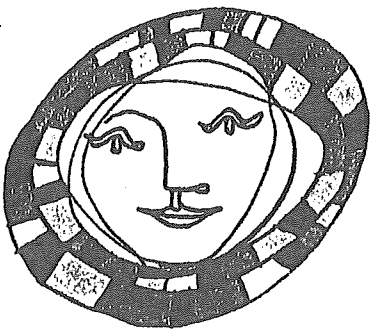
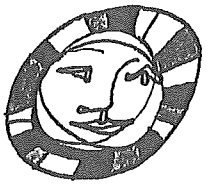
最新のがん手術





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はじめに

がんに対する手術療法は、がん治療、とくに固形がん治療の根幹をなしている。放射線治療と並んで局所療法の代表である。

周知のように近代外科はエーテルによる全身麻酔法（1846年）と石炭酸（1867年）などによる無菌手術法の出現をもって始まった。主要ながんについて、初めて摘出術が行われた年をみると、1881年にビルロートによる胃がん手術、1908年にはマイルズによる直腸がん手術が行われている。つまり1880年代から第1次世界大戦までの間に、現在行われている主要な手術法は開発されたと言ってよい。その後、難度の高い食道がん手術（1930年）、膵臓がん手術（1935年）、そして1955年に肝臓がん手術の最初の成功例が報告されている。

このように近代外科におけるがん手術史は、わずか100年前後しか経過していない。その後は移植外科に見られるように、免疫学や周術期栄養学の進歩や機械工学など最新テクノロジーの恩恵を受けながら、がん外科は確実に進歩しつつある。30年間の外科医としての自分史を振り返りながら、がん外科の進歩をたどってみたい。

がんの進展と手術の基本的考え方

がんは進行とともに発生した臓器内で発育する。つまり腫瘍径が増し、深部に進展する。深部進展が進むと臓器内にあるリンパ管、血管などの脈管にがん細胞が浸潤し、リンパ節転移や肝・肺転移などの遠隔転移を来す。また胸膜や腹膜に達すれば播種性進展を来す。国際的に進行度は、T（tumor）、N（lymph node metastasis）、M（distant metastasis）の3つのカテゴリーで表現される。

手術療法は局所療法であるがゆえに、主としてTとNに対峙する治療である。Mすなわち遠隔転移に対しては一般的には無力である。しかし、がん種によっては遠隔転移巣の切除が有効な固形がんもある。その代表は大腸がん肝・肺転移である。手術療法の原則は、原発巣に加え、一定量の正常組織と転移の起こりやすい所属リンパ節を含めて、一括切除（en-bloc resection）することである。リンパ節郭清とは、原発巣と周囲の血管および脂肪組織を一塊として取り除き、その中に含まれるリンパ管とリンパ節を系統的に切除する手術手技を言う。がん手術では原発巣や所属リンパ節領域に切り込むようなことがあってはならない。

Sentinel node navigation surgery

sentinelとは、衛兵つまり番人を意味する。Sentinel node（SLNs）とは、原発巣から最初に最も転移しやすいリンパ節のことであり、そうしたリンパ節が存在するとの仮説に基づき成り立っている概念である。

このリンパ節を手術中に取り出し、転移の有無を病理学的に検索し、転移がなければ所属リンパ節の郭清は施行せず、転移を認めれば郭清を行う。つまり機能温存や縮小手術の遂行を裏づける、術中のリンパ節検査法と位置づけることができる。パテントブルーなどの色素やテクネシウムなどの放射性物質が使用される。乳がんや

melanomaは原発巣から離れたところにSLNsがあるため、本法が縮小手術の裏づけに有効である。しかし、消化管など、リンパ流が豊富かつ原発巣の直下から始まる臓器では、腫瘍近傍に注射するため注射部位と重なり背景との区別が困難となり、有効性が劣る。胃がん、大腸がんなどへの検証の試みはあるが、縮小手術の裏づけとしての意義はあまりないと思われる。

むしろ、SLNs検索の意義は、stage Ⅰ/Ⅱをstage Ⅲにupstageし、補助療法の対象を広げることにより患者に利益をもたらすとの考え方にあり、欧米などでは消化器がんにも臨床応用されつつある。

臓器および機能温存手術

最近では画像診断の進歩により、がんの進行度は術前にかなり正確にわかるようになってきた。MRIはコントラスト分解能に優れ、目的とする領域の解剖学的指標が明瞭に示顕されるのでCTより精度は高い。一度に全身検索が可能なPET検査は進行がんに対する手術適応の検討に有用である。Micro-surgeryなど血管外科や放射線、化学療法などの進歩により、ほとんどすべてのがん種に対する温存手術が開発された。

乳がんにおける乳房温存術、舌がんや下咽頭がんなど頭頸部がんに対する機能温存手術、肺がんに対する気管・気管支温存手術、骨・軟部腫瘍に対する患肢温存手術、膀胱がんに対する自然排尿型新膀胱形成術など温存術の普及はほぼすべてのがん種に及ぶ。

本稿では胃がんと直腸がんを例にとり、機能温存手術の現状を概説する。

胃切除後の胸焼けやダンピング症候群を防止する目的で、幽門輪温存が可能な早期がん症例には、幽門輪温存胃切除術が行われる。また、胃切除範囲を縮小し、胃の運動を支配する迷走神経を確認し、これを選択的に温存するなど、機能温存手術が胃外科の分野では積極的に行われている。

直腸がんは、手術効果の大きな固形がんであることが知られている。1970年代から1980年代初めにかけて、わが国では直腸がんに対する拡大郭清が普及した。この術式は骨盤内を徹底的に郭清し、排尿や性機能を支配する自律神経を切除するものであった。そのため遠隔成績の改善は認められたが、反面、高頻度に排尿障害と性機能障害を合併した。その後、骨盤内自律神経に関する解剖と機能の理解の深まりと画像診断の進歩により、複雑な骨盤内自律神経を直視下に露出し、それらを意識的に温存する、わが国独自の直腸がんに対する手術法である自律神経温存術が誕生した。現在は直腸がん患者の多くがこの術式の恩恵を受けて、術前同様の排尿機能や性機能が温存され、著しく直腸がん術後のQOLは向上した。また自然肛門の温存にも多大の努力が払われている。

最近では、自動吻合器の開発により、直腸がんの9割近くに肛門括約筋温存手術が採用され、人工肛門回避という福音が患者にもたらされている。欧米では、術前放射線治療によるdownstaging効果を用い、肛門括約筋温存術の適応拡大が図られている。また、従来は肛門括約筋温存が禁忌と考えられていた歯状線直上の病変に対しても、括約筋の一部を切除する手術法（intersphincteric resection：ISR）が行われている。

低侵襲手術

患者に負担の少ない腹腔鏡手術、胸腔鏡手術、内視鏡的粘膜切除術などの低侵襲手術（Mimimaly Invasive Surgery：MIS）が登場した。1987年フランスのモーレが胆嚢摘出術に成功したのが腹腔鏡手術の最初である。

1990年代初めに、わが国においても胆嚢、結腸、直腸、胃、子宮、副腎、腎、前立腺、卵巣などほとんどすべての腹腔内がんに対し腹腔鏡手術が行われ、今日の普及を見ている。従来の開腹手術とは基本手技において異なっているため、この手術手技に慣れ、正確な技術を習得するためには、特別な教育環境が必要である。まず、通常の開腹手術でがん手術に必要な解剖と剥離層をしっかり学び、その後、動物を使って実技を練習し、熟練の指導者のもとで症例を集積する中で、手術時間が短縮され、安全な技術となるのである。施設によっては、腹腔鏡手術を傍大動脈リンパ節郭清術や進行結腸がんにまで適応を広げている。

進行結腸がんに関しては、開腹手術と腹腔鏡下結腸切除の無作為化比較試験が欧米で行われ、開腹手術に比較

し遜色のない成績が報告されている。わが国においても、大分大学の北野正剛教授を中心にJCOG大腸がん外科グループで、欧米同様の比較試験が開始された。

こうした新しい手術法を患者に説明するに当たっては、従来の開腹手術と腹腔鏡手術の長所、短所を偏りなくわかりやすく説明することが、外科医の重要な責務である。従来の開腹手術も低侵襲手術の影響を受け、できるだけ小範囲の創（ミニマム創）で前立腺や結腸の手術を試みている施設もある。

この手術法の今後解決しなければならない問題点を指摘しておきたい。開発、改良された手術器具のほとんどが使い捨てだということである。1例の手術を終えるとたくさんの医療用廃棄物が出る。経済効率ではなく、環境に配慮した re-usableな器具づくりが考慮されるべきである。同時に、手術器具のコストの問題も解決すべきである。現状のコストでは、保険などでコストカバーできる患者のみに適応範囲が狭まる可能性もあろう。

手術器具の進歩による手術時間の短縮と合併症の減少効果

消化管がんの手術で遭遇する合併症のうち、最も重篤なものは縫合不全である。

自動吻合器（器械吻合器）がなかった1970年代では、胃がんにおける胃全摘後の食道空腸吻合術や、直腸がんにおける低位前方切除術などは、吻合操作にずいぶん神経をつかった覚えがある。糸や針の材質、縫い幅、結紮の強さ、術野の展開など、自動吻合器が普及した現在では、想像もつかないような神経を擦り減らす局面であった。吻合に使う時間はリンパ節郭清に匹敵するものであった。

こうした細心の注意を払っても、縫合不全は一定の頻度で手縫い吻合の時代には起こった。1980年代に自動吻合器が普及し、1990年代にはさまざまな改良が行われ、現在の形に完成された。

以上のように、手術時間の短縮と合併症の減少への自動吻合器の貢献は計り知れない。欧米では、手縫い吻合ができない外科医が誕生していると数年前に聞き、冗談だと思っていた。それが、わが国においても現実となり

つつある。

腸閉塞など異常環境下での吻合では、手縫い吻合が必要である。次世代のための外科教育のあり方として考えさせられる課題である。

バイポーラー電気メス、高速振動を利用したハーモニックスカルペルや熱変性シーリング効果を利用したリガシュアーなどの一連の止血器械も続々と登場している。

私自身は、もはや古典的な外科医の群に分類されてもしかたないくらい、従来の鋏と結紮と電気メス一辺倒の外科医である。しかし、腹腔鏡手術用に開発された器械のうち、使い勝手の便利なものがあれば開腹手術に使いたいと思っているが、現在までのところ、そうした器具に遭遇していない。

がん外科領域における臨床試験

手術前後の補助療法や手術療法自体に関する臨床試験が、標準的治療法確立に向けて行われるようになったことは、"最近のがん外科の進歩"のひとつとして特筆されるべきである。

一般に術式の選択は、がんの進行度、経験、学習した内容などにより異なるが、学会での議論や論文等からの専門的知識で相違は埋まり、標準化に向かう。しかし、たとえば食道がん、胃がん、直腸がんにおけるリンパ節郭清度をとってみても、ある病変に対して拡大手術か縮小手術かのエビデンスに基づく判断は困難なことが多く、施設の方針や経験からリンパ節郭清度が選択される。

こうした術式の選択条件では、エビデンスに基づく外科学の構築は遅々として進まない。

そこで、優劣が決定していない術式に関しては、標準的術式と実験的術式とのランダム化比較試験が必要になってくる。胃がんにおいては傍大動脈リンパ節郭清、直腸がんにおいては側方郭清の功罪に関する臨床試験などが現在行われている。こうした手術療法に関する臨床試験では、品質管理、つまり手術の質（quality of surgery）の管理がきわめて重要で、決められた術野の写真判定などを用いる。

外科医にとって、自分の信ずる術式を遂行することに抵抗はないが、コンピュータが術式のランダム化割付けを行う臨床試験では、外科医の抵抗はかなり強い。私も当初は術式のランダム化割付けには、正直言って抵抗があった。しかし、グループ内での議論や倫理委員会の承認など、プロトコル作成までの過程を再考してみると、手術療法に関する臨床試験はきわめて倫理性が保障された術式の選択であることが理解できる。一方、従来の経験や医局の方針などに基づく術式の選択方法は、結局のところ倫理性において臨床試験に劣ると思われる。

補助化学療法の検証を行う臨床試験においても、手術の質の管理が、きわめて重要であることが最近指摘されている。

欧米では、直腸がんに対する放射線化学療法に関する大規模比較試験が多数行われている。直腸がん放射線治療の試験では、術前治療が有効であるとの報告が多いが、化学療法単独の試験では、有効であるとの結果が結腸がんに比較して少ない。その主な原因として局所再発率の高低が補助化学療法のパワーに影響を与えていることが指摘できる。

一般的に補助化学療法は、遠隔転移の抑制効果はあるが、局所再発にはそれほど有効ではないと言われている。局所再発率の低い試験では有効な結果が出やすく、局所再発率の高い試験では補助化学療法の影響が相殺される傾向がある。したがって、補助化学療法の検証は、低局所再発率で手術可能な研究グループが行うべきである。一方、局所コントロールに有効である直腸がん放射線治療では、補助療法とは逆に、bad surgeryにおいて有効性が出やすく、good surgery では有効性は出にくい。つまり、補助放射線化学療法の検証を行う場合でも、手術の質の管理がその臨床試験の成否の鍵となることを強調しておきたい。

(Yoshihiro Moriya)

2005. 2. 1受付　4. 15受理

症例報告

腹腔鏡下結腸切除術後に肺塞栓症をきたした1例

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キーワード：腹腔鏡下大腸切除術，肺塞栓症，術後合併症

患者は71歳，女性．下行結腸癌に対して腹腔鏡下下行結腸切除術を施行した．術中体位は頭高位，気腹圧10 mmHg，気腹時間は70分．肺塞栓症の予防として，術中・術後に下肢の間欠的空気加圧法を施行した．術後1日目，初回歩行時に胸部不快を訴え意識を消失した．低酸素血症をきたしており，肺塞栓症を疑いウロキナーゼ，ヘパリンの投与を開始した．肺血流シンチグラフィにおいて両側の多発欠損像を認め，肺塞栓症と診断した．抗凝固療法などにより全身状態は改善し，術後12日目に退院した．当院では2004年12月までに347例の腹腔鏡補助下大腸切除術を施行しているが，術後に肺塞栓症をきたした症例は本例のみ（0.29%）である．

はじめに

腹腔鏡下手術は胆嚢摘出術などの良性疾患や一部の悪性疾患に対して標準手術術式となりつつある．しかし，術中の体位や気腹による腹腔内圧の上昇が原因となり下肢の静脈還流障害をきたし，血栓形成や肺塞栓症を誘発する可能性が指摘されている[1]．また，開腹手術と比較して，腹腔鏡下手術で肺塞栓症の発症が高率との報告もある[2]．今回，われわれは腹腔鏡下大腸切除術後に肺塞栓症をきたした症例を経験したので，文献的考察を加えて報告する．

症　例

患　者：71歳，女性．

家族歴：母親が肺癌．

既往歴：6歳時に虫垂炎にて虫垂切除術．喘息にてテオフィリン®，高血圧にてブロプレス®を内服中である．

生活歴：喫煙歴なし，アルコール摂取せず．

現病歴：2003年2月に近医で施行された大腸内視鏡検査で，下行結腸に約2 cmの隆起性病変を指摘された．生検では高分化腺癌の診断で，内視鏡的粘膜切除術を施行された．しかし，病理学的に腫瘍の粘膜下浸潤を認め，追加腸切除目的で当院を受診した．

入院時現症：身長155 cm，体重52 kg，body mass index 21.6と標準体型であった．血圧162/76 mmHg，脈拍71/分・整．腹部に圧痛，腫瘤は認めなかった．また下肢に静脈瘤を認めなかった．

血液生化学検査：出血・凝固系検査も含め，特に異常所見は認めなかった．動脈血ガス分析ではroom airでPaO_2 81.4 mmHg，$PaCO_2$ 37.5 mmHgと異常を認めなかった．

術前検査所見：マスターダブル心電図にて陽性所見を認め，トレッドミル負荷心電図および心臓超音波検査を施行したが，虚血性変化は認めなかった．肺機能検査では肺活量2,040 m*l*（%VC：90%），一秒量1,560 m*l*（$FEV_{1.0\%}$：76.5%）と正常範囲内であった．

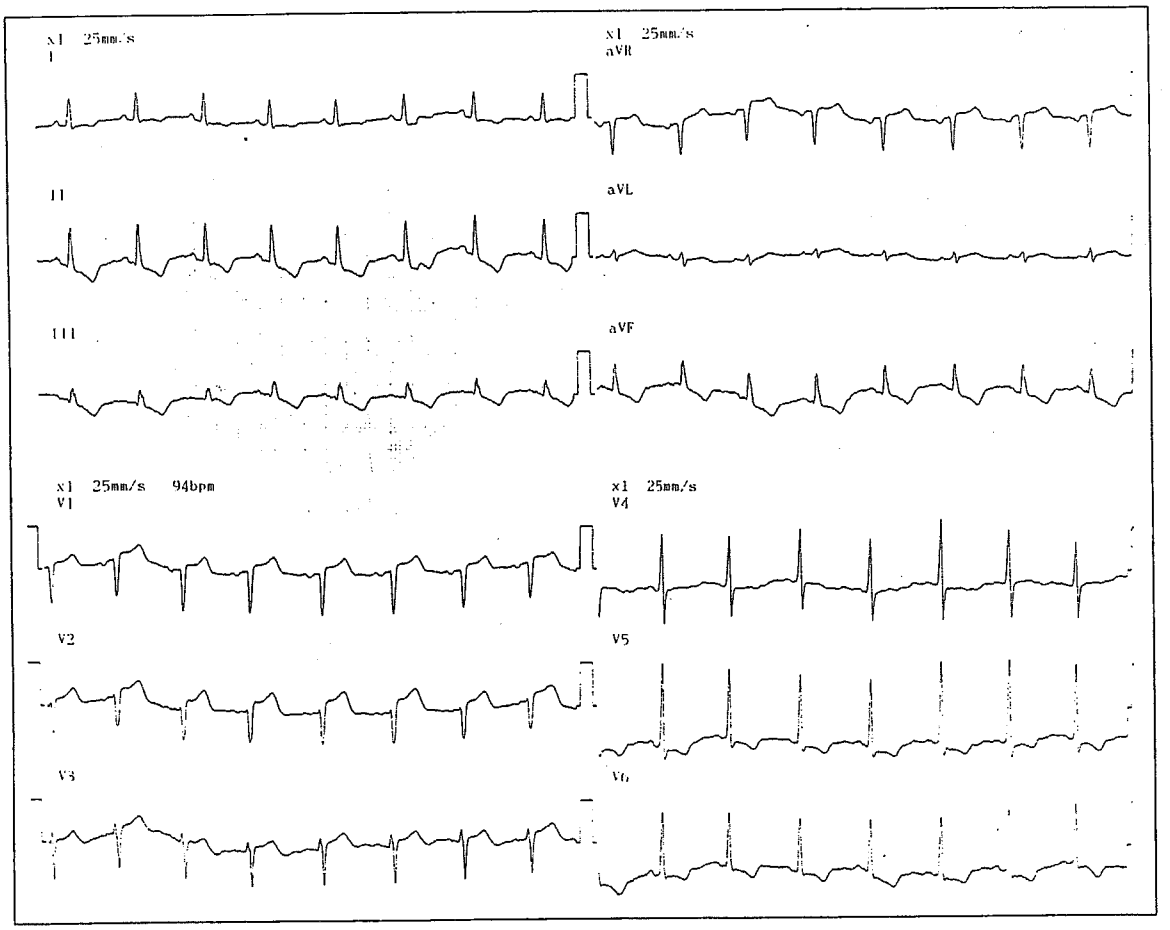

図1 肺塞栓発症直後の心電図
V2においてSTの上昇，II，III，aVF，V5，V6においてT波陰転化を認めた．

臨床経過

2003年5月，二酸化炭素による気腹下に腹腔鏡下下行結腸切除術（$D_1+\alpha$郭清）を施行した．気腹中の体位は主に右半側臥位，約25度の頭高位で行った．気腹圧は10 mmHg，手術時間165分，出血量25 g，気腹時間は約70分であった．術中に臨床的に問題となる低酸素血症や循環動態の変動は認めなかった．なお肺塞栓の予防として，術中より術後歩行開始まで下肢の間欠的空気加圧装置を使用した．

術後1日目の午前中までは全身状態に問題はなかったが，午後に歩行を開始した際，気分不快を訴えると同時に意識消失発作を起こした．意識は数分で回復したが，10 *l*/分の経鼻酸素投与下の血液ガス所見でPaO_2 42.9 mmHg，$PaCO_2$ 41.9 mmHgと著明な低酸素血症を認めた．血圧は143/63 mmHg，心拍数は98回/分・整であった．胸部X線写真では特に異常所見は認めなかった．臨床症状より肺塞栓症を疑い，直ちにウロキナーゼ24万単位を静脈内投与し，ヘパリンとの併用持続投与（ウロキナーゼ24万単位/日，ヘパリン1万単位/日）を開始した．また発症直後の心電図においてV2誘導でST上昇を認め（**図1**），さらに発症から4時間後に行ったトロポニンT検査において0.69 ng/m*l*と陽性を示したため，続発性の心筋梗塞を疑いミリスロールの持続投与も開始した．心エコー検査では心臓の動きに異常はなく，心房，心室内に血栓も認めなかった．低酸素血症により冠血管の攣縮を誘発しその結果，心

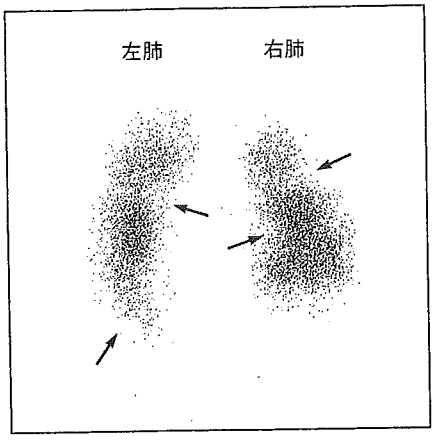

図2　肺塞栓発症後2日目の^{99m}Tc-MAA肺血流シンチグラム（背面像）
両肺の血流分布は不均一であり，多数の血流欠損部位を認めた（→）．

内膜下梗塞をきたしたのではないかと推測した．発症後2日目（3POD）に肺血流シンチグラムを施行し，両側に多発する血流低下部位を認め，急性肺塞栓症と診断した（**図2**）．下肢の静脈エコーにおいても静脈血栓像を認めた．

肺塞栓発症後の治療経過はおおむね良好に経過し，術後3日目に流動食を開始し，術後5日目からはワーファリン3 mg/日の内服を開始した．ウロキナーゼ，ヘパリンの併用投与は漸減し術後6日目に中止した．術後10日目の血液ガス所見では room air で PaO_2 79.2 mmHg，$PaCO_2$ 35.1 mmHg と回復し，術後12日目に退院となった．現在も外来通院中であるが，術後1年8か月を経過しているが肺塞栓症の再発は認めず，大腸癌も無再発生存中である．

考　察

肺塞栓症は重篤な術後合併症の一つであるが，日本人では欧米人に比して比較的まれな疾患とされてきた．しかし近年の生活様式の欧米化，診断技術の向上などにより肺塞栓症の報告例は増加し，その認知度は高まってきている[3~8]．2004年2月には肺塞栓症研究会，日本静脈学会など10の学会，研究会が参加して「肺血栓塞栓症/深部静脈血栓症（静脈血栓塞栓症）予防ガイドラインのダイジェスト版」が公表されている[9]．

一方，腹腔鏡下手術は一部の疾患で標準手術術式となっているが，術中の体位や気腹により深部静脈血栓を誘発し肺塞栓症を引き起こす懸念が報告されている[1]．最近，Sakon ら[2]は本邦における術後肺塞栓症の報告例をまとめ，外科手術全体における肺塞栓症の発生率が0.33%であるのに対し，内視鏡下手術では0.62%あったと報告している．当院においては2004年12月までに347例の腹腔鏡下大腸切除術を施行しているが，術後に肺塞栓症をきたした症例は本例のみ（0.29%）であった．また悪性腫瘍に対する手術における肺血栓塞栓症の発生率は非悪性腫瘍手術に比して高いとされており[2]，内視鏡下手術が悪性腫瘍に対し適応を拡大してきている現況では，肺血栓塞栓症の予防対策は重要性を増している．

腹腔鏡下手術における本症の予防法としては，手術手技に関して手術・気腹時間を短縮し，適度な気腹圧を保ち，過度の頭高位を避けるなどの留意点が挙げられる．特に過度で長時間の頭高位（reverse Trendelenberg position）は術後肺塞栓の危険因子とされており，腹腔鏡操作が長時間に及ぶ場合には，間欠的に体位を元に戻すなどの対策が必要である[10,11]．また弾性包帯や間欠的空気加圧装置の着用や抗凝固剤の使用なども推奨されている[12]．自験例では術中の体位が約25度の頭高位，気腹時間が約70分で，予防対策として術中，術後に間欠的空気加圧装置を着用していたにもかかわらず肺血栓塞栓症を発症した．抗凝固剤による予防法としては，8時間もしくは12時間ごとに未分画ヘパリン5,000単位を皮下注射する方法が簡便で実用的である．欧米の報告では，本法により致死的肺血栓塞栓症を含めた静脈血栓塞栓症のリスクを60~70%減少させるとの報告がある[13]．また急性肺血栓塞栓症や深部静脈血栓症を有する症例のうち，抗凝固療法の禁忌例や無効例に対して，下大静脈フィルターを肺血栓塞栓症の予防デバイスとして使用する場合がある[14]．

一方，肺血栓塞栓症を発症した際には速やかな対処が必要である．肺塞栓血栓症の致死率は約20%と高く，死亡例の約半数が発症1時間以内に死亡しているとされ[15]，本症を疑った場合には

直ちに治療を開始することが救命につながるといえる．治療法としては呼吸循環不全に対する全身管理とともに，ワーファリンやヘパリンなどの抗凝固療法やウロキナーゼや組織プラスミノーゲンアクチベータ（t-PA）などの血栓溶解療法が主体となる．これらの薬剤の投与に際しては，全身状態と出血傾向（特に術創からの出血や脳出血など）を考慮し，凝固機能検査などのモニタリングを行いつつ薬剤の投与量を決定する．外科的な血栓塞栓除去術はこれらの内科的治療が無効な場合に考慮する．特に慢性肺血栓塞栓症では内科的治療に限界があり，外科的治療が有効であり，最近では良好な手術成績が報告されている[16]．

おわりに

腹腔鏡下下行結腸切除後に肺塞栓症をきたした1例を経験した．肺塞栓症は重篤な合併症であり，予防対策が重要であるとともに，診断が疑われる場合には速やかな治療が必要である．腹腔鏡下手術では肺塞栓症の発症が開腹手術と比較して高率との報告があり，今後の症例の蓄積により術後の肺血栓塞栓症に対する有効な予防対策，治療法の確立が期待される．

なお，本論文要旨は第17回日本内視鏡外科学会総会にて発表した．

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Safety of Laparoscopic Intracorporeal Rectal Transection With Double-Stapling Technique Anastomosis

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Abstract: To assess the feasibility and analyze the short-term outcomes of laparoscopic intracorporeal rectal transection with double-stapling technique anastomosis, a review was performed of a prospective registry of 67 patients who underwent laparoscopic sigmoidectomy and anterior resection with intracorporeal rectal transection and double-stapling technique anastomosis between July 2001 and January 2004. Patients were divided into 3 groups: sigmoid colon/rectosigmoid carcinoma, upper rectal carcinoma, and middle/lower rectal carcinoma. A comparison was made of the short-term outcomes among the groups. The number of cartridges required in bowel transection was significantly increased in patients with middle/lower rectal carcinoma, and significant differences were observed in the length of the first stapler cartridge fired for rectal transection. Furthermore, mean operative time and blood loss were also significantly greater in the middle/lower rectum group; however, complication rates and postoperative course were similar among the 3 groups. No anastomotic leakage was observed. Laparoscopic intracorporeal rectal transection with double-stapling technique anastomosis can be performed safely without increased morbidity or mortality.

Key Words: laparoscopic low anterior resection, rectal transection, double-stapling technique, complication, colorectal carcinoma

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More than 10 years have passed since the first report of laparoscopic colectomy by Jacobs et al[1] in 1991. With regard to long-term oncological safety, which is the most important concern for laparoscopic surgery (LS) for malignancies, there have been no reports indicating that LS is inferior to conventional open surgery (OS).[2–5] On the other hand, because LS requires surgical techniques that are different from those of OS, even a surgeon with considerable experience in OS cannot readily perform LS.

In particular, LS for rectal carcinoma is very difficult surgery from a technical standpoint, and consequently many randomized, controlled trials have excluded patients with middle/lower rectal carcinoma. This is because of concerns over the safety of the procedure, ie, the risk of complications associated with the laparoscopic procedure and the risk of tumor cell spillage because of traumatic manipulation of the tumor. Previous studies have reported an anastomotic leakage rate of 5.7% to 21% in patients who underwent laparoscopic low anterior resection (Lap-LAR), and some authors have recommended a covering ileostomy as a routine in Lap-LAR cases.[6–12] It remains uncertain which cases of rectal carcinoma are appropriate for laparoscopic surgery.

Since our first laparoscopic colectomy for colorectal carcinoma in 1993, approximately 280 laparoscopic resections for colorectal malignancies have been carried out at our institution. Most of our early experience was confined to early (Tis or T1) colorectal cancer located at the cecum, ascending colon, sigmoid colon, or rectosigmoid due to technical problems and concerns regarding port site and peritoneal recurrences. In June 2001, we unified our surgical and postoperative management procedures and expanded our indications for laparoscopic colectomy to include advanced colorectal cancers (ie, T2 lesions and beyond) located anywhere in the colon and/or rectum.

In 1980, Knight and Griffen[13] described the double-stapling technique (DST), which offered great advantages in that it permitted low rectal anastomoses to be performed with great ease. The aim of the present study was to assess the feasibility and analyze the short-term outcomes of laparoscopic intracorporeal rectal transection with DST anastomosis, one of the most demanding and stressful techniques in laparoscopic colorectal surgery, in selected patients with sigmoid colon and rectal carcinoma, who all underwent LS at our hospital after June 2001.

PATIENTS AND METHODS

Patients

At the Division of Colorectal Surgery of the National Cancer Center Hospital in Japan, 156 nonrandomized consecutive patients underwent laparoscopic colorectal resections between July 2001 and January 2004. During this period, 67 patients were treated by laparoscopic sigmoidectomy and anterior resection with DST anastomosis. Because the safety of LS in cancer patients remains to be established, candidates for laparoscopic surgery were patients who were preoperatively diagnosed with T1 or T2. Additionally, LS cases also included patients with sigmoid colon or upper rectal carcinoma who were preoperatively diagnosed with T3 but wished to undergo LS, as well as those for which palliative resection was

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considered necessary. Exclusion criteria for LS were tumors larger than 6 cm, a history of extensive adhesions, severe obesity (body mass index >32 kg/m^2), intestinal obstruction, and refusal to undergo LS. The preoperative workup consisted of a clinical investigation, barium enema, total colonoscopy, chest x-ray, abdominal ultrasonography, and computed tomography.

LS was contraindicated for patients with preoperative diagnoses of T3 and T4 tumors in the middle and lower rectum because, with the current instrumentation, it was difficult to perform laparoscopic procedures without grasping and manipulating the bowel or mesorectum near the tumor; our concern was that this would result in accidental tumor spillage. Furthermore, lateral lymph node dissection combined with total mesorectal excision remains the standard surgical procedure for patients with T3 and T4 lower rectal carcinoma in Japan, and lateral lymph node dissection by laparoscopy is still an unexplored frontier.[14–16] As a result, some patients were found to have T3 cancer only after histopathological examination of the surgical specimens. Preoperative or postoperative radiation therapy was not performed in this series because of the low local recurrence rate in patients with T1–T3 lower rectal carcinoma without preoperative radiation.[14,16]

Patients were divided into 3 groups: sigmoid colon/rectosigmoid carcinoma, upper rectal carcinoma, and middle/lower rectal carcinoma. For the patients with rectal carcinoma, a primary rectal carcinoma was defined according to its distance from the anal verge as determined by colonoscopy. The tumors were grouped into lower rectum (0–7 cm), middle rectum (7.1–12 cm), and upper rectum (12.1–17 cm). We combined patients with middle and lower rectal carcinoma as a group because laparoscopic techniques for rectal transection and DST anastomosis were almost same: anastomosis located below peritoneal reflection.[7] Patients with lesions located within 2 cm of the dentate line who underwent laparoscopic intersphincteric rectal resection and hand-sewn coloanal anastomosis were excluded from the present study. This surgical technique has been described previously.[17] Conversion to open surgery was defined as any incision greater than 7 cm, excluding cases in which the incision was enlarged due to a large specimen size that could not be removed with a 7-cm incision.

Laparoscopic Technique

Laparoscopic resection techniques have previously been described, with minor modifications.[7,17] Initial port placement was performed using the open technique, and pneumoperitoneum was induced using carbon dioxide. Two 5-mm ports were then inserted in the left lower midabdominal and the left lower quadrant regions, and 2 other 12-mm ports were inserted in the mid-lower and the right midabdominal regions under laparoscopic guidance.

The left colon was initially mobilized laterally to medially until the left ureter and superior hypogastric nerve plexus were identified. The mobilization of splenic flexure was performed if necessary. Usually, Japanese patients have a long sigmoid colon, and if the surgeon preserves 1 or 2 arcades of marginal vessels of sigmoid colon by division of sigmoidal arteries between superior rectal artery and marginal vessels, mobilization of splenic flexure becomes unnecessary; thus, splenic mobilization was performed in only about 20% of our patients. Then, a window was made between the mesocolon containing the arch of the inferior mesenteric vessels and the superior hypogastric nerve plexus, starting at the bifurcation, with support from an assistant holding the sigmoid mesocolon ventrally under traction and to the left using a 5-mm bowel grasper through the left lower quadrant port. After the dissection, proceeding to the origin of inferior mesenteric artery, taking care not to injure the superior hypogastric nerve plexus and the roots of the sympathetic nerves, intracorporeal high ligation of the inferior mesenteric artery was performed. After cutting the inferior mesenteric vein and left colic artery, mobilization of the rectum and mesorectum was performed. The avascular plane between the intact mesorectum anteriorly and the superior hypogastric nerve plexus, right and left hypogastric nerves, and Waldeyer fascia posteriorly was entered by sharp dissection and extended down to the level of the levator muscle for middle and lower rectal carcinomas, taking care to protect the pelvic nerves. For proximal sigmoid colon carcinoma, the mesentery at the promontory was excised routinely using ultrasonic shears (laparoscopic coagulating shears [LCS], Ethicon Endo-Surgery Inc, Cincinnati, OH) or an endolinear stapler (Endo GIA Universal, Tyco Healthcare, Auto Suture Co, US Surgical Corp, Norwalk, CT). For rectosigmoidal and upper rectal lesions, mesorectal tissue extending down to 5 cm below the tumor was excised routinely using LCS. Middle and lower rectal tumors were treated by total mesorectal excision. Immediately before rectal transection, laparoscopic rectal clamping was performed just above the anticipated point of rectal transection, using a bowel clamping device (Fig. 1) introduced through the 12-mm mid-lower port. A distinct advantage of this device is that the bowel clamp at the head of the device can be easily bent intraabdominally without reducing the grasping strength. Rectal washout was performed routinely using 1000 mL of a 5% povidone-iodine solution. Rectal transection was then performed by a multiple-firing technique, using Endo GIA Universal staples, introduced through the 12-mm right midabdominal port.[18] If the rectal transection was not completed after the first cartridge, the stapler line for the second cartridge was carefully positioned on the anal side stapler line of the first cartridge. The third and fourth firings were performed in the same way. A 4- to 5-cm incision was then made over the mid-lower 12-mm port site, and the bowel was exteriorized under wound protection and divided with appropriate proximal clearance. After inserting the anvil head of the circular stapler into the end of the proximal colon, the proximal colon was internalized and the incision was closed. Intracorporeal anastomosis under a laparoscopic view was performed by means of the DST, using a circular stapler (ECS 29 or 33 mm, Ethicon Endo-Surgery Inc). After the insertion of the body of the circular stapler into the anus, the puncturing cone was pushed through the midpoint of the linear staple line. In patients in whom 2 or more linear stapler cartridges were used for rectal transection, the puncturing cone was pushed near the crossing point of the first and second stapler lines.

The anastomotic air leakage test was performed if the "doughnuts" were incomplete. Patients with a low anastomosis within 1 cm from the dentate line and incomplete doughnuts

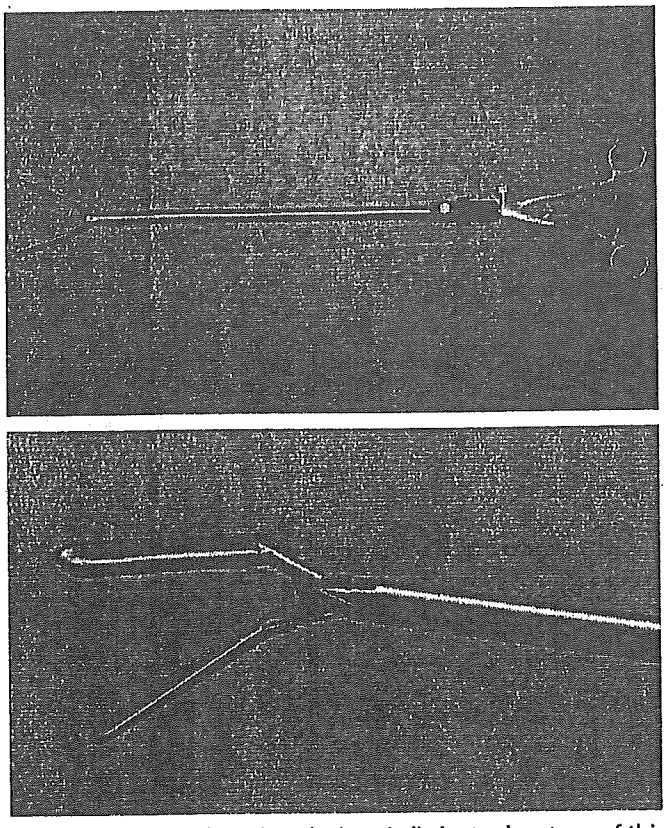

FIGURE 1. Bowel clamping device. A distinct advantage of this device is that the bowel clamp at the head of the device can be easily bent intraabdominally without reducing the grasping strength.

underwent a covering ileostomy. However, the decision to perform a protective ileostomy in this series was based on much looser criteria than those used in OS to avoid major anastomosis complications that could lead to a permanent stoma or a fatal outcome, especially in the early LS cases of lower rectal carcinoma.

Study Parameters

The parameters analyzed included gender, age, body mass index, prior abdominal surgery, operative time, operative blood loss, number of stapler cartridges fired and the length of the first stapler cartridge for rectal transection, conversion rate, days to resume diet, length of postoperative hospital stay, and both intraoperative and postoperative complications within 30 days of surgery. Pathologic staging was performed according to Duke's stage.

Statistical Analysis

Statistical analysis was performed using the χ^2 test, Kruskal-Wallis test with Bonferroni correction, and repeated-measure analysis of variance (ANOVA) with the Scheffe method when appropriate. A P value of <0.05 was considered significant.

RESULTS

The patient demographics are summarized in Table 1. No significant differences were observed in baseline characteristics among the 3 groups. In the middle/lower rectum group, anastomosis was performed <3 cm from the dentate line in 7 patients and >3 cm but below the peritoneal reflection in 3 patients. We performed an anastomotic air leakage test in 2 patients with lower rectal carcinoma and did not find any sign of air leakage; however, both patients underwent a protective ileostomy. Overall, a protective ileostomy was required in 4 patients, and a transverse coloplasty pouch was created in 1 patient.

The number of patients in relation to the number of stapler cartridges used for rectal transection in each group is shown in Table 2. The number of cartridges required during bowel transection was significantly increased in patients with middle/lower rectal carcinomas compared with the other groups. Similarly, significant differences were observed in the length of the first stapler cartridge fired for rectal transection (Table 3). In patients with middle/lower rectal carcinomas, the length of the first stapler cartridge was 45 or 30 mm, and it was 45 or 60 mm for proximal lesions.

Operative and postoperative results are shown in Table 4. Mean operative time and blood loss were significantly greater in the middle/lower rectum group. All the operations were completed laparoscopically. We did not experience any accidental intestinal perforations at or near the tumor site. Liquid and solid food was started at a median of 1 and 3 postoperative days in all groups. The median length of postoperative hospitalization was 8–9 days. No significant differences were observed in the postoperative course among the 3 groups. All patients were discharged home.

The postoperative complications are listed in Table 5. There were no perioperative mortality and no anastomotic leakage. Reoperation of a laparoscopic division of an adhesive band for a postoperative small bowel obstruction was necessary in 1 patient with sigmoid colon carcinoma. No significant differences were observed in complication rates among the 3 groups.

TABLE 1. Patient's Characteristics*

	Sigmoid Colon/ Rectosigmoid	Upper Rectum	Middle/Lower Rectum
No. of patients	36	21	10
Sex ratio (male:female)	22:14	10:11	8:2
Age (y)	59 (30–79)	59 (37–73)	60 (47–76)
Body mass index (kg/m^2)	23.5 (18.9–29.0)	24.1 (17.5–32.4)	23.8 (19.5–26.4)
Prior abdominal surgery (%)	6 (17)	5 (24)	5 (50)
Duke's stage			
A	27	16	7
B	1	0	0
C	7	3	3
D	1	2	0

*Values are means (range), $P > 0.05$.

TABLE 2. Number of Patients in Relation to the Number of Stapler Cartridges Fired for Rectal Transection*

No. of Stapler Cartridges Fired	Sigmoid Colon/Rectosigmoid†	Upper Rectum†	Middle/Lower Rectum
1	25	8	0
2	9	12	2
3	2	1	6
4	0	0	2

*$P < 0.01$ between groups, Kruskal-Wallis test.
†$P < 0.01$ versus middle, lower rectum/Boneferroni test.

DISCUSSION

In the present study, short-term outcomes were compared among different tumor sites in patients who underwent laparoscopic intracorporeal rectal transection with double-stapling technique anastomosis. The closer the tumor site was to the anus, the more the number of stapler cartridges needed for rectal transection increased and the use of a longer Endo GIA Universal stapler cartridge was significantly restricted, suggesting that rectal transection for Lap-LAR in patients with middle/lower rectal carcinomas may be a difficult and stressful procedure. In the present study, however, the complication rate did not increase despite lower anastomotic sites. With thorough and careful intracorporeal rectal transection and DST anastomosis, the safety of Lap-LAR may be established.

Minimum invasiveness is often noted as one of the merits of LS in comparison with OS for colorectal cancer.[19–23] But even recently, some studies have reported that minimal or no short-term benefits were found with LS compared with standard OS.[24–26] Reviewing these reports raises a question about the conversion rate. Even granting that LS has a lower surgical invasiveness than OS, there is a possibility that the treatment outcomes of LS will be contaminated by the treatment outcomes of OS, when the conversion cases are included in the LS group, based on the intention-to-treat principle. In the study by Weeks et al,[26] who reported a conversion rate of 25%, LS showed only minimal short-term quality-of-life benefits compared with OS in an intention-to-treat analysis, probably due to the high conversion rate. Moreover, they pointed out that patients assigned to laparoscopy-assisted colectomy who required intraoperative conversion to open colectomy had slightly poorer quality-of-life outcomes than patients who successfully underwent minimally invasive resection, and that the length of postoperative hospital stay in the LS group requiring conversion was longer than that in patients assigned to OS (7.4 vs. 6.4 days), although statistical analysis was not performed regarding these points. If the conversion patients did not show a worse outcome than those undergoing OS, patients who might benefit from LS should be considered as candidates for LS. Further studies are necessary to evaluate postoperative and oncological outcomes of patients assigned to laparoscopy-assisted colectomy who then require intraoperative conversion.

The results of the current study suggested that laparoscopic approaches to middle/lower rectal carcinoma do not compromise early postoperative recovery, such as days to oral feeding and length of hospitalization. Previous studies reported an anastomotic leakage rate of 5.7% to 21% in patients undergoing Lap-LAR.[6–12] Some authors have recommended a covering ileostomy as a routine step in Lap-LAR.[6,10,27] At present, patients with a preoperative diagnosis of T1–T2, middle/lower rectal carcinoma are required to decide whether they prefer to undergo OS or LS, after being given full information at our institution.

TABLE 3. Length of the First Stapler Cartridge Fired for Rectal Transection*

Length of the First Stapler Cartridge (mm)	Sigmoid Colon/Rectosigmoid†	Upper Rectum†	Middle/Lower Rectum
60	34	16	0
45	2	5	7
30	0	0	3

*$P < 0.01$ between groups, Kruskal-Wallis test.
†$P < 0.01$ versus middle/lower rectum, Boneferroni test.

TABLE 4. Operative and Postoperative Results

	Sigmoid Colon/Rectosigmoid	Upper Rectum	Middle/Lower Rectum
Operative time,* min (range)	221 (135–348)†	244 (190–328)‡	315 (190–392)
Blood loss,* mL (range)	29 (6–161)†	24 (10–198)†	124 (17–265)
Conversion	0	0	0
Liquid intake, d (range)	1 (1–4)	1 (1–3)	1 (1)
Solid food, d (range)	3 (2–5)	3 (3–4)	3 (2–4)
Hospital stay, d (range)	8 (7–12)	8 (7–11)	9 (7–17)

*$P < 0.01$ between groups, repeated-measure analysis of variance.
†$P < 0.01$ versus middle/lower rectum, Scheffe test.
‡$P < 0.05$ middle/lower rectum, Scheffe test.

TABLE 5. Morbidity and Mortality*

	Sigmoid Colon/ Rectosigmoid	Upper-Rectum	Middle/Lower Rectum
Mortality	0	0	0
Morbidity			
Wound sepsis	2	1	0
Bowel obstruction	1	0	1
Urinary tract infection	1	0	0
Abscess	0	0	1
Neurogenic bladder	0	1	0
Anastomotic leakage	0	0	0
Total	4	2	2

*$P > 0.05$.

In this study, the authors evaluated the safety of laparoscopic rectal transection using an endolinear stapler, which is one of the most technically difficult procedures in Lap-LAR. To date, we have not observed serious complications, such as anastomotic leakage. However, this surgical procedure remains technically difficult. We consider that this method should not be attempted if it is not performed by a laparoscopic surgical team with sufficient experience in LS. Regarding a surgical procedure that can be placed between OS and Lap-LAR, Vithiananthan et al[28] reported a hybrid method. In their procedure, they mobilized the left-sided colon and completed high ligation of the inferior mesenteric vessels with the use of the pneumoperitoneum, and then, from the inferior midline incision measuring 8 cm or longer, they performed rectal mobilization, mesorectal division, rectal transection, and anastomosis by DST using the OS tools. They noted that the mean incision length was 11.1 cm, which is longer than in Lap-LAR but shorter than in OS and that the patients treated with this method showed a significantly faster postoperative recovery than those treated with OS. Hand-assisted laparoscopic surgery may also be another treatment option.[29] However, compared with the standard Lap-LAR technique evaluated in this study, both of these methods may need a larger incision. With the surgeon's proficiency in the surgical procedure and the improvement in and development of instruments, the safety of standard Lap-LAR will probably be established; however, it is important to remember that this surgical technique cannot be employed at an early stage of the learning curve of laparoscopic surgery.

In conclusion, the findings of the present study demonstrate that laparoscopic intracorporeal rectal transection with DST anastomosis can be performed safely without increased morbidity or mortality. Even at present, there are few prospective, randomized trials investigating the short-term and oncological outcomes in patients with middle/lower rectal carcinoma, perhaps mainly because Lap-LAR has not been widely performed compared with LS for colon/upper rectal carcinoma due to the technical difficulties. The radical resection of middle/lower rectal cancers is a procedure that requires advanced technical skills in OS, to say nothing of Lap-LAR; however, we believe that use of Lap-LAR for middle/lower rectal carcinoma will expand with improvements in technology and surgeons' experience in the near future.

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A New Method for Isolating Colonocytes From Naturally Evacuated Feces and Its Clinical Application to Colorectal Cancer Diagnosis

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Background & Aims: **The early detection of colorectal cancer is desired because this cancer can be cured surgically if diagnosed early. The purpose of the present study was to determine the feasibility of a new methodology for isolating colonocytes from naturally evacuated feces, followed by cytology or molecular biology of the colonocytes to detect colorectal cancer originating from any part of the colorectum.** ***Methods:*** **Several simulation studies were conducted to establish the optimal methods for retrieving colonocytes from any portion of feces. Colonocytes exfoliated into feces, which had been retrieved from 116 patients with colorectal cancer and 83 healthy volunteers, were analyzed. Part of the exfoliated colonocytes was examined cytologically, whereas the remainder was subjected to DNA analysis. The extracted DNA was examined for mutations of the APC, *K-ras*, and p53 genes using direct sequence analysis and was also subjected to microsatellite instability (MSI) analysis.** ***Results:*** **In the DNA analysis, the overall sensitivity and specificity were 71% (82 of 116) of patients with colorectal cancer and 88% (73 of 83) of healthy volunteers. The sensitivity for Dukes A and B was 72% (44 of 61). Furthermore, the sensitivity for cancers on the right side of the colon was 57% (20 of 35). The detection rate for genetic alterations using our methodology was 86% (80 of 93) when the analysis was limited to cases in which genetic alterations were present in the cancer tissue.** ***Conclusions:*** **We have developed a new methodology for isolating colonocytes from feces. The present study describes a promising procedure for future clinical evaluations and the early detection of colorectal cancers, including right-side colon cancer.**

Colorectal cancer is one of the most common malignancies worldwide. In Japan, colorectal cancer is the third and second leading cause of death from cancer in men and women, respectively.[1] However, colorectal cancer is curable by surgical resection if diagnosed at a sufficiently early stage. This incentive has prompted investigators to develop new methods enabling the early diagnosis of colorectal cancer and has led to the introduction of cancer screening programs in many countries. For mass cancer screenings, a simple, economic, and noninvasive method of cancer detection is desired. The Hemoccult test is currently used in many countries for this purpose.[2–6] However, this test is nonspecific and is not sufficiently sensitive to detect early stage colorectal cancer, although a higher sensitivity has been reported for advanced-stage colorectal cancer.[7] Radioimmunoassays using tumor markers, such as carcinoembryonic antigen, also are not suitable for the detection of early cancer, although such tests can be used to monitor patients for an increasing tumor burden or tumor recurrence. Diagnosis by barium enema study and fiberoptic colonoscopy is accurate but time-consuming, expensive, and invasive. Therefore, an urgent need exists to establish a sensitive, reliable, and noninvasive method for the detection of colorectal cancer at an early stage.

To date, several screening methods for colorectal cancer based on the detection of mutated DNA in feces have been reported.[8–20] These methods, however, are time-consuming and are not sufficiently sensitive. The major reason for this inaccuracy is the fact that

Abbreviations used in this paper: APC, adenomatous polyposis coli; MSI, microsatellite instability; OMIM, Online Mendelian Inheritance in Man.

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nucleic acids in feces are derived from an enormous number and variety of bacteria and normal cells. Accordingly, the proportion of genes derived from cancer cells in feces is as low as 1%, at most.[9] This makes the application of gene-detecting methods difficult in clinical practice.

We previously reported that the expression of CD44 variants in exfoliated colonocytes isolated from feces according to the Percoll centrifugation method could serve as a noninvasive diagnostic marker for early colorectal cancer.[21] However, the repetition of the Percoll centrifugation method was found to distort the morphology of the exfoliated colonocytes. Accordingly, the sensitivity of this method also appeared to be unsatisfactory because of the low retrieval rate of the exfoliated colonocytes. Another study described a processing method that involved scraping or washing the stool's surface with a buffer to collect exfoliated colonocytes.[22] In the ascending colon, however, the feces remains unformed. Therefore, most cancer cells exfoliated from the walls of the ascending colon would be incorporated into the inner core of the feces during the course of its formation. Thus, recovering cancer cells that originated from the ascending colon might be difficult using methods that involve scraping or washing solid feces.

Under these circumstances, we succeeded in developing a new, very effective methodology that allows the simple isolation of exfoliated colonocytes from not only the surface but also the central portion of feces while maintaining the colonocytes' initial morphology. Currently, we are attempting to apply a molecular biologic tool to purified colonocytes exfoliated into feces to detect cells from early colorectal cancers, including right-side colon cancer.

Materials and Methods

Study Design

This was a prospective study conducted between December 2002 and August 2004. The study protocol was reviewed and approved by the Institutional Review Board of the National Cancer Center, Japan. Written informed consent was obtained from all patients and healthy volunteers. No modifications to the protocol procedures were made during the course of the study.

Study Population

A total of 116 patients with histologically confirmed colorectal cancer and 83 healthy volunteers were enrolled. The healthy volunteers consisted of 37 men and 46 women with no apparent abnormalities, such as adenoma or carcinoma (including hyperplastic polyps), found during a total colonoscopy performed at the National Cancer Center Research Center for Cancer Prevention and Screening. The median age of these volunteers was 58.4 years (range, 40–70 years). The characteristics of the patients and healthy volunteers are summarized in Table 1. All the patients with colorectal cancer had undergone surgical resection of their primary tumor at the National Cancer Center Hospital, Tsukiji, or at Hospital East, Kashiwa, Japan. The median age of the patients was 62.0 years (range, 32–82 years). There were 69 men and 47 women patients. The primary tumors were located in the following sites: rectum in 53 patients, sigmoid colon in 21 patients, descending colon in 7 patients, transverse colon in 6 patients, ascending colon in 23 patients, and cecum in 6 patients. The clinical stage of the patients according to Dukes' classification was as follows: Dukes' stage A in 30 patients, stage B in 31 patients, stage C in 53 patients, and stage D in 2 patients.

Table 1. Characteristics of Patients and Healthy Volunteers

Characteristic	Patient (N = 116)	Healthy volunteer (N = 83)
Age, *y*		
Mean	62.0	58.4
Range	32–82	40–70
Sex, no (%)		
Male	69 (59.5)	37 (44.6)
Female	47 (40.5)	46 (55.4)
DNA, ng/gram of stool		
Mean	570.8	175.3
Range	2.0–7462.8	0.2–1907.5
Tumor location, no (%)		
Cecum	6 (5.2)	
Ascending colon	23 (19.8)	
Transverse colon	6 (5.2)	
Descending colon	7 (6.0)	
Sigmoid colon	21 (18.1)	
Rectum	53 (45.7)	
Size, mm		
Mean	40.0	
Range	4.0–120.0	
Histology, no (%)		
W/D	55 (47.4)	
M/D	56 (48.3)	
P/D	2 (1.7)	
Mucinous carcinoma	2 (1.7)	
Carcinoid tumor	1 (0.9)	
Depth, no (%)		
T1	10 (8.6)	
T2	32 (27.6)	
T3	71 (61.2)	
T4	3 (2.6)	
Dukes' stage, no (%)		
A	30 (25.9)	
B	31 (26.7)	
C	53 (45.7)	
D	2 (1.7)	

W/D, Well-differentiated adenocarcinoma; M/D, moderately differentiated adenocarcinoma; P/D, poorly differentiated adenocarcinoma.

Stool Samples

Before surgical resection, stool samples were obtained from 116 patients with colorectal cancer. Stool sam-

ples were also obtained from 83 healthy volunteers a few weeks after they had undergone a total colonoscopy. Naturally evacuated feces from subjects who had not taken laxatives were used as stool samples. Each patient was instructed to evacuate into a polystyrene disposable tray (AS one, Osaka, Japan) measuring 5 × 10 cm in size at home and bring the sample to the reception counter at the outpatient clinic or the Cancer Prevention and Screening Center of the National Cancer Center. The samples were collected and transferred to a laboratory at which they were allowed to stand at room temperature. Preparation of the stool samples for examination was conducted within 1–6 hours after the evacuation.

Magnetic Beads

Dynabeads Epithelial Enrich are uniform, superparamagnetic, polystyrene beads (4.5-μm diameter) coated with a mouse IgG1 monoclonal antibody (mAb Ber-EP4) specific for the glycopolypeptide membrane antigen Ep-CAM, which is expressed on most normal and neoplastic human epithelial tissues (Dynal, Oslo, Norway). Ep-CAM is widely expressed in the highly proliferative cells of the intestinal epithelium, from the basal cells to cells throughout the crypts at the basolateral membranes, and only the apical membrane facing the lumen is negative. The development of adenomas has been reported to be associated with increased Ep-CAM expression, and Ep-CAM over expression (mAb GA733) has frequently been demonstrated in colorectal carcinomas.[23–25]

Simulation Studies

A series of simulation studies were conducted to establish the optimal conditions for retrieving HT-29 colorectal cancer cells from feces. Feces from healthy volunteers were divided into several portions, each of which was seeded with 100 μL HT-29 cells (1 × 10^6/approximately 5 g feces). The cells were retrieved under several different conditions as follows: use of a Hank's solution and 25 mmol/L Hepes buffer (pH 7.35); processed feces of 5, 10, or 30 g volume; filter with a pore size of 48, 96, 512, or 1000 μm; incubation of homogenized solution with magnetic beads at 4°C or room temperature; application of 20, 40, 80, 200, or 400 μL magnetic beads; incubation of homogenized solution with magnetic beads under gentle rolling at 15 rounds/minute in a mixer for 10, 20, 30, or 40 minutes; and the reaction time between the cell-magnetic bead complexes and a magnet on a shaking platform for 0, 2, 10, 20, 30, 40, 50, or 60 minutes. Finally, the cell retrieval rate calculated for the magnetic beads method under the conditions determined to be the most suitable for this simulation study was compared with that calculated for the Percoll centrifugation method. The retrieval rate was calculated by dividing the number of cells that bound to the retrieved beads by the number of cells initially added to the feces. The cells were counted using a NucleoCounter (ChemoMetec A/S, Allerød, Denmark).

Isolation of Exfoliated Cells From Feces

The procedure was conducted using the most suitable and optimal conditions determined by the simulation study (Figure 1). Approximately 5–10 g of naturally evacuated feces were used to isolate exfoliated cells. Feces were collected into Stomacher Lab Blender bags (Seward, Thetford, United Kingdom). The stool samples were homogenized with a buffer (200 mL) consisting of Hank's solution, 10% fetal bovine serum (FBS), and 25 mmol/L Hepes buffer (pH 7.35) at 200 rpm for 1 minute using a Stomacher (Seward). The homogenates were then filtered through a nylon filter (pore size, 512 μm), followed by division into 5 portions (40 mL each). Subsequently, 40 μL of magnetic beads were added to each homogenized solution portion, and the mixtures were incubated for 30 minutes under gentle rolling in a mixer at room temperature. The samples on the magnet were then incubated on a shaking platform for 15 minutes at room temperature. Colonocytes isolated from 5 tubes were smeared onto slides and then stained using the Papanicolaou method. The remainder of the samples was centrifuged, and the sediments were stored at −80°C until DNA extraction.

Extraction of DNA

Fresh tissue samples were obtained from the surgically resected specimens of 116 patients with colorectal cancer. The samples were snap frozen in liquid nitrogen within 20 minutes of their arrival at the pathologic specimen reception area and were stored in liquid nitrogen until analysis.

Genomic DNA was extracted from each tumor tissue specimen using a DNeasy kit (QIAGEN, Valencia, CA). Genomic DNA was also extracted from colonocytes isolated from feces using the SepaGene kit (Sanko-Junyaku, Tokyo, Japan).

Direct Sequence Analysis

Direct sequencing was conducted to identify mutations in the APC codon 1270–1594, in codons 12 and 13 of the K-*ras* gene, and in exons 5, 6, 7, and 8 of the p53 gene.

The PCR primers used in this study were as follows: APC (5′-AAACACCTCAAGTTCCAACCAC-3′, 5′-GGTAATTTTGAAGCAGTCTGGGC-3′); K-*ras* (5′-CTGGTGGAGTATTTGATAGTG-3′, 5′-CCCAAGGAAAGTAAAGTTC-3′); p53 exon 5 (5′-GCCGTCTTCCAGTTGCTTTAT-3′, 5′-CCAAATACTCCACACGCAAAT-3′); p53 exon 6 (5′-CATGAGCGCTGCTCAGATAG-3′, 5′-TGCACATCTCATGGGGTTATAG-3′); p53 exon 7 (5′-CTTGGGCCTGTGTTATCTCCTA-3′, 5′-AAGAAAACTGAGTGGGAGCAGT-3′); and p53 exon 8 (5′-ACCTCTTAACCTGTGGCTTC-3′, 5′-TACAACCAGGAGCCATTGTC-3′).

The sequence primers used in this study were as follows: APC (5′-CAAAAGGCTGCCACTTGCAAAG-3′, 5′-AAAATAAAGCACCTACTGCTG-3′, 5′-GAATCAGCCAGGCACAAAGC-3′); K-*ras* (5′-CTGGTGGAGTATTTGATAGTG-3′); p53 exon 5 (5′-CCAAATACTCCACACGCAAAT-3′); p53 exon 6 (5′-CATGAGCGCTGCTCAGATAG-3′); p53 exon 7 (5′-AAGAAAACTGAGTGGGAGCAGT-3′); and p53 exon 8 (5′-

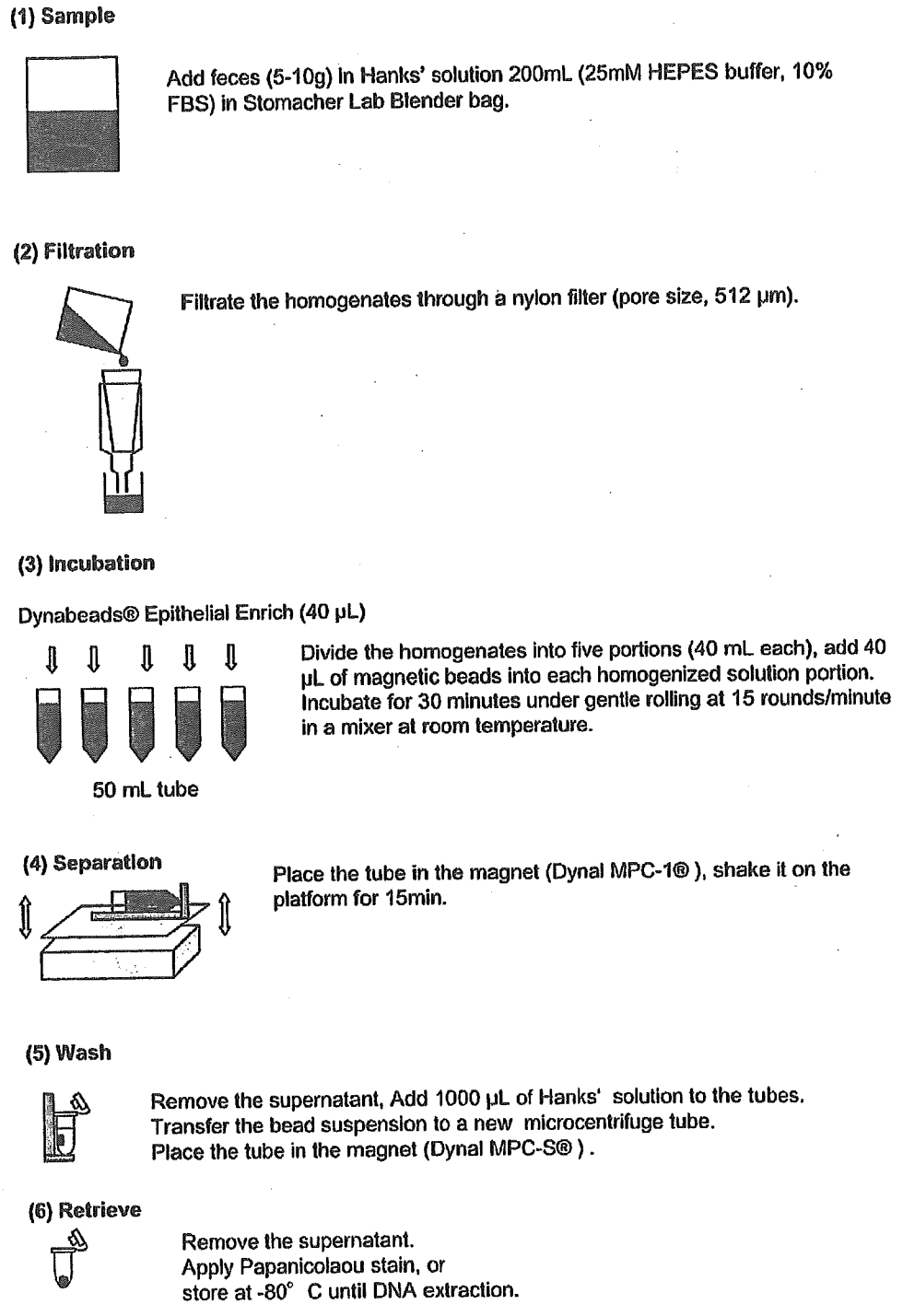

Figure 1. Schematic of procedure for isolating colonocytes from feces.

ACCTCTTAACCTGTGGCTTC-3′). Each fragment was sequenced by direct sequencing using the Big Dye Terminator v 3.1/1.1 cycle kit (Applied Biosystems, Forester City, CA).

All obtained sequences were aligned with previously published sequences (National Center for Biotechnology Information [NCBI] Genbank accession No. M74088 [APC], M54968 [K-*ras*], and X54156 [p53]) for each of the target genes and were analyzed using Phred/Phrp/DNASIS pro (Hitachi Software Engineering, Tokyo, Japan). The presence and nature of each mutation were confirmed by repeated PCR and sequencing.

BAT26

The BAT26 gene, an indicator of microsatellite instability (MSI), was amplified by PCR. Each fragment was elec-

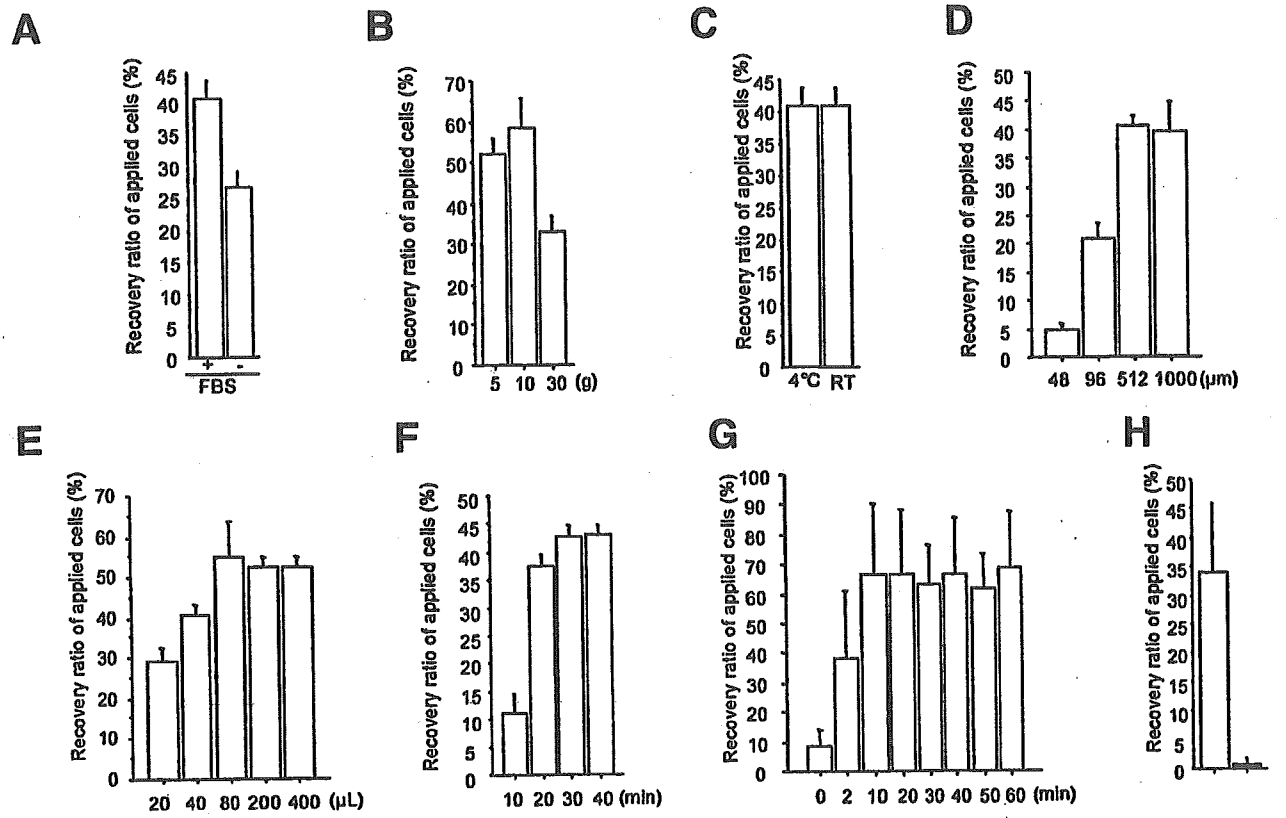

Figure 2. Simulation study to establish the optimal conditions for retrieving HT-29 colorectal cancer cells from feces and to compare the cell retrieval rates for the magnetic beads methods and the Percoll centrifugation method. Feces from healthy volunteers were divided into several portions, each of which was seeded with 100 μL HT-29 colorectal cancer cells (1×10^6/approximately 5 grams of feces). The procedure for retrieving the HT-29 cells was conducted under various conditions as follows: (*A*) homogenizing buffer with or without FBS; (*B*) stool weight (5, 10, or 30 g); (*C*) temperature during the cell-yielding procedure (4°C or room temperature); (*D*) filter pore size (48, 96, 512, or 1000 μm); (*E*) volume of applied magnetic beads (20, 40, 80, 200, or 400 μL); (*F*) incubation time of the homogenized solution with the magnetic beads under gentle rolling in a mixer (10, 20, 30, or 40 minutes); and (*G*) reaction time for the cells-magnetic bead complexes and the magnet on the shaking platform (0, 2, 10, 20, 30, 40, 50, or 60 minutes). The cell retrieval ratio (%) was calculated using the following formula: 100 × number of HT-29 cells retrieved/number of applied HT-29 cells. (*H*) Comparison of cell retrieval rates for the magnetic beads methods (*open column*) and the Percoll centrifugation method (*solid column*).

trophoresed using an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and then analyzed by GeneScan v 3.7 (Applied Biosystems). The PCR primers used in this study were 5′-TGACTACTTTTGACTTCAGCC-3′ and 5′-AACCATTCAACATTTTTAACCC-3′.

Cytology

Colonocytes isolated from feces were examined by 2 experienced cytotechnologists after Papanicolaou staining.

Study Blinding

We followed the guidelines of our medical institution for preparing blinded samples. Technicians processed the stool samples and prepared the slides for cytology and the cell pellets for DNA extraction. The samples were blinded to prevent the identification of individuals and the samples' origins. Two cytologists assessed the blinded samples, and the Life Science Group of Hitachi, Ltd, analyzed the DNA sequences.

Statistical Analysis

A Fisher exact test was used to compare all proportions. All reported P values are 2-sided. A value of $P < .05$ was considered statistically significant.

Results

Simulation Studies

The cell retrieval rate was found to decrease when Hank's solution without FBS was used, thus indicating the effectiveness of adding serum to the homogenizing buffer (Figure 2*A*). The cell retrieval rate was found to decrease when more than 30 g of feces were processed (Figure 2*B*). The cell retrieval rates were similar when incubation was conducted at room temperature and at 4°C (Figure 2*C*). Filtering of the stool suspension with the 48- or 96-μm filter resulted in significant clogging and thus hampered cell retrieval. However, a lot of fecal

residue remained after filtering with the 1000-μm filter, hindering the handling of the stool suspension thereafter. We therefore decided to use the 512-μm filter (Figure 2*D*). The dose of the magnetic beads applied was also examined. The cell retrieval rate increased in a dose-dependent manner up to 80 μL. In reality, a sufficient amount of genomic DNA derived from exfoliated colonocytes was obtained, even when 40 μL of magnetic beads were used (Figure 2*E*). Regarding the optimal incubation time of the magnetic beads for the complete binding of HT-29 cells to the beads, 30 minutes of incubation was found to be sufficient for the satisfactory binding of HT-29 cells to the beads (Figure 2*F*). For the retrieval of the cell-magnetic bead complexes on the magnet, a 10-minute reaction period was sufficient (Figure 2*G*).

The cell retrieval rates were 0.8% and 33.5% using the Percoll centrifugation method and the magnetic beads method, respectively, thus underscoring the advantage of the magnetic beads method (Figure 2*H*).

Cytology

Atypical cells were observed in colonocytes isolated from the feces of 32 of 116 patients with colorectal cancer, with a sensitivity rate of 28% (95% CI: 20–37; Table 2, Figure 3*A* and 3*B*). No atypical cells were observed in any of the 83 healthy volunteers, with a specificity rate of 100% (95% CI: 96–100). A significant difference ($P < .0001$) was found in the positivity rate between the patient group and the healthy volunteer group. The sensitivity rates for Dukes' A, B, and C or D colorectal cancers were 23% (7 of 30; 95% CI: 10–42), 32% (10 of 31; 95% CI: 17–51), and 27% (15 of 55; 95% CI: 16–41), respectively. No significant differences in the positivity rates were found among any of the stages. Furthermore, the sensitivity rates for cancers on the right side of the colon, including the cecum, ascending colon, and transverse colon, and for those on the left side of the colon, including the descending colon, sigmoid colon, and rectum, were 9% (3 of 35; 95% CI: 2–23) and 36% (29 of 81; 95% CI: 25–47), respectively. Therefore, the positivity rate was significantly higher for cancers on the left side of the colon ($P < .01$).

DNA Analysis

Overall analysis of stool samples. Sequence analysis showed distinct mutations in each of the analyzed genes in the tumor tissue and colonocytes isolated from feces (Figure 3*C–F*). Genetic alterations were observed in the colonocytes isolated from the feces of 82 of the 116 patients with colorectal cancer, yielding a sensitivity rate of 71% (95% CI: 62–79; Table 2). However, 10 of the 83 healthy volunteers were also positive for genetic alterations, producing a specificity value of 88% (95% CI: 79–94). A significant difference ($P < .0001$) was noted in the positivity rates of the patient group and the healthy volunteer group.

Genetic alterations were observed in 18 of the 30 patients with Dukes' A colorectal cancer, yielding a sensitivity rate of 60% (95% CI: 41–77). Furthermore, genetic alterations were observed among 26 of the 31 patients with Dukes' B colorectal cancer (84%; 95% CI: 66–95) and 38 of the 55 patients with Dukes' C or D colorectal cancer (69%; 95% CI: 55–81). No significant difference in sensitivity was found among any of the stages.

Genetic alterations were observed in colonocytes isolated from feces in 20 out of 35 patients with cancers originating on the right side of the colon (57%; 95% CI: 39–74) and in 62 out of 81 patients with cancers originating on the left side of the colon (77%; 95% CI: 66–85). No significant differences in the sensitivity rates were observed, although the sensitivity rate tended to be higher for cancers on the left side of the colon.

DNA analysis limited to colonocytes isolated from the feces of patients with colorectal cancer tissue involving genetic alterations. We assessed the performance of the present methodology for isolating cancer cells by examining the positivity rate of genetic alterations in colonocytes isolated from the feces of patients who showed alterations in their cancer tissues (Table 3). Among the 116 patients, a total of 93 (80%; 95% CI: 72–87) exhibited genetic alterations in the APC, K-*ras*, or p53 genes or BAT26 positivity in their cancer tissue: 51 patients exhibited APC mutations (44%, 95% CI: 35–53), 33 patients exhibited K-*ras* mutations (28%; 95% CI: 20–38), 62 patients exhibited p53 mutations (53%; 95% CI: 44–63), and 6 patients exhibited BAT26 positivity (5%; 95% CI: 2–11). Among the 93 patients with genetic alterations in their cancer tissues, the alterations were also successfully detected in colonocytes isolated from the feces of 80 patients (86%; 95% CI: 77–92). Among the 39 patients with Dukes' C or D advanced cancer who exhibited a genetic alteration in their cancer tissues, 36 patients exhibited genetic alterations in colonocytes isolated from their feces (92%; 95% CI: 79–98). Furthermore, genetic alterations were detected in colonocytes isolated from the feces of 18 of 24 patients with Dukes' A cancer (75%; 95% CI: 53–90) and 26 of 30 patients with Dukes' B cancer (87%; 95% CI: 69–96). No statistically significant difference was found among these 3 groups. In addition, genetic alterations could be detected in colonocytes isolated from the feces of 20 of 27 patients with cancers originating on the

Table 2. Incidences of Genetic Alterations of the APC, K-*ras*, p53, and MSI (BAT26) Genes as Well as Results From Cytology in all Patients and Healthy Volunteers

		Patient				Healthy volunteer	
		Tumor tissue		Isolated cell		Isolated cell	
	Marker	No.	Positivity (%) (95% CI)	No.	Sensitivity (%) (95% CI)	No.	Specificity (%) (95% CI)
Overall Patients (n = 116), healthy volunteers (n = 83)	Combined marker	93	80 (72–87)	82	71 (62–79)	10	88 (79–94)
	APC	51	44 (35–53)	47	41 (32–50)	1	99 (93–100)
	K-*ras*	33	28 (20–38)	33	28 (20–38)	1	99 (93–100)
	p53	62	53 (44–63)	45	39 (30–48)	6	93 (85–97)
	BAT26	6	5 (2–11)	4	3 (1–9)	3	96 (90–99)
	Cytology			32	28 (20–37)	0	100 (96–100)
Dukes' stage A (n = 30)	Combined marker	24	80 (61–92)	18	60 (41–77)		
	APC	14	47 (28–66)	11	37 (20–56)		
	K-*ras*	6	20 (77–39)	5	17 (6–35)		
	p53	6	20 (77–39)	9	30 (15–49)		
	BAT26	1	3 (1–17)	1	3 (1–17)		
	Cytology			7	23 (10–42)		
Dukes' stage B (n = 31)	Combined marker	30	97 (83–100)	26	84 (66–95)		
	APC	17	55 (36–73)	17	55 (36–73)		
	K-*ras*	10	32 (17–51)	9	29 (14–48)		
	p53	18	58 (39–75)	13	42 (25–61)		
	BAT26	2	6 (1–21)	1	3 (1–17)		
	Cytology			10	32 (17–51)		
Dukes' stages C and D (n = 55)	Combined marker	39	71 (57–82)	38	69 (55–81)		
	APC	20	36 (24–50)	19	35 (22–49)		
	K-*ras*	17	31 (19–45)	19	35 (22–49)		
	p53	27	49 (35–63)	23	42 (29–56)		
	BAT26	3	5 (1–15)	2	4 (0–13)		
	Cytology			15	27 (16–41)		
Right-sided colon cancer (n = 35)	Combined marker	27	77 (60–90)	20	57 (39–74)		
	APC	11	31 (17–49)	8	23 (10–40)		
	K-*ras*	16	46 (29–63)	12	34 (19–52)		
	p53	17	49 (31–66)	11	31 (17–49)		
	BAT26	2	6 (1–19)	1	3 (1–15)		
	Cytology			3	9 (2–23)		
Left-sided colon cancer (n = 81)	Combined marker	66	81 (71–89)	62	77 (66–85)		
	APC	40	49 (38–61)	39	48 (37–60)		
	K-*ras*	17	21 (13–31)	21	26 (17–37)		
	p53	45	56 (44–67)	34	42 (31–53)		
	BAT26	4	5 (1–12)	3	4 (1–10)		
	Cytology			29	36 (25–47)		

right side of their colon (74%; 95% CI: 54–89) and 60 of 66 patients with cancers originating on the left side of their colon (91%; 95% CI: 81–97). A statistically significant difference was found between the right- and left-side colon cancer patient groups ($P = .03$).

Discussion

We have devised a simple, highly reliable methodology for isolating colorectal cancer cells from nonlaxative-induced, naturally evacuated feces from most patients with colorectal cancer. To date, several methods of isolating colorectal cancer cells from feces have been reported.[21,22,26,27]

Our new funnel-shaped filter system extensively improved the filtration efficiency of the stool suspension by enlarging the filtration area and selecting the optimal pore size; the system was capable of filtrating the entire stool suspension without filter clogging. These properties permit the omission of centrifugation and simplify the overall process because all steps can be performed at room temperature. Furthermore, the use of serum successfully increased the cell retrieval rate. We presume that this increase may be attributed to the suppression of protease activity or the inhibition of nonspecific reactions of the antibodies on the bead surface. Consequently, our new methodology also allows the extraction of high-quality DNA or RNA from exfoliated colonocytes. Very recently, Imperiale et al compared a panel of fecal DNA markers and Hemoccult II as screening tests for colorectal cancer. It is worth noting that, in their study, colonoscopy as a reference standard was used