症例で身につける
消化器内視鏡シリーズ

Knack and pitfall of gastrointestinal endoscopy in Esophageal and gastric ESD with IT knife based on case study

食道・胃ESD

ITナイフによるESDの実際

第1章 治療法選択のための術前内視鏡診断
② 病変部の通常・色素内視鏡診断

1. 食道

森田周子，武藤 学

内視鏡治療の対象になる食道表在癌は内視鏡所見が乏しいことが多く，注意深く観察することが望ましい．粘液や残渣が多い場合には，丹念にガスコン水で洗浄し粘液を十分に除去した状態で観察する必要がある．ヨード色素内視鏡の際にも，粘液付着はヨードの染色性を低下させ診断の妨げになるため，粘液や残渣などの付着物は十分に洗い流しておく必要がある．

通常観察による病変の拾い上げ

食道表在癌は内視鏡所見に乏しいため白色光による通常観察では発見が難しい場合が多い．ヨード色素内視鏡による病変の拾い上げの有効性は広く認識されているが[1]，刺激性が強いためすべての被験者に行うには決して楽で安全な検査とは言いがたい．そこで，いかに病変の最初の拾い上げを通常観察で行うかが重要になってくる．

まず，観察しやすい環境作りが大切である．食道の粘液や残渣を十分に除去してわずかな変化を観察しやすくするために，咽頭麻酔前にガスコン® ドロップとプロナーゼ® を溶かした微温湯（2％ガスコン® ドロップ 20mL，プロナーゼ® 1/900g，炭酸水素ナトリウム 2/900g，蒸留水 10mL）を服用してもらい，食道に内視鏡を挿入した後も，ガスコン水にて食道壁を十分に洗浄する．

次に，粘液のない状態で食道を観察して，粘膜面の発赤，白苔付着の有無，凹凸不整，光沢の消失，正常血管網の消失を探す（図1 A，B）．食道表在癌の凹凸はごく浅い陥凹や低い隆起であり，送気量が多いと病変が平坦になって認識しづらくなる（図1 D）．そこで食道の観察時には軽度脱気するなど，送気量を変えながら観察したほうが病変を拾い上げやすい場合がある（図1 E）．

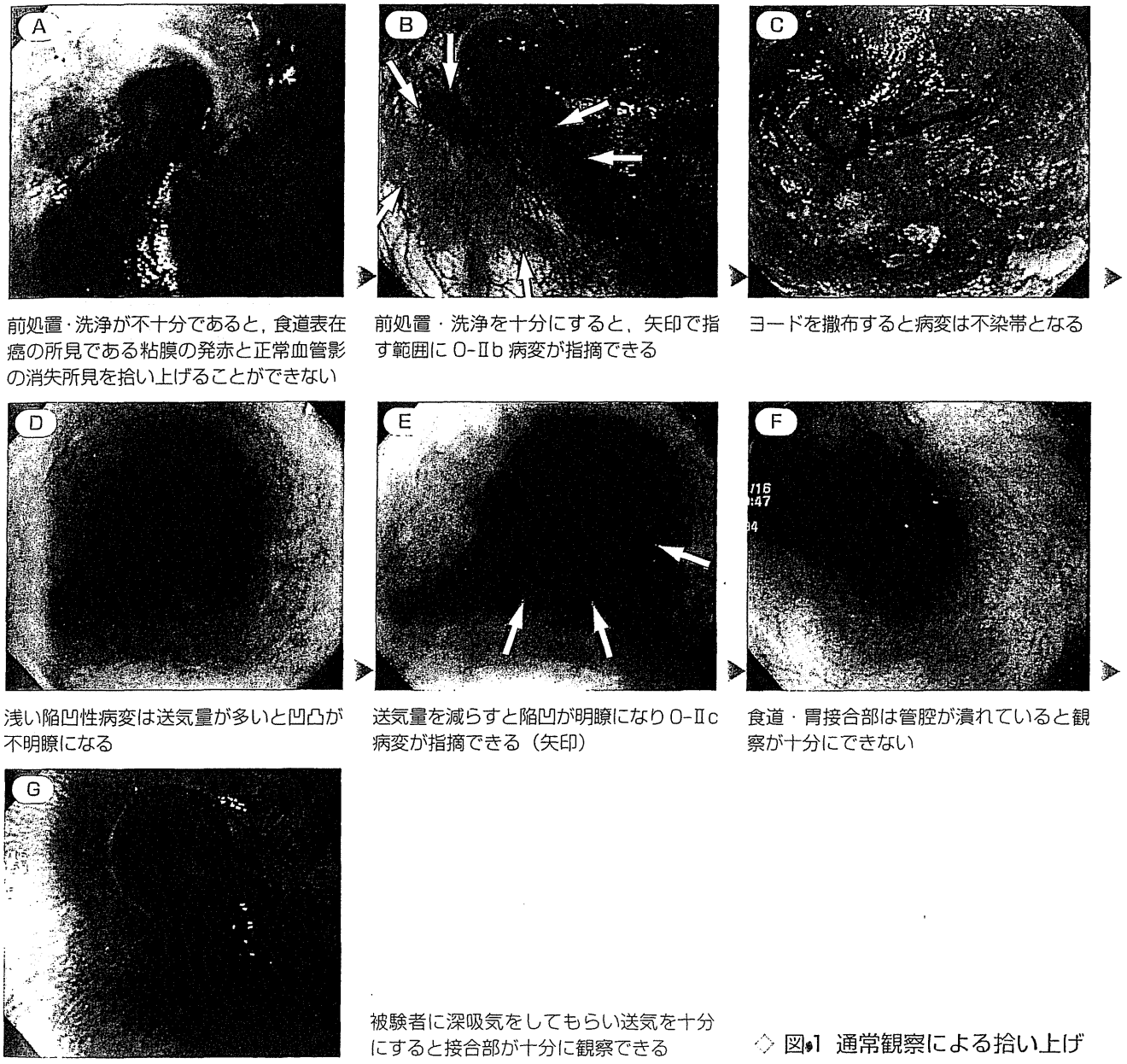
管腔を十分拡張させて観察することが難しい食道入口部と食道胃接合部では，呼吸を取り入れて観察する．食道入口部は，内視鏡を抜きながら観察し息を吐かせた状態にすると内腔が広がる瞬間があるのでそのタイミングを逃さず観察する．食道胃接合部では被験者に深吸気をしてもらうことで，食道粘膜と胃粘膜の連続性が観察しやすくなる（図1 F，G）．

頭頸部・食道癌の既往歴がある，アルコール多飲者であるといった高リスクの症例ではヨード染色を行う（図1 C）．

ヨード染色による詳細観察

病変の存在を疑う所見を認めた場合，癌かどうかの質的診断と，病変の範囲および深達度を診断するために詳細な観察をする必要がある．質的診断にはヨード染色法と基礎編-第1章-③-1で述べる narrow band imaging（NBI）が有用であるが，ここではヨード染色法について記載する．

粘液を十分に除去した後，1.5％ヨードを食道全体または部分的に撒布する（図1 C）．食道全体に撒布する理由は，多発癌の頻度が多いことがあげられる．また，ヨード液を撒布する際の注意点として，被検者の苦痛や誤嚥などのリスクを軽減するために過剰な量を撒布しないこ



A：前処置・洗浄が不十分であると，食道表在癌の所見である粘膜の発赤と正常血管影の消失所見を拾い上げることができない

B：前処置・洗浄を十分にすると，矢印で指す範囲に 0-IIb 病変が指摘できる

C：ヨードを撒布すると病変は不染帯となる

D：浅い陥凹性病変は送気量が多いと凹凸が不明瞭になる

E：送気量を減らすと陥凹が明瞭になり 0-IIc 病変が指摘できる（矢印）

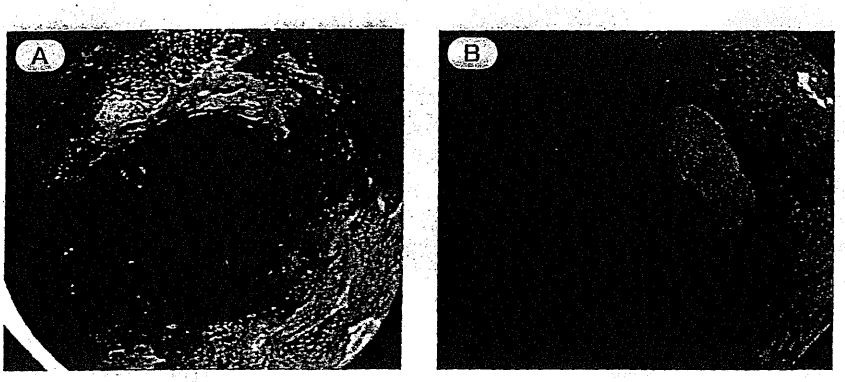
F：食道・胃接合部は管腔が潰れていると観察が十分にできない

G：被験者に深吸気をしてもらい送気を十分にすると接合部が十分に観察できる

◇ 図1 通常観察による拾い上げ

と，染色後は速やかに観察することがあげられる．われわれは，内視鏡を切歯 17 ～ 18cm まで引き抜いた後に，撒布チューブを鉗子口から肛門側に約 10 ～ 15cm 出してヨードを撒布しながら撒布チューブを鉗子口に引き戻す方法を推奨している．この場合，ヨード液は重力によって前壁側（9 時の方向）にたまりやすくなるので，後壁側（3 時の方向）に向けて撒布するのがまんべんなく染色するコツである．いったん目的の食道壁が染色されたら，引き続いて内視鏡を非染色部まで挿入し同じ作業を繰り返す．この行程により，2 ～ 3 回の撒布で食道全体が染色され，使用するヨード液も約 10mL 程度に抑えられる．逆に，撒布チューブを用いる場合でも胃食道接合部から撒布を始めると 20mL 以上の撒布量になることが多い．また，20 ～ 30mL のシリンジでヨード液を大胆に撒布し，不足した場合に追加撒布すると数 10mL の撒布量になるので，被検者へ与える苦痛は大きくなる．余分なヨード液は随時吸引して，観察の妨げにならないようにする．

ヨード撒布直後には正常上皮部ではヨードが茶色に発色してくるので，癌を示す境界明瞭なヨード不染の有無を観察する．また撒布から 3 ～ 4 分経過すると，癌部はピンク色に変化する pink color sign（PC sign）を呈するため，質的診断が容易になる[2]．この PC sign は感度・特



ヨード撒布から3～4分経過するとヨードがやや褪色しかけて，癌によるヨード不染帯はピンク色に変化する pink color sign（PC sign）が陽性となる

PC sign 陽性

◆図2 PC sign

異度が90％を超えるため質的診断にはきわめて有用である（**図2**）．

MEMO

多発ヨード不染を伴う場合は，不染の大きさや形態や境界の明瞭さ，不染なのか淡染なのか，PC sign の有無などを総合して，治療が必要な不染，生検すべき不染，経過観察が必要な不染かどうかを判断する．

注意 **食道表在癌にヨードを撒布すると，表層部のみが脱落して非腫瘍性上皮が再生するため基底層型癌になることがある[3]．基底層型癌はヨードで染色されるため，病変範囲を誤る可能性がある．そこで確実な内視鏡切除を行うためには，初回のヨード染色像を参考にして切除範囲を決める方がよい．**

深達度診断

内視鏡治療の適応になりうる壁深達度 SM1 までを深達度別に解説する（基礎編－第2章－1 図1参照）．

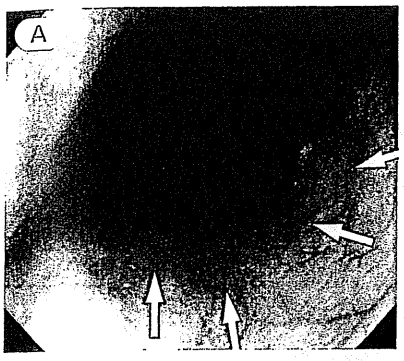
1 EP・LPM

EP（粘膜上皮）・LPM（粘膜固有層）の病変は平坦な 0-Ⅱb 病変（**図3 A**）と，ごく浅い陥凹性病変（0-Ⅱc：**図3 B**）と，高さが 1mm までの低い白色調の隆起性病変（0-Ⅱa）が含まれる（**図3 C**）．EP の大部分は 0-Ⅱb 病変である．0-Ⅱc 病変では辺縁隆起を伴っておらず，いずれも病変内は平坦であるか細顆粒状である．ヨードを撒布すると境界が明瞭な不染帯となり，不染帯は時間とともにピンク色になる PC sign が陽性になる．不染帯内部にはヨードに染まる非腫瘍扁平上皮が散在することが多い．また EP，LPM の病変では，送気量を調節すると畳み目ひだと呼ばれる粘膜筋板の収縮である横ひだが病変内部に観察できる（**図3 D**）．

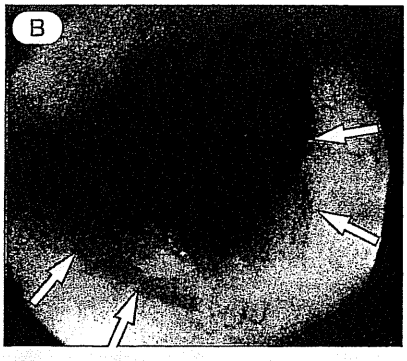
2 MM・SM1

深達度 SM1 とは，粘膜下層（SM）を3等分し，上 1/3 にとどまる病変であり，これは内視鏡的に切除された標本では粘膜筋板（MM）から 200 μm 以内の粘膜下層にとどまる病変と定義している[4]．

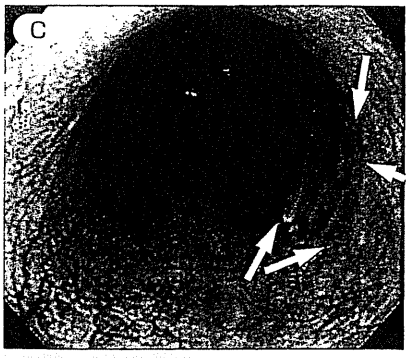
MM・SM1 の 0-Ⅱa 病変は，高さが 2mm くらいのやや高い隆起で，隆起の立ち上がりは比較的なだらかである．0-Ⅱc 病変は極軽度の辺縁隆起を伴う浅い陥凹で，陥凹内部には顆粒状の変化を伴う（**図3 E**）．ヨードを撒布すると境界が明瞭な不染帯となり，PC sign が陽性になる．MM，SM1 の病変では畳み目ひだが消失し（**図3 F**），輪状筋の収縮によって出現する縦ひだに太まりが出現する（**図3 G**）．



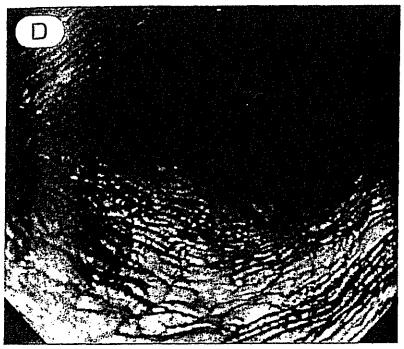
深達度 M1 ・ M2 の 0-Ⅱb 病変（矢印）



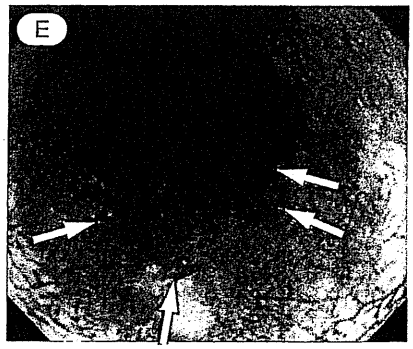
深達度 M1 ・ M2 の 0-Ⅱc 病変（矢印）



深達度 M1 ・ M2 の白色調の 0-Ⅱa 病変（矢印）

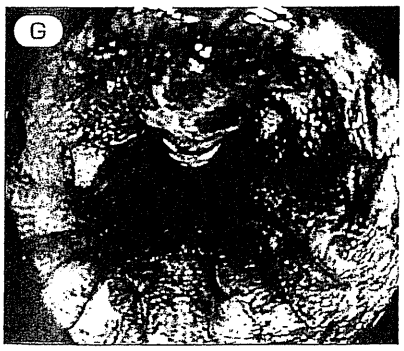


ヨード染色像．M1，M2 の病変では，送気量を調節すると畳み目ひだと呼ばれる粘膜筋板の収縮である横襞が病変内部に観察できる



深達度 M3 ・ SM1 の 0-Ⅱc 病変は極軽度の辺縁隆起を伴う浅い陥凹である（矢印）

ヨード染色像．深達度 M3，SM1 の病変では畳み目ひだが消失する



ヨード染色像．深達度 M3，SM1 の病変では輪状筋の収縮によって出現する縦ひだに太まりが出現する

図 3 深達度診断

ポイント☞

- 食道内の洗浄をしっかり行って，観察しやすくする
- 食道表在癌の所見である粘膜面の発赤，凹凸，光沢の消失，正常血管影の消失を探す
- ヨード染色法も併用して，範囲と深達度を正確に診断して内視鏡治療の適応を決定する

文　献

1）東野晃治，飯石浩康：色素内視鏡の基本．消化器内視鏡，18：660-664, 2006
2）大森 泰，横山 顯：危険なヨード不染帯所見 Pink Color sign の検討．Gastroenterol Endosc., 43：1613, 2001
3）小山恒男 他：表層拡大型食道表在癌の 1 例．胃と腸, 30：1055-1058, 1995
4）日本食道学会 編：「臨床・病理 食道癌取扱い規約 第 10 版」, p.13, 金原出版, 2007

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GASTROENTEROLOGY

Narrow-band imaging endoscopy with magnification is useful for detecting metachronous superficial pharyngeal cancer in patients with esophageal squamous cell carcinoma

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Key words
esophageal squamous cell carcinoma, head and neck cancer, magnification, narrow-band imaging, pharyngeal cancer.

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Abstract

Background and Aims: Head and neck cancers, especially pharyngeal cancers, as well as esophageal cancers frequently coexist either synchronously or metachronously, but most cases of pharyngeal cancer are detected at an advanced stage resulting in poor prognosis. The aim of this study is to evaluate the effectiveness of using narrow-band imaging (NBI) endoscopy with magnification for early detection of pharyngeal cancer on patients following their treatment for esophageal squamous cell carcinoma (SCC).

Methods: This case series was conducted at the National Cancer Center Hospital in Tokyo between April and October 2005 and included 424 consecutive patients for surveillance endoscopy who had previously undergone chemoradiotherapy (CRT) and/or surgery for esophageal SCC. Observation of the pharyngeal region was randomly conducted on 91 patients using NBI endoscopy with magnification (NBI group) and 333 patients using conventional white light endoscopy (control group).

Results: The detection rate for pharyngeal cancer was significantly higher using NBI endoscopy with magnification (10.9%; 10/91) compared with conventional endoscopy (1.2%; 4/333) ($P < 0.0001$). In particular, the detection rate in CRT patients was significantly higher in the NBI group (12.9%; 7/54) than the control group (0.5%; 1/191) ($P < 0.0001$). In addition, diagnostic sensitivity, specificity, accuracy, positive predictive value and negative predictive value for the NBI group were 100% (10/10), 97.5% (79/81), 97.8% (89/91), 83.3% (10/12) and 100% (79/79), respectively.

Conclusion: NBI endoscopy with magnification is a promising technique for detecting superficial pharyngeal cancer at an early stage in patients previously treated for esophageal SCC.

Introduction

According to the 'field cancerization' concept,[1] head and neck cancers, especially pharyngeal cancers, as well as esophageal cancers occurring from the same region of squamous epithelium, frequently coexist either synchronously or metachronously.[2–4] Because it is difficult to detect pharyngeal cancer at an early stage, however, most cases are detected at an advanced stage resulting in poor prognosis.[5] Patients surviving esophageal cancer undergo regular endoscopic examinations following surgery and/or chemoradiotherapy (CRT), but we continue to be confronted by detection of pharyngeal cancer at an advanced stage. Because prognosis is extremely poor with advanced hypopharyngeal cancer, early detection is vitally important.[6–10]

Esophageal cancer also is difficult to detect as a small or flat lesion, but early detection is possible by using Lugol solution that reveals the cancer as a Lugol-voiding lesion (LVL) white or pink in color.[11–14] This method causes severe mucosal irritation, however, leading to patient pain and discomfort. In addition, we cannot use Lugol solution to detect pharyngeal cancer because of possible aspiration to the patient's airway.

Given the problems associated with Lugol chromoendoscopy, new alternative methods of endoscopic diagnosis need to be developed and one of them, the narrow-band imaging (NBI) system, is attracting considerable attention. The usefulness of NBI in the gastrointestinal region has been reported[15–19] and some studies suggest its possible application in the diagnosis of esophageal and pharyngeal cancers.[20–23] Because oropharyngeal and hypopharyngeal mucosal sites also are squamous epithelium and cancers located there are usually squamous cell carcinoma (SCC), similar findings are likely regarding early cancer detection in those regions.[24,25]

Therefore, we performed NBI endoscopy with magnification on patients following their treatment for esophageal SCC to evaluate

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the effectiveness of the NBI system in the early detection of pharyngeal cancer possibly leading to an improved quality of life for such patients.

Methods

NBI system

The conventional video-endoscope system uses three broad-band optical filters covering all visible spectrum wavelengths. The NBI system narrows the bandwidth of spectral transmittance so the central wavelengths of the dedicated trichromatic optical filters are shifted to shorter wavelengths of 415 nm and 540 nm, respectively, with a bandwidth of 30 nm.[26,27] Mucosal surface structure is enhanced using NBI, thereby revealing more precise lesion information.

Patients

We screened for second primary pharyngeal cancer using either NBI endoscopy with magnification or conventional white light endoscopy between April and October 2005 in patients with esophageal SCC who had previously undergone surgery and/or CRT at the National Cancer Center Hospital (NCCH) in Tokyo. We examined a total of 424 consecutive patients which was the total number of patients receiving surveillance endoscopy during that period. Those patients had been followed up regularly at our institution after receiving earlier treatment. Patients who were of clinical or pathological stage IV and/or had a past history or record of treatment for head and neck cancer were excluded. If surveillance endoscopy was repeated in that period, we included only the initial one in this study. Endoscopies were performed using NBI with magnification on 91 patients as the NBI group while a control group of 333 patients received conventional endoscopies. The four NBI group endoscopists had a mean of 9.5 years of experience (range 5–15 years) while the eight control group endoscopists had a mean of 12.1 years of experience (range 5–25 years). The discrepancy in the size of the two groups was strictly the result of only one out of six endoscopic examination rooms at NCCH at that time being equipped with the NBI system. All room assignments for patients in both groups were randomly determined by the scheduling staff without any selection bias or input whatsoever from the endoscopists involved in this study. The detection rate for oropharyngeal and hypopharyngeal cancer was then evaluated in this study.

The instruments used were a magnifying endoscope with × 80 magnification (GIF-Q240Z; Olympus Optical, Tokyo, Japan) in the NBI group and conventional endoscope (GIF-Q240, H260; Olympus Optical) in the control group, a standard video-endoscope system (EVIS LUCERA; Olympus Optical) and an NBI system (Olympus Optical).

Examinations

None of the NBI group patients exhibited any symptoms in the pharynx and all of them underwent an NBI endoscopy as part of their regular examinations. Before the NBI, scopolamine butylbromide (20 mg) was administered i.v. if there were no contraindications to prevent sialorrhea. If a lesion was found during the NBI endoscopy, midazolam (2–5 mg) or pethidine (17.5–35 mg) was also administered i.v. to prevent gag reflex because of the lengthened procedure time.

Narrow-band imaging endoscopy without magnification was initially performed on the oropharyngeal and hypopharyngeal mucosal sites of the NBI group patients. We then added magnification if an abnormal mucosal area was identified during the examination. A brownish area and increased intraepithelial papillary capillary loops (IPCL) with irregularity were evaluated as endoscopic features of superficial pharyngeal cancer (Figs a–f).[21,24,28–30] Once a lesion was identified by NBI, we changed to the conventional view to determine if the lesion could be detected for comparative purposes. Lesions were then biopsied in the standard manner using NBI.

All the control group patients underwent a conventional endoscopic procedure without magnification or NBI with only scopolamine butylbromide being administered i.v. in accordance with our standard procedure. If a lesion was found during conventional endoscopy, midazolam or pethidine was administered similar to the NBI group.

Statistical analysis

All variables in this study were described in terms of mean, standard deviation (SD), median and range. In comparing baseline characteristics between the two groups, we used a Student's *t*-test for continuous variables and a χ^2-test for dichotomous variables. All statistical analyses were performed using SAS ver. 8.0 (SAS Institute, Cary, NC, USA). The *P*-values were two-sided and $P < 0.05$ was used to determine statistical significance.

Ethics

The NCCH ethics committee approved the study protocol and informed consent was obtained from all patients in the NBI group before they entered the study.

Results

There were no differences in patient characteristics between the two groups except for the number (Table 1). We identified a total of 18 superficial lesions in 14 of 424 patients (3.3%). All 14 patients were male. Fourteen lesions were detected in 10 of 91 patients (10.9%) in the NBI group and four lesions in four of 333 patients (1.2%) in the control group. The detection rate for pharyngeal cancer was significantly higher using NBI endoscopy with magnification (10.9%; 10/91) compared with conventional endoscopy (1.2%; 4/333) in esophageal SCC patients following treatment ($P < 0.0001$). The detection rate was also significantly higher among CRT patients in the NBI group (12.9%; 7/54) than in the control group (0.5%; 1/191) ($P < 0.0001$) (Table 2). In addition, diagnostic sensitivity, specificity, accuracy, positive predictive value and negative predictive value for the NBI group were 100% (10/10), 97.5% (79/81), 97.8% (89/91), 83.3% (10/12) and 100% (79/79), respectively. All lesions in both groups were determined to be SCC histologically.

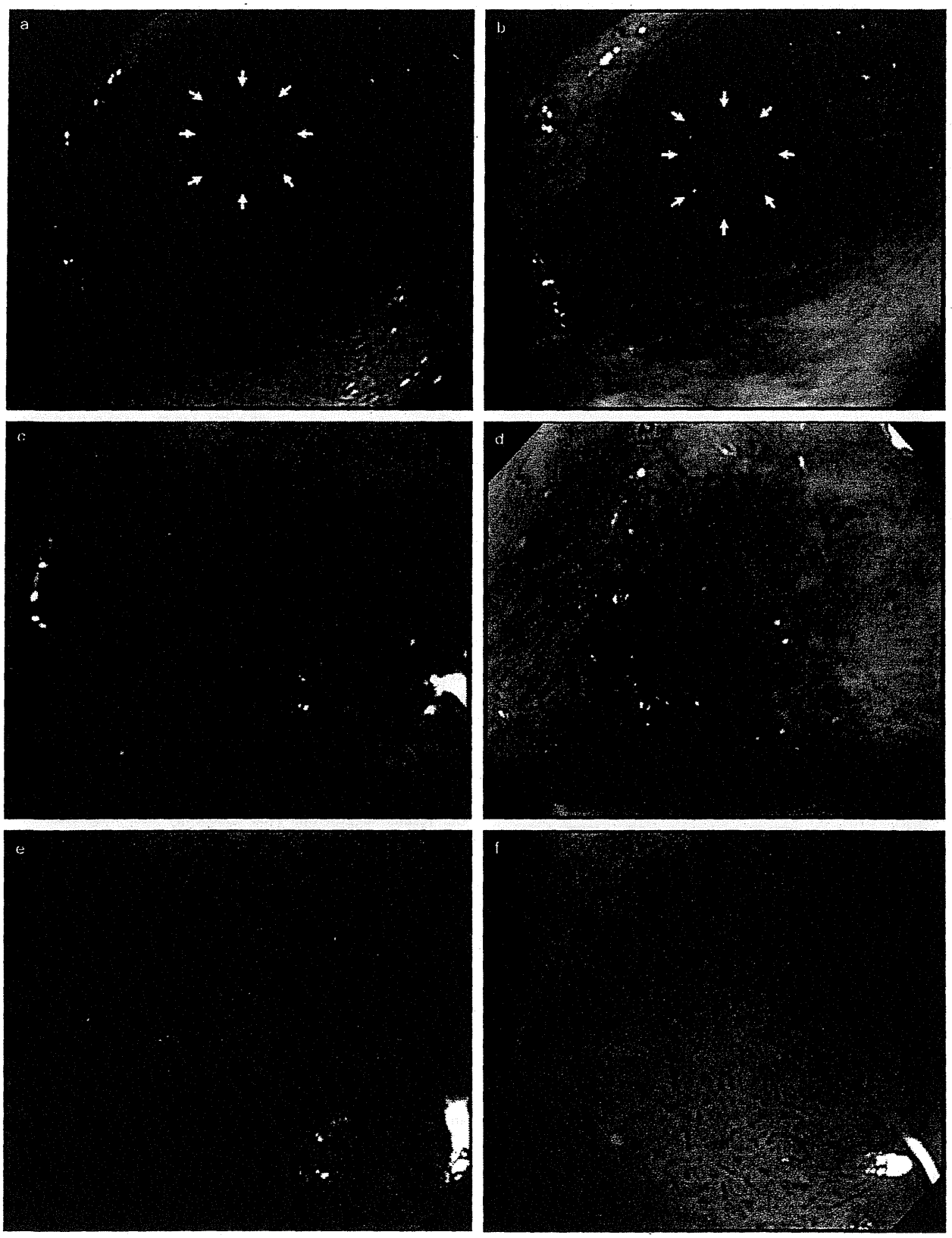

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Figure 1 Squamous cell carcinoma *in situ* in the right pyriform sinus of the hypopharynx (0-IIb; tumor diameter 10 mm). (a) Conventional endoscopy shows a slightly reddish area indicated by the arrows. (b) Narrow-band imaging endoscopy identifies a clearly demarcated brownish area indicated by the arrows. (c) Conventional endoscopy showing a close-up and slightly magnified view. (d) Narrow-band imaging endoscopy showing a close-up and slightly magnified view. The brownish area is clearly demonstrated compared with the surrounding mucosa. (e) Conventional endoscopy with magnification showing abnormal microvascular structures of the mucosa. (f) Narrow-band imaging endoscopy with magnification clearly demonstrates increased intraepithelial papillary capillary loops with irregularity on the surface of the lesion.

Table 1 Patients characteristics

	NBI	Control	*P*-value
No. of patients	91	333	–
Age (years; mean ± SD)	64.9 ± 7.9	66.1 ± 8.4	NS
Male : female	4.1:1	6.7:1	NS
CRT†/surgery (%)	59/41	57/43	NS

†Chemoradiotherapy includes three patients in the NBI group and five patients in the control group who received Radiotherapy only. CRT, chemoradiotherapy; NBI, narrow-band imaging endoscopy; NS, not significant; SD, standard deviation.

Table 2 Detection rate for pharyngeal cancer

	NBI	Control	*P*-value
Prior treatment†			
CRT	12.9% (7/54)	0.5% (1/191)	< 0.0001
Surgery	8.1% (3/37)	2.1% (3/142)	NS
Sex			
Male	13.7% (10/73)	1.4% (4/290)	< 0.0001
Female	0.0% (0/18)	0.0% (0/43)	–
Total	10.9% (10/91)	1.2% (4/333)	< 0.0001

†Initial treatment for primary esophageal cancer. CRT, chemoradiotherapy; NBI, narrow-band imaging endoscopy; NS, not significant.

Location

With the exception of five oropharynx lesions in the NBI group, all lesions in both groups were located in the hypopharynx and multifocal carcinoma was found in three patients (21%; 3/14). Eight of 14 (57%) lesions in the NBI group and two of four (50%) lesions in the control group were located on the right side (Table 3). The pyriform sinus was the most common primary site in both groups (12/18; 66%).

Macroscopic type and size

Using the esophageal cancer macroscopic classification,[31] six tumors were classified primarily as 0-IIa (43%; 6/14), five as 0-IIb (36%; 5/14), two as 0-IIc (14%; 2/14) and one as 0-I (7%; 1//14) in the NBI group and two as 0-IIc (50%; 2/4), one as 0-IIb (25%; 1/4) and one as 0-I (25%; 1/4) in the control group. The lesion size was 15.7 ± 6.8 mm (mean ± SD) in the NBI group and 17.5 ± 2.5 mm in the control group (Table 3).

Detection periods and number of examinations

The median period between the end of treatment for esophageal SCC and the detection of pharyngeal cancer was 27.6 months (range 7.1–143.5) in the NBI group and 101.0 months (range 11.0–134.5) in the control group. The median number of endoscopic examinations between initial examination and detection was 9.0 (range 2–31) in the NBI group and 8.5 (range 7–14) in the control group (Table 3).

Table 3 Characteristics of patients with pharyngeal cancer

	NBI	Control
Male/female	10/0	4/0
CRT/surgery	7/3	1/3
No. of lesions	14	4
Location†		
Oropharynx/hypopharynx	5/11	0/4
Right/center/left	8/5/3	2/1/1
Size (mm; mean ± SD)	15.7 ± 6.8	17.5 ± 2.5
0-IIa/0-IIb/0-IIc/0-I‡	6/5/2/1	0/1/2/1
Times of endoscopy (median; range)	9.0 (2–31)	8.5 (7–14)
Median period in months to pharyngeal cancer detection (range)	27.6 (7.1–143.5)	101.0 (11.0–134.5)

†Locations overlapped. ‡Macroscopic type using the esophageal cancer classification. CRT, chemoradiotherapy; NBI, narrow-band imaging endoscopy; NS, not significant; SD, standard deviation.

Discussion

Our results suggest that NBI endoscopy with magnification is superior to conventional endoscopy for the early detection of pharyngeal cancer in patients following treatment for esophageal SCC. We strongly recommend careful observation particularly in male and CRT patients. The visual recognition of lesions was significantly improved by being able to identify brownish areas and increased IPCL with irregularity as malignant disease. It was difficult to precisely diagnose simply from the existence of a brownish area, however, when an abnormal mucosal area was identified using NBI without magnification. Minute observation of the microvascular structure of the squamous epithelium for increased IPCL with irregularity was required for an accurate diagnosis.[21,28,29] Because NBI can be utilized in all endoscopes with such a system by pushing a single button, it is very easy to use and capable of becoming a valuable tool for endoscopic diagnosis particularly in the esophagus and pharynx.

In terms of esophageal cancer, careful follow up of patients who are completely cured becomes even more important with increased improvement in recent treatment outcomes.[32–34] Because it is necessary to detect pharyngeal cancer at an early stage to prevent a

poor prognosis,[35–40] NBI endoscopy with magnification is considered to be a positive advancement for ensuring a good prognosis through early detection.

Those patients, who previously underwent CRT, generally received surveillance endoscopy every 3–6 months following their treatment while surgical patients received such examinations once a year at our institution. Although it is difficult to precisely determine a suitable frequency from our results, we believe that 6–12 months is generally an acceptable interval.

Disadvantages of conventional endoscopy

Conventional endoscopy does not provide clear and comprehensible IPCL images. In addition, abnormal findings seen as reddish areas are less visually distinct than the brownish areas shown by NBI. Because Lugol chromoendoscopy cannot be performed in the pharynx because of the risk of aspiration, early detection is even more difficult as a result.

Visual recognition

In considering lesion size, the NBI group had slightly smaller lesions than the control group. In addition, the mean size of four lesions (10 ± 7.1 mm) in the NBI group which were undetected during the subsequent conventional view was smaller than that of the 10 lesions (20 ± 7.0 mm) which could be detected by careful examination during the subsequent conventional view. This suggests that NBI is particularly useful for detecting smaller lesions with no remarkable visual change.

There were more lesions detected on the right side in the NBI group. If a lesion was located on the left side, the standard endoscopic pathway, it is likely that it would have been easier to detect. A lesion on the right side is generally more difficult to detect, but use of the NBI system may help address this problem.

High-risk group

Since the estimated age standardized rate of cancer incidence (per 100 000) in the mouth and pharynx is less than five in Japan,[41] it is advisable to establish high-risk populations because screening for such cancer in all patients is virtually impossible as well as unnecessary. Careful examination of the pharynx only needs to be performed in high-risk populations including patients with esophageal cancer or multiple LVL in their background esophageal mucosa, heavy drinkers, smokers and men over 50 years of age.[42–44]

Future vision

Narrow-band imaging endoscopy with magnification should become a standard endoscopic diagnostic technique in the esophagus and pharynx regions in the future with the realistic expectation of a corresponding increase in the number of lesions detected at an early stage. Accordingly, we will need to determine diagnosis of NBI endoscopy with magnification based on observation of brownish areas and increased IPCL with irregularity and also diagnosis of the depth of invasion in the absence of muscularis mucosae in the pharynx region. In addition, we will have to investigate what kind of endoscopic treatment procedures are most feasible.

Limitations

The difference in the number of patients in the NBI group (91) and the control group (333) and the fact that our research was not based on a randomized control trial, are limitations of this study.

Conclusion

NBI endoscopy with magnification is a promising technique for detecting superficial pharyngeal cancer at an early stage in patients previously treated for esophageal SCC.

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References

1 Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer* 1953; **6**: 963–8.
2 Begg CB, Zhang ZF, Sun M *et al.* Methodology for evaluating the incidence of second primary cancers with application to smoking-related cancers from the Surveillance, Epidemiology, and End Results (SEER) program. *Am. J. Epidemiol.* 1995; **142**: 653–65.
3 Petit T, Georges C, Jung GM *et al.* Systematic esophageal endoscopy screening in patients previously treated for head and neck squamous-cell carcinoma. *Ann. Oncol.* 2001; **12**: 643–6.
4 Shibuya H, Wakita T, Nakagawa T *et al.* The relation between an esophageal cancer and associated cancers in adjacent organs. *Cancer* 1995; **76**: 101–5.
5 Japan Society for Head and Neck Cancer. *General Rules for Clinical Studies on Head and Neck Cancer*. Tokyo: Kanehara Syuppan, 2005.
6 Eckel HE, Staar S, Volling P *et al.* Surgical treatment for hypopharynx carcinoma: feasibility, mortality, and results. *Otolaryngol. Head Neck Surg.* 2001; **124**: 561–9.
7 Erkal HS, Mendenhall WM, Amdur RJ *et al.* Synchronous and metachronous squamous cell carcinomas of the head and neck mucosal sites. *J. Clin. Oncol.* 2001; **19**: 1358–62.
8 Johansen LV, Grau C, Overgaard J. Hypopharyngeal squamous cell carcinoma—treatment results in 138 consecutively admitted patients. *Acta Oncol.* 2000; **39**: 529–36.
9 Kraus DH, Zelefsky MJ, Brock HA *et al.* Combined surgery and radiation therapy for squamous cell carcinoma of the hypopharynx. *Otolaryngol. Head Neck Surg.* 1997; **116**: 637–41.
10 Wahlberg PC, Andersson KE, Biorklund AT *et al.* Carcinoma of the hypopharynx: analysis of incidence and survival in Sweden over a 30-year period. *Head Neck* 1998; **20**: 714–19.
11 Ina H, Shibuya H, Ohashi I *et al.* The frequency of a concomitant early esophageal cancer in male patients with oral and oropharyngeal cancer. Screening results using Lugol dye endoscopy. *Cancer* 1994; **73**: 2038–41.

12 Mori M, Adachi Y, Matsushima T *et al.* Lugol staining pattern and histology of esophageal lesions. *Am. J. Gastroenterol.* 1993; **88**: 701–5.
13 Muto M, Hironaka S, Nakane M *et al.* Association of multiple Lugol-voiding lesions with synchronous and metachronous esophageal squamous cell carcinoma in patients with head and neck cancer. *Gastrointest. Endosc.* 2002; **56**: 517–21.
14 Shiozaki H, Tahara H, Kobayashi K *et al.* Endoscopic screening of early esophageal cancer with the Lugol dye method in patients with head and neck cancers. *Cancer* 1990; **66**: 2068–71.
15 Machida H, Sano Y, Hamamoto Y *et al.* Narrow-band imaging in the diagnosis of colorectal mucosal lesions: a pilot study. *Endoscopy* 2004; **36**: 1094–8.
16 Nakayoshi T, Tajiri H, Matsuda K *et al.* Magnifying endoscopy combined with narrow band imaging system for early gastric cancer: correlation of vascular pattern with histopathology. *Endoscopy* 2004; **36**: 1080–4.
17 Sumiyama K, Kaise M, Nakayoshi T *et al.* Combined use of a magnifying endoscope with a narrow band imaging system and a multibending endoscope for en bloc EMR of early stage gastric cancer. *Gastrointest. Endosc.* 2004; **60**: 79–84.
18 Uedo N, Ishihara R, Iishi H *et al.* A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. *Endoscopy* 2006; **38**: 819–24.
19 Hirata M, Tanaka S, Oka S *et al.* Magnifying endoscopy with narrow band imaging for diagnosis of colorectal tumors. *Gastrointest. Endosc.* 2007; **65**: 988–95.
20 Hamamoto Y, Endo T, Nosho K *et al.* Usefulness of narrow-band imaging endoscopy for diagnosis of Barrett's esophagus. *J. Gastroenterol.* 2004; **39**: 14–20.
21 Yoshida T, Inoue H, Usui S *et al.* Narrow-band imaging system with magnifying endoscopy for superficial esophageal lesions. *Gastrointest. Endosc.* 2004; **59**: 288–95.
22 Nonaka S, Saito Y, Gotoda T *et al.* Narrow band imaging (NBI) system is promising device to detect superficial pharyngeal cancer at an early stage in patients with esophageal squamous cell carcinoma (SCC). *Gastrointest. Endosc.* 2006; **63**: AB250.
23 Nonaka S, Saito Y, Kozu T *et al.* Effectiveness of narrow-band imaging (NBI) in early detection of synchronous superficial pharyngeal cancer in patients with squamous cell carcinoma (SCC) before treatment—can NBI observation exceed endoscopic experience? *Gastrointest. Endosc.* 2007; **65**: AB339.
24 Muto M, Nakane M, Katada C *et al.* Squamous cell carcinoma in situ at oropharyngeal and hypopharyngeal mucosal sites. *Cancer* 2004; **101**: 1375–81.
25 Nonaka S, Saito Y. Endoscopic diagnosis of pharyngeal carcinoma by NBI. *Endoscopy* 2008; **40**: 347–51.
26 Gono K, Obi T, Yamaguchi M *et al.* Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J. Biomed. Opt.* 2004; **9**: 568–77.
27 Gono K, Yamazaki K, Doguchi N *et al.* Endoscopic observation of tissue by narrowband illumination. *Opt. Rev.* 2003; **10**: 211–15.
28 Inoue H, Honda T, Nagai K *et al.* Ultra-high magnification endoscopic observation of carcinoma in situ of the esophagus. *Dig. Endosc.* 1997; **9**: 16–18.
29 Kumagai Y, Inoue H, Nagai K *et al.* Magnifying endoscopy, stereoscopic microscopy, and the microvascular architecture of superficial esophageal carcinoma. *Endoscopy* 2002; **34**: 369–75.
30 Watanabe A, Tsujie H, Taniguchi M *et al.* Laryngoscopic detection of pharyngeal carcinoma in situ with narrowband imaging. *Laryngoscope* 2006; **116**: 650–4.
31 The Japan Esophageal Society. *Guide Lines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus.* Tokyo: Kanehara Syuppan, 2007.
32 Altorki N, Kent M, Ferrara C *et al.* Three-field lymph node dissection for squamous cell and adenocarcinoma of the esophagus. *Ann. Surg.* 2002; **236**: 177–83.
33 Ohtsu A, Boku N, Muro K *et al.* Definitive chemoradiotherapy for T4 and/or M1 lymph node squamous cell carcinoma of the esophagus. *J. Clin. Oncol.* 1999; **17**: 2915–21.
34 Peracchia A, Bonavina L, Ruol A *et al.* Esophageal cancer: a European perspective. *Recent Results Cancer Res.* 2000; **155**: 119–22.
35 Kumagai Y, Kawano T, Nakajima Y *et al.* Multiple primary cancers associated with esophageal carcinoma. *Surg. Today* 2001; **31**: 872–6.
36 Nagasawa S, Onda M, Sasajima K *et al.* Multiple primary malignant neoplasms in patients with esophageal cancer. *Dis. Esophagus* 2000; **13**: 226–30.
37 Natsugoe S, Matsumoto M, Okumura H *et al.* Multiple primary carcinomas with esophageal squamous cell cancer: clinicopathologic outcome. *World J. Surg.* 2005; **29**: 46–9.
38 Noguchi T, Kato T, Takeno S *et al.* Necessity of screening for multiple primary cancers in patients with esophageal cancer. *Ann. Thorac. Cardiovasc. Surg.* 2002; **8**: 336–42.
39 Shimizu Y, Tsukagoshi H, Fujita M *et al.* Head and neck cancer arising after endoscopic mucosal resection for squamous cell carcinoma of the esophagus. *Endoscopy* 2003; **35**: 322–6.
40 Watanabe A, Hosokawa M, Taniguchi M *et al.* Periodic pharyngolaryngoscopy detects early head and neck cancer and improves survival in esophageal cancer. *Ann. Thorac. Surg.* 2003; **76**: 1699–705.
41 Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int. J. Cancer.* 1999; **80**: 827–41.
42 Chyou PH, Nomura AM, Stemmermann GN. Diet, alcohol, smoking and cancer of the upper aerodigestive tract: a prospective study among Hawaii Japanese men. *Int. J. Cancer* 1995; **60**: 616–21.
43 Matsubara T, Yamada K, Nakagawa A. Risk of second primary malignancy after esophagectomy for squamous cell carcinoma of the thoracic esophagus. *J. Clin. Oncol.* 2003; **21**: 4336–41.
44 Muto M, Nakane M, Hitomi Y *et al.* Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. *Carcinogenesis* 2002; **23**: 1759–65.

Effect of Orally Administered Bovine Lactoferrin on the Growth of Adenomatous Colorectal Polyps in a Randomized, Placebo-Controlled Clinical Trial

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Abstract Lactoferrin (LF), a secreted, iron binding glycoprotein originally discovered as a component of milk, is found in a variety of exocrine secretions and in the secondary granules of polymorphonuclear leukocytes. Animal experiments have shown that oral administration of bovine lactoferrin (bLF) exerts anticarcinogenesis effects in the colon and other organs of the rat. The aim of this study was to determine whether oral bLF could inhibit the growth of adenomatous colorectal polyps in human patients. A randomized, double-blind, controlled trial was conducted in 104 participants, ages 40 to 75 years, with polyps ≤5 mm in diameter and likely to be adenomas. Participants were assigned to receive placebo, 1.5-g bLF, or 3.0-g bLF daily for 12 months. Target adenomatous polyps were monitored by colonoscopy. Ingestion of 3.0-g bLF significantly retarded adenomatous polyp growth in participants 63 years old or younger. Removal of adenomatous colorectal polyps is done as a preventative measure against colorectal cancer; however, polyps can be overlooked, and when detected, polypectomy is not always 100% effective in eradicating a polyp. Our study suggests that daily intake of 3.0 g of bLF could be a clinically beneficial adjunct to colorectal polyp extraction.

Colorectal cancer is one of the most frequent causes of death from cancer (1–5). Most colorectal cancers arise from benign adenomas (6). Adenoma formation and colorectal cancer incidence have been reported to be influenced by food elements and nutrition (7–10), making diet and dietary supplements factors in colorectal cancer. Bovine lactoferrin (bLF) isolated from cow milk has been studied for inhibition of colorectal and other cancers (11–15). Specifically, supplementation of bLF to the diet of azoxymethane-treated rats decreased the incidence of both colorectal cancer and aberrant crypt foci (12, 14, 15). Importantly, bLF has been reported to be well tolerated in clinical research (16, 17).

In the present study, we conducted a randomized controlled trial to evaluate whether a 1-year oral intake of bLF-containing tablets (Morinaga Milk Industry Co. Ltd.) inhibits the growth of colorectal polyps <5 mm in diameter with pit pattern III. Because polyps with pit pattern III have been reported to be histologically adenomatous in ~90% of the cases (18–20) and polyps <5 mm in diameter show a tendency to grow in size (21), our target lesion is suitable for the current study and measurement of polyp diameter is a promising surrogate end point. We found a statistically significant retardation of polyp growth in participants 63 years old or younger ingesting 3.0-g bLF daily over the course of 12 months.

Ingestion of bLF inhibits colorectal carcinogenesis in animal studies (12, 14, 15). In addition, ingestion of bLF enhances immune function, both in animal studies (22, 23) and in human patients (24). Finally, numerous studies have revealed the crucial role of the immune system in inhibiting the neoplastic process (25). Therefore, immunologic parameters associated with bLF ingestion were measured: interleukin-18 and IFNγ, because ingestion of bLF enhances expression of interleukin-18 and IFNγ in the mouse small intestine (22); T-cell subpopulation numbers, because ingestion of bLF reconstitutes T-cell populations in immunosuppressed mice (26); natural killer (NK) cell number and activity, because ingestion of bLF enhances NK cell activity in rats (12); and neutrophil number, because

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ingestion of bLF increases the number of neutrophil precursor cells in human patients (24, 27). In addition, because LF is an important component of the human immune system (23, 28–31), we also measured the levels of human lactoferrin (hLF) in the serum of trial participants.

At the end of the trial period, the target lesions were removed and examined. The number of neutrophils in these specimens was of particular interest: Several reports indicate that neutrophils can enhance tumor growth (32–35), but in rodents, ingestion of bLF attenuates the movement of neutrophils to the small intestine (36).

Materials and Methods

Trial profile

An outline of the trial profile is shown in Supplementary Fig. S1. The trial was initiated after approval by the Ethical Committee of the National Cancer Center Hospital, Tokyo, Japan, and continued until January 2006. This study is registered in the University Hospital Medical Information Network Clinical Trials Registry (Tokyo, Japan; no. C000000182). The Independent Data Monitoring Committee determined the trial should have approximately 105 participants. Between February 2002 and January 2005, 307 patients scheduled for colonoscopic examination at the National Cancer Center Hospital, Tokyo, Japan, were invited to join the trial; patients were approached before their examinations. Of these, 215 patients provided written informed consent.

The 215 potential trial participants underwent their scheduled colonoscopic examinations, and during the examination, potential target polyps were marked by injection of india ink close to the polyp and photographed. Patients were excluded from the trial who met any of the following criteria: no target lesion present; dairy product allergies; use of nonsteroidal anti-inflammatory drugs (NSAID) or statins for >50 d in the previous 3 mo (use of these drugs could affect polyp size refs. 37, 38); diagnosed as having cancer; a history of colectomy within the previous 3 y; inflammatory bowel disease, familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer; or active infection such as hepatitis B or C. Patients with a history of cancer were included in the trial only if they were diagnosed as being cured of cancer and unlikely to suffer a relapse for at least 1 y. Ultimately, 108 participants ages 40 to 75 y with ≤5-mm-diameter polyps showing a pit pattern III were enrolled in the trial; the classification of pit patterns was based on Kudo's classification (39).

Participants were randomized (Randomization Center; Japan Clinical Research Support Unit, Tokyo, Japan) and assigned to one of three treatment groups (the placebo group, the 1.5-g bLF group, or the 3.0-g bLF group). Trial participation commenced within 30 d after colonoscopic examination. At commencement, patients underwent their initial trial interviews.

Participants took six tablets orally everyday for 12 mo. One tablet (1.5 g) contained bLF at 0, 250, or 500 mg. In addition, the tablets contained carbohydrate (D-sorbitol, maltitol, and corn starch), but not fat or dietary fiber. The caloric value of six tablets was 36 kcal for all groups. Tablets were designed to be indistinguishable from each other in appearance, smell, and taste. Good compliance was defined as having taken two thirds or more of the tablets prescribed, and poor compliance as having taken less than two thirds of the tablets prescribed. Intake of any product containing bLF was prohibited throughout the entire study period. Participants were verbally instructed to continue with their usual food (especially fat and fiber), alcohol, and supplement intake at trial commencement and 3, 6, and 9 mo, although they were not requested to record their meals during the trial period. Treatment assignments and participant assessments were not revealed to investigators, participants, or the sponsor over the study period.

Colonoscopy

Endoscopists performed initial and final total colonoscopic examinations with zoom colonoscopes (CF-240ZI, PCF-240ZI, and CF-200Z, Olympus). During the initial examination, before the commencement of the trial, all lesions detected by colonoscopy were observed at 100-fold magnification after spraying 0.2% indigo carmine dye over the lesion to easily differentiate polyp from normal mucosa and to more clearly observe the pit patterns of the polyps. Of these, ≤5-mm-diameter polyps with pit pattern III at the most proximal sites of the right colon (cecum to transverse colon) and the left colon (descending colon to rectum) were identified as target lesions. The classification of pit patterns was based on Kudo's classification (39). This classification system assigns colonic lesions into six categories: types I and II are designated nonneoplastic; types IIIS, IIIL, IV, and V are designated neoplastic. Type IIIS shows small tubular or roundish pits; type IIIL shows large tubular or roundish pits. Macroscopically, intramucosal polypoid growths (pedunculated polypoid lesions and sessile and broad-based polypoid lesions) and nonpolypoid growths showing infiltration of tumor cells below the submucosal layer were excluded from being used as target lesions because they would most probably show malignant characteristics (40–43). These lesions and >5-mm-diameter polyps with pit pattern III were removed at the time of detection. A total of 119 lesions in the full analysis set (FAS) population (104 participants) with pit pattern III were identified as target polyps.

Target polyps were photographed and marked by injection of india ink close to the polyp. Target polyp size was estimated using both open biopsy forceps (44–46) and a 5-mm paper disc and open scale forceps (see Supplementary data for a description of the use of the 5-mm paper disc to estimate polyp size). At the end of the trial period, target polyps were identified by location and the presence of the india ink marker. The size and pit pattern of the polyps were measured by endoscopy, and the polyps were excised for examination. (All other premalignant and malignant growths were also removed at this time.) Target polyps were immediately fixed in phosphate-buffered neutral formalin, embedded in paraffin, and stained with H&E.

The Endoscopic Data Adjudication Committee (Drs. Akasu and Gotoda) evaluated the size of the polyps and pit pattern by photographs. Excised lesions were histopathologically diagnosed by a board-certified pathologist.

Assessment of safety

To assess safety, medical health checkups and evaluation of clinical findings through diary writing and patient interviews were done at trial commencement and every 3 mo. Safety analyses were done for all participants who took at least one tablet.

Immunologic parameters

Peripheral blood was collected from participants at trial commencement (before treatment), 3 mo, 6 mo, 9 mo, and 12 mo. Parameters to be measured were selected based on the results of previous experimental studies (12, 14, 15, 47). ELISA, lymphocyte subset (CD4, CD8, CD16, and CD56) measurement, and NK cell activity were measured as described below.

ELISA. Peripheral blood was collected from trial participants as noted above. ELISA for hLF was done as follows. Microtiter plates were coated with a mouse anti-hLF antibody (mouse, clone: 2B8, IgG1, Advanced Immuno Chemical) and incubated at 5°C overnight. To block the wells, 250 μL of 0.5% gelatin in PBS were added to the wells and the plate was incubated at 37°C for 1 h. One hundred microliters of sample were applied to the blocked wells and the plate was incubated at 5°C overnight. Captured hLF was detected using horseradish peroxidase–labeled polyclonal antibodies against hLF (rabbit; Cappel) and visualization was done using *o*-phenylenediamine (Sigma). The minimal detectable concentration of hLF was ~200 pg/mL; the minimal detectable concentration of bLF was >20 μg/mL. An ELISA specific for bLF using a specific monoclonal

antibody against bLF, developed by Morinaga Milk Industry, was done similarly. Briefly, microtiter plates were coated with capture antibody (anti-bLF rabbit polyclonal, Morinaga Milk Industry) and blocked, and then 100-μL sample was applied. Captured bLF was detected using a biotin-labeled anti-bLF polyclonal antibody (rabbit; Nacalai, Japan) and visualized with horseradish peroxidase–labeled streptavidin (Zymed) and *o*-phenylenediamine. The minimal detectable concentration of bLF was ~500 pg/mL; the minimal detectable concentration of hLF was >20 μg/mL. An ELISA kit (minimum detection limit, 25.0 pg/mL; Medical & Biological Laboratories Co. Ltd.) was used to measure mature human interleukin-18. Human IFNγ levels were determined by SRL, Inc.

Lymphocyte subsets (CD4, CD8, CD16, and CD56). Peripheral blood was collected from trial participants as noted above. Blood samples were diluted with saline, and lymphocytes were separated from the diluted blood samples using Ficoll-Conray solution (relative density, 1.077; IBL). Fluorescence-activated cell sorting was used to isolate the different lymphocyte subsets. The following antibodies were used for immunofluorescence staining of lymphocytes: two-color antihuman CD4-FITC (T4, Beckman Coulter), CD8-RD1 (T8, Beckman Coulter), CD16-FITC (Becton Dickinson), and CD56-RD1 (Beckman Coulter). Relative fluorescence intensities of single- or two-color staining were then measured with a FACSCalibur (Becton Dickinson).

NK cell activity. Peripheral blood was collected from trial participants and lymphocytes isolated using Ficoll-Conray solution as noted above. The entire lymphocyte population (containing effector cells) was washed twice with PBS. Cell killing activity was then measured using K-562 cells labeled with ^{51}Cr as target cells. Target cells (1×10^6/mL, 10 μL) and effector cells (1×10^6/mL, 200 μL) were mixed and incubated at 37°C, 5% CO_2 for 3.5 h. The medium was then removed and clarified by centrifugation, and soluble ^{51}Cr released by killed K-562 cells was measured with a gamma-counter (1470 Wizard, Perkin-Elmer Life and Analytical Sciences).

Polymorphonuclear leukocyte infiltration into target polyps

At the end of the trial period, a final colonoscopic examination was done and target polyps (and all other premalignant and malignant growths found) were removed. Target polyps were immediately fixed in buffered formalin, embedded in paraffin, and stained with H&E, followed by histopathologic examination. Polymorphonuclear leukocytes (PMN) were counted in the stroma of adenomatous polyps. Only polyp sections containing at least five atypical adenoma glands and mucosa propria to a depth equal to at least the average gland diameter (d) were used. PMNs in the stroma of five glands and the underlying mucosa propria to depth d were counted. The area was measured using an image analysis system (Image Processor for Analytical Pathology, Sumika Technos Corp.).

Statistical analyses

Fifteen participants had two target polyps; these participants were assessed according to the change in the average of the diameter of the two polyps. Polyp assessment was done based on the Response Evaluation Criteria in Solid Tumors (48). Analysis of covariance and Dunnett's multiple comparison test were used to compare the change in polyp size in the LF-treated groups with that in the placebo group, using the initial polyp size as a covariate. The assumption for analysis of covariance was checked, and there was no interaction between baseline and treatment. To check the interaction between treatment outcome and each prognostic factor (age, sex, presence or absence of previous colectomy, and site of target lesion), multiple regression analysis was done with the following four variables: treatment, sum of target-lesion diameters by colonoscopy at trial commencement, factor, and factor-by-treatment interaction. For grouping by age, trial participants were divided into two groups: participants at or below the overall median age of the trial participants (≤63) and participants above the overall median age of the trial participants (>63). For prognostic factors showing significant interaction with treatment outcome, subgroup analyses were done separately in each subgroup population. The data for NK activity and hLF levels were analyzed using Dunnett's test. Pearson coefficients of correlation were calculated to determine degrees of association between different variables. Levels of significance were set at 0.05 (two-sided) for all statistical analyses. All calculations were conducted using SAS version 8.2 (SAS Institute).

Results

Disposition of subjects

The trial profile is shown in Supplementary Fig. S1 and detailed in Materials and Methods. Briefly, over the course of ~3 years (February 2002 to January 2005), patients scheduled for a colonoscopic examination were approached before their examinations and invited to join the trial. Each of the 215 patients who agreed to join the trail provided written informed consent and underwent their scheduled examination. During this examination, potential target polyps were marked and photographed for further evaluation by the Endoscopic Data Adjudication Committee. Each of these patients also underwent a pre-trial interview to determine their suitability for inclusion in the trial. For each trial participant, trial commencement began within 30 days after their initial colonoscopic examination.

Of the 215 patients who initially agreed to join the trial, 108 patients met the trial criteria and agreed to continue with the trail. These 108 patients were each enrolled and randomized into one of three treatment groups (the placebo group, the 1.5-g bLF group, or the 3.0-g bLF group). After randomization into treatment groups, two participants originally enrolled into the trial were excluded: One participant assigned to the placebo group did not have a target polyp, as judged by the Endoscopic Data Adjudication Committee after further evaluation of the photographs taken during the initial colonoscopic examination, and during the initial trial interview, one participant assigned to the 3.0-g bLF group was found to have used statins. Therefore, the initial trial population consisted of 106 participants. Table 1 shows the characteristics of this population at trial commencement. The overall age (mean ± SD) was 62.4 ± 6.9 years. Eighty-seven subjects (82.1%) were men. A total of 121 polyps with pit pattern III were identified by colonoscopy in this population, and the estimated diameter (mean ± SD) was 3.5 ± 0.9 mm. All those (n = 27) with a history of colectomy had undergone it as a result of colorectal cancer.

Two participants withdrew from the trial after commencement (both in the placebo group). The FAS population for the trial, therefore, consisted of 104 participants. The per-protocol set (PPS) population included 102 participants after exclusion of two from the FAS population who used NSAIDs (both in the 3.0-g bLF group). The FAS population was used to analyze the effects of bLF treatment on target polyp size, and the PPS population was used to analyze the effects of bLF treatment on immunologic parameters; see Supplementary data for a brief explanation of our use of the FAS and PPS population data.

Compliance

In the FAS population (n = 104), tablet intake rates (mean ± SD) were 92.1 ± 9.0% in the placebo group (n = 33), 94.3 ± 5.0% in the 1.5-g bLF group (n = 37), and 92.1 ± 9.3% in the 3.0-g

Table 1. Characteristics of the initial trial population at trial commencement

	Placebo group (n = 35)	1.5-g bLF group (n = 37)	3.0-g bLF group (n = 34)
Age (y), mean ± SD	63.0 ± 6.4	61.4 ± 7.3	63.0 ± 6.8
Male, n (%)	29 (82.9)	29 (78.4)	29 (85.3)
Height (cm), mean ± SD	165.4 ± 7.4	164.5 ± 7.0	164.4 ± 7.8
Weight (kg), mean ± SD	64.2 ± 7.6	65.9 ± 11.9	63.9 ± 9.0
Previous colectomy, n (%)	10 (28.6)	8 (21.6)	9 (26.5)
Alcohol consumption, n (%)	26 (74.3)	28 (75.7)	26 (76.5)
Smoking, n (%)	5 (14.3)	13 (35.1)	8 (23.5)
Target-lesion diameters (mm) by colonoscopy, mean ± SD	4.0 ± 1.4	3.8 ± 1.4	4.1 ± 1.7
Site of target lesions by colonoscopy			
Right colon, n (%)	23 (65.7)	23 (62.2)	20 (58.8)
Left colon, n (%)	7 (20.0)	9 (24.3)	9 (26.5)
Right and left colon, n (%)	5 (14.3)	5 (13.5)	5 (14.7)

bLF group (n = 34). There was poor compliance (as defined in Materials and Methods) from two participants: one participant of two withdrawing consent from the placebo group (tablet intake rate, 54.6%) and one participant of two excluded from the 3.0-g bLF group due to NSAID use (tablet intake rate, 51.7%).

Pit patterns and histologic diagnosis

A total of 119 polyps in the FAS population with pit pattern III were identified by colonoscopy as target polyps. The pit pattern of two (both in the 3.0-g bLF group) changed into pit pattern I (regular round crypts, normal mucosa) at 12 months. All the others showed pit pattern III. Of these lesions, 91 were histologically diagnosed: 31 in the placebo group, 30 in the 1.5-g bLF group, and 30 in the 3.0-g bLF group. (Twenty-eight polyps were used up during RNA extraction; the analysis of the RNA is ongoing and not part of this report.) Eighty-nine (97.8%) were adenomas and two were hyperplasias (both in the 1.5-g bLF group), verifying our identification of polyps with pit pattern III as a predictor of adenoma.

Safety

One participant in each of the three groups had an adverse event for which a causal relationship with tablets could not be determined: A mild decrease in triacylglycerol levels was observed in a participant in the placebo group; a mild increase in alkaline phosphatase levels was observed in a participant in the 1.5-g bLF group; and a moderate increase in total bilirubin levels (with a mild increase observed at trial commencement) was observed in a participant in the 3.0-g bLF group. Levels of alkaline phosphatase and total bilirubin spontaneously returned to normal after the end of the study treatment. In the 3.0-g bLF group, lung metastases from colorectal cancer were observed in one participant and liver metastases from colorectal cancer were observed in a second participant (both participants had a history of colon cancer). Because both were diagnosed with metastases within 1 week before the end of the treatment and were found to have no laboratory abnormalities associated with them, the treatment was continued and completed. No other serious adverse events occurred during this study, and the safety of the treatment was confirmed.

Efficacy of bLF treatment on polyp size

A comparison of the change in polyp size among treatment groups in the FAS population is shown in Table 2. Although the differences between LF-treated groups and the placebo group were not significant (P = 0.098), some reduction in polyp diameter in the 3.0-g bLF group (−0.2 mm, 4.9% regression) was observed, whereas an increment in polyp size was observed in the placebo group (+0.2 mm, 5.0% increase). Similar results were obtained in the analysis of the PPS population (Table 2).

A comparison of the change in polyp size among groups and in subgroups (age or sex) is shown in Table 2. Multiple regression analysis revealed both age-by-treatment (P = 0.034) and sex-by-treatment (P = 0.043) interactions. Significant retardation of target polyp diameter was found in participants ≤63 years of age ingesting 3.0 g of bLF per day (P = 0.006; Table 2) and possibly in female participants ingesting 3.0 g of bLF per day (P = 0.019; Table 2). The number of female participants, however, was small; therefore, whereas the retardation of target polyp diameter was statistically significant, the effect of bLF in women needs to be confirmed in trials with a larger number of female participants.

The number and site of target lesions and the presence or absence of previous colectomy showed nonsignificant interaction with treatment outcome (P = 0.39 and P = 0.57, respectively).

Efficacy of bLF treatment on immunologic parameters

The effect of bLF ingestion on polyp size was equivalent in the FAS and PPS populations (Supplementary Table S1); therefore, the PPS population was used to examine the effect of bLF treatment on immunologic parameters (see Supplementary data for a brief explanation of our use of the FAS and PPS population data).

Ingestion of 3.0-g bLF resulted in significantly elevated serum hLF levels (P < 0.001; Fig. 1A), suggesting that ingestion of 3.0-g bLF affected the immune system. Multiple regression analysis revealed age-by-treatment interaction on serum levels of hLF (P < 0.001). As with the effect of bLF on polyp size, bLF significantly affected serum levels of hLF only in participants ≤63 years of age. In the ≤63 years age subgroup (n = 54),

serum hLF levels changed by −1.63 ± 10.69 ng/mL in the placebo group and by 25.43 ± 19.35 ng/mL in the 3.0-g bLF group ($P < 0.001$); in the ≥64 years age subgroup ($n = 48$), serum hLF levels changed by −3.18 ± 5.69 ng/mL in the placebo group and by 5.25 ± 14.16 ng/mL in the 3.0-g bLF group ($P = 0.079$). Analysis of serum hLF levels in participants ingesting 3.0-g bLF revealed that in this group, induction of hLF weakened with aging ($r = -0.642$, $P < 0.001$; Fig. 1B). Serum levels of (ingested) bLF were below the limit of detection in all groups (data not shown).

Ingestion of 1.5-g bLF increased NK cell activity ($P = 0.048$; Fig. 2); however, the increase in NK cell activity in participants ingesting 3.0-g bLF did not attain statistical significance ($P = 0.058$). There was no age-by-treatment interaction on NK cell activity ($P = 0.911$).

For all other immunologic parameters measured, no differences between LF-treated groups and the placebo group were observed (data not shown).

Changes in polyp size and possible associated factors

As noted above, the effect of bLF ingestion on polyp size was equivalent in the FAS and PPS populations. Therefore, the PPS population was used to examine correlations between changes in target polyp size and NK cell activity, serum hLF levels, and PMN infiltration into target polyps.

Participants with growth-retarded polyps had significantly higher NK cell activity compared with participants with growing polyps ($P = 0.037$; Supplementary Fig. S2A). In addition, increased NK cell activity correlated with increases in the $CD16^+/CD56^+$ subset of NK cells in the blood ($r = 0.371$,

Table 2. Analysis of the growth of polyps by colonoscopy

	Baseline,* mm ± SD	Change,† mm ± SD (%)	95% CI,‡ mm	P§
Variable				
Full analysis set ($n = 104$)‖				
Placebo group ($n = 33$)	4.0 ± 1.4	0.2 ± 0.8 (5.0)		
1.5-g bLF group ($n = 37$)	3.8 ± 1.4	0.1 ± 0.8 (2.6)	−0.70, 0.26	0.490
3.0-g bLF group ($n = 34$)	4.1 ± 1.7	−0.2 ± 1.3 (−4.9)	−0.91, 0.07	0.098
Per-protocol set ($n = 102$)				
Placebo group ($n = 33$)	4.0 ± 1.4	0.2 ± 0.8 (5.0)		
1.5-g bLF group ($n = 37$)	3.8 ± 1.4	0.1 ± 0.8 (2.6)	−0.70, 0.26	0.500
3.0-g bLF group ($n = 32$)	4.1 ± 1.8	−0.2 ± 1.3 (−4.9)	−0.95, 0.05	0.081
Subgroup				
Age ($n = 104$)				
≤63-year-old ($n = 55$)¶				
Placebo group ($n = 18$)	3.9 ± 1.5	0.5 ± 0.8 (12.8)		
1.5-g bLF group ($n = 21$)	4.0 ± 1.6	−0.1 ± 0.5 (−2.5)	−1.08, 0.03	0.066
3.0-g bLF group ($n = 16$)	4.0 ± 1.7	−0.4 ± 1.3 (−10)	−1.41, −0.22	0.006
≥64-year-old ($n = 49$)				
Placebo group ($n = 15$)	4.1 ± 1.3	−0.1 ± 0.8 (−2.4)		
1.5-g bLF group ($n = 16$)	3.5 ± 1.2	0.3 ± 1.0 (8.6)	−0.66, 1.02	0.840
3.0-g bLF group ($n = 18$)	4.1 ± 1.8	−0.1 ± 1.3 (−2,4)	−0.82, 0.80	>0.950
Sex ($n = 104$)				
Male ($n = 85$)				
Placebo group ($n = 27$)	3.9 ± 1.4	0.2 ± 0.8 (5.1)		
1.5-g bLF group ($n = 29$)	3.9 ± 1.5	0.1 ± 0.9 (2.6)	−0.66, 0.39	0.790
3.0-g bLF group ($n = 29$)	4.1 ± 1.9	−0.1 ± 1.2 (−2.4)	−0.72, 0.33	0.600
Female ($n = 19$)				
Placebo group ($n = 6$)	4.6 ± 1.2	0.3 ± 0.9 (6.5)		
1.5-g bLF group ($n = 8$)	3.4 ± 0.6	0.1 ± 0.4 (2.9)	−2.06, 0.73	0.410
3.0-g bLF group ($n = 5$)	3.7 ± 0.5	−1.1 ± 1.4 (−29.7)	−3.10, −0.29	0.019

Abbreviation: 95% CI, 95% confidence interval.

*Baseline refers to the polyp measurements obtained during the initial examination.

†Difference between initial polyp measurements (baseline) and month 12 values. Percentages show the ratio of mean change to mean baseline values.

‡95% CI with Dunnett's adjustment using the baseline value as a covariate.

§*P* values versus placebo, calculated by Dunnett's test using the baseline value as a covariate.

‖Two participants in the placebo group who withdrew from the trial after commencement were excluded from the full analysis set population. As a result, 104 participants were analyzed.

¶The overall median age of the trial participants is 63 y.

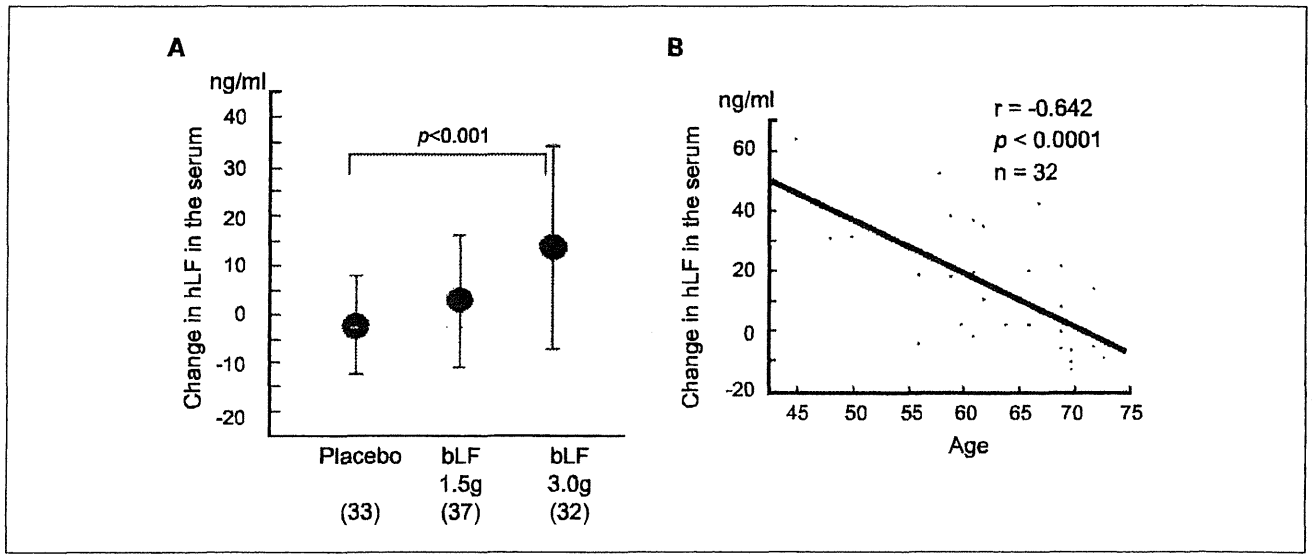

Fig. 1. Serum levels of hLF were measured every 3 mo during the trial period. Changes in serum hLF over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) are shown (see Supplementary data for a description of the PPS population). *A*, effect of bLF ingestion on serum levels of hLF in the PPS population. *B*, correlation between age and hLF induction in PPS members ingesting 3.0-g bLF. The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

$P < 0.001$; Supplementary Fig. S2B). Our data suggest that higher NK cell activity may be associated with retardation of target polyp growth.

Participants with growth-retarded polyps had higher levels of hLF in their serum than participants with growing polyps ($r = -0.279$, $P = 0.005$; Fig. 3A). Our data suggest a possible negative correlation between serum hLF levels and polyp growth; however, because bLF ingestion affects both polyp growth and serum hLF in the ≤63 years age subgroup, the association between hLF and polyp growth is currently unknown.

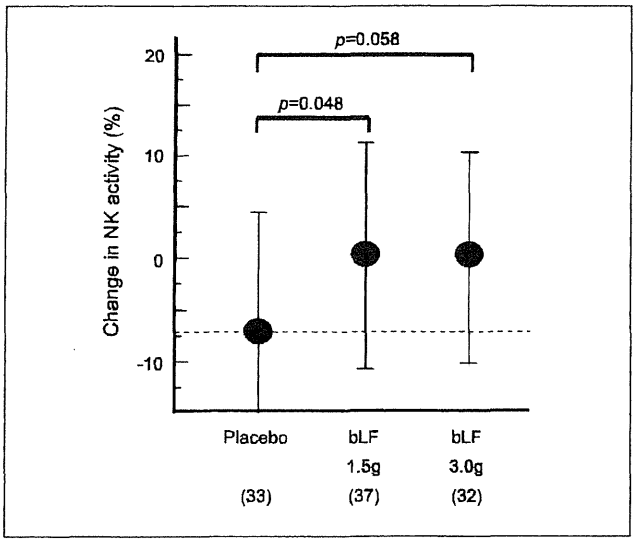

Fig. 2. NK cell activity in the serum was measured every 3 mo during the trial period. Changes in NK cell activity over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) are shown (see Supplementary data for a description of the PPS population). The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

Growth-retarded polyps contained a lower density of PMNs compared with faster-growing polyps. A total of 91 target polyps were histologically diagnosed, and the density of PMNs in the stroma surrounding the target polyps could be determined in 88 of these polyps, defining a subgroup referred to as the PPS population with countable PMNs (PCP). In the PCP subgroup, the stroma surrounding growth-retarded polyps contained a lower density of PMNs than the stroma surrounding growing polyps ($r = 0.440$, $P < 0.001$; Fig. 3B). There was no statistically significant age-by-treatment interaction on PMN infiltration into target polyps ($n = 88$, $P = 0.819$).

Finally, PCP subgroup participants in whom ingestion of bLF resulted in induction of serum hLF levels by more than 5 ng/mL had a lower density of PMNs in the stroma surrounding the target polyps than PCP subgroup participants in whom ingestion of bLF resulted in induction of serum LF levels by 5 ng/mL or less ($P = 0.021$; Fig. 3C). These results suggest a possible negative correlation between serum levels of hLF and PMN infiltration into the stroma surrounding adenomatous polyps; however, because the variables being measured were not independent, the actual association between hLF and PMN infiltration into the target polyp is currently unknown.

Discussion

Diet and dietary supplements are factors in colorectal cancers (7–10). When used as a dietary supplement, bLF isolated from cow milk decreases the incidence of both colorectal cancer and aberrant crypt foci in animal models (12, 14, 15). We conducted a randomized, double-blind, controlled trial with patients ages 40 to 75 years with ≤5-mm-diameter adenomatous colorectal polyps to determine whether supplementation of bLF to the human diet had an effect on these

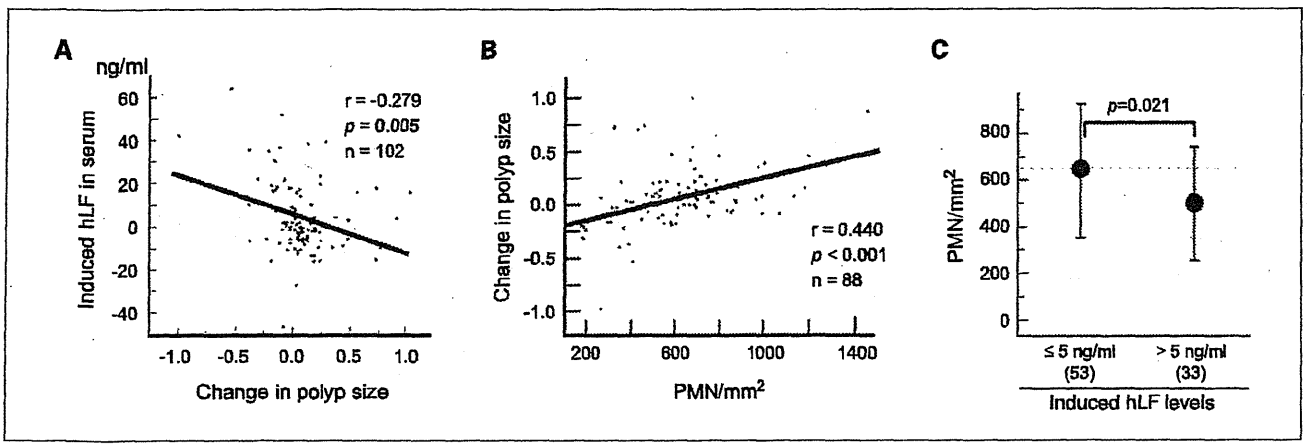

Fig. 3. Comparison of the change in target polyp size, change in serum levels of hLF, and the density of PMNs in the stroma of the target polyps. *A*, changes in serum hLF over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) were determined (see Supplementary data for a description of PPS) and the change in hLF levels was compared with the change in the size of the targets polyps in these participants. *B*, a total of 91 target polyps from the PPS population were histologically diagnosed. The density of PMNs in the stroma surrounding the target polyps could be determined in 88 of these polyps; this defines a PPS subgroup referred to as the PPS population with countable PMNs (PCP) subgroup. The change in polyp size was compared with the density of PMNs at the target polyp in the PCP subgroup. *C*, PMN counts at the target polyps of PCP members in whom ingestion of bLF resulted in induction of serum hLF by 5 ng/mL or less and participants in whom ingestion of bLF resulted in induction of serum hLF by more than 5 ng/mL. Relative change in polyp size (*A* and *B*) was determined as [(size at the end of the trial/initial size) − 1]; this method normalizes the changes in polyp sizes, with negative values representing regressing polyps and positive values representing growing polyps. In *C*, 5 ng/mL was the mean change in serum hLF levels among the participants during the course of this study (i.e., $\frac{\sum_{i=1}^{n}(hLF_{after}-hLF_{before})i}{n}$ = 5 ng/mL. The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

polyps. Participants were assigned to receive placebo, 1.5-g bLF, or 3.0-g bLF daily for 12 months. In sum, a 1-year oral intake of 3.0 g of bLF per day induced statistically significant retardation of colorectal adenomatous polyp size in participants 63 years old or younger.

The beneficial effect of bLF may be more prominent in women than in men (see Table 2). However, because of the small number of women taking part in the present study, the retardation of polyp size in the 3.0-g bLF group versus the placebo group, although statistically significant, is not a reliable finding. Further studies with an increased number of participants are warranted.

The exact mechanisms by which bLF affects colorectal polyps in humans were not directly addressed in this clinical trial. One possibility is that oral intake of bLF affects human immunologic activities, and that this in turn retards polyp growth; immune modulation mediated by oral administration of bLF has been shown in animal models (12, 22, 47, 49, 50). Notably, at the time of writing of this article, a study sponsored by the National Cancer Institute[8] is recruiting participants to examine the effects of talactoferrin, a recombinant form of hLF that has been successfully tested in phase II clinical trials with patients with refractory metastatic renal cell carcinoma (51), on the immune system and its effectiveness in treating non–small cell lung cancer.

Intake of 3.0 g of bLF increased serum hLF levels (Fig. 1A). Because hLF is an important component of the innate immune system (23, 28–31), the increase in hLF levels suggests that ingestion of bLF did affect the immune system. Importantly, increased serum hLF correlated well with retardation of polyp size (Fig. 3A). However, whereas ingestion of bLF induced serum hLF levels and serum hLF levels correlated with retardation of polyp size, a statistically significant association between bLF ingestion and retardation of polyp size was obtained only in participants 63 years old or younger. Intriguingly, the ability of bLF ingestion to elevate serum hLF weakened with aging (Fig. 1B and C), suggesting that the effect of bLF on immune function may weaken with age, and this weakening may be the reason that bLF inhibition of adenomatous polyp growth seems to be age dependent.

The mechanisms by which ingested bLF exerts its effects on the immune system are currently being studied in several laboratories (see ref. 23). Possibly, ingested bLF peptides interact with gut-associated lymphoid tissue. Several pathways have been proposed, but the exact cell types and the receptors with which ingested bLF interacts remain undefined.

Another consideration is that LF is an iron chelator (23), and the bLF used in this study was approximately 10% to 20% iron saturated. Consequently, this bLF had a high iron chelating ability. Tumor cells, like all cells, require iron, and removal of iron from the tumor environment results in regression of tumor growth (52, 53). However, the ability of bLF peptides to chelate iron after passage of the bLF-containing tablets through the stomach and small intestine has not been determined, and due to the nature of this trial, we were unable to measure iron levels in the target polyp environment. Therefore, the effect, if any, of the iron chelating ability of bLF on target polyp growth is unknown, but it is a possible factor.

LF is found in a variety of exocrine secretions (e.g., tears, nasal exudate, saliva, bronchial mucus, gastrointestinal fluids, bile, cervicovaginal mucus, and seminal fluid); it is also a major component of the secondary granules of neutrophils and is released by activated neutrophils (see ref. 23). Therefore, should ingested bLF cause activation of neutrophils, this would result in elevated serum hLF levels. Due to the nature of this study, however, we could not directly measure neutrophil activity in the serum samples obtained

[8] Available from: http://ClinicalTrials.gov; identifier NCT00923741.

from the participants in the trial. Consequently, bLF-mediated induction of serum hLF levels via activation of neutrophils is hypothetical, and it remains a possibility that induction of serum hLF proceeds wholly or in part by other routes.

The induction of serum hLF is most likely due to the effect of ingested bLF on the immune system. Whether serum hLF itself caused regression of polyp growth or was simply a consequence of bLF treatment is not known, and the exact relationship between tumor growth and serum hLF levels was not elucidated in this study. The major difficulty in determining whether serum hLF affects polyp growth is the lack of knowledge of possible mechanisms by which serum hLF could affect polyp growth.

NK cells are a principal effector of immunosurveillance against tumors (54). The effect of bLF on NK cell activity in this study was inconsistent. There was a tendency for NK cell activity to increase in participants ingesting bLF, and the increases were statistically significant in participants ingesting 1.5 g of bLF per day, but not in participants ingesting 3.0 g of bLF. Importantly, participants with growth-retarded polyps had higher NK cell activity than participants with growing polyps. Therefore, whereas this study did not establish a relationship between ingestion of bLF and NK cell activity, it remains a reasonable possibility that ingestion of bLF is associated with increased NK cell activity, and that this increased NK cell activity may have retarded polyp growth. A second trial with an increased number of participants extended over a longer period of time is needed to resolve these points.

Infiltration of PMNs into a tumor site can enhance tumor growth (32–35). In this study, participants with higher induction of serum hLF levels had both retarded polyp growth (Fig. 3A) and a lower density of PMNs in the stroma surrounding their target polyps (Fig. 3C). In rodents, oral administration of recombinant human LF attenuates neutrophil migration to the intestine (36). Therefore, in our study, bLF acting directly in the colon or possibly through serum hLF may have inhibited PMN infiltration into the target area.

In summary, ingestion of bLF had two significant effects: First, it resulted in regression of polyp growth in participants ≤63 years of age; second, it resulted in increased serum hLF levels in participants ≤63 years of age. In addition, induction of serum hLF was statistically associated with decreased infiltration of PMNs into the target area, and decreased infiltration of PMNs into the target area correlated with decreased polyp growth. Finally, enhanced NK cell activity was associated with decreased polyp growth, and there was a tendency (not consistent statistically) for NK cell activity to be enhanced in participants ingesting bLF. Taken together, our findings suggest that ingested bLF inhibits the growth of adenomatous colon polyps and that this inhibition proceeds via bLF modulation of immune system function.

Colonoscopy with clearing of neoplasms by polypectomy significantly reduces colorectal cancer; however, colorectal cancer incidence after clearing colonoscopy is appreciable (55). Factors considered to be involved in colorectal cancer that arise after clearing colonoscopy include detection failures during colonoscopy and incomplete polyp extraction (55, 56). Agents associated with retardation of adenomas are likely to reduce colorectal cancer risk because the cumulative incidences of progression from ≥10-mm-diameter polyps to cancer at 5, 10, and 20 years are reported to be 2.5%, 8%, and 24%, respectively (57), significantly higher than those for 2- to 5-mm-diameter polyps (58). Therefore, a supplement effective in the retardation of polyp growth would be a clinically useful adjunct to colorectal polyp extraction. Cyclooxygenase-2 inhibitors have been shown both to decrease the incidence of sporadic colorectal polyps (38) and to induce regression of colorectal polyps already present (44, 45); however, these drugs can have severe adverse effects (38, 44). Similarly, NSAIDs can be beneficial, but because of possible severe adverse effects, the U.S. Preventive Services Task Force recommends against their routine use to prevent colorectal cancer in individuals at average risk (59, 60). bLF is well tolerated in preclinical (61) and clinical research studies (16, 17), and no severe adverse effects related to bLF were observed in the present trial. Our study suggests that daily intake of 3.0 g of bLF could be a useful adjunct to colorectal polyp extraction.

Disclosure of Potential Conflicts of Interest

T. Kozu, G. Iinuma, Y. Saito, T. Akasu, D. Saito, and T. Kakizoe report receiving funding from the Morinaga Milk Industry Co. Ltd. in accordance with the provisions of their respective institutions; Y. Ohashi reports receiving consultancy fees from Morinaga Milk Industry. D.B. Alexander, M. Iigo, and H. Tsuda report no potential conflict of interest.

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References

1. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007;18:581–92.
2. Kuriki K, Tajima K. The increasing incidence of colorectal cancer and the preventive strategy in Japan. Asian Pac J Cancer Prev 2006;7:495–501.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
4. Rim SH, Seeff L, Ahmed F, King JB, Coughlin SS. Colorectal cancer incidence in the United States, 1999-2004: an updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. Cancer 2009;115:1967–76.
5. Toyoda Y, Nakayama T, Ito Y, Ioka A, Tsukuma H. Trends in colorectal cancer incidence by subsite in Osaka, Japan. Jpn J Clin Oncol 2009;39:189–91.
6. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. Cancer 1975;36:2251–70.
7. Kim YS, Young MR, Bobe G, Colburn NH, Milner JA. Bioactive food components, inflammatory targets, and cancer prevention. Cancer Prev Res 2009;2:200–8.
8. Wirfalt E, Midthune D, Reedy J, et al. Associations between food patterns defined by cluster analysis and colorectal cancer incidence in the NIH-AARP diet and health study. Eur J Clin Nutr 2009;63:707–17.
9. Reedy J, Mitrou PN, Krebs-Smith SM, et al. Index-based dietary patterns and risk of colorectal cancer: the NIH-AARP Diet and Health Study. Am J Epidemiol 2008;168:38–48.

10. Marshall JR. Prevention of colorectal cancer: diet, chemoprevention, and lifestyle. Gastroenterol Clin North Am 2008;37:73–82, vi.
11. Masuda C, Wanibuchi H, Sekine K, et al. Chemopreventive effects of bovine lactoferrin on *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced rat bladder carcinogenesis. Jpn J Cancer Res 2000;91:582–8.
12. Sekine K, Ushida Y, Kuhara T, et al. Inhibition of initiation and early stage development of aberrant crypt foci and enhanced natural killer activity in male rats administered bovine lactoferrin concomitantly with azoxymethane. Cancer Lett 1997;121: 211–6.
13. Tanaka T, Kawabata K, Kohno H, et al. Chemopreventive effect of bovine lactoferrin on 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in male F344 rats. Jpn J Cancer Res 2000;91: 25–33.
14. Tsuda H, Fukamachi K, Xu J, et al. Prevention of carcinogenesis and cancer metastasis by bovine lactoferrin. Proc Jpn Acad Ser B 2006;82:208–15.
15. Tsuda H, Sekine K, Nakamura J, et al. Inhibition of azoxymethane initiated colon tumor and aberrant crypt foci development by bovine lactoferrin administration in F344 rats. Adv Exp Med Biol 1998;443: 273–84.
16. Tanaka K, Ikeda M, Nozaki A, et al. Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. Jpn J Cancer Res 1999;90:367–71.
17. Ueno H, Sato T, Yamamoto S, et al. Randomized, double-blind, placebo-controlled trial of bovine lactoferrin in patients with chronic hepatitis C. Cancer Sci 2006;97:1105–10.
18. Kudo S, Rubio CA, Teixeira CR, Kashida H, Kogure E. Pit pattern in colorectal neoplasia: endoscopic magnifying view. Endoscopy 2001;33: 367–73.
19. Su MY, Ho YP, Chen PC, et al. Magnifying endoscopy with indigo carmine contrast for differential diagnosis of neoplastic and nonneoplastic colonic polyps. Dig Dis Sci 2004;49:1123–7.
20. Togashi K, Konishi F, Ishizuka T, Sato T, Senba S, Kanazawa K. Efficacy of magnifying endoscopy in the differential diagnosis of neoplastic and nonneoplastic polyps of the large bowel. Dis Colon Rectum 1999;42:1602–8.
21. Hofstad B, Vatn MH, Andersen SN, et al. Growth of colorectal polyps: redetection and evaluation of unresected polyps for a period of three years. Gut 1996;39:449–56.
22. Iigo M, Alexander DB, Long N, et al. Anticarcinogenesis pathways activated by bovine lactoferrin in the murine small intestine. Biochimie 2009; 91:86–101.
23. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J. Lactoferrin structure and functions. Adv Exp Med Biol 2008;606:163–94.
24. Zimecki M, Wlaszczyk A, Wojciechowski R, Dawiskiba J, Kruzel M. Lactoferrin regulates the immune responses in post-surgical patients. Arch Immunol Ther Exp (Warsz) 2001;49:325–33.
25. de la Cruz-Merino L, Grande-Pulido E, Albero-Tamarit A, Codes-Manuel de Villena ME. Cancer and immune response: old and new evidence for future challenges. Oncologist 2008;13:1246–54.
26. Artym J, Zimecki M, Kruzel ML. Reconstitution of the cellular immune response by lactoferrin in cyclophosphamide-treated mice is correlated with renewal of T cell compartment. Immunobiology 2003;207:197–205.
27. Zimecki M, Spiegel K, Wlaszczyk A, Kubler A, Kruzel ML. Lactoferrin increases the output of neutrophil precursors and attenuates the spontaneous production of TNF-α and IL-6 by peripheral blood cells. Arch Immunol Ther Exp (Warsz) 1999;47: 113–8.
28. Kruzel ML, Actor JK, Boldogh I, Zimecki M. Lactoferrin in health and disease. Postepy Hig Med Dosw (Online) 2007;61:261–7.
29. Lonnerdal B, Iyer S. Lactoferrin: molecular structure and biological function. Annu Rev Nutr 1995; 15:93–110.
30. Spadaro M, Caorsi C, Ceruti P, et al. Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells. FASEB J 2008;22:2747–57.
31. Ward PP, Paz E, Conneely OM. Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci 2005;62:2540–8.
32. Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res 2005;65: 8896–904.
33. van den Tol MP, ten Raa S, van Grevenstein WM, van Rossen ME, Jeekel J, van Eijck CH. The post-surgical inflammatory response provokes enhanced tumour recurrence: a crucial role for neutrophils. Dig Surg 2007;24:388–94.
34. Wada Y, Yoshida K, Tsutani Y, et al. Neutrophil elastase induces cell proliferation and migration by the release of TGF-α, PDGF and VEGF in esophageal cell lines. Oncol Rep 2007;17:161–7.
35. Wislez M, Antoine M, Rabbe N, et al. Neutrophils promote aerogenous spread of lung adenocarcinoma with bronchioloalveolar carcinoma features. Clin Cancer Res 2007;13:3518–27.
36. Dial EJ, Dohrman AJ, Romero JJ, Lichtenberger LM. Recombinant human lactoferrin prevents NSAID-induced intestinal bleeding in rodents. J Pharm Pharmacol 2005;57:93–9.
37. Kyzer SD, Gordon PH, Wang E. Immunohistochemical analysis of statin in colorectal adenocarcinoma, polyps, and normal mucosa. Dis Colon Rectum 1996;39:546–51.
38. Rostom A, Dube C, Lewin G, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med 2007;146: 376–89.
39. Kudo S, Tamura S, Nakajima T, Yamano H, Kusaka H, Watanabe H. Diagnosis of colorectal tumorous lesions by magnifying endoscopy. Gastrointest Endosc 1996;44:8–14.
40. Kudo S, Kashida H, Tamura T, et al. Colonoscopic diagnosis and management of nonpolypoid early colorectal cancer. World J Surg 2000;24: 1081–90.
41. Shimoda T, Ikegami M, Fujisaki J, Matsui T, Aizawa S, Ishikawa E. Early colorectal carcinoma with special reference to its development *de novo*. Cancer 1989;64:1138–46.
42. Ajioka Y, Watanabe H, Kazama S, et al. Early colorectal cancer with special reference to the superficial nonpolypoid type from a histopathologic point of view. World J Surg 2000;24:1075–80.
43. Kashida H, Kudo SE. Early colorectal cancer: concept, diagnosis, and management. Int J Clin Oncol 2006;11:1–8.
44. Arber N, Kuwada S, Leshno M, Sjodahl R, Hultcrantz R, Rex D. Sporadic adenomatous polyp regression with exisulind is effective but toxic: a randomised, double blind, placebo controlled, dose-response study. Gut 2006;55:367–73.
45. Matsuhashi N, Nakajima A, Fukushima Y, Yazaki Y, Oka T. Effects of sulindac on sporadic colorectal adenomatous polyps. Gut 1997;40:344–9.
46. Nusko G, Mansmann U, Kirchner T, Hahn EG. Risk related surveillance following colorectal polypectomy. Gut 2002;51:424–8.
47. Baveye S, Elass E, Mazurier J, Spik G, Legrand D. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin Chem Lab Med 1999;37:281–6.
48. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92: 205–16.
49. Wang WP, Iigo M, Sato J, Sekine K, Adachi I, Tsuda H. Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. Jpn J Cancer Res 2000;91:1022–7.
50. Iigo M, Shimamura M, Matsuda E, et al. Orally administered bovine lactoferrin induces caspase-1 and interleukin-18 in the mouse intestinal mucosa: a possible explanation for inhibition of carcinogenesis and metastasis. Cytokine 2004; 25:36–44.
51. Jonasch E, Stadler WM, Bukowski RM, et al. Phase 2 trial of talactoferrin in previously treated patients with metastatic renal cell carcinoma. Cancer 2008;113:72–7.
52. Nie G, Chen G, Sheftel AD, Pantopoulos K, Ponka P. *In vivo* tumor growth is inhibited by cytosolic iron deprivation caused by the expression of mitochondrial ferritin. Blood 2006;108:2428–34.
53. Freitas I, Boncompagni E, Vaccarone R, Fenoglio C, Barni S, Baronzio GF. Iron accumulation in mammary tumor suggests a tug of war between tumor and host for the microelement. Anticancer Res 2007;27:3059–65.
54. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. Oncogene 2008;27:5932–43.
55. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008;58:130–60.
56. van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. Am J Gastroenterol 2006;101:343–50.
57. Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL. Natural history of untreated colonic polyps. Gastroenterology 1987;93:1009–13.
58. Knoernschild HE. Growth rate and malignant potential of colonic polyps: early results. Surg Forum 1963;14:137–8.
59. U.S. Preventive Services Task Force. Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: recommendation statement. Am Fam Physician 2007;76: 109–13.
60. U.S. Preventive Services Task Force. Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2007;146:361–4.
61. Tamano S, Sekine K, Takase M, Yamauchi K, Iigo M, Tsuda H. Lack of chronic oral toxicity of chemopreventive bovine lactoferrin in F344/DuCrj rats. Asian Pac J Cancer Prev 2008;9:313–6.