and tumor budding. The strong correlation suggests that IMP3 plays an important role in epithelial-mesenchymal transition.

Biopsy specimens are used increasingly as treatment options and prognostic evaluation in patients with CRC [18-23]. In the last few years, a number of studies have demonstrated high concordance between biopsy and resection specimens in CRC using IHC and molecular testing. Shia et al [18] analyzed the immunohistochemical staining patterns for MLH1, MSH2, MSH6, and PMS2 in paired biopsy and resec-

tion specimens in 70 carcinomas of the tubular gastrointestinal tract, and confirmed that biopsy samples are as reliable as resection specimens in predicting mismatch repair protein abnormality. Fadhil et al [19] used PCR to detect KRAS, BRAF, PIK3CA and TP53 mutations in CRC, and show biopsies are adequately representative of the tumor as a whole. To the best of our knowledge, no other study has attempted to evaluate and correlate IMP3 expression between biopsy and subsequent resection specimens. In our data, we showed a highly concordance (90.1%) of IMP3 expression between paired biopsy and resection specimens, and found a strong linear correlation between the percentage of IMP3-positive cells in the biopsy and resection specimen ( $r = 0.629$ ;  $P < 0.001$ ). The results suggest that biopsy specimens can be used to predict IMP3 status.

Preoperative chemotherapy and radiotherapy are more effective than similar postoperative treatment for oesophageal, gastric, and rectal cancers. The FOxTROT phase 3 studies are currently investigated in primary colon cancer [6]. If the FOxTROT trial is successful, then preoperative chemotherapy may become routine in the management of locally advanced CRC. Accurate preoperative staging is essential in determining the optimal therapeutic planning for individual patients. Currently, radiographic staging is standard of care in the pre-operative setting, but sub-optimal sensitivity and specificity for detection of lymph nodal metastasis have been reported [23]. Therefore, a biomarker for better risk stratification in CRC helps avoid over- or under-treatment. Here, our study explored the correlation of the expression of IMP3 and the clinicopathological characteristics in biopsy specimens of CRC patients. The results demonstrated that positive expression of IMP3 in biopsy specimens was significantly associated with lymph node metastasis ( $P = 0.004$ ), TNM stage ( $P = 0.005$ ) and tumor budding ( $P = 0.001$ ). We also found that IMP3 expression in the biopsy had a higher sensitivity than CT scan for lymph nodal metastases, suggesting that IMP3 expression in the biopsy is of higher value in detecting lymph nodal metastases. Our study results showed that IMP3 expression in biopsy specimens of CRC patients could be used as the potential marker for lymph node metastasis and TNM stage.



A discrepancy was found between biopsy and resection specimens in 7 cases (9.9%). Of the 7 cases, 5 (71.4%) were false-negative (IMP3 negative in biopsy specimens and IMP3 positive on the resection specimen), whereas 2 (28.6%) were false-positive. We recognized in our study that the most common type of discrepancy arose from false negative cases. The discordance may be caused by multiple factors such as intra-tumor heterogeneity or tissue processing. A small percentage of samples would inevitably be scored as falsely negative, thereby inappropriately excluding these patients from preoperative chemotherapy and radiotherapy. On the basis of our study, we would advocate performing IMP3 immunohistochemistry in combination with radiographic staging to reduce the false-negative rate of patients who may benefit from preoperative chemotherapy and radiotherapy.

In conclusion, the current study has found a high rate of concordance between IMP3 expression on biopsy and resection specimens. Our results firstly confirm that IMP3 expression in biopsy specimens of CRC patients was significantly associated with lymph node metastasis and TNM stage. IMP3 expression in biopsy specimens could be used to predict lymph node metastasis and TNM stage in CRC patients.

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# Disclosure of conflict of interest

None.

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