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Accuracy of Multi-Slice Spiral Computed Tomography for Preoperative Tumor Node Metastasis (TNM) Staging of Colorectal Carcinoma

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Background: With the advances in imaging technologies, multi-slice spiral computed tomography (MSCT) has demonstrated superiority in the diagnosis and staging of colorectal carcinoma. In the current study, preoperative TNM staging of colorectal carcinoma by using MSCT was conducted and compared with the corresponding postoperative pathological examination findings, in order to evaluate the accuracy of preoperative MSCT for TNM staging.



Material/Methods: Combinations of biphasic or triphasic enhanced-phase MSCT scans were obtained for 76 patients with colorectal carcinoma, and the TNM stage was determined based on imaging reconstruction from various angles and perspectives to display the size, location, and affected range of tumors. The preoperative TNM stage was compared with the postoperative pathological stage, and the consistency between the 2 methods was tested by the κ test using SPSS 17.0 software.

Results: Among the different combinations of enhanced-phase MSCT scanning, triphasic MSCT imaging, comprising the arterial, portal venous, and delayed phases, showed the highest accuracy rates, at 81.6% (62/76), 82.89% (63/76), and 96.1% (73/76) for T, N, and M staging, respectively, with κ values of 0.72, 0.65, and 0.56, respectively, indicating consistency with the postoperative pathological staging.

Conclusions: Combined MSCT scanning comprising the arterial phase, portal venous phase, and delayed phase showed satisfying consistency with the postoperative pathological analysis results for TNM staging of colorectal carcinoma. Thus, MSCT is an important clinical value for improving the accuracy of TNM staging and for planning the appropriate colorectal cancer treatment.

MeSH Keywords: **Colorectal Neoplasms • Neoplasm Staging • Tomography Scanners, X-Ray Computed**

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Background

Colorectal carcinoma is a major malignancy threatening human health [1] and is the fifth most common cause of cancer death in China, with a continuously increasing annual incidence [2]. Surgery is still the main therapeutic method for treating colorectal carcinoma, but the 5-year survival rate is remains unsatisfactory, largely due to delayed diagnosis and limited medical resources [3]. Accurate assessment of preoperative tumor (T) size, lymph node (N) involvement, and metastasis (M) staging is suggested for the evaluation of tumor invasion and local or remote (liver) metastasis, and might be helpful in selecting the optimal therapeutic strategy and for achieving ideal therapeutic outcomes [4].

Multi-slice spiral computed tomography (MSCT) is a high-speed scanning technology that is capable of both thin-layer and volume scanning, with high spatial resolution and a powerful post-processing workstation. MSCT can directly visualize the lesion and its surrounding affected lumen or organs by multi-planar reconstruction, with 100% accuracy [5]; therefore, it is considered one of the most valuable preoperative examinations for achieving accurate staging of colorectal carcinoma and for selecting the optimal therapeutic method [6]. In the current study, we aimed to determine the accuracy of combined enhanced-phase (arterial phase, portal venous phase, and delayed phase) MSCT scanning in the preoperative staging of colorectal carcinomas by comparing the MSCT findings with the corresponding postoperative pathological examination results.

Material and Methods

Patients

The medical information on 76 colorectal carcinoma patients, including 40 men and 36 women, with an average age of 54 years (range, 32–74 years), was reviewed for inclusion in the current study. Patients who were diagnosed on the basis of the postoperative pathological findings and who had undergone preoperative MSCT scanning between 2013 and 2014 were included. The patients were informed of their disease conditions and each patient signed an informed consent form for MSCT examination and for inclusion in the study. The inclusion criteria were as follows: patients identified to have colorectal carcinoma, as confirmed by colonoscopy examination; patients without serious heart and lung disease and iodine allergies; and patients who underwent preoperative MSCT examination within 1 week before the operation. The exclusion criteria were as follows: patients without pathological data and complete imaging data, and patients with distant metastasis at diagnosis. The interval between imaging examination

and surgery ranged between 1 and 7 days, with a median interval of 3.5 days.

MSCT examination

The GE Lightspeed VCT 64-slice CT scanner (GE Healthcare, Milwaukee WI, USA) was used in the current study, along with the GE AW4.3 CT post-processing workstation. The patients were on a liquid diet the day before the procedure; magnesium sulfate or phenolphthalein was administered for intestinal cleaning, and it was suggested that water be consumed 1.0 h before the procedure, to fill the bladder with urine. For each patient, a total of 10 mg of anisodamine (654-2) was intramuscularly injected 10–15 min before the procedure. The patients were placed in the right lateral position and injected with 600–800 mL warm water through the anus; subsequently, the patients were placed in the supine position for MSCT scanning. Iohexol (300 mg/mL), a non-ionic iodine contrast agent used to enhance MSCT imaging, was injected through the antecubital vein using a high-pressure syringe, at a speed of 3.5 mL/s. The scanning procedure was divided into the arterial phase (25–28 s after injection), portal venous phase (50–60 s after injection), and equilibrium phase (120 s after injection), and the patients were scanned from the umbilical level to the inferior margin of the pubic bone union. The original dataset of volume scanning was transferred to the AW4.3 CT workstation, and the 3D reconstructed images were analyzed from various angles, planes, and perspectives using multi-planar reconstruction, volume rendering, surface-shaded display, and CT virtual endoscopy to observe the colorectal carcinoma lesion or metastasis thereof. The lesion was observed as a focused target in the transverse-sagittal-coronal position, and the location, shape, size, and margin of the lesion were recorded, as well as the range and depth of the involved colorectal lumen wall, fat layer of the outer membrane, and adjacent or remote lymph nodes in terms of the size, number, density, shape, and enhancement degree.

Image description and analyses

The images were inspected by 2 independent qualified attending doctors from the Imaging Diagnosis Department in a double-blinded manner. The description of the image comprised: 1) location and size of the colorectal carcinoma, 2) invasion range of the colorectal carcinoma, 3) enhancement feature of the colorectal carcinoma, 4) lymph nodes with edema or enhancement features, 5) number and size of the affected lymph nodes, and 6) metastasis of the tumor.

Preoperative staging by CT

The tumors were staged with MSCT using the tumor-node-metastasis (TNM) staging system [7,8]. Accordingly, the tumors

Table 1. Staging criteria.

Tx	Primary tumor unable to be evaluated
Primary tumor (T)	T0 No primary tumor
	Tis Carcinoma <i>in situ</i>
	T1 Tumor invasion of the mucosa
	T2 Tumor invasion of the inherent muscle layer
	T3 The tumor penetrating the intrinsic muscle layer to the lower layer, or affecting the paraproctium without peritoneal covering
	T4a Tumor penetrating peritoneal layer
	T4b Tumor invasion or adherence to other structures
Local lymph node (N)	Nx Local lymph nodes unable to be evaluated
	N0 No local lymph nodes metastasis
	N1 1–3 local lymph nodes metastasis
	N2 More than four local lymph nodes metastasis
Remote metastasis (M)	Mx Remote metastasis unable to be evaluated
	M0 No remote metastasis
	M1 Remote metastasis

were classified as stage $\leq T2$ in cases of local or circumferential thickening of the rectal wall with enhancement, smooth and clear surroundings of the tumor with a clear fat gap, and deformed colorectal wall at the lesion, without stenosis; stage T3 in the presence of rectal wall thickening or the tumor mass protruding into the cavity, irregular tumor shape without a clear fat gap, a few cable-like and nodule-like images with low density, and no sign of surrounding organs affected; and stage T4 in cases of rectal wall irregular thickening or the tumor mass protruding into or outside of the cavity, with stenosis and enhanced fat density, and with high-density cable-like and nodule-like images protruding or affecting the surrounding organs. Regional lymph nodes were classified as stage N0 if there was no lymph node edema or lymph nodes with a diameter larger than 1.0 cm; stage N1 if there were 1–3 lymph nodes with a diameter larger than 1.0 cm or accumulation of more than 1 normal-sized lymph node; and stage N2 if more than 3 lymph nodes with a diameter larger than 1.0 cm were found. Cases without remote metastasis were classified as stage M0, while those with remote metastasis affecting the liver, lungs, peritoneum, and retroperitoneal lymph nodes were classified as stage M1.

Postoperative staging by pathological examination

Staging according to the colonoscopy biopsy or postoperative pathology test was regarded as the criterion standard; the staging criteria were adopted from the American Joint Committee

on Cancer/Union for International Cancer Control Rectal Cancer TNM Staging System (7th Edition, 2010) (Table 1) [9].

Statistical analysis

The TNM stage of each patient was determined by preoperative MSCT and postoperative pathological examination. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform κ tests for consistency analysis. The κ value ranged between 0 and 1. A $\kappa \geq 0.75$ indicated good consistency, $\kappa \geq 0.4$ indicated fair consistency, and $\kappa < 0.4$ indicated poor consistency [10].

Results

T staging by MSCT

The comparison of the T stage between the preoperative assessment and pathological analysis is shown in Figure 1. After MSCT scanning, 23 (30.26%) of the 76 cases were staged as T1–2, 23 (30.26%) were staged as T3, and 30 (39.48%) were staged as T4. Using plain scanning, the tumor presented as soft tissue density; with enhanced scanning, the density uniformly increased and was slightly greater than that of the surrounding tissues. Fourteen (60.86%) cases pathologically staged as T1–2, 10 (43.48%) cases staged as T3, and 20 (66.67%) cases staged as T4 were accurately staged with arterial plus portal venous phase-MSCT scanning (Table 2). For arterial phase plus

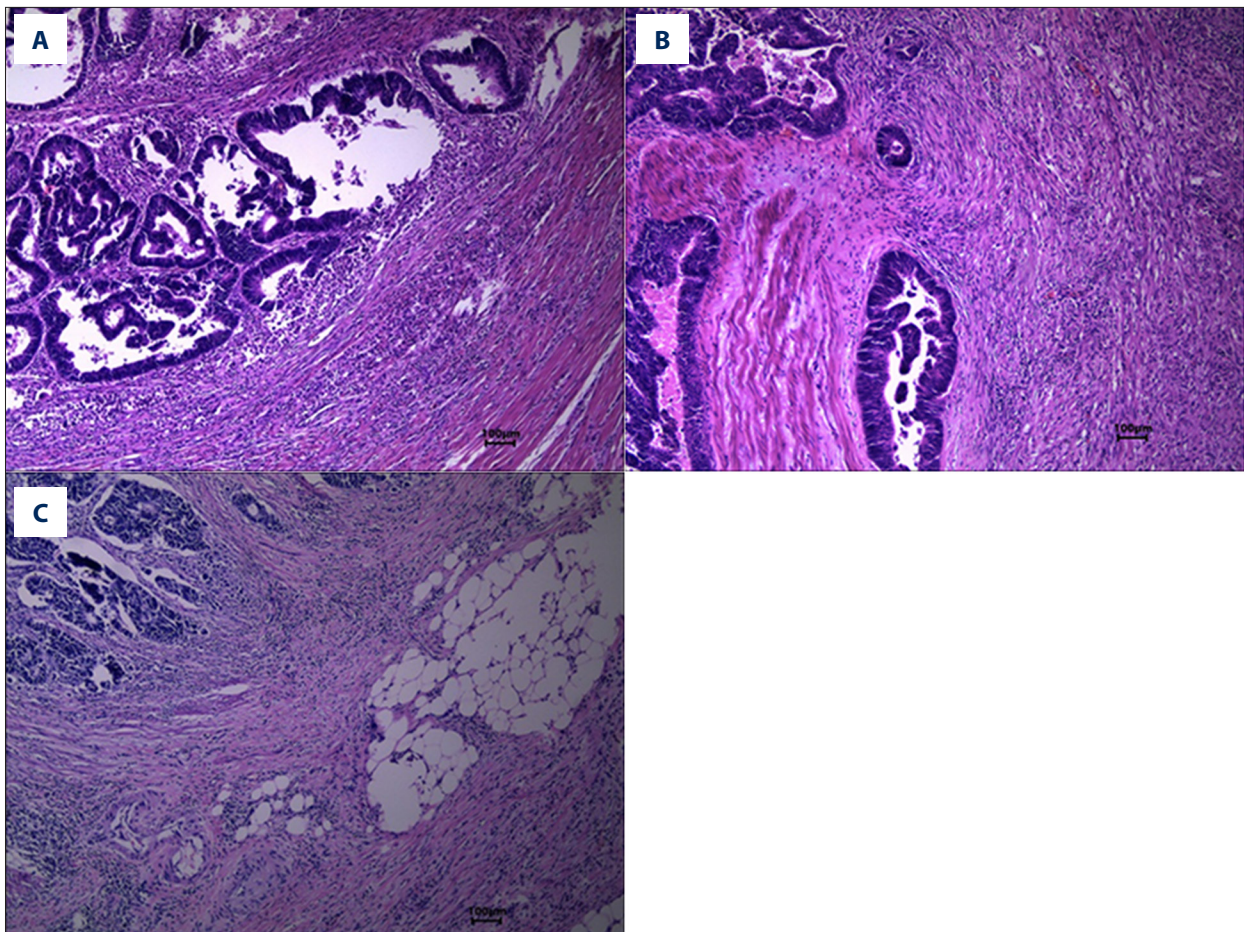


Figure 1. Representative pathological tissue slices. (A) Tumor invasion limited to the bowel wall. (B) A tumor invading the serous layer. (C) A tumor invading the adventitia.

Table 2. Comparison of T staging for in situ carcinoma by different phase combination pattern of computed tomography (CT) scanning and postoperative pathological examination.

Enhanced phase combination	Pathological staging	T1-2 (n=23)	T3 (n=23)	T4 (n=30)	N0 (n=49)	N1 (n=23)	N2 (n=4)	M0 (n=74)	M1 (n=2)
Arterial + portal	Consistent	14	10	20	42	15	3	71	1
	Inconsistent	9	13	10	7	8	1	3	1
Arterial + delayed	Consistent	18	13	26	38	13	2	70	1
	Inconsistent	5	10	4	11	10	2	4	1
Arterial + portal + delayed	Consistent	20	15	27	44	16	3	71	2
	Inconsistent	3	8	3	5	7	1	3	0

delayed phase-MSCT scanning, 18 (78.26%) cases staged as T1-2, 13 (56.52%) cases staged as T3, and 26 (86.67%) cases staged as T4 were accurately staged (Table 2). As shown in Figure 2, this combination was conducive for the diagnosis of T staging. Moreover, 20 (86.95%) cases staged as T1-2, 15 (65.21%) cases staged as T3, and 27 (90%) cases staged as

T4 were accurately staged with combined arterial phase, portal venous phase, and delayed phase-MSCT scanning (Table 2 and Figure 3), with an accuracy of 81.6% (62/76) for T staging in colorectal carcinoma. This result was consistent with the staging by postoperative pathological examination according to the κ test ($\kappa=0.72, P<0.001$). The results of 3 (13.04%)

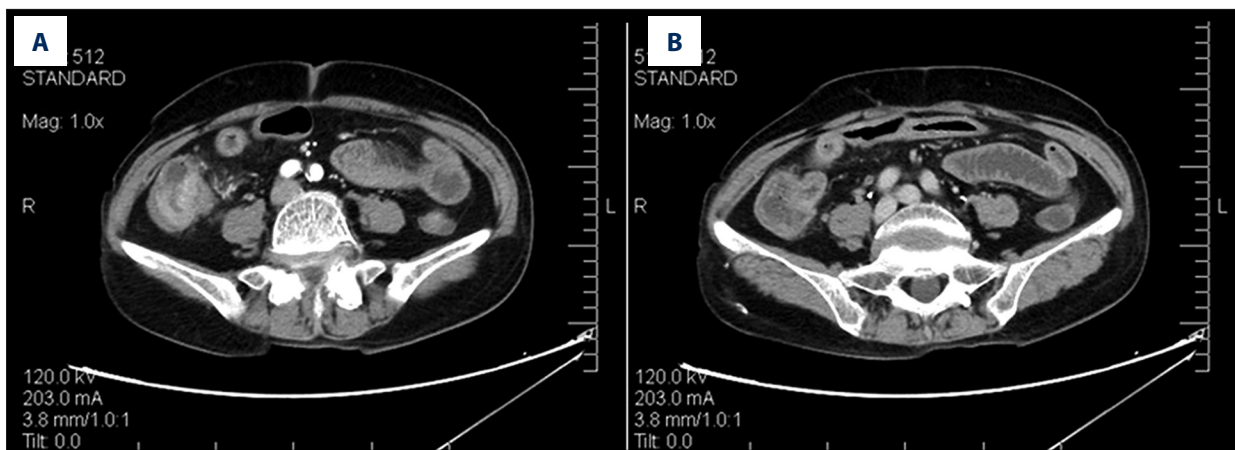


Figure 2. T staging by enhanced arterial phase (A) and delayed phase multi-slice spiral computed tomography scanning (B). The images show a tumor located in the hepatic flexure of the colon.

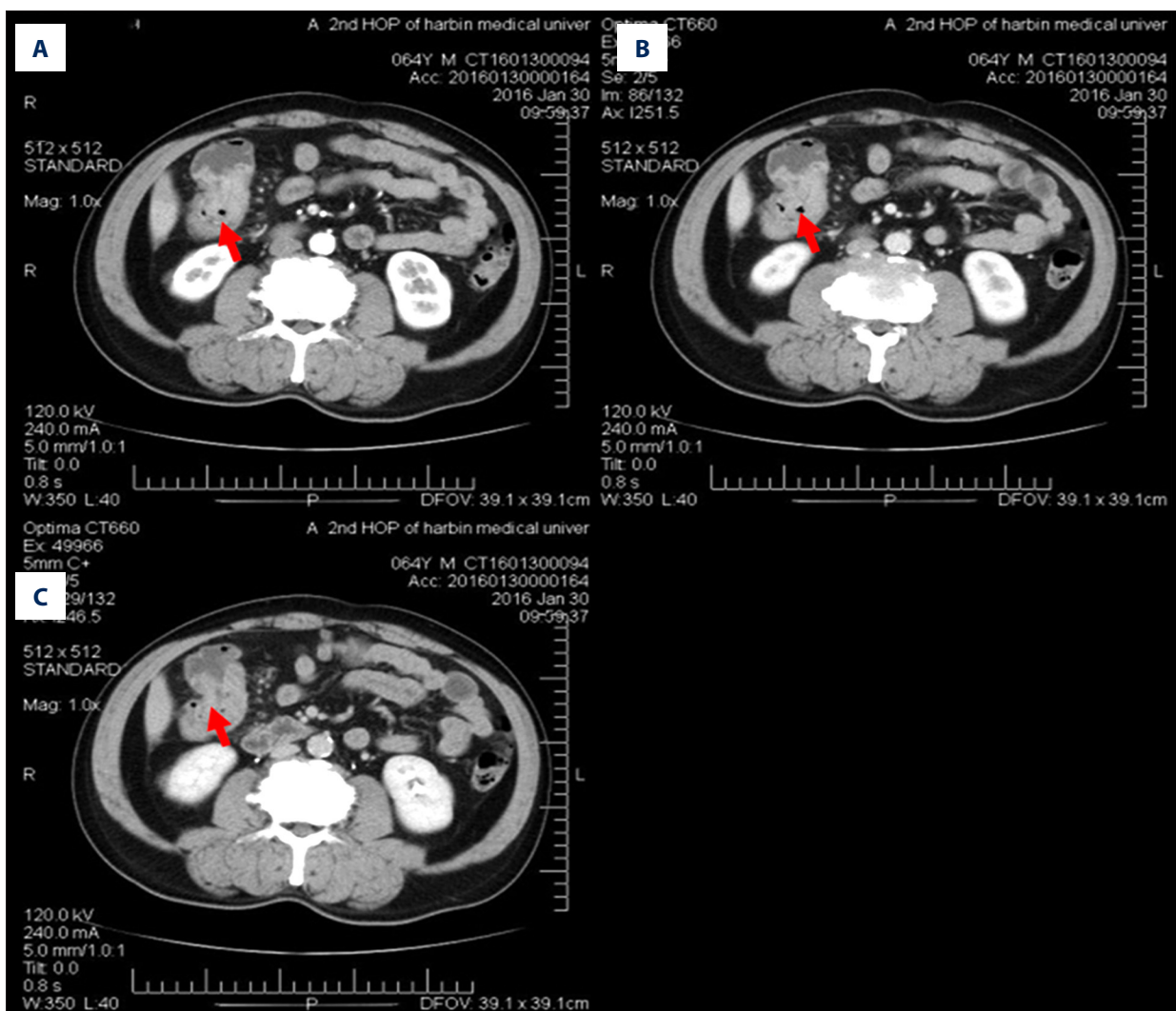


Figure 3. T staging and N staging by enhanced arterial phase (A), portal venous phase (B), and delayed phase multi-slice spiral computed tomography scanning (C). The images show a tumor in the hepatic flexure of the colon. The number and size of the lymph nodes are increased around the rectus (arrow).

Table 3. Comparison of T staging for *in situ* carcinoma by combined MSC To farterial phase, portal venous phase and delayed phase with postoperative pathological examination.

CT staging	n	Pathological staging ≤			Positive prediction (%)	Sensitivity (%)	Specificity (%)
		T2	T3	T4			
≤T2	23	20	2	1	86.9 (20/23)	83.3 (20/24)	94.2 (49/52)
T3	23	4	15	4	65.2 (15/23)	75.0 (15/20)	85.7 (48/56)
T4	30	0	3	27	90.0 (27/30)	84.4 (27/32)	93.2 (41/44)

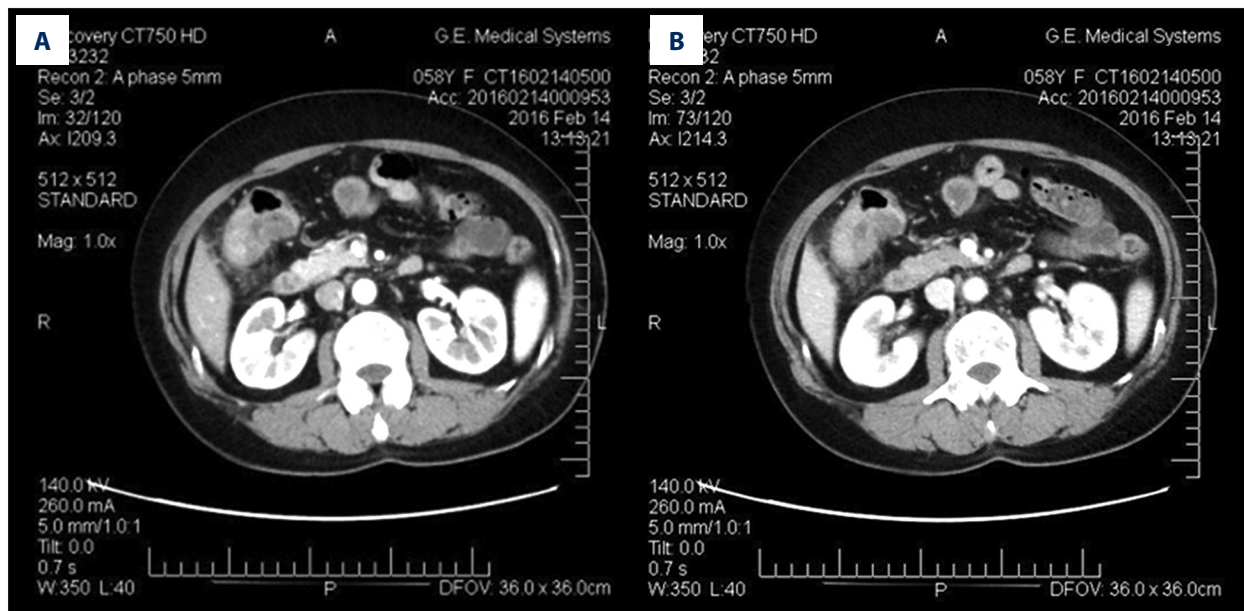


Figure 4. N staging by enhanced arterial phase (A) and portal venous phase multi-slice spiral computed tomography (B). The images show a clear tumor in the hepatic flexure of the colon and metastatic lymph nodes.

cases at stage ≤T2 by triphasic MSCT were inconsistent with the pathological analysis results, including 2 and 1 at stage T3 and T4, respectively. With regard to all colon segments, the positive predictive value, sensitivity, and specificity of this combined MSCT scanning for T staging of colorectal tumors were 86.9%, 83.3%, and 94.2% for stage ≤T2 tumors; 65.2%, 75%, and 85.7% for stage T3 tumors; and 90%, 84.4%, and 93.2%, respectively, for stage T4 tumors (Table 3).

N staging by MSCT

Based on the MSCT scan analysis, 49 (64.47%) cases were defined as N0, 23 (30.26%) cases were defined as N1, and 4 (5.27%) cases were defined as N2. For the 49 cases defined as N0, 42 (85.71%) cases staged with biphasic MSCT imaging consisting of the arterial and portal venous phases (Table 2, Figure 4) were accurately staged by histopathological evaluation. Using the biphasic MSCT imaging protocol consisting of the arterial and delayed phase phases, the results of 38 (77.55%) cases were consistent with those of the histopathological

examination (Figure 1, Table 2). For the triphasic MSCT imaging (arterial, portal venous, and delayed phases), 44 (89.79%) cases were precisely staged (Table 2, Figure 3). Fifteen (65.2%) cases staged as N1 in the histopathological evaluation were exactly staged with arterial plus portal venous phase-MSCT scanning; the corresponding numbers were 13 (56.52%) and 16 (69.57%) when using arterial phase combined with delayed phase-MSCT scanning and the combination of arterial phase, portal venous phase, and delayed phase-MSCT, respectively (Table 2). Regarding the 4 cases staged as N2 by MSCT scanning, 3 (75%) cases staged as N2 in the histopathological evaluation were accurately staged with arterial plus portal venous phase-MSCT scanning, which was the same as the result from the triphasic MSCT scanning (arterial phase, portal venous phase, and delayed phase). Only 1 case was incorrectly evaluated to be at the N1 stage by MSCT examination, with a lymph node diameter larger than 1.0 cm, thus fulfilling the criteria of N2 by MSCT, but the number was ignored. Three cases of N1 and 2 cases of N2 disease were mistakenly diagnosed by MSCT as stage N0.

Table 4. Comparison of N staging for *in situ* carcinoma by combined MSCT of arterial phase, portal venous phase and delayed phase with postoperative pathological examination.

CT staging	n	Pathological staging			Positive prediction (%)	Sensitivity (%)	Specificity (%)
		N0	N1	N2			
N0	49	44	3	2	89.79 (44/49)	88.0 (44/50)	80.8 (21/26)
N1	23	6	16	1	69.56 (16/23)	80.0 (16/20)	87.5 (49/56)
N2	4	0	1	3	75.0 (3/4)	50.0 (3/6)	98.6 (69/70)

Similar to the T staging, the combination of arterial phase, portal venous phase, and delayed phase-MSCT for N staging in colorectal carcinoma showed the highest accuracy, at 82.89% (63/76), which was consistent with that of the postoperative pathological examination according to the κ test ($\kappa=0.65$, $P<0.001$). As shown in Table 4, the positive predictive value, sensitivity, and specificity of this combined MSCT scanning for N staging of colorectal tumors were 89.4%, 88%, and 80.8% for stage N0 tumors; 69.6%, 80%, and 87.5% for stage N1 tumors; and 75%, 50%, and 98.6%, respectively, for stage N2 tumors.

M staging of lymph nodes by MSCT

Regarding the 74 cases staged as M0 by MSCT examination, 71 (95.94%) were accurately staged with combined arterial and portal venous phase-MSCT and with combined arterial phase, portal venous phase, and delayed phase-MSCT (Table 2). Using combined arterial phase and delayed phase-MSCT, 70 (94.59%) cases were accurately staged (Table 2). The 2 cases staged as M1 with triphasic MSCT scanning were consistent with the histopathological features (Figure 1). The combination of arterial, portal venous, and delayed phasic MSCT for remote metastasis of colorectal carcinoma showed the highest accuracy, at 96.1% (73/76) (Table 2, Figure 5), which was consistent with the staging by postoperative pathological examination according to the κ test ($\kappa=0.56$, $P<0.001$). Two patients showed micro-metastases in the mesentery and greater retina, and 3 patients showed remote metastases in the liver. The positive predictive value, sensitivity, and specificity of this combined MSCT scanning in the M staging of colorectal tumors were 96%, 100%, and 40% for stage M0 tumors, and 100%, 40%, and 100%, respectively, for stage M1 tumors (Table 1).

Discussion

Colorectal cancer is one of the most common digestive tract malignancies in China. Preoperative TNM staging of colorectal cancer contributes to ensuring appropriate surgical planning. The application of imaging methods for preoperative TNM staging of colorectal cancer is important, with great value in

planning the clinical treatment. Currently, various imaging technologies are of great help for TNM staging of colorectal carcinoma, including MSCT [11]. Previous reports have revealed that MSCT can accurately reveal a local infiltration lesion and suggest suspected T3 lesions at the early stage ($\leq T2$) [12,13]. Moreover, MSCT can exclude T2, N1, and N3 tumors, which is helpful in designing the optimal treatment to increase the tumor excision rate and reduce the risk of intraoperative metastasis. In this study, combinations of enhanced-phase (arterial phase, portal venous phase, and delayed phase) MSCT scanning were used for preoperative TNM staging to evaluate the value of MSCT in the diagnosis and staging of colorectal carcinoma.

The accuracy of MSCT for tumor staging in colorectal carcinoma mainly depends on the resolution of each layer of the colorectal wall. However, reports regarding the accuracy of MSCT for preoperative staging of colorectal cancer have shown highly varying rates, ranging from 60% to 98% [14–17]. In this study, biphasic MSCT imaging – comprising the arterial plus portal venous phase or arterial plus delayed phase – and triphasic MSCT imaging – comprising a combination of the arterial, delayed, and portal venous phases – were used. As it is impossible to distinguish the mucosa from the submucosa of the colon wall with tomographic means, stage T1 and T2 tumors cannot be separated from each other using CT [7]. Therefore, stage T1 and T2 tumors were combined into stage $\leq T2$ in this study. In a previous study, using biphasic MSCT imaging comprising the arterial and portal phases in the preoperative T staging of colorectal cancer, accuracy rates of 92% for stage $\leq T2$ tumors, 88% for stage T3 tumors, and 100% for stage T4 tumors were reported [14]. In our study, the accuracy of T staging by biphasic MSCT imaging comprising the arterial and portal phases was 57.89% (60.86% for stage $\leq T2$, 43.48% for T3, and 66.67% for T4). Upon adding the delayed phase to the aforementioned phases, the accuracy for T staging was increased to 81.6% (86.95% for stage $\leq T2$, 65.21% for T3, and 90% for T4). This rate was inconsistent with the accuracy rates found in previous studies [18,19], further highlighting the highly varying accuracy rates of MSCT for TNM staging of colorectal carcinoma. Moreover, in this study, 3 patients with stage $\leq T2$ disease were overrated by MSCT, 2 of whom had an inflammatory response and effusion from the

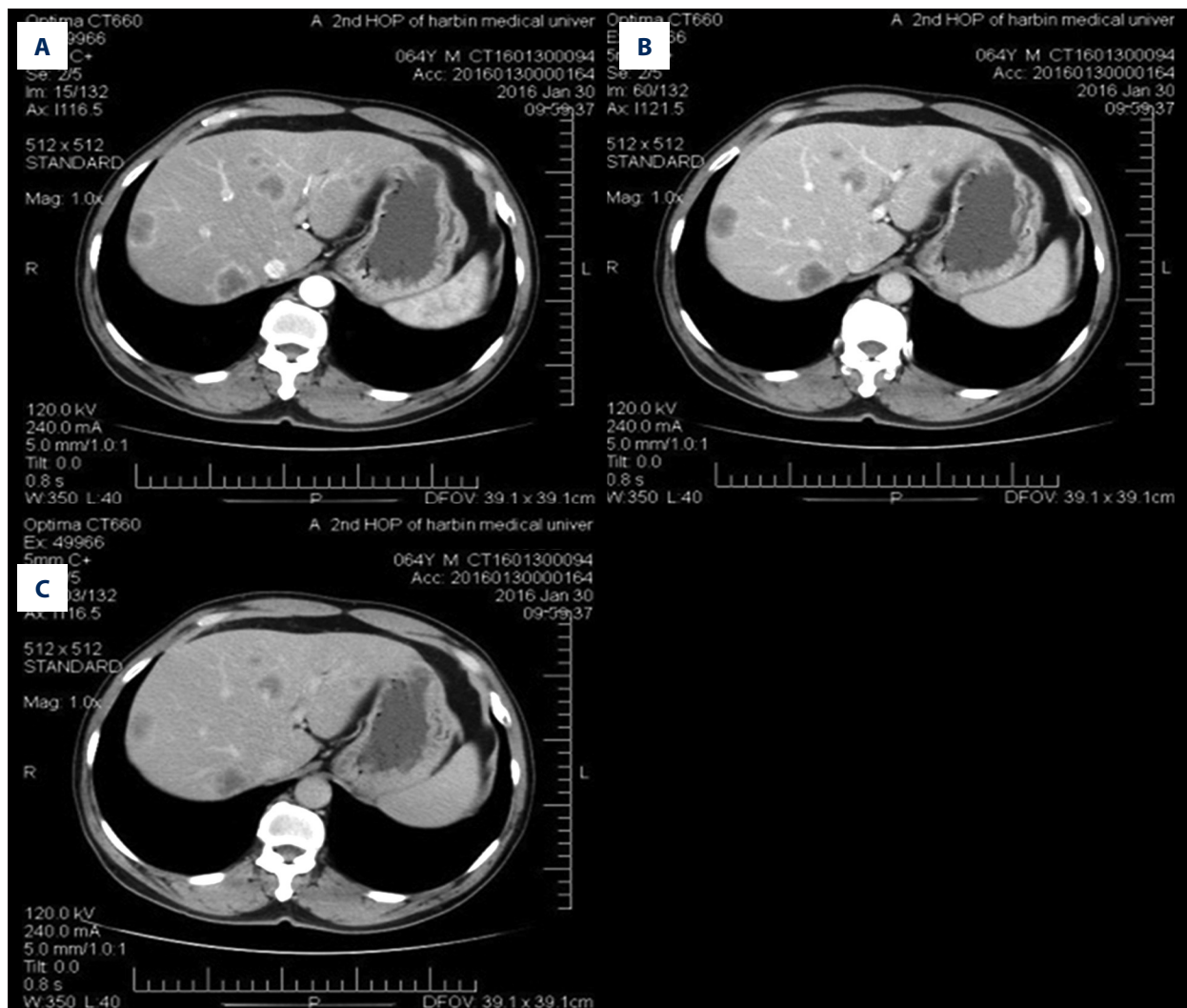


Figure 5. M staging by enhanced arterial phase (A), portal venous phase (B), and delayed phase multi-slice spiral computed tomography scanning (C). The images show multiple hepatic metastases.

surrounding fat gap, as well as cable-like images within the fat gap of the middle and lower section of the colon; these were overrated as stage T3. The third patient had a large tumor adhesion to the surrounding organs and was overrated as stage T4. This result suggests that carcinomatous infiltration should be considered if the lesion is ≤ 1.0 mm or if it is < 1.0 mm from the mesorectal fascia when there is an inflammatory response or proliferation. Of note, the accuracy of diagnosing T3 tumors by MSCT was relatively low in this study, which may be attributed to the difficulty MSCT has in differentiating an inflammatory response or edema from moderate infiltration of the tumor or to clearly visualize micro-infiltration and irregular nodules. Therefore, the serosa membrane and mesorectal gap should be emphasized to improve the accuracy of T3 staging. On the other hand, the accuracy on T4 staging by triphasic MSCT was relatively high (90%) and this was consistent with the previously published results of 80–95% [20],

indicating that triphasic MSCT is of great clinical value for assessing colorectal carcinoma at the moderate and late stages.

Assessment of the existence of lymph node involvement in colorectal carcinoma is vital in terms of forecasting the prognosis of the disease and for planning the treatment protocol. Markedly different accuracy rates, ranging from 55% to 95%, have been obtained in previous studies carried out for the detection and staging of lymph nodes using MSCT [21,22]. Using single-phase-MSCT, the accuracy rates of N staging of colorectal carcinoma was 59% for stage N0, 80% for stage N1, and 89% for stage N2 in a previous study, indicating that single-phase multi-detector CT is not sufficient for accurate N staging [23]. In the study by Filipponi et al., the accuracy rates of N staging for colorectal carcinoma were 85% for stage N0, 83% for stage N1, and 93% for stage N2 by using a biphasic protocol including the arterial and portal phases [7]. In

the present study, the biphasic protocol including the arterial and portal phases was also conducted for colorectal carcinoma scanning, which resulted in accuracy rates of 85.17% for stage N0, 65.21% for stage N1, and 75% for stage N2. After adding the delayed phase to the aforementioned phases, the accuracy rates were increased to 89.79%, 69.56%, and 75% for N0, N1, and N2, respectively. This result further confirms the highly different accuracy rates of MSCT for the detection and staging of lymph nodes.

Herrera-Ornelas et al. [24] reported that 86% of metastatic lymph nodes had a diameter of <1.0 cm, with some even were <0.5 cm in size, which may result in misdiagnosis using the current criteria. In the present study, 3 patients with N1 and 2 patients with N2 disease were mistakenly diagnosed by MSCT as having stage N0, which was largely because the diameter of the lymph node was <1.0 cm, with low density, especially in the center, compared to the surrounding area. On the other hand, 7 patients with larger lymph nodes showing enhanced density were overrated by MSCT, and the postoperative pathological examinations showed that these enlarged lymph nodes were actually inflamed. Although it has been suggested that a diameter of 0.3 cm be used as the criterion for lymph node metastasis to increase the diagnostic sensitivity, the misdiagnosis rate was still increased and led to overtreatment in 2 previous studies [25,26]. Thus, there are some limitations when using MSCT for the diagnosis of lymph node metastasis, as MSCT barely differentiates inflammatory reactive lymph nodes from reactive lymph nodes with fibrous hyperplasia showing tumor invasion. In addition, it is not easy to determine the presence of micro-metastasis within lymph nodes <1.0 cm in size by MSCT, suggesting that a combination of various imaging technologies should ideally be used for N staging. For example, when the lymph nodes are close to the colorectal wall, ultrasound can be used to determine their nature [27] and magnetic resonance imaging should be considered when it is difficult to differentiate inflammatory reactive lymph nodes from those with tumor metastasis [28].

Remote metastasis is commonly found in colorectal carcinoma patients at the time of primary diagnosis. Therefore, evaluation of the affected organs is important. The liver is the most common organ for remote metastasis [29], followed by the lungs and bones, while metastasis to other organs is seldom reported. In this study, the accuracy of M0 staging by biphasic

MSCT comprising the arterial and portal phases and triphasic MSCT was 95.94%. The 2 cases defined as M1 were accurately staged by triphasic MSCT, and the accuracy for remote metastasis of colorectal carcinoma by triphasic MSCT was as high as 96.1%. Mesentery and greater retina micro-metastasis were found in 2 patients, and liver metastasis was found in 3 patients by triphasic MSCT. The metastasis within the liver that was undetected by MSCT was small and lacked metastasis features, without density enhancement at the edge. The other 2 metastasized tumors in the mesentery and greater retina were missed by MSCT because of resolution or inspection errors for tumors that were small or close to the abdominal wall. This result suggests that atypical small metastases or metastases in the mesentery and greater retina are not well detected by MSCT because of its limits in resolution or enhancement. Therefore, it is necessary to improve the MSCT technology to allow for accurate diagnosis of small lesions.

There are some inherent limitations to this study because of its retrospective and single-center nature. Other limitations of this study include the relatively small sample size and the fact that evaluation of inflammatory reactive lymph nodes was not conducted in all patients.

Conclusions

Triphasic MSCT scanning comprising a combination of the arterial phase, portal venous phase, and delayed phase showed satisfying consistency with the postoperative pathological analysis results for TNM staging of colorectal carcinoma. This finding indicates that MSCT is an important and accurate method for planning appropriate colorectal carcinoma therapy.

Conflict of interest

The authors declare that they have no actual or potential conflicts of interest.

Institutional Review Board statement

The study was approved by the Ethics Committee of the Second Hospital of Harbin Medical University in China (Approval ID: 2014-058 and ID: KY2016-028).

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