J Med **345**: 638–646, 2001.
18) Kodaira S, Kikuchi K, Yasutomi M, *et al*: Postoperative adjuvant chemotherapy with mitomycin C and UFT for curatively resected rectal cancer: results from the cooperative project No. 7 group of the Japanese Foundation for Multidisciplinary Treatment of Cancer. *Int J Clin Oncol* **3**: 357–364, 1998.
19) Kato T, Ohashi Y, Nakazato H, *et al*: Efficacy of oral UFT as adjuvant chemotherapy to curative resection of colorectal cancer: multicenter prospective randomized trial. *Langenbecks Arch Surg* **386**: 575–581, 2002.
20) Akasu T, Moriya Y, Yoshida Y, *et al*: Adjuvant oral uracil and tegafur (UFT) improves survival after complete mesorectal excision (ME) for pathologic TNM stage III rectal cancer (RC): Results of the National Surgical Adjuvant Study (NSAS)-Colorectal Cancer (CC) 01 randomized trial. *Proc ASCO* **23**: 251, (abstr 3524) 2004
21) Mori T, Takahashi K, Yasuno M, *et al*: Radical resection with autonomic nerve preservation and lymph node dissection techniques in lower rectal cancer surgery and its results: the impact of lateral lymph node dissection. *Langenbecks Arch Surg* **383**: 409–415, 1998.
22) Akasu T and Moriya Y: Abdominopelvic lymphadenectomy with autonomic nerve preservation for carcinoma of the rectum: Japanese experience. Surgery for gastrointestinal cancer: a multidisciplinary approach (ed by Wanebo HJ), Lippincott-Raven, Philadelphia, 1996, pp 667–680.

日本大腸肛門病会誌 59：367-372，2006

原　著　I

外肛門括約筋に浸潤あるいは近接する直腸癌の術前MRI診断

石井　正之　結縁　幸子[1)]　山口　茂樹　森田　浩文　齊藤　修治
大田　貢由　森本　幸治　奥本　龍夫　橋本　雅彦
静岡県立静岡がんセンター大腸外科，同　画像診断科[1)]

外肛門括約筋に浸潤あるいは近接する肛門管近傍の下部直腸癌症例を正確に診断することはIntersphincteric resectionの適応決定に重要である．今回われわれは直腸切断術症例の病理組織所見と術前MRI所見を対比し，外肛門括約筋に浸潤あるいは近接する直腸癌の診断にMRIが有用であるか検討した．方法：直腸切断術を施行した26例を対象とした．病理組織学的に腫瘍先進部から外肛門括約筋内側縁までの距離を測定し，1mm以下の場合を外肛門括約筋近傍浸潤と定義した．MRI T2強調画像において外肛門括約筋浸潤に関する診断基準を定め，組織学的所見との対比を行った．結果：組織学的に外肛門括約筋近傍浸潤例は7例，括約筋内浸潤例は2例であった．MRIによる括約筋内浸潤あるいは近傍浸潤診断は感度89%，特異度82%であった．結語：MRIは外肛門括約筋に浸潤あるいは近接する直腸癌の診断に有用である．

索引用語：直腸癌，Intersphincteric resection，外肛門括約筋浸潤，MRI

背　景

Circular staplerなどのDeviceや手技の発達により低位直腸癌症例において自然肛門を温存する機会が多くなった．しかし肛門管近傍の直腸癌に対しては永久的人工肛門をともなう直腸切断術（以下，APR）が行われることが多い．近年Intersphincteric resection（以下，ISR）を行うことによって肛門管近傍の直腸癌に対しても自然肛門を温存することが可能となってきた[1-3]．肛門機能を可能な限り温存しつつ局所再発を抑えるためには，外肛門括約筋内へ浸潤する症例，または外肛門括約筋に近接するため括約筋間を剝離するとmarginが確保できない症例はISRの適応外となる可能性が高い．しかし外肛門括約筋への浸潤の有無を術前に評価しISRの適応の参考とする診断法として何が優れているかはまだ明らかではない．一方，直腸癌の術前深達度診断にmagnetic resonance imaging（以下MRI）が有用であることが報告されており[4-7]，ISRの適応決定に関する診断方法としても期待される．今回われわれはAPR症例の病理所見と術前MRI所見を対比し，外肛門括約筋浸潤症例あるいは非浸潤症例の術前診断にMRIが有用であるか否かを検討したので報告する．

対　象

術前大腸内視鏡における生検で腺癌と診断された直腸癌症例のうち，2002年9月から2005年8月までにAPRが行われた症例26例を対象とした．その内訳は男性15例女性11例，年齢は44歳から84歳（平均64.5歳）であった．術前放射線治療が行われた症例は除外した．

病理組織学的検討

摘出標本は腫瘍反対側で切除後すぐに切開し，48時間10%ホルマリンにて固定を行った．病変部の連続切片を作製し，ヘマトキシリン・エオジン染色（HE染色）を行った後に病理組織学的検討を行った．検討項目は歯状線から腫瘍下縁の距離，最大腫瘍径，組織型，深達度，外肛門括約筋内への癌浸潤の有無，肛門管レベルにおける腫瘍先進部と外肛門括約筋内側との距離である．距離の測定は外肛門括約筋と腫瘍を含むプレパラートで行い，その内で最短距離を採用した．外肛門括約筋内に浸潤を認めな

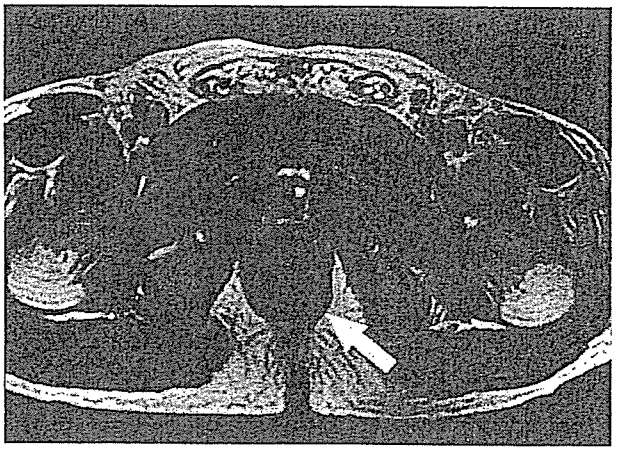

Fig. 1 Axial T2-weighted image demonstrated the increased signal intensity at the external anal sphincter muscle that was adjacent to the tumor. (arrow)

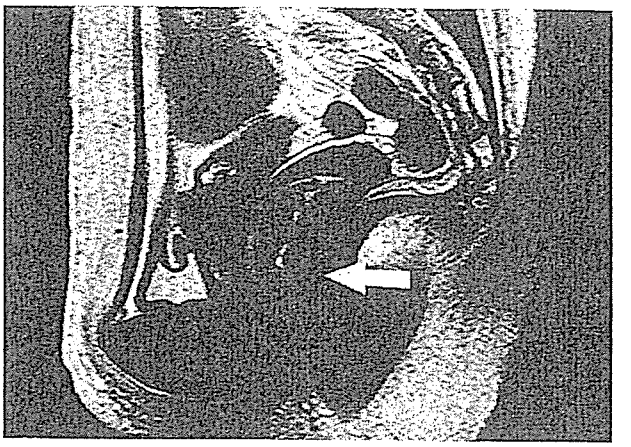

Fig. 2 Sagital T2-weighted image demonstrated the irregularity of the border between the advancing edge of the tumor and the external anal sphincter muscle. (arrow)

Table 1 Patients' Profile (n=26)

Age (yr)	64.5 (44-84)
Gender (male : female)	15 : 11
Histological classification	
well	7
moderately	15
mucinous	3
other	1
Depth of tumor invasion	
sm	1
mp *	7
a1 *	6
a2 *	10
ai *	2
Mean tumor size (cm)	4.9 (2.0-7.5)
Pathology	
ESI positive	2
paraESI positive	7
ESI and paraESI negative	17

ESI : External anal sphincter invasion
paraESI : para External anal sphincter invasion
well : well diferentiated adenocarcinoma
moderately : moderately diferentiated adenocarcinoma
mucinous : mucinous carcinoma
* : Depth of tumor invasion at lower rectum

いが肛門管レベルでの腫瘍先進部と外肛門括約筋内側縁との距離が1mm以下の場合に外肛門括約筋近傍浸潤と定義した.

MRIの検討

今回の検討にはGE社製GENESIS SIGNA, 1.5Tおよびphasedarraycoilを使用して, T2強調画像(撮像条件：TR/TE=3,000/102msec, Echo Train Length=13, FOV=250mm, matrix=224×320, スライス厚=5mm, ギャップ=1mm, 加算回数4回)を矢状断, 冠状断, 軸位断で撮影した. 腸管洗浄を目的とした前処置や抗コリン剤は使用しなかった. MRIの診断は同一の放射線科医が病理結果や他の画像所見に関する情報なしで対象症例の術前MRI画像を読影した. MRIにおいて以下の①②のいずれかの所見を認めた場合に外肛門括約筋浸潤陽性と定めた. ①T2強調画像において腫瘍に接する外肛門括約筋内の信号に変化がある(Fig. 1), ②腫瘍と外肛門括約筋との境界が不整である(Fig. 2). 切除標本における外肛門括約筋内浸潤あるいは近傍浸潤の有無とMRIにおける浸潤診断とを比較検討し, 浸潤あるいは近傍浸潤に関する術前MRIの感度・特異度・正診率を求めた. またMRI診断における陽性例と陰性例の間で肛門管レベルでの腫瘍先進部と外肛門括約筋内側縁との距離に違いが存在するかを比較検討した.

統計学的検討はMann-Whitney U testを用いて行い, $p<0.05$の場合に有意差ありと判断した.

結　果

歯状線から腫瘍下縁の距離は平均0.3cmで, 全例2cm以下であった. 対象症例の背景因子をTable 1に示す. 外肛門括約筋内に癌の浸潤を認めた症例は2例であり, 浸潤を認めなかったが肛門管レベルで癌先進部と外肛門括約筋内側縁との距離が1mm以下の近傍浸潤を認めた症例は7例であった(Fig. 3, 4). 外肛門括約筋内浸潤, 近傍浸潤ともに陰性の症

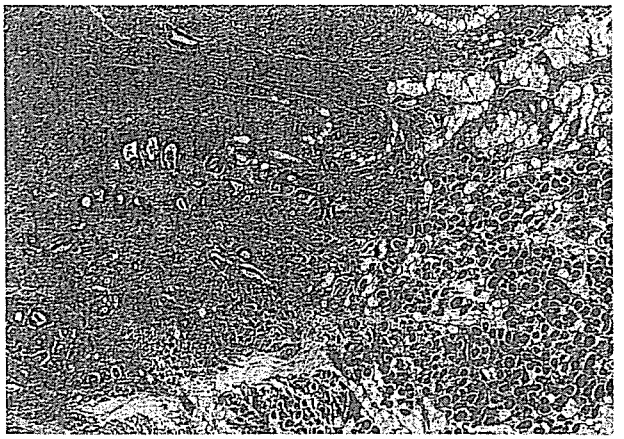

Fig. 3　Histological findings showed moderately differentiated adenocarcinoma infiltrating the striated muscle.

Table 2　Comparision of MRI and pathology reporting of external anal sphincter involvement

	Pathology (ESI + paraESI)	
External anal sphincter invasion on MRI	positive	negative
positive	8	3
negative	1	14

MRI = magnetic resornance imaging
ESI : External anal sphincter invasion
paraESI : para External anal sphincter

例は17例であった．

術前MRI診断と病理学的所見との比較をTable 2に示す．MRIにて外肛門括約筋浸潤陽性と診断した症例は11例であり，このうち2症例は組織学的に外肛門括約筋内浸潤を認めた．実際に外肛門括約筋内浸潤を認めたのはこの2症例のみであった．またMRIにて浸潤陽性と診断された残り9症例のうち6例には組織学的に近傍浸潤を認めた．逆に組織学的に外肛門括約筋内浸潤あるいは近傍浸潤を認めた症例は9例あり，このうち8例は術前MRIにおいて浸潤陽性として診断されていた．術前MRIは外肛門括約筋内浸潤あるいは近傍浸潤のある症例を感度89%，特異度82%，正診率85%で診断可能であった．病理標本上，筋内浸潤，近傍浸潤ともに陰性であるがMRIにて浸潤陽性とされた3症例では腫瘍と外肛門括約筋との間に線維化と炎症細胞浸潤を認めた．また病理標本上，近傍浸潤陽性であったがMRIで陰性と診断された症例では腫瘍と外肛門括

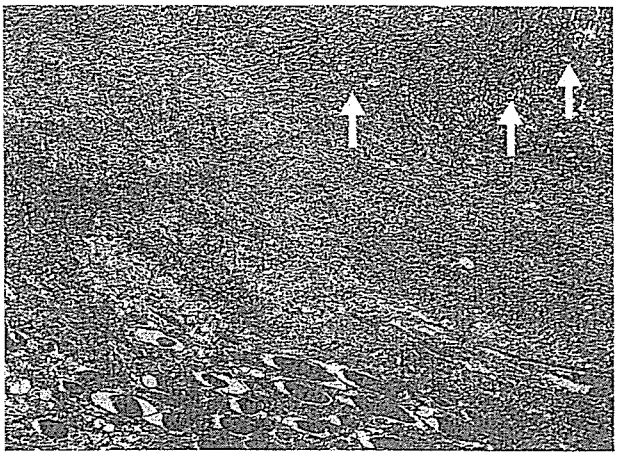

Fig. 4　Histological findings showed the fibrosis between tumor (arrow) and the striated muscle. The distance between the tumor and the inter rim of the external anal sphincter muscle was 0.8mm.

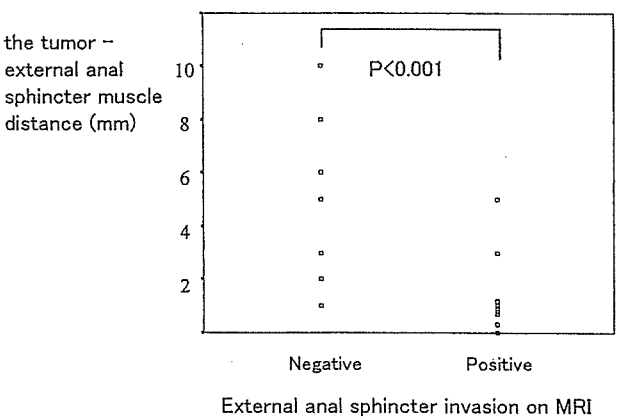

Fig. 5　Correlation between the tumor-external anal sphincter muscle distance and the external anal sphincter muscle invasion on MRI.

約筋との間にほとんど反応性の組織学的変化を認めなかった．

肛門管レベルでの腫瘍先進部と外肛門括約筋内側縁間の距離とMRI診断との関係をFig. 5に示す．MRIにて浸潤陽性とされた症例では腫瘍先進部と外肛門括約筋内側縁間の距離の中央値は0.9mm，陰性症例では5.0mmであった．術前MRIにて浸潤陽性と診断された症例は陰性症例と比較して，統計学的有意差（$p<0.001$）をもって腫瘍と外肛門括約筋との距離が近いことが判明した．

考　察

ISRは肛門管近傍に浸潤する直腸癌においても肛

門温存を可能とする術式であるため本邦でも行われつつある[8,9]．ISRでは外肛門括約筋を温存しつつ内肛門括約筋を切除するため，今まで肛門側断端における距離の不足から直腸切断術が行われていた症例でも肛門温存が可能となった．しかし肛門機能は外肛門括約筋のみで保持されることになるために外肛門括約筋を出来る限り温存する必要がある．そのため適応は外肛門括約筋に浸潤が無い症例に限られるが，外肛門括約筋浸潤に関する診断方法は確立されていない．今回の検討で，術前MRIは外肛門括約筋内に浸潤および近接している症例を診断することがほぼ可能であり，外肛門括約筋浸潤あるいは近接症例の術前診断に有用であることが示唆された．

直腸癌手術において，その剝離面と腫瘍先進部との距離が1mm以下の症例ではそれ以上の症例に比べて有意に局所再発が増加する[10,11]．そのため欧米では剝離面に癌浸潤が存在しない場合でも腫瘍先進部との距離が1mm以下の症例では周囲剝離断端（Circumferential Resection Margin：以下CRM）浸潤陽性とすることが多い[12,13]．一方病理学的に直腸癌が外肛門括約筋内に浸潤することは今回の検討も含めて頻度は少なく[8]，近接するに留まっていることが多い．しかし外肛門括約筋を完全に温存した場合には1mm以下に近接した症例はCRM浸潤陽性に準じたものとなり，根治性を損なう可能性が高いと思われる．外肛門括約筋の温存の可能性を考える場合，1mm以下に近接した症例は広義の外肛門括約筋浸潤として扱うべきと考える．

近年，MRIにより深達度診断のみでなく，直腸癌切除の際CRM浸潤陽性となる症例を術前に診断することができ，その正診率は90%以上であることが報告されている[13,15]．肛門管近傍の直腸癌においても同様の正確さで外肛門括約筋浸潤あるいは近接した症例を診断できることが望ましいが，今回の検討ではその正診率は85%とやや低くなっている．今回の診断が諸家のCRM浸潤陽性診断に比べて正診率が低いのは，肛門管近傍の解剖学的特性が影響していると考えられる．肛門管近傍の直腸は肛門管上部にて屈曲しているため外肛門括約筋と腫瘍との最短距離を描出するには，限られたDimensionでの画像では限界があると思われる．またPeschaudらは下部直腸癌ではCRM浸潤に関する診断が上中部に比べて困難であることを報告しており，その理由として下部直腸では直腸間膜内の脂肪層が菲薄することを挙げている[16]．肛門管上部の屈曲および間膜内脂肪層の菲薄化が上中部直腸癌のCRM浸潤の診断に比べ外肛門括約筋浸潤の診断をより困難にしている．

MRIにおける浸潤陽性所見は組織学的にどのような所見を捉えているのだろうか．MRI陽性症例のうち外肛門括約筋内に癌の浸潤を認めた症例は2例のみであった．その他の陽性症例の大部分では外肛門括約筋の内部や近傍に炎症細胞の浸潤や線維化を認め，腫瘍周囲の炎症が外肛門括約筋に近接しているために起こる反応性変化をMRIにて観察していると考えられる．腫瘍周囲の炎症が強い症例では腫瘍と外肛門括約筋が離れていても外肛門括約筋に変化を及ぼすため偽陽性になる可能性がある．反対に周囲に炎症所見が非常に乏しい場合には，偽陰性となる可能性もある．今回の診断基準や撮影方法のみでは癌の浸潤と炎症による反応性変化を鑑別することは困難と思われる．

直腸癌の外肛門括約筋浸潤のMRI診断に関する報告は現在までに2編のみである[15,16]．いずれも外肛門括約筋内への浸潤を術前MRIで100%予測することが可能と報告している．しかし癌と外肛門括約筋との距離をMRI診断と対比した報告は今回の検討が初めてのものであり，MRIが外肛門括約筋近接症例の診断にも有用であることが判明したことはISRの適応決定を行う上でも有意義と思われる．今後他の診断方法との併用や新たなMRIの診断基準により外肛門括約筋浸潤および近接症例の診断の精度を向上させることは，特にISRの適応決定をより正確に行うために必要である．

なお，本研究は平成17年度科学研究費補助金の交付を受けて行った（基盤研究（C）課題番号：17591426）「直腸癌肛門温存手術の適応に関する検討」の一環として行われたものである．

文　献

1) Schiessel R, Karner-Hanusch J, Herbst F, et al : Intersphincteric resection for low rectal tumors. Br J Surg 81 : 1376–1378, 1994
2) Rullier E, Zerbib F, Laurent C, et al : Intersphincteric resection with excision of internal anal sphincter for conservative treatment of very low rectal cancer. Dis Colon Rectum 42 : 1168–1175, 1999
3) Renner K, Rosen HR, Novi G, et al : Quality of life after surgery for rectal cancer : Do we still need a permanent colostomy? Dis Colon Rectum 42 : 1160–1167, 1999
4) Brown G, Richards CJ, Newcombe RG, et al : Rectal carcinoma : Thin-section MR Imaging for staging in 28 patients. Radiology 211 : 215–222, 1999
5) Drew PJ, Faarouk R, Turnbull LW, et al : Preoperative magnetic resonance staging of rectal cancer with an endorectal coil and dynamic gadolinium enhancement. Br J Surg 86 : 250–254, 1999
6) Gualdi GF, Casciani E, Guadalaxara A, et al : Local staging of rectal cancer with transrectal ultrasound and endorectal magnetic resonance imaging : comparison with histologic findings. Dis Colon Rectum 43 : 338–345, 2000
7) Akasu T, Iinuma G, Fujita T, et al : Thin-section MRI with a phased-array coil for preoperative evaluation of pelvic anatomy and tumor extent in patients with rectal cancer. AJR 184 : 531–538, 2005
8) 白水和雄，緒方　裕，荒木靖三：下部直腸癌，肛門管癌に対する括約筋切除をともなう新しい肛門温存術の可能性―病理組織学的研究―．日本大腸肛門病会誌　57 : 315–323, 2004
9) Saito N, Ono M, Sugito M, et al : Early results of intersphincteric resection for patients with very low rectal cancer : An active approach to avoid a permanent colostomy. Dis Colon Rectum 47 : 459–466, 2004
10) Adam IJ, Mohamdee MO, Martin IG, et al : Role of circumferential margin involvement in the local recurrence of rectal cancer. Lancet 344 : 707–11, 1994
11) De Haas-Kock DF, Baeten CG, Jager JJ, et al : Prognostic significance of radial margins of clearance in rectal cancer. Br J Surg 83 : 781–85, 1996
12) Wibe A, Rendedal PR, Svensson E, et al : Prognostic significance of the circumferential resection margin following total mesorectal excision for rectal cancer. Br J Surg 89 : 327–334, 2002
13) Brown G, Radcliffe AG, Newcombe RG, et al : Preoperative assessment of prognostic factors in rectal cancer using high-resolution magnetic resonance imaging. Br J Surg 90 : 355–364, 2003
14) Blomqvist L, Rubio C, Holm T, et al : Rectal adenocarcinoma : assessment of tumour involvement of the lateral resection margin by MRI of resected specimen. Br J Radiol 72 : 18–23, 1999
15) Beets-Tan RGH, Beets GL, Vliegen RF, et al : Accuracy of magnetic resonance imaging in prediction of tumour-free resection margin in rectal cancer surgery. Lancet 357 : 497–504, 2001
16) Peschaud F, Cuenod CA, Benoist S, et al : Accuracy of magnetic resonance imaging in rectal cancer depends on location of the tumor. Dis Colon Rectum 48 : 1603–1609, 2005
17) Urban M, Rosen HR, Hölbling N, et al : MR Imaging for the preoperative planning of sphincter-saving surgery for tumors of the lowerthird of the rectum : Use of intravenous and endorectal contrast materials. Radiology 214 : 503–508, 2000
18) Holzer B, Urban M, Hölbling N, et al : Magnetic resonance imaging predicts sphincter invasion of low rectal cancer and influences selection of operation. Surgery 133 : 656–661, 2003

Preoperative Assessment of External Anal Sphincter Invasion and Para-external Anal Sphincter Invasion in Rectal Cancer Using Magnetic Resonance Imaging

M. Ishii[1], S. Yuen[2], S. Yamaguchi[1], H. Morita[1], S. Saito[1], M. Oota[1], K. Morimoto[1], T. Okumoto[1], and M. Hashimoto[1]

Division of Colon and Rectal Surgery[1], Diagnostic Radiology[2], Shizuoka Cancer Center

PURPOSE : The aim of this study was to assess the accuracy of preoperative magnetic resonance imaging for the prediction of external anal sphincter invasion and para-external anal sphincter muscle invasion in lower rectal cancer patients.

METHOD : Twenty-six patients with lower rectal cancer who underwent curative abdomino-perineal resection were investigated. The para-external anal sphincter invasion was defined when tumor was observed within 1 mm from the inter rim of the external anal sphincter muscle. We established the criteria for external anal sphincter invasion on magnetic resonance imaging. Preoperative magnetic resonance imaging was compared with pathological results prospectively.

RESULT : External anal sphincter invasion was seen in only two patients, and para-external anal sphincter invasion was seen in 7 of the 26 patients histologically. Accuracy of preoperative magnetic resonance imaging for detecting external and para-external anal sphincter invasion was a sensitivity of 89% and a specificity of 82%.

CONCLUSION : Preoperative magnetic resonance imaging could be useful for detecting external and para-external anal sphincter invasion in rectal cancer patients.

(2006年3月16日受付)
(特別掲載)

Reprinted from GUT, July 2006, Vol 55, p 1007-1011

GASTROINTESTINAL CANCER

Positron emission tomography scanning is not superior to whole body multidetector helical computed tomography in the preoperative staging of colorectal cancer

H Furukawa, H Ikuma, A Seki, K Yokoe, S Yuen, T Aramaki, S Yamaguchi

Gut 2006;55:1007–1011. doi: 10.1136/gut.2005.076273

See end of article for authors' affiliations

Correspondence to: Dr H Furukawa, Division of Diagnostic Radiology, Shizuoka Cancer Centre Hospital, 1007, Nagaizumi-cho, Suntogun, Shizuoka, 411-8777, Japan; h.furukawa@scchr.jp

Revised version received 27 September 2005
Accepted for publication 7 December 2005
Published online first 16 December 2005

Background: The role of positron emission tomography with the glucose analogue [18F] fluoro-2-deoxy-D-glucose (FDG-PET) in the initial staging of disease in patients with primary colorectal cancer (CRC) has not been adequately assessed.
Aims: To evaluate the additional value of FDG-PET as a staging modality, complementary to routine multidetector row computed tomography (MDCT) in patients with CRC.
Methods: Forty four patients with CRC underwent preoperative MDCT and FDG-PET. The accuracy of intraoperative macroscopic staging was also investigated compared with histopathological diagnosis. All FDG-PET images were evaluated with respect to detectability of the primary tumour, lymph node involvement, and distant metastases. Both MDCT and FDG-PET diagnoses and treatment plan were compared with surgical and histopathological results.
Results: Thirty seven patients underwent surgery. Tumour detection rate was 95% (42/44) for MDCT, 100% (44/44) for FDG-PET, and 100% (37/37) for intraoperative macroscopic diagnosis. Pathological diagnosis of T factor was T1 in five, T2 in four, T3 in 24, and T4 in four cases. Concordance rate with pathological findings of T factor was 57% (21/37) for MDCT and 62% (23/37) for macroscopic diagnosis. Lymph node involvement was pathologically positive in 19 cases. Regarding N factor, overall accuracy was 62% (23/37) for MDCT, 59% (22/37) for FDG-PET, and 70% (26/37) for macroscopic diagnosis. For all 44 patients, FDG-PET findings resulted in treatment changes in only one (2%) patient.
Conclusion: FDG-PET is not superior to routine MDCT in the initial staging of primary CRC.

Colorectal cancer (CRC) is an important cause of morbidity and mortality in Japan as well as in other countries.[1] The prognosis of CRC directly relates to extramural tumour spread, ability to achieve surgical clearance, and the presence of lymph node and distant metastases.[2 3] Optimal management of individual patients requires detailed assessment of the locoregional and distant extent of disease.

Conventional preoperative staging of CRC has been abdominal computed tomography (CT) and chest radiography to rule out liver, lung, or lymph node metastases and invasion of the surrounding organs, respectively. The introduction of multidetector row CT (MDCT) has provided high resolution imaging and shortened examination time.[4] This becomes an effective diagnostic technique in the evaluation of preoperative staging of CRC.

Positron emission tomography with the glucose analogue [18F] fluoro-2-deoxy-D-glucose (FDG-PET) is a sensitive diagnostic test that images tumours based on increased utilisation of glucose by tumour cells.[5 6] FDG-PET has been demonstrated to be more sensitive than conventional imaging in the detection of recurrent or metastatic CRC.[7–13] One meta-analysis revealed an overall sensitivity of 97% and an overall specificity of 76% for FDG-PET in detecting recurrent CRC.[14]

However, reports of FDG-PET in the staging of primary CRC are few.[15–18] Also, these studies had several limitation: patient numbers were small,[15 16] or diagnostic accuracy of FDG-PET was compared with conventional abdominal CT[17] or CT was performed at a different hospital.[18] Comparison of state of the art FDG-PET with CT using variable techniques and qualities is not meaningful. Thus the role of FDG-PET in the initial staging of disease in patients with primary CRC has not been fully investigated to date.[19]

The purpose of this study was to prospectively evaluate the additional value of FDG-PET as a staging modality complementary to routine MDCT in patients with primary CRC. In patients undergoing surgery, the accuracy of intraoperative macroscopic staging was also investigated compared with histopathological diagnosis, as well as preoperative imaging results. All studies were performed in one Japanese hospital.

METHODS

Patients

Between September 2002 and January 2004, 44 consecutive patients with CRC who approved of this study were enrolled after giving written informed consent in accordance with the regulations of the institutional review board. There were 33 men and 11 women with a mean age of 61.4 years (range 38–82). The primary tumour originated from the right colon (n = 2), sigmoid colon (n = 4), or rectum (n = 38). Histological diagnosis was performed in all patients by colonoscopy. All patients underwent preoperative MDCT and FDG-PET within one month (median 9 days (range 0–26)).

Abbreviations: PET, positron emission tomography; FDG-PET, [18F] fluoro-2-deoxy-D-glucose-positron emission tomography; CRC, colorectal cancer; CT, computed tomography; MDCT, multidetector row computed tomography

MDCT

The diagnosis and location were established by barium enema and/or colonoscopy before CT scanning. The time interval between barium enema and/or colonoscopy and MDCT was ⩽1 week. No specific preparation, such as laxatives, enema, or oral contrast agents, was performed before MDCT examination.

We used an Aquiline 16 CT scanner (Toshiba Medical Systems, Tokyo, Japan). For imaging of the whole body, we used the 16 high resolution central detectors. From these detectors we selected a 2 mm slice thickness and reconstructed the data at 5 mm intervals. Other parameters were a 0.5 second helical rotation time, 135 kVp, and 300 mAs. Iopamidol 100 ml (Iopamiron; Nihon Schering, Tokyo, Japan) was administered through a peripheral venous line at 3 ml/s using a power injector (Autoenhance A-50; Nemoto Kyorindo, Tokyo, Japan). CT scanning began 120 seconds after the start of injection of the contrast medium and scan data were acquired from the neck to the upper femur within one breathhold in approximately 20 seconds. Multiplanar reformation was reconstructed by a freestanding workstation (ZAIO, Tokyo, Japan) if diagnostic radiologists considered it necessary.

FDG-PET

Patients fasted for at least four hours before the examination. Patients received an intravenous injection of 200–250 MBq of [18F] fluoro-2-deoxy-D-glucose and then rested for approximately 60 minutes before undergoing imaging. Image acquisition was performed with use of an Advance NXi (GE Medical Systems, Milwaukee, Wisconsin, USA). Two dimensional emission scanning from the groin to the base of the skull (6–7 bed positions) was performed, lasting five minutes per bed position, in combination with a transmission scan lasting 1.5 minutes per bed position (transmission scanning time was corrected to allow for decay of the transmission sources). Data acquired were reconstructed by iterative ordered subsets expectation maximisation (21 subsets, two iterations).

Image analysis

At first, MDCT images were prospectively evaluated by two radiology physicians in consensus. They were assessed for detectability of the tumour, depth of tumour infiltration (T factor), regional lymph node involvement (N factor), and distant metastasis (M factor).

T factor on MDCT was defined by a modified TNM stage: tumour confined to the bowel wall was classified as T1 or T2. T1 was defined as an intraluminal elevated mass without thickening of the bowel wall. T2 was defined as thickening of the bowel wall (>5 mm) without invasion into the surrounding tissue. Tumour exposed out of the bowel wall but with no extension to the surrounding organs was considered as T3. Tumour infiltration into adjacent organs was considered T4. Lymph nodes were considered positive when the short axis was greater than 1 cm in diameter or there were clusters of three or more smaller nodes (each <1 cm). Lesions in the liver not characteristic of a cyst or haemangioma were considered suspicious of metastases. Also in the lung, pulmonary nodules without calcification were regarded as suspicious of metastases.

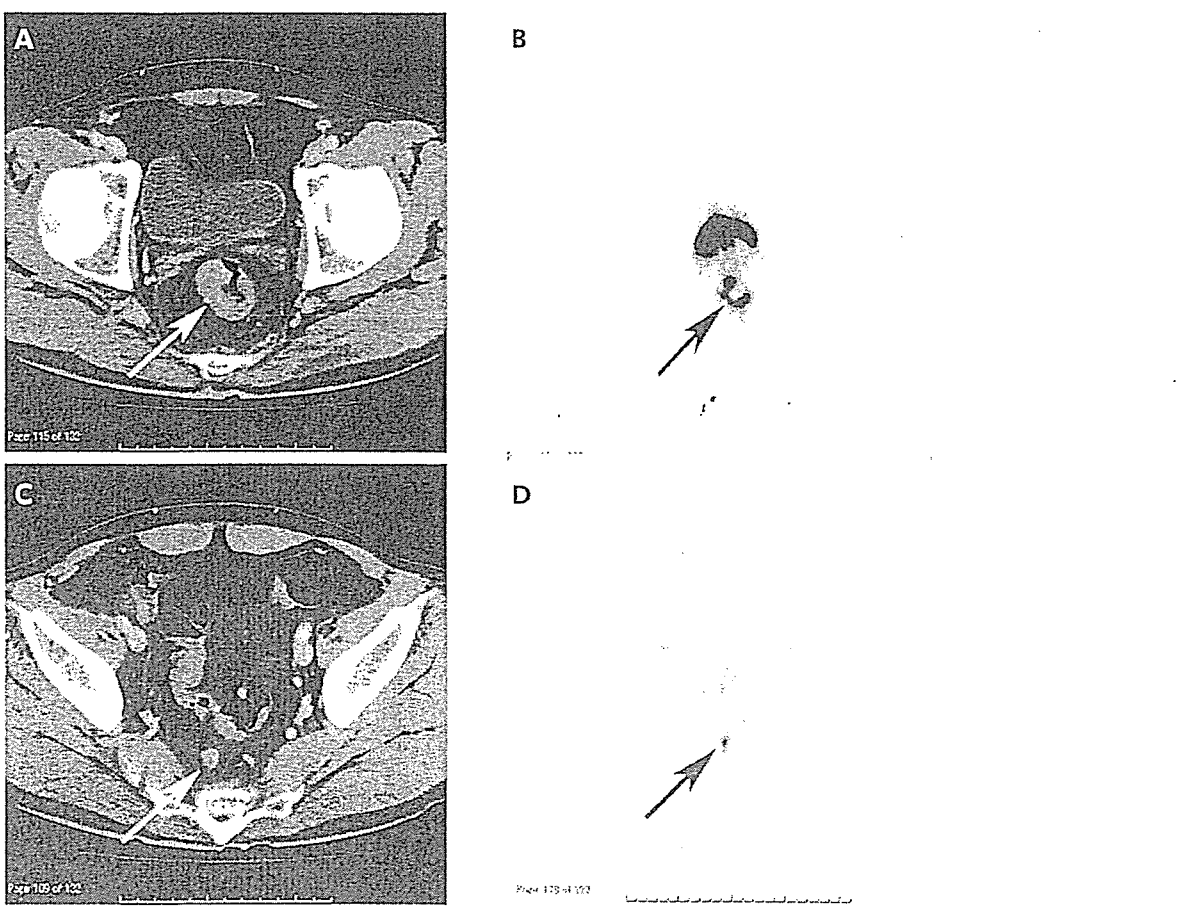

Figure 1 (A) Primary rectal tumour was exposed to the rectal wall but no extension to the pelvic side walls on computed tomography (CT) (arrow). (B) At the same level as (A), avid uptake was demonstrated on positron emission tomography (PET) (arrow). (C) A superior rectal lymph node was greater than 1 cm on CT (arrow). (D) At the same level as (C), PET showed uptake corresponded to the lymph node demonstrated on CT (arrow). This case was preoperatively diagnosed as TNM stage T3N1 and confirmed at surgery and on histopathological examination.

Table 1 Comparisons of MDCT and macroscopic diagnosis in staging depth of tumour invasion with colorectal carcinoma

Pathological staging	MDCT diagnosis					Macroscopic diagnosis			
	Tx	T1	T2	T3	T4	T1	T2	T3	T4
T1	2	0	2	1	0	1	4	0	0
T2	0	0	1	3	0	1	0	3	0
T3	0	0	5	17	2	0	3	18	3
T4	0	0	0	1	3	0	0	0	4

MDCT, multidetector row computed tomography.

All FDG-PET images were interpreted with knowledge of the patient's medical history and MDCT findings, and were evaluated with respect to detectability of the primary tumour, lymph node involvement, and distant metastases by two nuclear radiology physicians. T factor was not evaluated because the layers of intestinal wall and neighbouring structures cannot be differentiated on FDG-PET. Uptake higher than background was considered to be increased. Physicians interpreted the FDG-PET images by visually correlating the FDG-PET and MDCT images (fig 1). This approach was chosen because it represents the routine practice of combined reading of FDG-PET and MDCT images in our hospital. On the basis of their visual correlation, physicians assigned a TNM stage on FDG-PET. Regarding N factor, we chose to analyse the imaging studies on a nodal station bases and not on an individual lymph node basis. It seemed impossible for us to make a precise correlation between individually sampled and mapped lymph nodes on imaging studies.

Preoperative staging decision

Both MDCT and FDG-PET results were presented at the colorectal cancer conference, comprising surgeons, medical oncologists, endoscopists, nuclear radiology physicians, and radiation oncologists. All conference members confirmed the MDCT and FDG-PET findings. When a clear differentiation between different tumour stages on MDCT and FDG-PET was not possible, both stages were noted and confirmed after surgery. Based on the consensus of the conference, patients were divided into two groups. Patients considered as unresectable were referred to the Division of Gastrointestinal Medical Oncology, where chemotherapy, chemoradiotherapy, or best supportive care was performed. If unresectable factors were negative, the patient was admitted to a surgical ward and curative resection was attempted. In our hospital, neoadjuvant therapy was not routinely performed. These decisions on diagnosis and treatment plan were recorded and compared with surgical and pathological results.

Macroscopic diagnosis

For the 37 patients who proceeded to surgery, detection of the primary tumour, its depth of invasion, lymph node status, and liver metastases were macroscopically diagnosed either during surgery or through a node collection and classification procedure immediately after resection. These procedures were performed with knowledge of the preoperative imaging findings.

Data analysis

Resected specimens were examined by pathologists without knowing the preoperative MDCT and FDG-PET findings. The diagnostic accuracy of MDCT, FDG-PET, and macroscopic diagnosis of T and N factors were assessed using the histopathological findings as the gold standard. Comparison of diagnostic and pathological parameters was performed using the McNemar test. The level of statistical significance was determined at 5% in all cases.

RESULTS

All 44 patients tolerated both MDCT and FDG-PET examinations without any complications. Based on both MDCT and FDG-PET, 10 lesions of distant metastases were revealed in five patients and defined as unresectable: three bone metastases, three lung metastases, two liver metastases, and two distant lymph node metastases. MDCT showed eight of these 10 lesions; one each of bone and distant lymph node metastasis were missed. FDG-PET showed nine of the 10 lesions; one lung metastasis was missed. These five patients did not undergo surgical resection. Two patients were refused any anticancer treatment and left our hospital although their tumours were potentially resectable. Thus the remaining 37 patients were defined as resectable and underwent surgery. As expected, all lesions were resected with regional lymph node dissection.

The tumour detection rate was 95% for MDCT, 100% for FDG-PET, and 100% for intraoperative macroscopic diagnosis. The two cases which were not detected on MDCT were 0.7 cm and 1.8 cm adenocarcinomas, both limited to the submucosal layer. Regarding T factor, concordance rate with pathological findings was 57% for MDCT and 62% for macroscopic diagnosis (table 1). The difference was not significant ($p = 0.813$). In three of seven cases, tumours were diagnosed as T4 at surgery but histopathologically with no evidence of invasion to the adjacent organ. In contrast, in one case, MDCT showed no evidence of invasion to the adjacent organ but the tumour was found to have invaded the vagina

Table 2 Comparisons of MDCT, PET, and macroscopic diagnosis in staging lymph node metastasis with colorectal carcinoma

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value(%)	Accuracy (%)
MDCT	58 (11/19)	67 (12/18)	65 (11/17)	60 (12/20)	62 (23/37)
PET	37 (7/19)	83 (15/18)	70 (7/10)	43 (15/27)	59 (22/37)
Macroscopic diagnosis	68 (13/19)	72 (13/18)	72 (13/18)	68 (13/19)	70 (26/37)

MDCT, multidetector row computed tomography; PET, positron emission tomography.

at surgery and combined resection was performed. Invasion was confirmed histopathologically.

Regarding N factor, overall accuracy was 62% for MDCT, 59% for FDG-PET, and 70% for macroscopic diagnosis (table 2). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated as 58%, 67%, 65%, and 60%, respectively, for MDCT; 37%, 83%, 70%, and 43%, respectively, for FDG-PET; and 68%, 72%, 72%, and 68%, respectively, for macroscopic diagnosis. Macroscopic diagnosis showed a slightly higher accuracy but values were not significantly different between these modalities ($p = 0.624$ for MDCT *v* macroscopic diagnosis; $p = 0.466$ for FDG-PET *v* macroscopic diagnosis).

Of the 44 patients, FDG-PET findings resulted in treatment changes in only one (2%) patient who had bone and distant lymph node metastases detected only by FDG-PET. Although MDCT detected lung metastases that were not demonstrated on FDG-PET in one case, the patient had other distant metastases and the treatment plan was not influenced by the MDCT findings.

DISCUSSION

CT examination is an established method for staging colorectal carcinoma. However, recent studies have shown low accuracy rates due to considerably low sensitivity for detection of lymph node metastases and for local tumour extension.[20–23] MDCT is expected to improve diagnostic accuracy by scanning a wider region with better resolution. To evaluate the usefulness of FDG-PET, we compared the diagnostic accuracy of FDG-PET with this up to date technology.

The efficacy of imaging techniques is usually evaluated by retrospective reading by radiologists blinded to the clinical information. However, this is not practical and many physicians often feel that actual diagnostic results are different from the results reported. We wished to evaluate the usefulness of FDG-PET in the clinical setting. Recently, integrated PET-CT scanners have been introduced into the clinical situation.[24] Using this technique, PET, CT, and integrated PET-CT images are displayed together on the monitor. This type of PET scanner and reading style will become routine. Thus in this study, FDG-PET images were interpreted with knowledge of the patient's medical history and MDCT images.

For detection of a primary tumour, FDG-PET was positive in all 44 lesions but MDCT gave two false negative lesions. We did not use any preparation before the MDCT studies. If sufflation of air or water into the bowel cavity and administration of antiperistaltic drug had been performed, the detection rate might have been improved. In contrast, FDG-PET was positive in all lesions, including those that were negative with MDCT. Such high sensitivity confirms the results of previous reports.[15–17] The injected dose that we used was lower than the conventional dose reported. However, we had confirmed in our preliminary study that image quality with this dose did not deteriorate. This may have been due to differences in the physique of Japanese and Western patients. We should try to reduce radiation exposure while preserving diagnostic accuracy.

CT studies over the last decade showed accuracy rates of 41–82% in T staging.[20–23] Our result (57%) was comparable with these reports. Even with the improved imaging resolution of MDCT, it is still difficult to discriminate bowel wall layers as conventional single detector spiral CT. MDCT did not demonstrate satisfactory results for diagnosis of N factor, as reported in previous studies.[25–27] Microscopic metastasis or uninvolved swelling of lymph nodes results in misdiagnosis. As long as a diagnosis is made based on the size of lymph nodes, a certain percentage of false positive and negative lymph nodes is unavoidable. In this study, FDG-PET had low sensitivity (37%) and high specificity (83%), as reported in previous studies.[16–18] FDG-PET was no better than MDCT. The high false negative rate was attributed to limited spatial resolution, which was a disadvantage in detecting micrometastases, and the proximity of the dose to the primary tumour to lymph node metastases.

The accuracy of intraoperative macroscopic diagnosis was superior but not significantly different from that of MDCT and FDG-PET. By palpation and inspection, lymph nodes in the immediate vicinity of the primary tumour could be differentiated more easily than by MDCT or FDG-PET. Moreover, macroscopic diagnosis was made with knowledge of the MDCT and FDG-PET findings. Nevertheless, accurate diagnosis of lymph node metastasis is difficult, even at surgery.

FDG-PET has the advantage of studying the whole body at one examination and synchronous tumours have been identified on FDG-PET. However, MDCT can also scan the whole body in a shorter time than FDG-PET. In this study, distant metastases revealed only 10 lesions in five patients. While patient numbers were too small to compare the usefulness of the diagnostic modalities, MDCT and FDG-PET showed various corresponding metastatic lesions.

In assessing the influence of FDG-PET findings on clinical management, changes in therapeutic decision making were made in only 2% (1/44) of cases, which is less than in other investigations. The incidence of management alterations due to FDG-PET was reported as 16–50%.[8 13 18 28 29] The reason may be selection of patients in the other studies as many were already known to have advanced disease and FDG-PET was performed to detect recurrences or metastases.

The results of this study suggest that the diagnostic accuracy of FDG-PET for the initial staging of CRC was not superior to routine MDCT and was not influential in terms of patient management. We believe routine evaluation of patients with a suspicion of CRC by FDG-PET is not necessary; it should be performed on selected patients who have suggestive but inconclusive metastatic lesions with other modalities.

ACKNOWLEDGEMENTS

This work was supported by the Foundation for Promotion of Cancer Research in Japan.

Authors' affiliations

H Furukawa, H Ikuma, A Seki, K Yokoe, S Yuen, T Aramaki, Division of Diagnostic Radiology, Shizuoka Cancer Centre Hospital, Shizuoka, Japan

S Yamagushi, Division of Colorectal Surgery, Shizuoka Cancer Centre Hospital, Shizuoka, Japan

Conflict of interest: None declared.

REFERENCES

1 Nomura K, Sobue T, Honma I, *et al.* Mortality from malignant neoplasms by age group and sex in Japan. In: *The editorial board of the cancer statistics in Japan. Cancer statistics in Japan 2003.* Tokyo: Foundation for Promotion of Cancer Research, 2003:40–1.
2 Adam IJ, Mohamdee MO, Martin IG, *et al.* Role of circumferential margin involvement in the local recurrence of rectal cancer. *Lancet* 1994;344:707–11.
3 de Haas-Kock DF, Baeten CG, Jager JJ, *et al.* Prognostic significance of radial margins of clearance of rectal cancer. *Br J Surg* 1996;83:781–5.
4 Hu H, He HD, Foley WD, *et al.* Four multidetector-row helical CT: image quality and volume coverage speed. *Radiology* 2000;215:55–62.
5 Som P, Atkins HL, Bandoypadhyay D, *et al.* A fluorinated glucose analog, 2-fluoro-2-deoxy-D-glucose (F-18): nontoxic tracer for rapid tumor detection. *J Nucl Med* 1980;21:670–5.
6 Flier JS, Mueckler MM, Usher P, *et al.* Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science* 1987;235:1492–5.

7 **Topal B**, Flamen P, Aerts R, *et al.* Clinical value of whole-body emission tomography in potentially curable colorectal liver metastases. *Eur J Surg Oncol* 2001;**27**:175–9.
8 **Arulampalam T**, Costa D, Visvikis D, *et al.* The impact of FDG-PET on the management algorithm for recurrent colorectal cancer. *Eur J Nucl Med* 2001;**28**:1758–65.
9 **Delbeke D**, Vitola JV, Sandler MP, *et al.* Staging recurrent metastatic colorectal carcinoma with PET. *J Nucl Med* 1997;**38**:1196–201.
10 **Flamen P**, Stroobants S, Van Cutsem E, *et al.* Additional value of whole-body positron emission tomography with fluorine-18-2-fluoro-2-deoxy-D-glucose in recurrent colorectal cancer. *J Clin Oncol* 1999;**17**:894–901.
11 **Johnson K**, Bakhsh A, Young D, *et al.* Correlating computed tomography and positron emission tomography scan with operative findings in metastatic colorectal cancer. *Dis Colon Rectum* 2001;**44**:354–7.
12 **Lai DT**, Fulham M, Stephen MS, *et al.* The role of whole-body positron emission tomography with [18F]fluorodeoxy-glucose in identifying operable colorectal cancer metastases to the liver. *Arch Surg* 1996;**131**:703–7.
13 **Ogunbiyi OA**, Flanagan FL, Dehdashti F, *et al.* Detection of recurrent and metastatic colorectal cancer: comparison positron emission tomography and computed tomography. *Ann Surg Oncol* 1997;**4**:613–20.
14 **Huebner PH**, Park KC, Shepherd JE, *et al.* A meta-analysis of the literature for whole body FDG PET detection of recurrent colorectal cancer. *J Nucl Med* 2000;**41**:1177–89.
15 **Gupta NC**, Falk PM, Frank AL, *et al.* F-18 fluorodeoxyglucose (FDG) PET for preoperative staging of colorectal carcinoma (abstr). *J Nucl Med* 1992;**33**:975.
16 **Abdel-Nabi H**, Doerr RJ, Lamonica DM, *et al.* Staging of primary colorectal carcinomas with fluorine-18 Fluorodeoxyglucose whole-body PET: correlation with histopathologic and CT findings. *Radiology* 1998;**206**:755–60.
17 **Mukai M**, Sadahiro S, Yasuda S, *et al.* Preoperative evaluation by whole-body 18F-fluorodeoxyglucose positron emission tomography in patients with primary colorectal cancer. *Oncol Rep* 2000;**7**:85–87.
18 **Kantorová I**, Lipská L, Bělohlávek O, *et al.* Routine 18F-FDG PET preoperative staging of colorectal cancer: comparison with conventional staging and its impact on treatment decision making. *J Nucl Med* 2003;**44**:1784–8.
19 **Arulampalam THA**, Costa DC, Loizidou M, *et al.* Positron emission tomography and colorectal cancer. *Br J Surg* 201, **88**:176–89.
20 **Thoeni RF**. Colorectal cancer: radiological staging. *Radiol Clin N Am* 1997;**35**:457–8.
21 **Hundt W**, Braunschweig R, Reiser M. Evaluation of spiral CT in staging of colon and rectum carcinoma. *Eur J Radiol* 1999;**9**:78–84.
22 **Angelelli G**, Macarini L, Lupo L, *et al.* Rectal carcinoma: CT staging with water as contrast medium. *Radiology* 1990;**177**:511–14.
23 **Chiesura-Corona M**, Muzzio PC, Giust G, *et al.* Rectal cancer: CT local staging with histopathologic correlation. *Abdom Imaging* 2001;**26**:134–8.
24 **Cohade C**, Osman M, Leal J, *et al.* Direct comparison of 18F-FDG PET and PET/CT in patients with colorectal carcinoma. *J Nucl Med* 2003;**44**:1797–803.
25 **Matsuoka H**, Nakamura A, Masaki T, *et al.* Preoperative staging by multidetector-row computed tomography in patients with rectal carcinoma. *Am J Surg* 2002;**184**:131–5.
26 **Klinna C**, Eibel R, Matzek W, *et al.* Staging of rectal cancer: diagnostic potential of multiplanar reconstructions with MDCT. *AJR Am J Roentgenol* 2004;**183**:421–7.
27 **Flippone A**, Ambrosini R, Fuschi M, *et al.* Preoperative T and N staging of colorectal cancer: accuracy of contrast-enhanced multidetector-row CT colonography-initial experience. *Radiology* 2004;**231**:83–90.
28 **Boykin KN**, Zibari GB, Lilien DL, *et al.* The use of FDG-positron emission tomography for the evaluation of colorectal metastases of the liver. *Am Surg* 1999;**65**:1183–5.
29 **Ruers TJ**, Langenhoff BS, Neeleman N, *et al.* Value of positron emission tomography with (F-18)fluorodeoxyglucose in patients with colorectal liver metastases: a prospective study. *J Clin Oncol* 2002;**20**:388–95.

Original Paper

Colonic J-Pouch Decreases Bowel Frequency by Improving the Evacuation Ratio

H Kimura MD, H Shimada MD, H Ike MD, S Yamaguchi MD, Y Ichikawa MD
M Kikuchi MD, S Fujii MD, S Ohki MD
Second Department of Surgery, Yokohama City University School of Medicine, Yokohama, Japan
Corresponding Author: Dr Hideaki Kimura, Second Department of Surgery, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa, Yokohama 236-0004, Japan
Tel: +81 45 787 2650, Fax: +81 45 782 9161, E-mail: kfc04274@nifty.com

KEY WORDS:
Colonic J-pouch; Defecography; Transit study; Evacuation ratio; Contraction ratio

ABSTRACT

Background/Aims: To compare the functional outcome of ultra-low anterior resection for rectal cancer with colonic J-pouch reconstruction with that of straight reconstruction.

Methodology: Twenty-three patients who underwent ultra-low anterior resection with or without J-pouch reconstruction underwent bowel transit study, videodefecography, and answered a questionnaire survey 4 months and 1 year after surgery. Eleven healthy subjects underwent similar testing as controls.

Results: Patients with a J-pouch had less frequent stools than patients with straight reconstruction 4 months after surgery ($p<0.05$), but the two groups were similar at 1 year. Bowel transit time was similar at both study points. The evacuation ratio was higher after J-pouch than straight reconstruction 4 months after surgery ($p<0.05$). However, the ratio improved in the straight group, and no difference existed at 1 year. Colonic contraction was seen only near the anastomosis 4 months after surgery, but the contraction proximal to the anastomosis improved over the next 8 months.

Conclusions: J-pouch reconstruction facilitates evacuation by improving the evacuation ratio. Although straight anastomosis caused excessive stool frequency 4 months after surgery, colonic function continued to improve and was comparable with J-pouch and straight reconstruction 1 year after surgery because the contraction ratio proximal to the anastomosis improved.

INTRODUCTION

Generally, ultra-low anterior resection for rectal cancer avoids the need to create a permanent colostomy, but it often results in excessive stool frequency which decreases the quality of life. It has been reported that functional outcome after low anterior resection for rectal cancer can be improved by the construction of a colonic J-pouch. However the reasons for this improvement are poorly understood. Therefore construction of a colonic J-pouch is a controversial procedure, and its use varies from institution to institution.

This study compared the functional outcome of ultra-low anterior resection with and without colonic J-pouch reconstruction using new indicators of bowel function termed the evacuation ratio and the contraction ratio.

METHODOLOGY

Between April 1999 and March 2001, ultra-low anterior resection with the primary anastomosis (<4cm above the dentate line) was performed in 23 rectal cancer patients. Patients were assigned randomly to the J-pouch with a 5-cm limb (n=13) or to the straight anastomosis (n=10) by a computer-generated table of random numbers. Informed consent was obtained from all patients.

Patients in the two groups were similar with respect to age, gender, distance between the anastomosis and the dentate line, nerve preservation, and Dukes stage **(Table 1)**.

Bowel function was evaluated by a bowel transit study, a videodefecography, and a questionnaire administered before surgery, 4 months and 1 year after surgery.

Bowel Transit Study

Twenty radiopaque markers within a gelatin capsule (Sitzmarks: Konsyl Pharmaceuticals, U.S.A.) were ingested, and a plain film of the abdomen was taken 8, 24, 32, 48, and 96 h after ingestion. The half-dose transit method was used, and segment transit time for each segment of the colon was calculated before surgery and 4 months after surgery. Each segment was defined as follows; Ascending colon: A, Transverse colon: T, Descending colon: D, Sigmoid colon and (neo) Rectum: SR.

Videodefecography

Thick barium sulfate of standardized consistency and viscosity was introduced into the (neo) rectum using a caulking gun injector until the contrast reached the sacral promontory (approximately 120g). Evacuation was videotaped fluoroscopically with the

Hepato-Gastroenterology 2006; 53:854-857

patient in the sitting position.

The weight of infused contrast (W1) and evacuated contrast in 1 minute (W2) were recorded. The evacuation ratio was given by calculating W2/W1 x 100(%). The contraction ratio (CR) was the post-evacuation diameter of the colon divided by the pre-evacuation diameter (x 100(%)) which were calculated by lateral view of pelvic X-ray. The CR was calculated 5, 10, and 15cm above the anastomosis (CR5, CR10, and CR15, respectively).

Videodefecography was taken only postoperation (4 months and 1 year after surgery) because the examination was prevented by the existence of the tumor preoperation, so eleven healthy volunteers underwent the same examination and served as normal controls.

Questionnaire Survey

A questionnaire was administered 4 months and 1 year after surgery inquiring as to the number of bowel movements per day, fecal soiling, and urgency.

Student's *t* test was used for intergroup comparisons. *P* values less than 0.05 were considered significant.

RESULTS

1. Relationship between Bowel Frequency and Results of the Examination

The patients were divided into two groups to evaluate a relationship between bowel transit time and bowel frequency: high frequency (>5 bowel movements per day) and low frequency. The left colonic transit time was longer in patients in the high bowel frequency group than those in the low **(Figure 1)**.

There was a tendency towards an inverse correlation between the evacuation ratio by videodefecography and the number of bowel movements per day. The patients with a low evacuation ratio tended to have more frequent stools **(Figure 2)**.

2. Comparison of Colonic Function between J-Pouch and Straight Reconstruction

Patients who underwent J-pouch reconstruction had fewer stools per day than patients who received straight reconstruction 4 months after surgery, but the two groups were similar at 1 year. Soiling and urgency were similar at both sampling points **(Table 2)**.

The bowel transit time was longer postoperatively than it was preoperatively, especially in the left colon (D, SR), and that was similar in the two groups postoperatively **(Figure 3)**.

Patients with a J-pouch had a higher evacuation ratio (71%) than patients with a straight reconstruction (48%) 4 months after surgery. At 1 year, however, the two groups were similar **(Table 3)**.

The contraction ratio at different distances proximal to the anastomosis shows that powerful contractions occurred only near the anastomosis (CR5). The CR5 in J-pouch patients was higher than it was in patients with a straight reconstruction. One year after surgery, CR5, CR10 and CR15 in straight reconstruction patients both were higher than they were 4 months after surgery **(Table 3)**.

TABLE 1 Patients' Characteristics

	Type of reconstruction		
	J-pouch (n=13)	Straight (n=10)	
Age (yr)	55±10	64±9	(ns)
Gender ratio (M:F)	7:6	6:4	(ns)
Distance from dentate line (cm)			
anterior wall	2.6±1.2	2.4±1.2	(ns)
posterior wall	2.0±1.5	2.3±1.2	(ns)
Nerve preservation			
hypogastric nerve	complete : 4, partial : 0	complete : 4, partial : 0	(ns)
pelvic plexus	complete : 5, partial : 7	complete : 6, partial : 3	(ns)
Dukes stage (A:B:C:D)	6:1:5:1	3:1:6:0	(ns)

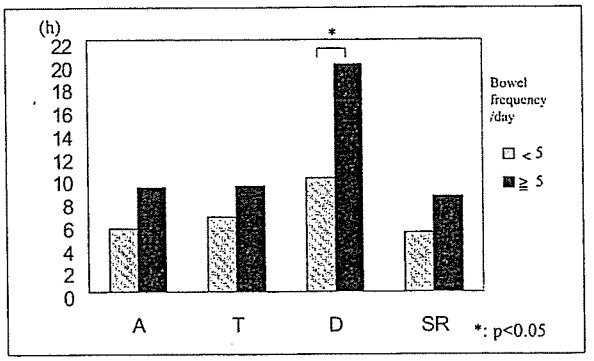

FIGURE 1 Segmental transit time between high and low bowel frequency group. Each segment was defined as follows; Ascending colon: A, Transverse colon: T, Descending colon: D, Sigmoid colon and (neo) Rectum: SR. The left colonic transit time was longer in patients in the high bowel frequency group (>5 bowel movements per day) than in patients in the low.

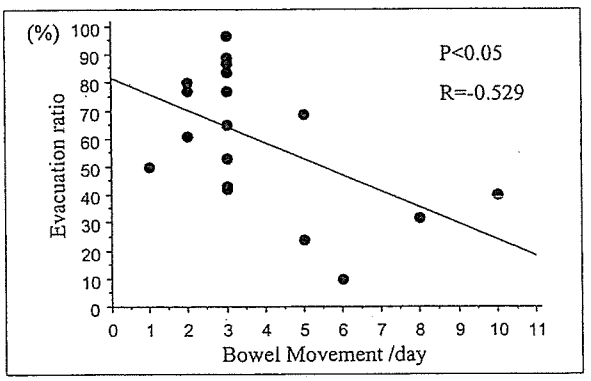

FIGURE 2 Relationship between evacuation ratio and bowel frequency. There was a tendency towards an inverse correlation between the evacuation ratio and the number of bowel movements per day. The patients with a low evacuation ratio tended to have more frequent stools.

DISCUSSION

The introduction of stapling devices has improved the safety of ultra-low anterior resection for rectal cancer. However, the ability of the rectum to function as a stool reservoir decreases in proportion to the amount of rectum removed, and conventional low anterior resection can result in dyschezia. Lazorthes

TABLE 2 Postoperative Functional Results

	Stool frequency (/day)		Soiling (%)		Urgency (%)	
	J-pouch	Straight	J-pouch	Straight	J-pouch	Straight
4M	3.0*	6.2*	31	20	0	20
8M	3.2**	6.2**	15	0	0	0
1Y	2.7	2.5	0	0	0	0

*, **: $p<0.05$.
J-pouch (n=13), Straight (n=10).

TABLE 3 Evacuation Ratio and Contraction Ratio

	Period	ER (%)	CR5 (%)	CR10 (%)	CR15 (%)
Control		89±12*1	50±16*2	31±15*3*4*5	33±5*6*7
J-pouch	4M	71±19	52±24	16±10*3	4±4*6
	1Y	77±19	50±15	5±2*4	14±4
Straight	4M	48±21*1	31±10*2	7±4*5	3±3*7
	1Y	70±24	47±21	21±3	12±10

*1-6 $p<0.05$.
ER: Evacuation ratio, CRx: contraction ratio x cm above the anastomosis.
Control values were derived from 11 healthy volunteers.

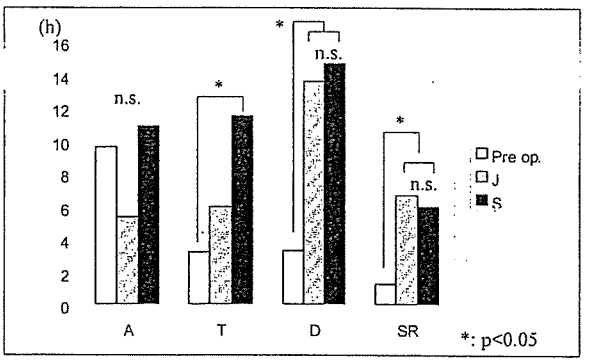

FIGURE 3 Segment transit time between J-pouch and straight reconstruction. The bowel transit time was longer postoperatively than it was preoperatively, especially in the left colon (D, SR). Bowel transit time was similar in patients with J-pouch and straight reconstruction.

(1) and Parc (2) reported in 1986 that colonic J-pouch improves postoperative rectal function. Hida *et al.* (3) recommended that a J-pouch be constructed when the anastomosis is <8cm from the anal verge because a satisfactory functional outcome can be obtained with straight reconstruction when the distance is >9cm. In recent years, a small J-pouch, with a 5 or 6-cm limb, has been recommended because it is difficult to evacuate a large pouch (4-7). Based on these recommendations, we used a J-pouch reconstruction with a 5-cm limb when the anastomosis was <4cm above the dentate line in this study.

It has been reported that J-pouch reconstruction improves rectal compliance (8), reduces the frequency of strong contractions (9), and increases the anorectal pressure gradient (9,10), all of which improve function.

We used a colonic transit study with radiopaque markers to evaluate motor activity of the colon. The reproducibility of this method has been validated (11). The postoperative bowel transit time was longer than in healthy controls, especially in the left colon, and corresponded to excessive stool frequency. These results suggest that the bowel frequency is related to the transit time of the left side colon.

In videodefecography, evacuation ratio and contraction ratio were obtained to evaluate the evacuation function of the colonic segment above the anastomosis. Also patients with a low evacuation ratio tended to have more frequent stools (12). Decreased motor activity of the colon proximal to the anastomosis prolongs the transit time and decreases the evacuation ratio (13). Denervation (14), poor blood supply (15), and the appearance of strong contractions (15,16) are thought to be causes of decreased motor activity after low anterior resection, and contribute to dyschezia.

The motor activity proximal to the anastomosis was decreased at 4 months regardless of whether a J-pouch was constructed. Contraction of the colon occurred only near the anastomosis. However the evacuation ratio was higher when a J-pouch was constructed. These results show that the J-pouch does not improve colonic transit time, but decreases stool frequency by facilitating fuller evacuation during each bowel movement.

It has been reported that the advantages of J-pouch over straight reconstruction are short-term and that the functional results are similar after 1 or 2 years (17,18). Our study reproduced this finding. Interestingly, the CR10 and CR15 were both higher at 1 year than they were 4 months after surgery in both groups. Improvement has been attributed to recovery of the nerve function (18) and reduction in the frequency of strong contraction (15), but further study is needed to clarify the cause of dyschezia after ultra-low anterior resection.

CONCLUSIONS

Decreased motor activity of the colon proximal to the anastomosis increases stool frequency after ultra-low anterior resection. Colonic J-pouch reconstruction did not improve colonic transit time, but did decrease stool frequency secondary to an improvement in the evacuation ratio. Stool frequency in patients with a straight reconstruction decreased during the first year after surgery because the contraction ratio proximal to the anastomosis improved.

REFERENCES

1 **Lazorthes F, Fages P, Chiotasso P, Lemozy J, Bloom E:** Resection of the rectum with construction of a colonic reservoir and coloanal anastomosis for carcinoma of the rectum. Br J Surg 1986; 73:136-138.
2 **Parc R, Tiret E, Frileux P, Moszkowski E, Loygue J:** Resection and coloanal anastomosis with colonic reservoir for rectal carcinoma. Br J Surg 1986; 73:139-141.
3 **Hida J, Yasutomi M, Maruyama T:** Indications for colonic J-pouch reconstruction after anterior resection for rectal cancer. Dis Colon Rectum 1998; 41:558-563.
4 **Hida J, Yasutomi M, Maruyama T:** Horizontal Inclination of the longitudinal axis of the colonic J-pouch. Dis Colon Rectum 1999; 42:1560-1568.
5 **Lazorthes F, Gamagami R, Chiotasso P:** Prospective,

randomized study comparing clinical results between small and large colonic J-pouch following coloanal anastomosis. Dis Colon Rectum 1997; 40:1409-1413.
6 **Hida J, Yasutomi M, Fujimoto K:** Functional outcome after low anterior resection with low anastomosis for rectal cancer using the colonic J-pouch - Prospective randomized study for determination of optimum pouch size. Dis Colon Rectum 1996; 39:986-991.
7 **Seow-Choen F:** Colonic pouches in the treatment of rectal cancer. Br J Surg 1996; 83:881-882.
8 **Hallbook O, Nystrom PO, Sjodahl R:** Physiologic characteristics of straight and colonic J-pouch anastomoses after rectal excision for cancer. Dis Colon Rectum 1997; 40:332-338.
9 **Ho YH, Tan M, Leong AFPK:** Ambulatory manometry in patients with colonic J-pouch and straight coloanal anastomoses: Randomized, controlled trial. Dis Colon Rectum 2000; 43:793-799.
10 **Romanos J, Stebbing JF, Humphreys MMS:** Ambulatory manometric examination in patients with a colonic J pouch and in normal controls. Br J Surg 1996; 83:1744-1746.
11 **Nam YS, Pikarsky AJ, Wexner SD:** Reproducibility of colonic transit study in patients with chronic constipation. Dis Colon Rectum 2001; 44:86-92.
12 **Kikuchi M:** Defecographic assessment after sigmoidectomy or anterior resection of rectum. Jpn J Gastroenterol Surg 2000; 33:455-461. (In Japanese with English abstract)
13 **Faucheron JL, Dubreuil A:** Rectal akinesia as a new cause of impaired defecation. Dis Colon Rectum 2000; 43:1545-1549.
14 **Nakamura F, Morita T, Konn M:** Clinical evaluation by means of radiopaque markers of colonic transit after low anterior resection of cancer. J Jpn Soc Coloproctol 1995; 48:193-205.
15 **Kurihara H:** The mechanism of bowel movement disorder after low anterior rectal resection: Time-course physiological analyses of colon oral to anastomosis. J Jpn Soc Coloproctol 1994; 47:121-132.
16 **Fujita M, Oya M:** Prolonged manometric study on motor activity of the neorectum after low anterior resection of rectal cancer. J Jpn Soc Coloproctol 2000; 53:20-26.
17 **Joo JS, Latulippe JF, Albaz O:** Long-term functional evaluation of straight coloanal anastomosis and colonic J-pouch: Is the functional superiority of colonic J-pouch sustained? Dis Colon Rectum 1998; 41:740-746.
18 **Ho YH, Seow-choen F, Tan M:** Colonic J-pouch function at six months versus straight coloanal anastomosis at two years: randomized controlled trial. World J Surg 2001; 25:876-881.

Int J Clin Oncol (2006) 11:385–389
DOI 10.1007/s10147-006-0590-5

ORIGINAL ARTICLE

Kenji Katsumata · Tetsuo Sumi · Yasuharu Mori
Masayuki Hisada · Akihiko Tsuchida · Tatsuya Aoki

Detection and evaluation of epithelial cells in the blood of colon cancer patients using RT-PCR

Received: August 18, 2005 / Accepted: May 25, 2006

Abstract
Background. As a mode of colorectal cancer recurrence, liver metastasis plays an important role. One of the factors reported to predict liver metastasis is the detection of trace amounts of tumor cells in the blood. For this purpose, cancer cell-induced cytokeratins (CKs) are generally identified, using the reverse transcriptase-polymerase chain reaction (RT-PCR). In the present study, we aimed to detect trace amounts of tumor cells, based on CK20, in the circulating venous blood, and we examined pathological factors, liver metastasis, and prognosis.
Methods. The subjects were 57 colorectal cancer patients who had undergone operation. We examined the cancer-induced marker (CK20) in circulating venous blood by RT-PCR and investigated the relationships between this marker, pathological factors, and prognosis.
Results. Detection ratio of CK20 mRNA was 42.1%, and CK20 was significantly correlated with the pathological factor of lymph node metastasis($P = 0.037$). The 5-year survival rate for CK20-positive patients was 62.5% and that for the CK20-negative patients was 87.5%; there was a significant difference($P = 0.048$) between the two groups. Recurrence was recognized in six patients; two were positive for CK20 and four were negative for CK20.
Conclusions. These findings indicate that CK20 is strongly related to lymph node metastasis and prognosis, suggesting its usefulness for the diagnosis of colorectal cancer recurrence. However, CK20 did not predict liver metastasis.

Key words Colorectal cancer · Cancer cells in circulating venous blood · Cytokeratin 20 · RT-PCR

K. Katsumata (✉) · T. Sumi · Y. Mori · M. Hisada · A. Tsuchida · T. Aoki
The Third Department of Surgery, Tokyo Medical University, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0022, Japan
Tel. +81-3-3342-6111; Fax +81-3-3340-4575
e-mail: k-katsu@tokyo-med.ac.jp

Introduction

The primary strategy in treating colorectal cancer is complete resection of the lesion; this sometimes includes wide resection of surrounding organs, extended lymph node dissection, and procedures that consider the patient's quality of life (QOL). However, after radical resection, some patients have recurrences, which are presumed to be due to residual micrometastatic foci. However, with classical morphological diagnostic methods, it is difficult to detect micrometastases; therefore, genetic diagnosis is being investigated. Real-time polymerase chain reaction (PCR) is generally used for this purpose, with genes for cytokeratins (CKs) and carcinoembryonic antigen (CEA) – regarded as cancer cell-induced genes – generally being identified using reverse transcriptase-PCR (RT-PCR). However, the detection rates vary, and there are also problems with false-positive and false-negative results depending on the target genes and the detection technology. In the present study, we aimed to detect trace amounts of tumor cells, based on the detection of CK19, CK20, and CEA mRNA in circulating venous blood.

Patients and methods

The subjects were 57 patients with colorectal cancer who had undergone surgical resection of the carcinoma. There were 29 men and 28 women with a mean age of 66.1 ± 10.9 years. The degree of cancer progression, by Dukes classification, was Dukes A (7 patients), Dukes B (26 patients), Dukes C (14 patients), and Dukes D (10 patients). Findings for cancer differentiation were: well-differentiated adenocarcinoma, 38 patients; moderately differentiated adenocarcinoma, 17 patients; and mucinous adenocarcinoma, 2 patients (Table 1) .The observation period ranged from 70 months to 84 months. The study was approved by the institution's ethics committee. The significance of the study and its safety were explained to the patients, and we obtained their informed consent.

Specimens for measurement were prepared as follows. Samples of circulating venous blood were collected during surgical resection of the carcinoma; the initial 5 ml of the sample, and the syringe, were discarded and the remaining 15 ml was employed.[1]

CK19 and CK20 were measured, using RT-PCR, as follows. Monocytes were separated from 5 ml of the blood specimen, and total RNA was extracted using acid quanidinium thiocynate-phenol-chlorotorm Nippon Gene Tokyo Japan (AGPC) and Isogen (Nippon Gene, Tokyo, Japan). From the total RNA, cDNA was synthesized, using both reverse transcriptase and random primer (25 mM Tris-Cl, pH 7.5; 75 mM KCl; 3 mM $MgCl_2$; 10 mM dithiothreitol [DTT]; 0.5 mM dNTP; 1 nM random primer; 1 U/μl RNase inhibitor; and 1 U/μl Super Script II RTase (GIBCO BRL Life Technologies, CA, USA).

The preparation was amplified using a CK19 amplification primer set (5′-AGC TAA CCA TGC AGA ACC TCAa-3′ and 5′-CTT CAG GCC TTC GAT CTG CAT-3′). If CK19 existed in the test product, the band was observed at 383 bp.

The preparation was also amplified using a CK20 amplification primer set (5′-CAG ACA CAC GGT GAA CTA TGG-3′ and 5′-GAT CAG CTT CCACTG TTA GACG-3′) in order to induce a PCR reaction (95°C/30 s, 60°C/30 s, and 72°C/30 s: 40 cycles of amplification). The PCR product was separated using electrophoresis with 25% agarose, and photographed under UV irradiation. If CK20 existed in the test product, the band was observed at 371 bp.[2]

CEA was measured using RT-nested PCR, as used to prevent a non-specific amplification. Total RNA was extracted using Isogen, and cDNA was synthesized, using 1.0 μg of the total RNA, at 40°C for 60 min. The synthesized cDNA was added to 50 μl of first-PCR solution (10 × Ampli Taq Gold Gold Enzyme ABI Calfornia USA) PCR solution, 0.2 mM dNTP, 30 pmol outer forward primer [CEA: 5′-GGA CCT ATG CCT GTT TTG TCT C-3′], 30 pmol reverse primer [CEA: 5′-GTT GCA AAT GCT TTA AGG AAG AAGC-3′], and 1.0 U Ampli taq gold PCR solution] and 35 cycles of PCR were carried out (one cycle: 95°C/30 s, 60°C/30 s, and 72°C/30 s). After completion of the PCR reaction, 5.0 μl of the first-PCR amplification product was added to 50 μl of the second-PCR solution (10 × Ampli taq gold PCR solution, 0.2 mM dNTP, 30 pmol inner forward primer [CEA: 5′ TTC TCC TGG TCT CTC AGC TGG-3′], 30 pmol reverse primer, and 1.0 U Amplitaq gold PCR solution], and 30 cycles of the PCR reaction were carried out (one cycle: 95°C/30 s, 60°C/30 s, and 72°C/30 s). After the reaction was completed, the PCR amplification product was electrophoresed with 3% agarose gel and ethidium bromide staining was performed; then the amplified product was confirmed using a UV transilluminator. If CEA existed in the test product, the band was confirmed at 145 bp. As an internal standard, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was detected.

Statistical analysis was done using the *t*-test, the χ^2 test, multivariate analysis by the proportional-hazard model, Spearman's test, and the Kaplan-Meier 5-year survival test. Significance was defined as $P < 0.05$.

Table 1. Patients' characteristics

Sex (M/F)	M29/F28
Age (years)	66.1 ± 10.9
Stage	Dukes A, 7; Dukes B, 26; Dukes C, 14; Dukes D, 10
Histology	Well 38 Moderate 17 Mucinous adenocarcinoma 2

Results

CEA, CK19, and CK20 were measured by each examination in healthy volunteers. CEA showed false-positive in 4 patients (57.1%) of 7 of the volunteers, but there were no false-positive results for CK19 and CK20 in 14 volunteers.

Preliminary analysis was carried out in 40 patients. CK19 was not a remarkable prognostic factor because its positive rate was very low and it was not relevant to tumor progression. The positive rates for CK20 and CEA were very similar (Table 2) and the identical ratio between CEA and CK 20 was 62.8% We also recognized some false-positive results for CEA by RT-PCR and decided to measure CK20 and to investigate this factor further of the patients.

CK20 in circulating blood was detected in 24 (42.1%) of the 57 patients. No correlation was observed between CK20 expression and the pathological factors of tumor progression, lymphatic invasion, vessel invasion, or liver metastasis. A correlation was seen between CK20 expression and the presence or absence of lymph node metastasis ($P = 0.037$; Table 3). There were two patients with recurrence in the CK20-positive group (11.8%). One patient had hepatic metastasis 28 months after operation, and the other had pulmonary metastasis at 26 months after operation. They died at 43 months and 34 months, respectively, after operation. There were four patients with recurrence in the CK-

Table 2. Relationship between cancer cells in blood and staging

	CK19		CK20		CEA	
	Positive	Negative	Positive	Negative	Positive	Negative
Dukes A	1	3	2	2	1	3
Dukes B	1	18	7	12	9	10
Dukes C	3	7	6	4	4	6
Dukes D	0	7	3	4	3	4
Positive rate	12.5%		45.0%		42.5%	

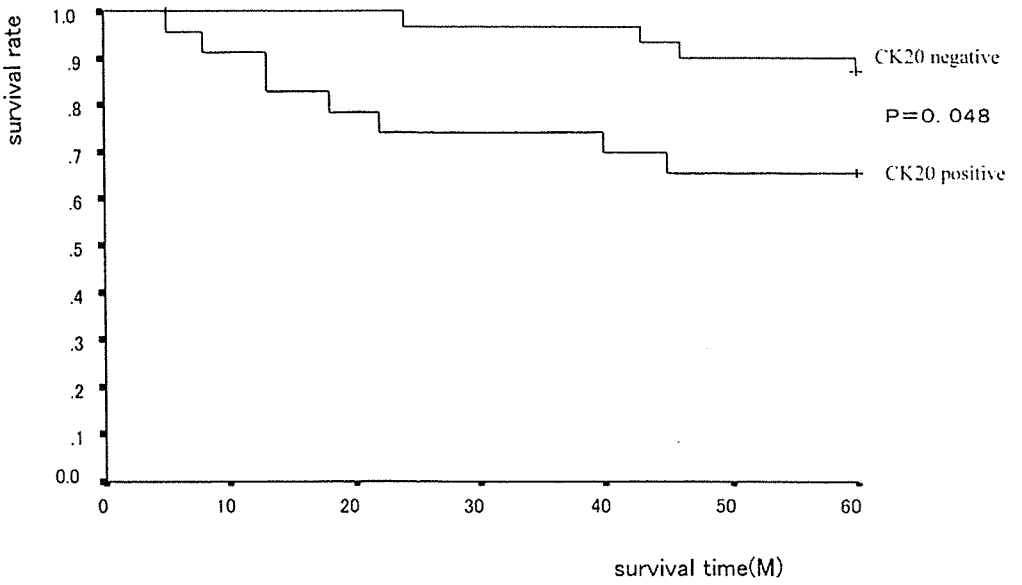

Fig. 1. Comparison of 5-year survival curves between CK20-negative and CK20-positive patients. *m*, moths. The 5-year survival rate for CK20 positive patients was 62.5% and CK negative patients was 87.5%. There was a significant difference ($P = 0.047$)

Table 3. Relationship between CK20 mRNA and pathological factors

		CK20-positive (n = 24, 42.1%)	CK20-negative (n = 33, 57.9%)	
Dukes	A	2	5	
	B	8	18	
	C	8	6	$P = 0.065$
	D	6	4	
Lymphatic invasion	Negative	20	20	
	Positive	4	13	
Vessel invasion	Negative	2	6	
	Positive	22	27	
Lymph node metastasis	Negative	10	24	
	Positive	14	9	$P = 0.037$
Liver	H0	18	29	
metastasis	H1	0	2	$P = 0.067$
	H3	6	2	

20-negative group (14.3%). Two patients had pulmonary metastasis, which occurred at 25 and 26 months after operation. One of the other two patients had a hepatic metastasis (10 months after operation) and the other had a brain metastasis at 25 months. The four patients died at 34, 53, 43, and 46 months, respectively, after operation. There were no differences between the two groups in recurrence rates.

The 5-year survival rate in the CK20-positive group was 65.2% and that in the CK20-negative group was 87.5%. There was a significant difference ($P = 0.048$) between the two groups (Fig. 1).

In the multivariate analysis, tumor progression and lymph node metastasis correlated with the prognosis ($P = 0.044$). Lymphatic invasion ($P = 0.940$),vessel invasion ($P = 0.313$), CK20 ($P = 0.632$), and CEA in serum ($P = 0.237$) showed no correlation with prognosis (Table 4).

Table 4. Analysis of maximum likelihood estimates for recurrence of colorectal cancer

Variable[a]	χ^2	$Pr^b > \chi^2$	Hazard ratio
Stage	4.063	0.044*	8.300
Wall-infiltrating	0.248	0.619	0.525
Lymphatic invasion	0.006	0.940	1.079
Vessel invasion	1.097	0.313	0.472
CK20	0.230	0.632	1.636
CEA > 2.5/CEA ≦ 2.5	1.400	0.237	0.245

Multivariate analysis by proportional-hazard model
[a] Lymph node metastasis was excluded from this model because it had a linear correlation with stage
[b] Pr, provability

Discussion

It has been reported that, even when no lymph node metastasis is observed pathologically, immunostaining examination may show that there is micrometastasis in the lymph nodes, which is related to cancer recurrence and prognosis.[3] It is difficult to prove that there is micrometastasis in the liver. In patients whose tumor cells are detected in circulating blood, metastasis in distant organs, including the liver, occurs at a high incidence, indicating that tumor cell detection in circulating blood can serve as an index of cancer recurrence. Few molecular biology studies have shown can-

cer cells in circulating blood, or the relationship between the micrometastasis in the liver and macroscopic recurrence or prognosis, although some studies[4-6] have shown the prognostic usefulness of detecting tumor cells in circulating blood. Nevertheless, some patients with obvious metastasis show no evidence of tumor cell presence in circulating blood, based on molecular biology.[7] To identify tumor cells in circulating blood, RT-PCR diagnosis, using monoclonal antibodies, is now being tried, i.e., using antibodies specific to epithelial and tumor cells. Tumor markers for detecting tumor cells in circulating blood are classified into markers of genetic alteration, markers of tissue-specific forms, markers of cancer-specific forms, and markers of viral transformation. Tumor markers for colorectal cancer reportedly include K-ras and p53 point mutation in the genetic alteration group; CKs-18, 19, and 20 for the tissue-specific forms; and CEA for the cancer-specific form. However, when RT-PCR is extremely sensitive, these tumor markers may yield false-positive results. Therefore, it is reportedly necessary to employ several procedures, e.g., to decrease the degree of amplification as well as using different gene index procedures.[8] There are other methods for detecting tumor cells in circulating blood, using either mutant-allele-specific amplification (MASA) or PCR-restriction fragment length polymorphism (RFLP) analysis; these procedures have lower sensitivity but higher specificity for genetic alterations.[9] These methods are reportedly useful for detecting K-*ras* gene alterations in pancreatic cancer.[10] At present, real-time PCR is usually carried out for these tumor markers to measure. But, unfortunately, when we measured tumor markers, it could not be done, because it had not been developed yet.

Of the tissue-specific forms of representative tumor markers, such as CK18, CK19, and CK20, CK20 is reportedly the most representative of gastrointestinal epithelium. However, PCR-single-strand conformation polymorphism (SSCP) showed force positive results. Therefore in the present study, we useless improved PCR-SSCP reported by Gunn Jeremy[11] and accuracy became to be higher.[2] Our results showed that CK19 had a low positive rate and that the positive rates for CK20 and CEA were very similar. Because there were no false-positive cases in the CK20 group, we judged that the specificity of CK20 for colon cancer was high. Previously, tumor markers were measured only in Dukes C patients in order to examine the relation with metastasis. However the detection of these markers in Dukes A and B patients has not been clarified because there are small amount of patients in Dukes A and B. Therefore, measurements at all stages are needed. We found a correlation between CK20 and lymph node metastasis and prognosis, indicating that this marker expression was specific to colorectal cancer. Many reports have shown no relationship between tumor markers and cancer recurrence or prognosis,[5,6] although one report[12] indicated that there were differences in malignant grade between patients with and without tumor marker expression. Thus, no conclusion has been established on this subject to date.

CEA, in contrast to the CKs, is an epithelial tumor marker which appears nonspecifically in epithelial tumors, although this marker has been confirmed in normal gastrointestinal membrane, indicating that CEA may appear in most patients with digestive-tract cancers.[13] Our present study showed that CK20 and CEA positive rates were very similar and there was no relationship between CEA in serum and CEA mRNA in the blood by Spearman's test ($P = 0.297$). But there were some false-positive cases in our healthy volunteers and there were no relationships between CEA and the pathological factors we examined.

In our study, we examined the existence of tumor cells using RT-PCR, but it became apparent that metastasis depends on the numbers of tumor cells and their features, and on factors at the metastatic organ site. Reportedly it is possible to determine tumor cell count using a real-time PCR assay.[14] Tumor cells reportedly spread in the blood from the primary focus due to the dysfunction of either cadherin or catenin. It has also been reported that tumor cell spread in the blood is related to patient prognosis.[15] Another report has shown that tumor cells spreading in the blood appear to be destroyed within 24h by mechanical stress and by immunocytes, whereas, in metastasis from gastric and colorectal cancers to the liver, cadherin is still expressed, suggesting that the tumor cells form tumor masses in order to ensure their survival.[16] It has been reported that tumor cell spread in the blood results in the following processes, in association with various factors: tumor cell agglutination induced by cadherin and SLX; adhesion to endothelium induced by endotherial leucocyte adhesion molecule-1 (ELAM-1), sialyl Lewis (SLX), and intercellular adhesion molecule-1 (ICAM-1); and target organ infiltration induced by cadherin.[17,18] In general, the procedures for quantifying tumor cells in blood are being improved using real-time PCR, but no reports have yet clarified the features of the tumor cells themselves. Some reports show a relationships between tumor cells detected in blood, cancer recurrence, and patient prognosis. We also examined these relationships, and although there were no conclusive findings associated with recurrence, there were significant differences in pathological factors and prognosis between patients who were CK20-positive and those who were CK2-negative.

Conclusion

Our study indicated that CK20 in the blood of colorectal cancer patients was a specific marker for this disease and was useful for determining prognosis. But it was not an independent prognostic factor. If a correlation between CK20 in the blood and cancer recurrence were established by other studies, our findings could be better evaluated.

References

1. Wharton RQ, Patel H, Jonas SK, et al. (2000) Venesection needle coring increases positive results with RT-PCR for detection of