上記のように分類した。

西村らはKleinらの病型分類に加えて、消化管壁全層に好酸球浸潤がみられるTransmural diseaseを加えるのが妥当であると述べている[11]。アンケート調査では生検での好酸球の粘膜浸潤で診断した症例が多く、Kleinの分類における粘膜優位型が多いことを示唆する事実とも考えられる。

【病変部位の考察】

食道病変を有する例は多くなく、eosinophilic esophagitis合併例はむしろ少数と考えられている[12]。アンケート調査においても食道病変は9%と少数であった。

【画像所見の考察】

X線所見は病型によって異なる。

①Predominant mucosal layer diseaseでは所見がないこともあるが、多くは十二指腸、小腸のKerckring皺襞の腫大、潰瘍、管腔狭小化、分泌亢進、易刺激性、攣縮がみられる。

②Predominant muscle layer diseaseでは①のX線所見に加え、管状狭窄、壁硬化、蠕動不良を認める。

③Predominant subserosal layer diseaseでは①、②と同様のX線所見を呈することもあるが、X線像に異常がみられないこともある[4]。

アンケート調査ではこれらの所見を確認、区別することはできなかった。

内視鏡では粘膜面の変化に乏しく浮腫、発赤、皺襞の密度上昇以外に異常としてみられないことが多い。

今回のアンケート調査ではびらん、発赤、浮腫などの非特異的所見が約半数にみられた。

腹痛や嘔吐などの症状が強い時期には腹部CTや腹部超音波検査が有用である。

アンケート調査ででも約60%に腸管壁の肥厚がみられた。

【生検の考察】

組織学的には浮腫と好酸球を主体とした炎症細胞浸潤が特徴的である。生検での好酸球浸潤は60%程度で証明されるにすぎない。また文献上、強拡大1視野に20個以上の好酸球を認めるものを優位と捉えているが、明確な基準はない[12)~14)]。粘膜優位型以外の病型では生検での診断率は低下する。尚、病変の主座が粘膜にあっても、病変が非連続性に分布することがあるため、内視鏡上正常に見える粘膜も含め数ヵ所からの生検が望まれる。

アンケート調査での最も高頻度（95%）の所見で、診断の根拠となっている。

【鑑別診断】

末梢血中の好酸球増多や消化管に好酸球浸潤を来たす疾患が鑑別となる。寄生虫感染、hypereosinophilic synodrome、アレルギー性肉芽腫性血管炎（Churg-Strauss症候群）などが挙げられる。また画像診断上ループス腸炎やアニサキス症、アミロイドーシス、シェーンライン・ヘノッホ紫斑病などが挙げられる。

その他、類似のX腺を呈するも疾患としてキャンピロバクター腸炎、サルモネラ腸炎などの感染性腸炎、虚血性小腸炎、NSAIDs腸炎、Crohn病、放射線性腸炎などが挙げられている[14]。

【治療と予後】

自然治癒例も存在するが、治療の主体はステロイドの投与と対症療法である。一般にPSL20～40mg/日で投与される症例が多い。数週～数ヶ月にわたって投与されているが、漸減に関しての一定の基準はない。抗アレルギー薬である抗ヒスタミン薬や抗ロイコトリエン薬などが有効であるともいわれている[6]。PSL中止後の約55%に再発するという報告もあり、抗アレルギー薬による維持療法に期待がかかる[15]。

繰り返す炎症により穿孔や狭窄を来たし、手術に至った症例もあるため、早期診断により治療と再燃を反復しないための維持療法が望まれる。

D. 結論

好酸球性胃腸炎の明確な診断基準、治療指針は定められていない。比較的なまれな疾患であり、特異的所見に乏しいことがその要因とも考える。本邦、海外にて過去数十年にわたり報告例はあるものの本症の病態解明を含めた進歩にはやや乏しいものがある。再発を繰り返すこと、不可逆的な変化により手術症例もあることを考慮すると、診断や治療の明確な指針が望まれ、早期の診断や治療が求められる。Talleyの診断基準は多くの文献で引用されており、有用と考えられるものの、除外する疾患の明確な基準が定められていない。この点に関しては今後の検討の

課題とも言えるであろう。またKleinの病型分類は臨床の現場で病型分類の推測こそできるものの、治療方針に大きな影響は与えない。現段階ではステロイドの投与が一般的に用いられており、ほとんどの症例では著効する。診断的治療として用いられることもあると考える。予後は良好であるが、治療に関しての病勢を示すマーカーなどはまだ存在しておらず、診断も含めサイトカインとしてIL-5、IL-13、eotoxinなどに今後の期待がかかる。

文　献

1)Talley NJ, et al. Eosinophilic gastroenteritis: A clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. Gut 31; 54-58, 1990

2)Klein NC, et al. Eosinophilic gastroenteritis. Medicine 49; 299-319, 1970

3)上尾太郎,他. 好酸球性胃腸炎. 胃と腸 38(4):553-558, 2003

4)山本智文,他. 好酸球性胃腸炎. 小腸疾患の臨床:307-312, 2004

5)Yan BM,Shaffer EA. Primary eosinophilic disorders of the gastrointestinal tract. Gut 58:721-732, 2009

6)市川仁志,他. 好酸球性胃腸炎. Mebio 26(11):30-35, 2009

7)Shiner M,et al. The small intestinal mucosa in cow's milk allergy.Lancet 2:136-140, 1975

8)Hogan SP, et al. Review article:the eosinophilic as a therapeutic target in gastrointestinal disease. Aliment Pharmacol Ther 20:1231-1240, 2004

9)Rothenberg ME. Eosinophilic gastrointestinal disorders(EGID). J Allergy Clin Immunol 113:11-28, 2004

10)森田智子,他. 好酸球性胃腸炎の一症例.消化管の臨床10:75-78, 2004

11)西村浩,他. 好酸球性胃腸炎の1例及び本邦60例の文献的考察. Gastroenterol Endosc 31:2196-2204,1989

12)H.E. Oh , R chetty. Eosinophilic gastroenteritis: a review. Journal of Gastroenterology. 43:741-750, 2008

13)白井敏博,他. 好酸球性胃腸炎. アレルギーの臨床 25(14), 2005

14)檜沢一興,他. 小腸炎症性疾患とその鑑別診断. 小腸疾患の臨床:105-108, 2004

15)Naylor AR. Eosinophilic gastroenteritis. Scott Med J 35:163-165, 1990

E. 健康危険情報

なし

F. 知的財産権の出願・登録状況

1. 特許取得

特になし

2. 実用新案登録

特になし

3.その他

特になし

厚生労働科学研究費補助金難治性疾患克服研究事業
好酸球性食道炎/好酸球性胃腸炎の疾患概念確立と治療指針作成のための臨床研究
分担研究報告書

再発性の好酸球性胃腸炎に対するシクロスポリンを用いた治療の試み

研究分担者　松本主之　九州大学病院消化管内科　講師

研究要旨

再発性に経過した好酸性胃腸炎に対してシクロスポリンによる治療を試みた。患者は33歳、女性である。10行/日以上の水様下痢を主訴に来院、末梢血の好酸球増加に加え、胃・十二指腸の生検で好酸球の浸潤を認めたため好酸球性胃腸炎と診断した。プレドニゾロンの投与で一旦好酸球数は正常化し症状も消失したが、同薬の減量に伴う再発を繰り返した。ロイコトリエン拮抗薬や抗ヒスタミン薬の併用投与は効果が無かったが、シクロスポリンを併用したところ奏功し、プレドニゾロンの減量が可能となった。

研究協力者

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A. はじめに

好酸球性胃腸炎は、多彩な消化器症状がみられる原因不明の疾患で、組織学的に消化管壁に血管炎を伴わない浮腫と好酸球の浸潤を特徴とする。好発部位は胃と小腸であるが、全消化管に好酸球浸潤を認めることがあり、再発を繰り返す症例も少なくない。今回我々は、再発性の胃腸炎に対してシクロスポリンの投与を試みた。

B. 症例

患者：30歳代、女性。

主訴：下痢、腹痛。

既往歴：28歳時より筋緊張性頭痛あり、非ステロイド性消炎鎮痛薬を屯用している。気管支喘息や食物アレルギーの既往なし。

家族歴・生活歴：特記事項なし。

現病歴：2009年1月中旬より10行/日以上の水様下痢と腹痛が出現したため同年2月に近医を受診した。末梢血の白血球数17450/μL、好酸球数9550/μL（54.7%）の好酸球増多に加え、上部消化管内視鏡検査において胃体部に発赤した粘膜を認めた。同部位からの生検で好酸球の集簇を認めたため好酸球性胃腸炎と診断された。プレドニゾロン40mg/日による治療を開始したところ、治療開始直後より好酸球数の正常化および消化器症状が消失した。しかし、同薬剤を5mg/日まで減量したところ、症状が再発がみられプレドニゾロンの増量を要し、さらにプレドニゾロンを20mg/日まで減量したところ再発がみられたため入院した。

入院時現症：異常なし。

検査成績：前医より処方されたプレドニゾロン15mg/日を内服中であったが、白血球数は13280/μLで好酸球数は3600/μL（27.1%）と著明に増加していた。頻回の下痢に起因すると思われる軽度の低蛋白血症を認めたが、炎症反応は陰性で貧血はなかった。上部消化管内視鏡検査では食道、胃および十二指腸球部には異常を認めなかった。しかし、十二指腸第二部の粘膜は粗米造であった（図1）。小腸造影X線検査では、空腸にKerckring皺壁のわずかな腫大を認めた。大腸内視鏡検査では終末回腸と全大腸に明らかな異常を指摘できなかった。また、腹部CTでは胃前庭部と十二指腸第二部に軽度の壁肥厚を認めるのみであった。消化管各所より生検を施行したところ、十二指腸第二部の生検組織に好酸球の集簇を認めた（図2）。

慢性好酸球性白血病の可能性を考え、骨髄穿刺を施行したが、骨髄像および染色体分析で異常は指摘出来なかった。以上より好酸球性胃腸炎と診断した。

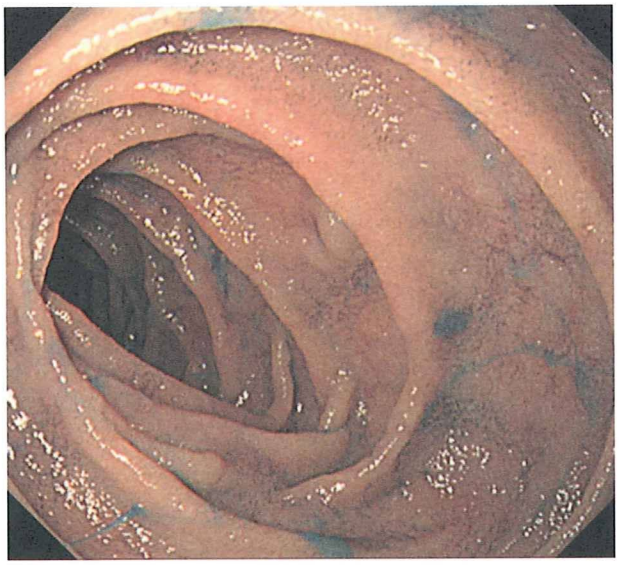

図1　十二指腸内視鏡所見

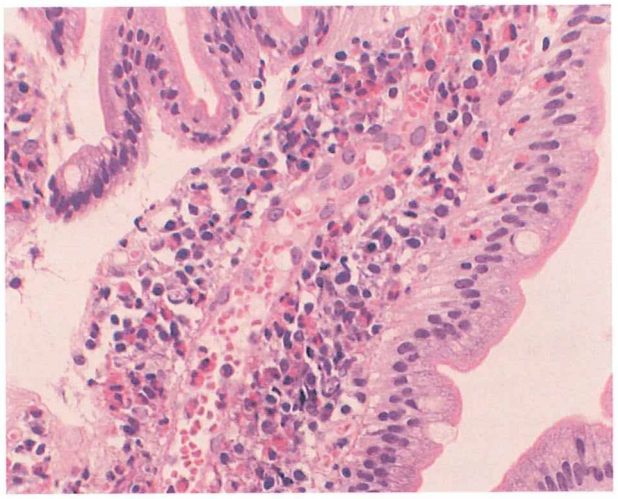

図2　十二指腸生検病理組織所見
十二指腸粘膜内に好酸球の浸潤あり

経過：ロイコトリエン拮抗薬や抗ヒスタミン薬を併用しながらプレドニゾロンの減量を試みたが、同剤を20mg/日まで減量すると再発した。そこで、患者の同意を得た上で、2010年1月よりシクロスポリンの経口投与を試みることとした。シクロスポリンの目標血中濃度を150～200ng/mLに設定して投与量を調節しながらプレドニゾロンを漸減したところ、症状の再発や好酸球増多を認めないまま10mg/日まで減量可能となった。

C. 考察

好酸球性胃腸炎は、発症頻度が10万人あたり1-20人と比較的まれな疾患で、20～50歳代の男性に好発する。診断基準として、Talleyら[1]が報告した１）消化管症状、２）消化管生検での好酸球浸潤、あるいは末梢血好酸球増多と特徴的なX線所見、３）アレルギー疾患など明らかな好酸球増多の原因となる他疾患がない、の３点が挙げられている。自験例は、基礎疾患となるアレルギー疾患の存在がないことより、従来の診断基準を満たしていたが、本症の診断基準に関しては本研究班において見直されるものと考えられる。

一方、本症は好酸球の主な浸潤部位と臨床症状により、predominant mucosal layer disease、predominant muscle layer disease、predominant subserosal layer diseaseの3つの病型に分類されている[2]。自験例は他院で施行された治療開始前の内視鏡検査で発赤した胃粘膜を認めたこと、下痢や低蛋白血症が主症状であったこと、腸管狭窄はなく腹水貯留も明らかでなかったことなどから、predominant mucosal layer diseaseとするのが妥当であろう。ただし、本研究班の調査で明らかとなったように、近年では食道病変の存在が問題となっており、病型に関しても更なる検討が必要と思われる。

本疾患に対する治療法の中心は、副腎皮質ステロイドと抗アレルギー薬の投与、および対症療法である。一方、高度の腸管狭窄を有する症例や消化管穿孔を来した症例では外科的切除も選択肢となる。しかしながら、これらの治療に一旦は反応するものの、再発を繰り返す症例が少なくない。自験例においても、当初ステロイドに対する反応は良好であったが、同剤の減量とともに再発し治療法の選択に難渋した。

本症と同様に好酸球増多を特徴とする疾患群として、好酸球増多症候群（hypereosinophilic syndrome; HES）がある。HESは、寄生虫感染やアレルギー性疾患などの基礎疾患がなく、1500/μL以上の高度な好酸球増加が6ヶ月以上持続して認められ、臓器障害を伴う疾患群の総称であり[3]、一部は好酸球性胃腸炎と重複する。自験例は、発症直後に好酸球性胃腸炎の治療が開始されたためHESの診断基準を満たさなかっ

たが、その後の経過からHESに準じた治療法を選択すべきと考えた。現在までに、HESに対する内科的治療として遺伝子異常をターゲットとした抗体製剤、ハイドレア、ビンクリスチンなどの抗腫瘍薬、インターフェロン、シクロスポリンなどの免疫調整薬の有効性が報告されいる[4]。自験例では好酸球に遺伝子異常を認めなかったこと、若年女性であること、さらに費用を考慮の上シクロスポリンを選択したところ、有効性が確認できた。

シクロスポリンは、T細胞においてカルシニューリンの活性化を阻害することにより強力な免疫抑制作用を示す薬剤である。本剤は好酸球が産生するインターロイキン（IL-）2やIL-5を抑制し、再生不良性貧血や乾癬、臓器対宿主病の治療目的で使用されることが多い。一方、HESに対する有効性を示した報告も散見される[5,6]。しかし、好酸球性胃腸炎に対するシクロスポリンの効果を検討した報告は皆無に等しい。自験例は難治・再発性の好酸球性胃腸炎に対する治療の可能性を示唆した症例であり、今後同様の症例の集積が重要と思われる。

文献

1) Talley NJ, Shorter RG, Phillips SF, et al. Eosinophilic gastroenteritis: A clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. Gut 31: 54-58, 1990
2) Klein NC, Hargrove RL, Sleisenger MH, et al. Eosinophilic gastroenteritis. Medicine 49: 299-319, 1970
3) Chusid MJ, Dale DC, West BC, et al. The hypereosinophilic syndrome: analysis of 14 cases with review of the literature. Medicine 54: 1-27, 1975
4) Tefferi A. Modern diagnosis and treatment of primary eosinophilia. Acta Haematol 114: 52-60, 2005
5) Nadarajah S, Krafchik B, Roifman C, et al. Treatment of hypereosinophilic syndrome in a child using cyclosporine: implication for a primary T-cell abnormality. Pediatrics 99: 630-633, 1997
6) Zabel P, Schlaak M. Cyclosporine for hypereosinophilic syndrome. Ann Hematol 62: 230-231, 1991

D. 健康危険情報

なし

E. 知的財産権の出願・登録状況

1. 特許取得
   特になし
2. 実用新案登録
   特になし
3.その他
   特になし

厚生労働科学研究費補助金難治性疾患克服研究事業
好酸球性食道炎/好酸球性胃腸炎の疾患概念確立と治療指針作成のための臨床研究
分担研究報告書

本邦における好酸球性食道炎の実態

研究分担者　坂本長逸　日本医科大学消化器内科学　教授

研究要旨
本邦での好酸球性食道炎の報告はきわめて稀である。好酸球性食道炎症例をアンケート調査および系統的なデータベースを使用して検索し、本邦における好酸球性食道炎の実態を明らかにした。

研究協力者
岩切勝彦（日本医科大学消化器内科学・准教授）

A. 研究目的

近年欧米では多数の好酸球性食道炎の報告が行われているが、本邦での好酸球性食道炎の報告はきわめて稀である。今回、われわれが経験した好酸球性食道炎の1例を含め、アンケート調査および系統的なデータベースを使用して検索を行い過去5年間に診断された計36例の本邦の好酸球性食道炎患者の実態を明らかにし、欧米での報告と比較検討する。

B. 自験例

症例は70歳の女性。2年前より胸部不快感が出現し、数ヶ月前より嚥下困難、胸やけ、咽頭部痛がみられるようになった。近医を受診し行われた上部消化管内視鏡検査において、逆流性食道炎(LA分類 grade M)が指摘され、プロトンポンプ阻害薬（lansoprazole 30mg）を8週間処方され内服したが症状の改善はみられなかった。嚥下困難、咽頭部痛のため食事摂取が十分に行われず当院耳鼻咽喉科に入院となった。喉頭鏡検査では異常所見は認めなかったが、頸部食道造影検査において一過性に中部から下部食道にかけての狭小化が観察され、食道運動異常症を疑い当科に転科となった。当科転科時には嚥下困難が強く食事摂取はできない状態であった。既往歴に気管支喘息（テオフィリン内服治療中）、糖尿病（インスリン治療中）、狭心症がある。転科翌日に上部消化管内視鏡検査を施行したところ、空気挿入直後に一過性に多数のリング状（しわ状）の所見が観察された。食道粘膜は浮腫状で血管透見は不良であった。胃食道接合部の食道粘膜は一部白色混濁していたが明らかな食道粘膜傷害は認めなかった。ヨード染色後再度多数のリング状所見が一過性に出現した。嚥下困難、食道内の多数のリング状所見より、好酸球性食道炎を疑い中部食道から生検を行った。胃・十二指腸には特記所見はみられなかった。胸・腹部CT検査では中部から下部食道に連続する全周性の食道壁の肥厚を認めたが、その他の消化管に壁の肥厚は認めなかった。組織生検では食道粘膜に25以上/HPF（high power field）以上の著明な好酸球を認めたが、胃・十二指腸からの生検では異常な好酸球はみられなかった。以上の結果より好酸球性食道炎と診断した。入院時の採血で好酸球数は1279/μl（白血球数 8700/μl）と上昇していたが、IgE値は正常範囲内であった。ダニ・花粉・室内塵・食品・真菌・動物・昆虫等の57項目のアレルゲン検索を行ったが全て陰性であった。また虫卵・原虫等も陰性であった。

好酸球性食道炎に対する治療としてプレドニゾロン30mg経口投与を開始したところ、翌日より嚥下困難は改善を認め、4日目には症状ほぼ消失し食事摂取が可能となった。末梢血の好酸球数も内服開始1週後には正常化した。また1ヶ月

後の上部消化管内視鏡検査で食道壁の浮腫は改善し、リング状の内視鏡所見もみられず、組織生検でも好酸球は観察されなかった。嚥下困難は消失したものの、1か月後CT検査では食道壁の肥厚の改善はわずかであった。3か月後のCT検査においても食道壁の肥厚は持続していたが、6か月後のCTでは食道壁の肥厚は消失していた。現在プレドニゾロン2.5mgに減量し外来にて経過観察中である。

C. 本邦の好酸球性食道炎患者の実態

1　性別、年齢

性別の記載があった35症例中28人（80%）は男性であり、女性は7人であった。36人の平均年齢は50.8歳であった。海外でのレビューでの成績でも男性が76.9%と多く、平均年齢は38.7歳であった。

2　症状

今回の調査で報告された好酸球性食道炎患者36例中の16例（44.4%）が嚥下障害を認め、2例(5.7%)が胸やけを有していた。嚥下障害や胸やけ以外の症状はその他症状としたが19例の患者がその他症状を有していた。症状を認めなかったのは36例中の1例のみであった。海外のレビューでは、嚥下障害(93%)、食物の詰まり（61.9%）、胸やけ（23.6%）が主な症状であるとされている。

3　アレルギー疾患の有無

36例中の29例においてアレルギー性疾患の有無に関する回答が得られた。29例中の16例（55.1%）の患者が何らかのアレルギー疾患を有していたが、この結果は海外でのレビューでの報告（51.6%）とほぼ同様な結果であった。

4　末梢血での好酸球増多の頻度

36例中の29例において末梢血での好酸球数が測定されたが、5%以上であった症例は22例（75.9%）にみられ、500/μl異常を認めた症例は14例48.3%であった。海外でのレビューでは30.8%に好酸球増多があったと報告されている。成人での好酸球性食道炎では、total IgEは60-69%において上昇がみられたと報告されている。

5　好酸球性食道炎の食道内視鏡所見

35例において食道内視鏡所見に関する回答が得られた。16例(45.7%)では内視鏡的な異常所見は認められなかった。9例(25.7%)において縦走する溝状の所見（linear furrowing）、8例(22.9%)において白斑（white specks）、6例(17.1%)においてリング状（気管支輪様）所見（transient or fixed circular rings）、1例(2.9%)において狭窄がみられた。海外のレビューでは、好酸球性食道炎患者においてリング状の内視鏡所見が49.2%、白斑が15.8%、狭窄が39.7%、狭小化した食道が5.4%、食道粘膜の脆弱・浮腫が59.3%に観察されたことが報告されている。また、内視鏡的な異常所見がみられなかった症例は8.8%であったと報告されている。

6　好酸球性食道炎の診断

食道生検による好酸球性食道炎の診断基準が十分に示されていた症例は36例中の10例であり、食道生検での20以上/HPFの好酸球または20以上の好酸球として診断されていた。現在、欧米における好酸球性食道炎の診断は、①食道に起因すると考えられる症状を有する、②胃や十二指腸粘膜に好酸球増多がないが、食道生検において15-20以上/HPFの好酸球を認める、③高容量のPPI投与も症状の改善がない、④食道pHモニタリングにおいても異常な食道内酸曝露を認めないことにより行われている。

7　CT・超音波内視鏡による食道壁の肥厚の有無

今回報告された36例中22症例においてCT検査が施行され、14症例において食道壁の肥厚がみられた。

8　好酸球性食道炎の治療

36例中の24症例に対してステロイド治療が行われ、効果判定が記載されていた15症例中14症例（93.3%）において改善がみられた。海外におけるレビューにおいても、コルチコステロイドの全身投与、局所投与の有効性が示されている。コルチコステロイドの全身投与は症状を１週以内に組織所見を４週以内に改善することが報告されている。またコルチコステロイドの吸入療法も即効性に症状軽減をもたらし有効率は95%であったと報告されている。

D. 結論

好酸球性食道炎患者は特徴的な症状（嚥下困難）、食道内視鏡所見（縦走溝、多数のリング状所見、白斑）を有することが多い。その他末梢血での好酸球増多やCT、超音波検査における食道壁肥

厚が認められることもある。診断は食道生検による20以上/HPFの好酸球の存在で行われ、治療にはプレドニゾロンが有効である。本邦の好酸球性食道炎も欧米で報告されている好酸球性食道炎と多くの共通点を有していた。

参考文献

1. Sgouros SN, Bergele C, Mantides A. Eosinophilic Esophagitis in adults: What is the clinical significance? Endoscopy 2006; 38: 515-520
2. Glenn T, Furuta C, Liacouras CA, et al. Eosinophilic esophagitis in children and adults: A systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology 2007; 133: 1342-1363

E. 健康危険情報

なし

F. 研究発表

1. Futagami S, Shindo T, Kawagoe T, Horie A, Shimpuku M, Kusunoki M, Miyake K, Gudis K, Iwakiri K, Itho T, Sakamoto C. Migration of eosinophils and CCR2-/CD68-double positive cells into the duodenal mucosa of patients with post-infectious functional dyspepsia. Am J Gastroenterol, in press
2. Futagami S, Hamamoto T, Shimpuku M, Nagoya H, Kawagoe Y, Horie A, Shindo T, Gudis K, Sakamoto C. Celecoxib inhibits CD133-positive cell migration via reduction of CCR2 in Helicobacter pylori-infected Mongolian gerbils. Digestion. 81(3):193-203, 2010
3. Nagoya H, Tanaka S, Tatsuguchi A, Mitsui K, Ehara A, Kobayashi T Fujimori S, Sakamoto C. Rare cause if obscure gastrointestinal bleeding due to pyogenic granuloma in the ileum detscted by capsule endoscopy and treated with double balloon endoscopy. Dig Endosc. 22(1):71-73, 2010
4. Iwakiri K, Hoshihara Y, Kawami N, Sano H, Tanaka Y, Umezawa M, Kotoyori M, Nomura T, Miyashita M, Sakamoto C. The appearance of rosette-like esophageal folds("esophageal rosette")in the lower esophagus after a deep inspiration is a characteristic endoscopic finding of primary achalasia. J Gastroenterol. 2010
5. Tanaka Y, Iwakiri K, Kawami N, Sano H, Umezawa M., Kotoyori M, Hoshihara Y, Nomura T, Miyashita M, Sakamoto C. Predictors of a better outcome of pneumatic dilatation in patients with primary achalasia. J Gastroenrerol. 45(2):153-158, 2010
6. Futagami S, Iwakiri K, Shindo T, Kawagoe T, Horie A, Shimpuku M, Tanaka Y, Kawami N, Gudis K, Sakamoto C. The prokinetic effect of mosapride citrate combined with omeprazole therapy improves clinical symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying. J Gastroenterol. 2009 Dec
7. Miyake K, Kusunoki M, shindo T, Ueki N, Kawagoe T, Gudis K, Tatsuguchi A, Futagami S, Tsukui T, Sakamoto C. Duodenogastric reflux induced by endoscopic submucosal dissection. Endoscopy. 41(11):934-940, 2009 Nov

G. 知的財産権の出願・登録状況

1. 特許取得

特になし

2. 実用新案登録

特になし

3.その他

特になし

Ⅲ．研究成果の刊行に関する

一覧表

研究成果の刊行に関する一覧表

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Furuta K, Adachi K, Ohara S, Morita T, Tanimura T, Koshino K, Kinoshita Y	Acid inhibition on first and seventh days after starting administrations of two different proton pump inhibitors in Japanese subjects : Rnadomized two-way crossover study	J Int Med Res			in press
Koshino K, Adachi K, Furuta K, Ohara S, Morita T, Nakata S, Tanimura T, Miki M, Kinoshita Y	Effects of mosapride on esophageal functions and gastroesophageal reflux	J. Gastroenterol Hepatol			in press
Rahman F, Kadowaki Y, Ishihara S, Tobita H, Imaoka H, Fukuhara H, Aziz M, Furuta K, Amano Y, Kinoshita Y	Fibroblast derived HB-EGF promotes Cdx2 expression in esophageal squamous cells	Lab Invest			in press
Yoshida K, Furuta K, Adachi K, Ohara S, Morita T, Tanimura T, Nakata S, Miki M, Koshino K, Kinoshita Y	Effects of anti-hypertensive drugs on esophageal body contraction	World J Gastroenterol			in press
Fukuhara H, Kadowaki Y, Ose T, Aziz MM, Ishihara S, Takasawa S, Kinoshita Y	In vivo evidence for the role of RegÖüin gastric regeneration: transgenic overexpression of RegÖü accelerates the healing of experimental gastric ulcers	Lab Invest			in press
Otani A, Amano Y, Koshino K, Takahashi Y, Mishima Y, Imaoka H, Moriyama I, Ishimura N, Ishihara S, Kinoshita Y	Is autofluorescence imaging ebdoscopy useful for the determination of invasive depth in gastric cancer?	Digestion	81	96-103	2010
Komazawa Y, Amano Y, Yuki M, Fukuhara H, Mishiro T, Mishiro T, Shizuku T, Kioshita Y	Oolong tea is useful for lens cleansing in transnasal small-caliber esophagogastrodoudenoscopy	Endoscopy	42	104-108	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Akitake R, Watanabe T, Zaima C, Uza N, Ida H, Tada S, Nishida N, Chiba T	Possible involvement of T helper Type 2 responses to toll -like receptor ligands in IgG4-related sclerosing disease	Gut			in press
Tanaka J, Saga K, Kido M, Nishiura H, Akamatsu T, Chiba T, Watanabe N	Proinflammatory Th2 cytokines induce production of thymic stromal lymphopoietin in human colonic epithelial cells	Dig Dis Sci			in press
Kido M, Tanaka J, Aoki N, Iwamoto S, Nishiura H, Chiba T, Watanabe N	Helicobacter pylori promotes the production of thymic stromal lymphopoietin by gastric epithelial cells and induces dendritic cell-mediated inflammatory Th2 responses	Infect Immun	78	108-114	2010
Tanaka J, Watanabe N, Kido M, Saga K, Akamatsu T, Nishio A, Chiba T	Human TSLP and TLR3 ligand promote differentiation of Th17 cells with central memory phenotype under Th2-polarizing conditions	Clin Exp Allergy	39	89-100	2009
Fujiwara M, Miyamoto S, Iguchi K, Matsunaka T, Sakashita H, T suruyama T, Kanegane H, Marusawa H, Nakase H, Chiba T	Acute Epstein-Barr virus infection presenting as servere gastroenteritis without infectious mononucleosis-like manifestations	Clin J Gastroenterol	2	398-403	2009
Futagami S, Shindo T, Kawagoe T, Horie A, Shimpuku M, Kusunoki M, Miyake K, Gudis K, Iwakiri K, Itho T, Sakamoto C	Migration of eosinophils and CCR2-/CD68-double positive cells into the duodenal mucosa of patients with post-infectious functional dyspepsia	Am J Gastroenterol			in press
Futagami S, Hamamoto T, Shimpuku M, Nagoya H, Kawagoe Y, Horie A, Shindo T, Gudis K, Sakamoto C	Celecoxib inhibits CD133-positive cell migration via reduction of CCR2 in Helicobacter pylori-infected Mongolian gerbils	Digestion	81(3)	193-203	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nagoya H, Tanaka S, Tatsuguchi A, Mitsui K, Ehara A, Kobayashi T Fujimori S, Sakamoto C	Rare cause if obscure gastrointestinal bleeding due to pyogenic granuloma in the ileum detscted by capsule endoscopy and treated with double balloon endoscopy	Dig Endosc	22(1)	71-73	2010
Iwakiri K, Hoshihara Y, Kawami N, Sano H, Tanaka Y, Umezawa M, Kotoyori M, Nomura T, Miyashita M, Sakamoto C	The appearance of rosette-like esophageal folds(Ågesophageal rosetteÅh)in the lower esophagus after a deep inspiration is a characteristic endoscopic finding of primary achalasia	J Gastroenterol			in press
Tanaka Y, Iwakiri K, Kawami N, Sano H, Umezawa M., Kotoyori M, Hoshihara Y, Nomura T, Miyashita M, Sakamoto C	Predictors of a better outcome of pneumatic dilatation in patients with primary achalasia	J Gastroenrerol	45(2)	153-158	2010
Futagami S, Iwakiri K, Shindo T, Kawagoe T, Horie A, Shimpuku M, Tanaka Y, Kawami N, Gudis K, Sakamoto C	The prokinetic effect of mosapride citrate combined with omeprazole therapy improves clinical symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying	J Gastroenterol			in press
Miyake K, Kusunoki M, shindo T, Ueki N, Kawagoe T, Gudis K, Tatsuguchi A, Futagami S, Tsukui T, Sakamoto C	Duodenogastric reflux induced by endoscopic submucosal dissection	Endoscopy	41(11)	934-940	2009

Ⅳ. 研究成果の刊行物・別刷

Original Paper

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Is Autofluorescence Imaging Endoscopy Useful for Determining the Depth of Invasion in Gastric Cancer?

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Key Words
Autofluorescence imaging endoscopy · Gastric cancer · Invasion depth

Abstract
Background/Aims: Newly developed autofluorescence (AF) imaging (AFI) endoscopy can detect AF emitted by the gastrointestinal wall and may reliably detect tumors or inflammation that block AF. However, the efficacy of AFI endoscopy has not been evaluated for diagnosing the depth of tumor invasion in gastric cancer. ***Methods:*** AF endoscopic images were split into three bands (R, G and B) and expressed as grayscale values. AF indices, defined as the ratio of the G band image grayscale value to that of the R band, were calculated preoperatively. Correlations of AF indices with invasion depth and tumor thickness were assessed. AF indices were calculated preoperatively for 72 gastric cancer lesions without ulceration in 67 patients. The invasion grade of the lesions was classified histologically into 5 groups: M, SM, MP, SS and SE. ***Results:*** The mean tumor AF indices for each depth stage were 0.99, 0.77, 0.75, 0.74 and 0.61, respectively. A statistically significant difference was found between group M and the other groups. ***Conclusion:*** AFI endoscopy may reliably determine the depth of gastric cancer tumor invasion, although an expanded study comprised of larger numbers of subjects and different types of cancers may be required to clearly demonstrate its validity.

Introduction

An endoscopic diagnostic system that detects reflective autofluorescence has been tested [1], and a preliminary device has been constructed with the purpose of evaluating lesions in the gastrointestinal tract [2]. Newly developed autofluorescence imaging (AFI) endoscope can be used easily and consistently to detect autofluorescence emitted from the gastrointestinal wall, with relative precision [3]. Initially, AFI endoscopy was anticipated to have promising clinical applications, and several endoscopists have documented its clinical value [4, 5]. However, adequate and satisfactory evidence for the value of AFI endoscopy has, regrettably, not been found in the endoscopic screening of gastric neoplasms, as reported by several investigators [6–8]. Yet in the colon [9–13] and Barrett's esophagus [14–18], the superiority of AFI

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endoscopy over conventional endoscopy was observed not only for the detection of lesions but also for the endoscopic differentiation of tumors.

The natural endogenous autofluorescence of the gastrointestinal wall originates from collagen in the submucosal layer and other endogenous fluorophores, such as nicotinamide, adenine dinucleotide, flavin and porphyrins [9, 19, 20]. Some lesions may show a reflective autofluorescence pattern that is uniquely different from that of the normal gastrointestinal wall. With the AFI endoscope, lesions such as tumors and inflammation block autofluorescence from the gastrointestinal submucosal and deeper layers, and are displayed as a magenta color on the AFI, showing decreased autofluorescence, in contrast to the bright green color of the normal mucosa [21, 22]. Therefore, changes in autofluorescence intensity have the potential to reveal hidden tumor thickness, thereby allowing estimation of the depth of tumor invasion. However, no studies have been published on the relationship between autofluorescence intensity and invasion depth of gastrointestinal tract tumors. Our aim in the present study was to evaluate the efficacy of the AFI endoscope in determining the depth of tumor invasion in cases of gastric cancer.

Patients and Methods

From April 2007 to March 2008, 67 patients (M/F 38/29, mean age 72.5, range 56–89 years), with a total of 72 non-ulcerated gastric cancer lesions, who had undergone preoperative AFI endoscopic examination were enrolled in the present study. The evaluated lesions consisted of 27 of the flatly elevated type (0-IIa, 0-IIa + IIc like, or type 2), 36 of the mildly depressed type (0-IIc like), and 9 of the protruded type (0-I, 0-I + IIa like or type 1). Gastric cancers with ulceration were excluded, as ulcerative exudates show an extremely bright AFI, thus hampering accurate tumor evaluation and occasionally leading to overdiagnosis of the tumor and tumor extension.

Endoscopy

A high-resolution endoscope with an AFI system (GIF-Q260FZ, Olympus Medical Systems Corp., Tokyo, Japan) was used. The AFI system incorporates another exclusively charged coupled device for autofluorescence. For AFI endoscopy, excitation light (390–470 nm) for the induction of autofluorescence (500–630 nm) and green light (550 nm) for the reflectance image were provided by a xenon arc lamp via the rotation filter. Normal mucosa emits a vivid autofluorescence characterized by a bright green image. Tumors show a magenta color, as the tumor tissue diminishes autofluorescence. Five expert endoscopists with board certification from the Japan Gastroenterological Endoscopy Society recorded the AFI photographs of the lesions with the accompanying background mucosa. To avoid the halation that can develop, the photographs were taken directly in front, in the most optimal position possible.

Numerical Analysis of Reflective Autofluorescence

Endoscopic images that consist of three separate band images, red (R), green (G) and blue (B), are usually subjected to gamma processing for conversion to the linear tone of grayscale values. Gamma processing is well known for interrupting the numerical analysis of the image. The effect of gamma processing is removed by de-gamma processing software (ImageJ ver 1.33, National Institutes of Health, USA) to convert the values back to the original tone scale before numerical image analysis. The images are then split into R, G and B bands, and expressed as grayscale values. The G band image shows the intensity of the autofluorescence, and the R and B bands show the reflectance image for green light (fig. 1). In this AFI endoscopic system, the grayscale value of the B band is intentionally assigned to be a twofold grayscale value of R band to produce a clearer autofluorescence image, and the grayscale value of the B band is automatically determined by that of the R band. The grayscale value of the G band is decreased by blockade of the reflective autofluorescence from the submucosal layer at the lesion [6, 23–25]. The grayscale values of the R and B bands are unchanged by the mucosal thickness and/or the autofluorescence intensity, as they are determined by the absorption feature of hemoglobin [7]. However, the grayscale value of the G band showed various values with differing conditions, which shift with changes in the distance and/or angle of the photographic subject. Therefore, we ascertained that the autofluorescence (AF) index for the area of interest constantly indicated the intensity of autofluorescence in an area of interest based on results of an in vitro study with a porcine stomach. Measurement of the AF index (described below) was done by another expert endoscopist (A.O.) who did not have prior knowledge of the histological findings of the tumors.

AF Index Determination Based on the Porcine Stomach Study

We first prepared the following three AF indices to evaluate the autofluorescence intensity of an experimental tumor in a porcine stomach: a raw value of the G band grayscales of the tumor (a); the ratio of the G band grayscales of the tumor to that of the background (a/b), and the ratio of the G band grayscale to the R band grayscale of the tumor (G/R of a; fig. 2). After insertion into the submucosal layer of the porcine stomach of semi-interception material (polyvinyl chloride) against autofluorescence as an experimental tumor, G and R band grayscales were measured in both the tumor and the background at different distances, ranging from 1 to 5 cm, and the 3 indices were calculated. We prepared 3 porcine stomachs with experimental tumors and carried out endoscopic observation in the following ways: a vertical view and a slant view at an approximately 45° angle. Then, the most suitable marker indicating the autofluorescence intensity was determined by in vitro analysis with the porcine stomach.

Histological Analysis

The invasion depth and the thickness of the tumors were evaluated by an expert pathologist who had no prior knowledge of the endoscopic AFI results and AF indices of the tumors. The invasion grade of the tumors was determined histologically by examination of the resected specimens and classified into the following five groups: M, SM, MP, SS and SE [26]. Tumor invasion was de-

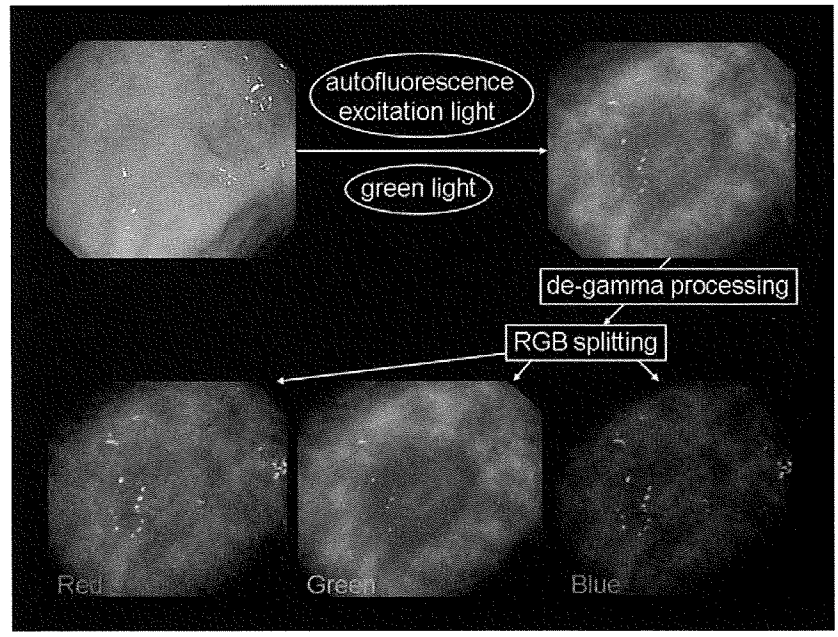

Fig. 1. Numerical analysis of endoscopic images. Early gastric cancer with 0-IIa + IIc form is shown in the lesser curvature of the gastric antrum. The autofluorescence image was obtained after autofluorescence excitation and usual green lights were irradiated. After de-gamma processing of the autofluorescence image drawn by a false green color, it was split into images with R, G and B grayscales. De-gamma conversion is needed to process the RGB image signal into linear values to analyze the endoscopic autofluorescence image numerically. Among the three images, the value of the G grayscale indicates the intensity of autofluorescence.

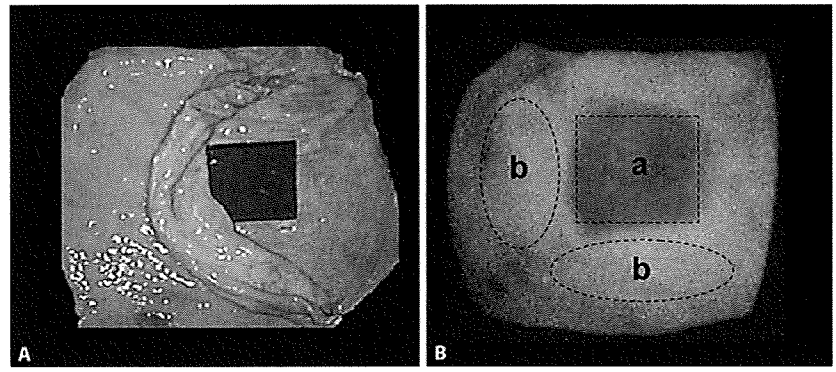

Fig. 2. An experimental tumor that can mildly block autofluorescence was set into the submucosa of a porcine stomach (**A**), and then endoscopic autofluorescence images were taken at different distances (1, 2, 3, 4 and 5 cm). The values of the G grayscale of the tumor (area a) and the background (area b) were obtained using calculating software (**B**).

fined as invasion of the mucosa or muscularis mucosa (M), submucosa (SM), muscularis propria (MP), subserosa (SS) and penetration of serosa (SE). The mean thickness of the tumor was measured with a maximum of 9 points, with each of 3 points assigned to 3 different sections of a lesion (fig. 3). Finally, the relationships between the AF index and the invasion depth or tumor thickness were investigated.

Statistical Analysis

Determination of the most available AF index was done by the Bartlett test in the porcine stomach study. The statistical analysis of the relationship between the AF index and the invasion depth of the tumor was performed with the Wilcoxon signed-rank test only when the Friedman test showed significant differences. The correlation between the thickness of the tumor and the AF index was evaluated with Spearman's analysis. The diagnostic value of the AF index for the invasion depth of the tumor was evaluated by the receiver operator characteristic (ROC) curve. p values of <0.05 were considered significant. All statistical analyses were performed with Statistical Analysis Software (SPSS, version 12.0 for the PC, SPSS Japan Inc.).

Results

The raw values of the G and B bands and the background grayscale of the experimental tumor at various distances were measured by the vertical and slant observation view in 3 porcine stomachs, and the ratio of the G band grayscale of the tumor to that of the background and the ratio of the G band grayscale to the R band gray-

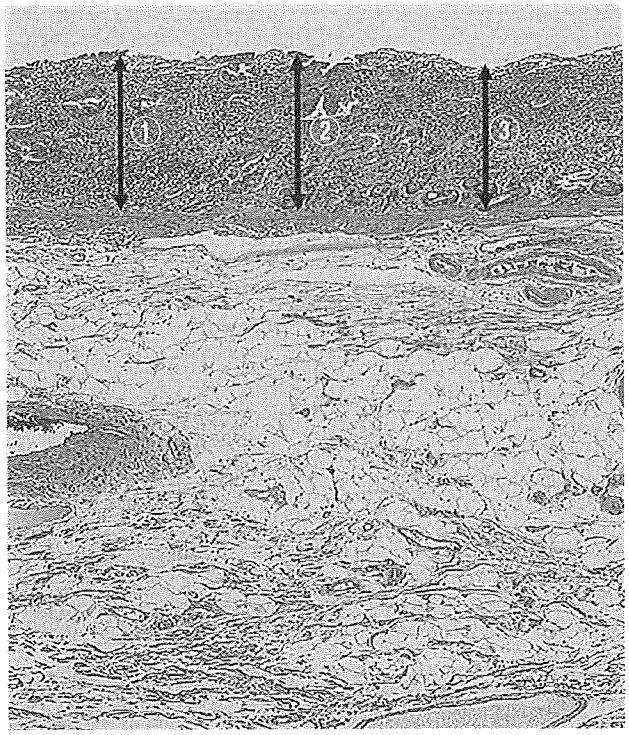

Fig. 3. The thickness of the tumor was measured at three points in three different sections of the specimen, and the mean value was calculated. In the present study, none of the lesions had vastly different thicknesses from one part to the next, as tumors of the depressed type were excluded.

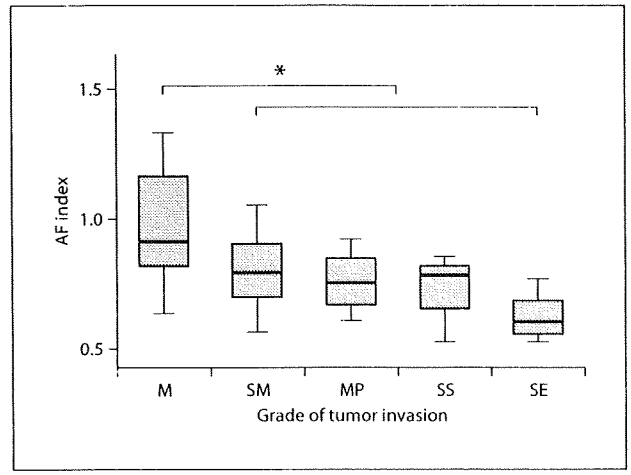

Fig. 4. Relationship between the autofluorescence (AF) index and the invasion depth of 72 gastric cancers. Smaller indices showed deeper invasion. In particular, the AF index of the depth M group was significantly larger than those of the remaining groups (*$p < 0.05$). Each box shows data from the 25th to the 75th percentiles; the bolded line in the box is the median value. The T-line above each box represents the 90th percentile, and the nadir line under each box represents the 10th percentile.

scale of the tumor were calculated as shown in table 1. Among the 3 indices, the raw value of the G band grayscale of the experimental tumor, the ratio of the G band grayscale of the tumor to that of the background, and the ratio of the G band grayscale to the R band grayscale of the tumor, the G/R grayscale ratio of the tumor showed the minimum variance in the observation distances by both the vertical and slant observation views ($p < 0.001$). These results suggest that the G/R grayscale ratio is an excellent marker for autofluorescence intensity, as it is not influenced by the observation distance or the observation angle. Therefore, the G/R grayscale ratio was used as the AF index to evaluate the autofluorescence intensity of the tumor in the present study.

A pilot study done to evaluate the AF index clinically also found it to be an excellent marker for autofluorescence intensity. Three gastric cancer lesions were prepared for measurement of parameters possibly revealing the autofluorescence intensity of the lesion. Lesion A was depth M cancer (0-IIa); B was depth SM massive (0-IIa + IIc) and C was depth SS (elevated type). The autofluorescence intensity of each lesion was measured with the AFI endoscope at approximately near, middle and far distances. The reproducibility of several parameters was compared with the G/R grayscale ratio of the tumor (the AF index). Standard deviation values of the G/R grayscale ratio of the tumor showed significantly smaller variance compared with raw values of G band grayscales of the tumor and the index of G lesion/G background. The most suitable marker as the AF index was also clinically proven to be the G/R grayscale ratio of the tumor in the analysis of the human stomach, as shown in table 2.

The histological types of gastric cancer of the enrolled patients were 38 well-differentiated adenocarcinomas, 11 undifferentiated (diffuse), and 13 mixed. No relationship was found between histological type and autofluorescence intensity. Of the 72 lesions analyzed, the histologically determined invasion depth of the gastric tumors included 29 in the M group, 22 in the SM group, 6 in the MP group, 7 in the SS group, and 8 in the SE group. The relationship between the AF indices and the invasion depth of the tumors is shown in figure 4. Mean AF indi-

Table 1. Relationship between G band grayscale values of experimental tumor lesions, the G band grayscale ratio of the tumor to the background and the G/R grayscale ratio of the tumor at different distances by vertical and slant view observation of the autofluorescence imaging endoscope in the porcine stomach

Porcine letter	Distance cm	Grayscale values			Indices	
		G tumor	R tumor	G background	G tumor/ G background	G tumor/R tumor
A	1	38.25 (37.89)	36.55 (36.12)	50.32 (46.25)	1.046 (0.819)	1.046 (1.049)
	2	36.33 (35.67)	34.68 (33.21)	52.11 (58.33)	0.697 (0.611)	1.047 (1.060)
	3	34.66 (33.21)	34.22 (33.98)	56.88 (65.23)	0.609 (0.509)	1.012 (0.977)
	4	34.56 (32.58)	32.18 (33.76)	65.23 (66.32)	0.529 (0.491)	1.073 (0.965)
	5	32.37 (32.78)	32.22 (31.22)	57.36 (70.25)	0.564 (0.466)	1.004 (1.049)
B	1	42.47 (41.56)	41.44 (40.23)	55.23 (60.23)	0.769 (0.690)	1.025 (1.033)
	2	41.36 (40.65)	40.74 (39.66)	63.55 (54.56)	0.651 (0.745)	1.015 (1.025)
	3	39.22 (39.88)	38.22 (39.37)	46.78 (46.02)	0.838 (0.867)	1.026 (1.013)
	4	39.32 (37.98)	38.56 (37.88)	40.33 (65.22)	0.975 (0.582)	1.020 (1.003)
	5	38.22 (38.35)	38.11 (38.66)	60.12 (44.32)	0.636 (0.865)	1.003 (0.992)
C	1	32.27 (29.33)	29.33 (29.02)	66.75 (65.36)	0.483 (0.449)	1.100 (1.010)
	2	31.80 (29.58)	28.25 (28.56)	49.04 (51.02)	0.648 (0.580)	1.126 (1.036)
	3	29.55 (30.52)	27.32 (28.33)	70.85 (46.80)	0.417 (0.652)	1.082 (1.077)
	4	28.55 (27.30)	26.24 (26.58)	44.36 (43.25)	0.644 (0.631)	1.088 (1.027)
	5	28.78 (26.98)	26.75 (25.10)	45.66 (68.12)	0.630 (0.396)	1.076 (1.075)
SD	A	2.195 (2.299)	1.836 (1.755)	5.798 (5.798)	0.202 (0.144)	0.028*(0.045)*
	B	1.740 (1.514)	1.558 (0.912)	9.565 (8.977)	0.140 (0.121)	0.009*(0.016)*
	C	1.732 (1.532)	1.231 (1.637)	12.49 (11.18)	0.107 (0.113)	0.019*(0.029)*
Variance	A	4.821 (5.286)	3.372 (3.080)	33.62 (353.6)	0.044 (0.021)	0.001 (0.002)
	B	3.030 (2.294)	2.430 (0.832)	91.46 (80.59)	0.020 (0.015)	<0.001 (<0.001)
	C	3.001 (2.348)	1.516 (2.682)	156.1 (125.1)	0.011 (0.013)	<0.001 (<0.001)

Values by slant view observation are shown in parentheses. G lesion = G band grayscale value of the tumor; R lesion = R band grayscale value of the tumor; G background = G band grayscale value of the background; SD = standard deviation.

* $p < 0.001$, compared to raw values of the G band grayscale of the tumor and the index of G lesion/G background.

ces and standard deviations were 0.99 ± 0.31, 0.77 ± 0.21, 0.75 ± 0.05, 0.74 ± 0.17 and 0.61 ± 0.03 in the M, SM, MP, SS and SE groups, respectively. The mean AF index of the M group was statistically significantly larger than those of the remaining groups ($p < 0.05$). However, the mean AF index of the SM group did not show a significant difference in comparison with those of the advanced stage groups (MP, SS and SE). The area under the ROC curve in the diagnostic value of the AF index for gastric cancer invading within the mucosal layer was 0.87 (95% CI 0.79–0.95), as shown in figure 5. In this field, the cutoff value of the AF index was decided as 0.82. When the cutoff value of the AF index for the diagnosis of the tumor invasion grade of gastric cancer with depth M was set at 0.82, the diagnostic sensitivity was 0.82 and the specificity was 0.65.

On the other hand, thicker tumors had weaker autofluorescence intensity, i.e., a lower AF index. A statistically significant negative correlation ($r = -0.024$, $p = 0.011$) was found between these two parameters. The relationship between AF indices and mean tumor thickness is shown in figure 6.

Discussion

Conventional endoscopy for gastric cancer in its incipient stages was thought to be a useful tool that could yield information regarding tumor detection, tumor characteristics, and perhaps, the extent of tumor invasion. In particular, the necessity for assessing the invasion depth of early gastric cancer is a very important fac-

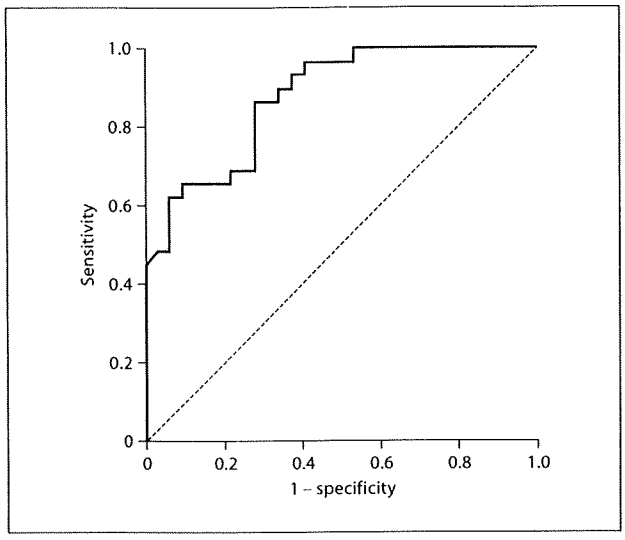

Fig. 5. The receiver operator characteristic (ROC) curve of the diagnostic value of AFI endoscopy for gastric cancer invading within the mucosal layer (M). The area under the ROC curve was 0.87 (95% CI 0.79–0.95).

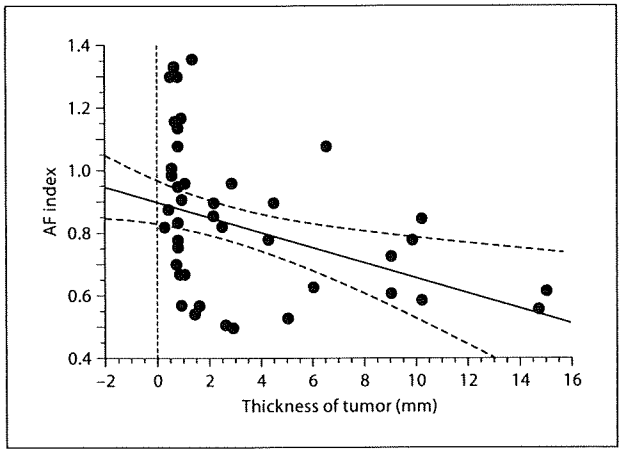

Fig. 6. Relationship between the autofluorescence (AF) index and the mean thickness of 72 gastric cancers. The AF index was also related to the mean thickness of the tumor (r = −0.024, p = 0.011). Dotted lines show the ranges of a 95% confidence interval.

tor in deciding to undertake an endoscopic therapeutic procedure [27, 28]. The diagnostic method with the best outcome for evaluating tumor invasion is endoscopic ultrasonography (EUS) [29–32]. However, even with EUS, there are notable limitations involving the diagnosis of

Table 2. Relationship between G band grayscale values in preventative cases with gastric cancer, the G band grayscale ratio of the tumor to the background and the G/R grayscale ratio of the tumor at different distances in the stomach

Lesion	Distance	Grayscale values			Indices	
		G tumor	R tumor	G background	G tumor/G background	G tumor/R tumor
A	Near	57.65	49.48	60.21	0.957	1.165
	Middle	48.26	41.35	62.11	0.749	1.167
	Far	21.02	18.06	26.23	0.801	1.163
B	Near	52.64	61.49	53.72	0.979	0.856
	Middle	41.06	47.94	54.77	0.821	0.856
	Far	19.24	22.39	26.64	0.722	0.859
C	Near	72.78	113.89	66.32	1.097	0.639
	Middle	32.82	51.12	39.97	0.801	0.642
	Far	29.04	46.09	49.05	0.592	0.630
SD	A	19.02	16.30	20.18	0.108	0.002*
	B	16.95	19.85	15.94	0.129	0.001*
	C	24.23	37.77	13.38	0.253	0.006*
Variance	A	361.9	265.9	407.6	0.012	<0.001
	B	287.6	394.2	254.2	0.017	<0.001
	C	587.3	1427	179.1	0.064	<0.001

G lesion = G band grayscale value of the tumor; R lesion = R band grayscale value of the tumor; G background = G band grayscale value of the background; SD = standard deviation. Lesion A = 0-IIa type cancer, depth M; lesion B = 0-IIa+IIc type cancer, depth SM-3; lesion C = elevated type cancer, depth SS.

* $p < 0.001$, compared to raw values of the G band grayscale of the tumor and the index of G lesion/G background.

the depth of tumor invasion [33]. For example, in tumors of the protruded type, depth of invasion diagnosis is prevented due to central attenuation of the ultrasound [34, 35]. Accordingly, there is no reliable method available that renders a thorough diagnosis with respect to the depth of tumor invasion.

In recent studies, there has been little evidence supporting the efficacy of AFI endoscopy for the early detection of small gastric neoplasms [6–8]. Abe et al. [6] reported that the overall detection rates of gastric cancer with the light-induced fluorescence endoscopy system were 92.3 and 100% in the early and advanced stage. Their system was composed of a mercury lamp with 437 nm of excitation blue-light wavelength, and 490–560 or ≥630 nm of detection wavelengths of the autofluorescence. On the other hand, the AFI system used in this

study was composed of a xenon arc lamp with 390–470 nm blue-light wavelengths for excitation and 550 nm of green-light wavelength for the reflective image of the autofluorescence. Based on the differences between the two systems, the image quality for the detection of gastric cancer may be different. Consequently, all gastric cancer could be detected with the AFI endoscopy system used in this study, even if the tumor stage is very early or the histological finding is undifferentiated. Moreover, AFI endoscopy may play another role for cases with gastric cancer, such as in the diagnosis of tumor invasion depth. However, no reports have affirmed the validity of AFI endoscopy for the diagnosis of tumor invasion depth. Among the endogenous fluorophores, the most important contributor is the collagen of the submucosal layer [20, 23–25]. The emission spectrum of collagen is about 350–450 nm [36, 37], and these waves are excited mainly by the AFI system used in the present study. When tumor occupation of the submucosal layer is significantly large, the autofluorescence detected by the AFI endoscope diminishes remarkably. In contrast, tumors in the superficial mucosal layer may not show decreased autofluorescence. Therefore, the tumor invasion depth quite possibly influences the interference of the emission of autofluorescence, and subsequently the intensity of autofluorescence may indicate the degree of tumor invasion. Moreover, the AF index defined as the best marker for autofluorescence intensity was easily detectable, and its reliability was elucidated by both analyses of an animal model and a human stomach.

In the present study, gastric cancer with an invasion depth of M could be discriminated from those with other invasion depths. AF indices (G/R ratio) were not influenced by the distance or the angle of observation or by the variations in the autofluorescence intensity of the background mucosa. The index consisted of the ratio of the autofluorescence intensity to the grayscale value of the R band image in the area of interest after de-gamma processing of the endoscopic images, and the latter subsequently showed a constant value in every condition. We found in the present study that there is a marked difference in AF indices between tumors with an invasion depth of M and other grades. This difference may be derived from residual collagen fibers in the submucosal layer that have not yet been replaced by cancer cells when the carcinoma occupies only the superficial mucosal layer. For the endoscopic treatment of early gastric cancer, it is important to decide whether the tumor invades within M or the superficial layer of the submucosa, SM-1 [27, 28]. However, the differences in AF indices cannot be evaluated between cancers with M and SM-1 invasion depths, as there was no case of gastric cancer with SM-1 invasion during this study period. In the present study, most cases with SM invasion showed patterns of massive invasion (SM-2 or SM-3). Therefore, it follows that further study may elucidate whether the AF index can discriminate tumors within the M group from those within the SM-1 group. Because the diagnostic specificity is not high, a definite conclusion stating that the autofluorescence intensity of gastric cancer decides the grading of invasion depth cannot be made. However, because the diagnostic sensitivity is high, AFI endoscopy indeed plays an important role in the diagnosis of invasion depth in the present study. Further developments of the AFI system may more closely contribute to the diagnostic yield for tumor invasion.

One of the critical issues to bear in mind is the histological subtype of the gastric cancer. Autofluorescence in signet ring cell carcinoma was reported to show an atypical pattern due to the presence of collagen production induced by the cancerous lesion [38]. In addition, gastric cancers with ulceration were excluded from the present study because whitish, ulcerative mucosal exudates show an extremely high AF index that interferes with the assessment of the lesion's autofluorescence intensity. It has also been reported in the analysis with an EUS study that ulcerative changes decrease the diagnostic accuracy of invasion depth in cases of early gastric cancer [39]. It may be necessary to improve the current diagnostic method or to create a novel system to analyze invasion depth adequately in cases of gastric cancer with ulceration.

In conclusion, AFI endoscopy may be helpful for determining tumor invasion depth in gastric cancers exhibiting no ulceration. However, to demonstrate the validity of this method as a diagnostic tool, an expanded study comprised of larger numbers of patients and lesions of varying types is required.

References

1 Takehana S, Kaneko M, Mizuno H: Endoscopic diagnostic system using autofluorescence. Diagn Ther Endosc 1999;5:59–63.
2 Mayinger B, Horner P, Jordan M, Gerlach C, Horbach T, Hohenberger W, Hahn EG: Endoscopic fluorescence spectroscopy in the upper GI tract for the detection of GI cancer: initial experience. Am J Gastroenterol 2001; 96:2616–2621.
3 Mayinger B, Horner P, Jordan M, Gerlach C, Horbach T, Hohenberger W, Hahn EG: Light-induced autofluorescence spectroscopy for tissue diagnosis of GI lesions. Gastrointest Endosc 2000;52:395–400.