3）生検・病理組織検体の採取に工夫が必要である。

非上皮性腫瘍の確定診断において，特に悪性腫瘍が疑われる場合には病理組織診断が必須である。しかし，表層に腫瘍が露呈していない粘膜下腫瘍では，生検陽性率が低く，上皮の薄い潰瘍縁を狙撃生検するなど，検体採取に工夫が必要である。生検による組織診断が不十分な場合には，ボーリング生検，EMRなどによる検体採取を検討する。

4）全身性疾患では大腸以外の消化管も精査する。

悪性リンパ腫のような全身性疾患では，臨床病期を確定するために，上部消化管と小腸の検索が必要である。

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## Colonic Elevated Lesions without Apparent Borders


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We present cases of malignant lymphomas, metastatic colon cancer, and mucosal prolapse syndrome to illustrate elevated colonic lesions with unclear margins. We describe the process of endoscopic diagnosis. When we encounter elevated colonic lesions, we have to differentiate an epithelium-derived neoplasm from non-epithelial lesions like subumucosal tumors or inflammatory lesions. If the case is an elevated lesion with unclear margin and smooth surface, we suspect non-epithelial lesion, and we can rule out epithelium-derived neoplasms by detecting a non-neoplastic pit pattern. It is important to make a differential diagnosis of non-epithelial lesions from such findings as the following; location, solitary or multifocal appearance, size, color, shape, hardness, surface properties, mobility, and existence of depression or ulceration. Although pathological diagnosis is essential to make a definite diagnosis of non-epithelial lesions, the diagnostic rate of biopsy is low. So we have to devise strategies for obtaining proper diagnostic pathological specimens. For malignant lymphoma, the therapeutic approach is determined based on the histological type, so we have to make an accurate pathological diagnosis with enough specimens in order to avoid the effects of crash artifacts.



**key words**: malignant lymphoma, metastatic colon cancer, mucosal prolapse syndrome


## Legends to Figures

Figure 1　Case 1 of diffuse large B-cell lymphoma: 70-year-old, female.
a, b. Colonoscopy revealed a large submucosal tumor in the ascending colon.
A shallow and regular-shaped ulcer was

detected at the top of the lesion.
c. Histological examination of the biopsy specimen revealed medium to large size lymphocyte infiltration.
The lesion was diagnosed as diffuse large B-cell lymphoma.

Figure 2 Case 2 of MALT lymphoma: 73-year-old, male.
a. Colonoscopy revealed a solitary submucosal tumor, 15 mm in diameter, in the sigmoid colon.
b. Indigocarmine dye spraying image showed a shallow depression at the top of the lesion.
c. Magnifying endoscopic view revealed non-neoplastic pit pattern.
d. EUS revealed a well-demarcated inhomogeneous hypoechoic mass in the third layer.
e. Microscopic view. The mass was composed of lymphocyte infiltration localized in the submucosal layer.
f. Histological examination revealed medium-size atypical lymphocyte infiltration. The lesion was diagnosed as marginal zone B-cell lymphoma of MALT type.

Figure 3 Case 3 of mantle cell lymphoma: 66-year-old, male.
a, b. Colonoscopic image of the cecum (a) and rectum (b). Multifocal elevated lesions with central erosion were detected throughout the large intestine.
c. Magnifying image of a nodule showed non-neoplastic pit pattern.
d. Histological diagnosis of the biopsy specimen was malignant lymphoma.
e, f. Upper-GI endoscopic image revealed giant folds in the gastric fornix and mural thickening with erosive changes in the gastric antrum.
g. Per-anal double balloon endoscopy revealed multinodular SMT-like lesions in the terminal ileum.

Figure 4 Case 4 of ovarian cancer metastasis to the rectum: 43-year-old, female.
a, b. Colonoscopic image: Extramural compression was noted at the lower rectum (a). An elevated lesion with multiple reddish granulation was noted in the middle rectum (b).
c. Magnifying image of the elevated lesion demonstrated non-neoplastic pit pattern.
d. Histologic examination of rectal biopsy specimen revealed adenocarcinoma arranged like sheets in the submucosal layer.
The surface epithelium was normal. These findings are compatible with submucosal invasion of ovarian cancer.
e, f. Contrast enhancement CT of the pelvis revealed a large tumor composed of cystic and solid components. Disseminating tumor diffusely involved the rectal wall.

Figure 5 Case 5 of mucosal prolapse syndrome: 58-year-old, female.
a. Colonoscopy revealed ill-demarcated reddish elevated lesion at the lower rectum.
b. After indigocarmine dye spraying, granular and nodular surface pattern became evident. But the margin of the lesion was still obscure.
c. Magnifying image revealed non-neoplastic pit pattern.
d. EUS revealed a multifocal cystic non-echoic area in the submucosa. These findings were compatible with the diagnosis of colitis cystica profunda.
e. Histological examination of the EMR specimen revealed fibromuscular obliteration in lamina propria mucosae.

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主 題

# NBIによる食道小扁平上皮癌の存在診断

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北村陽子 堀田欣一 宮田佳典

**要旨** 食道小扁平上皮癌32病変を遡及的に検討した結果，存在診断の感度は白色光66％，NBI 84％，ヨード染色100％であった．ヨード染色は炎症を惹起するため表層が非腫瘍性上皮で再生され，内視鏡治療の妨げになることがある．一方，NBIは全く非侵襲的であり，食道小扁平上皮癌の存在診断に有用と思われた．しかし，32病変中1病変はNBIでは存在診断困難であったが，白色光では診断可能であったことから，食道小扁平上皮癌の存在診断には白色光とNBIの併用が重要と思われた．一方，ヨード染色の感度は100％であり，咽頭・食道癌治療後などのハイリスクグループに対するサーベイランスではいまだにヨード染色が必須と思われた．

*Key words* : **食道小扁平上皮癌 存在診断 NBI ヨード染色**

## はじめに

従来，食道の通常観察で扁平上皮癌(squamous cell carcinoma ; SCC)を疑った場合，ヨード不染やPCサイン(pink color sign)[1)]で内視鏡診断し，生検にて確定診断を行っていた．しかしヨード液は頸部食道には撒布できず，さらにヨード染色は炎症を惹起するため粘膜表層が非腫瘍で覆われ，治療する際に病変の存在診断と境界診断を誤る場合がある[2)]．また不必要に過多な生検採取は病変を分断する恐れがあり，粘膜下層への線維化も来すため内視鏡治療の妨げになる．

このようにヨード染色や生検は侵襲的な診断方法であるが，最近開発されたNBI(narrow band imaging)は侵襲がなく，食道小扁平上皮癌の存在診断に有用であると報告されている[3)4)]．また拡大機能を併用することにより，食道表面の微細血管を観察し，癌の鑑別診断や深達度診断が可能となる[5)~8)]．このためNBIはヨード染色や生検に代わるものとして期待されている．

そこで，本稿では食道小扁平上皮癌の存在診断におけるNBIの有用性を検討した．なお食道小扁平上皮癌を10 mm以下の癌と定義した．

## 観察方法

食道の内視鏡観察は前処置としてプロナーゼを使用し，切歯列から20～25 cmで貯留した唾液や粘液を除去するためにGascon®水約100 mlで洗浄を行う[9)]．

内視鏡挿入時と抜去時のどちらかをNBIで観察するが，NBIに頼りすぎると白色光(white light ; WL)での診断能が低下するため，NBIで病変を発見した場合はWLでも観察し，それぞれの所見を比較することが大事である．また，頸部食道は挿入時の観察が困難であるため，抜去時にNBIで観察する．

ヨード染色を施行すると表在癌表層部が変性脱落し，非腫瘍性上皮で覆われることがあるため，WLまたはNBI拡大観察にて癌と診断できた場合はヨード染色を施行しないほうがよい．

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**Table 1** Sensitivity of three methods

| | | | |
|---|---|---|---|
| WL | 66 % | n.s. | $p = 0.01$ |
| NBI | 84 % | n.s. | |
| Iodine | 100 % | | |

*n.s.* : not significant.

**Table 2** Comparison of WL and NBI

| | WL | |
|---|---|---|
| | positive | negative |
| NBI | | |
| positive | 63 % (20/32) | 19 % (6/22) |
| negative | 3 % ( 1/32) | 15 % (5/32) |

## WL・NBI・ヨード染色による食道小扁平上皮癌の発見感度

### 1. 対象と方法

2005年5月～2008年12月にEMRC (endoscopic mucosal resection using a cap) またはESD (endoscopic submucosal dissection) を施行した術前未治療の食道小扁平上皮癌29例33病変のうち，SM癌1病変を除外した32病変〔深達度T1a-EP 29病変，T1a-LPM 3病変，腫瘍長径中央値(範囲) 3 (0.8～10) mm〕を対象とした．

WL像，ヨード内視鏡像，NBI内視鏡像をretrospectiveに見直し，それぞれのsensitivityを検討した．WLでは境界明瞭な色調変化や隆起，陥凹を，NBI通常観察では境界明瞭な茶色領域(brownish area)を，ヨード内視鏡では不整形な不染に注目して診断した．

### 2. 結　果

WLでのsensitivityは66％(21/32)，NBIでは84％(27/32)，ヨード染色では100％であった(**Table 1**)．NBIで発見可能だがWLでは発見できなかった症例は6例19％であり，逆にWLで発見可能だがNBIでは発見できなかった症例は1例3％であった(**Table 2**)．

### 症　例

**〔症例1〕** WL，NBIともに存在診断が可能であった症例．

WLでMt左壁に不整形な発赤陥凹性病変を認めた(**Fig. 1 a**)．NBI通常観察にて同病変はbrownish areaを呈し，その境界はより明瞭となった(**Fig. 1 b**)．WL拡大観察では拡張した血管が密に存在する領域があった(**Fig. 1 c**)．NBI拡大観察ではこの変化がWL拡大観察より明瞭に観察された(**Fig. 1 d**)．ヨード染色では境界明瞭な不整形の不染帯を呈した(**Fig. 1 e**)．以上よりSCC, T1a-EPと診断し，ESDにて一括切除を施行した．病理組織学的所見では全層性のSCCで，深達度はT1a-EPであった(**Fig. 1 f**)．最終診断は，SCC，T1a-EP，ly0，v0，LM(－)，VM(－)，0-IIb，10 × 9 mmであった(**Fig. 1 g**)．

**〔症例2〕** WLで診断が可能だったが，NBIでは診断が不可能であった症例．

WLにてMt後壁に白色調の扁平隆起性病変を認めた(**Fig. 2 a**)．NBI通常観察では明らかなbrownish areaは認めなかった(**Fig. 2 b**)．ヨード染色では境界明瞭で不整形な不染帯を呈した(**Fig. 2 c**)．以上よりヨード染色の所見を重視し0-IIa型扁平上皮癌と診断し，ESDにて一括切除を施行した．病理組織学的所見では基底層型のSCCであり，一部粘膜固有層へ進展していた(**Fig. 2 d**)．最終診断は，SCC，T1a-LPM，ly0，v0，HM0，VM0，0-IIc，7 × 6 mmであった(**Fig. 2 e**)．

**〔症例3〕** WLでは存在が診断できず，NBIでは診断が可能であった症例．

WLにて指摘できなかったが(**Fig. 3 a**)，NBI観察にてLt右壁に境界明瞭な小brownish areaを認めた(**Fig. 3 b**)．ヨード染色では明らかな小不染帯を呈した(**Fig. 3 c**)．以上よりSCC，T1a-EPと診断し，EMRCにて一括切除した．病理組織学的所見では核異型が上層部まで広がる全層性SCCで，深達度はT1a-EPであった(**Fig. 3 d**)．最終診断は，SCC，T1a-EP，ly0，v0，HM0，VM0，0-IIa，1.5 × 1.5 mmであった(**Fig. 3 e**)．

**〔症例4〕** WLとNBIともに存在診断ができなかった症例．

食道癌内視鏡治療後サーベイランスにてWLとNBIでは病変を指摘できなかったが(**Fig. 4 a, b**)，

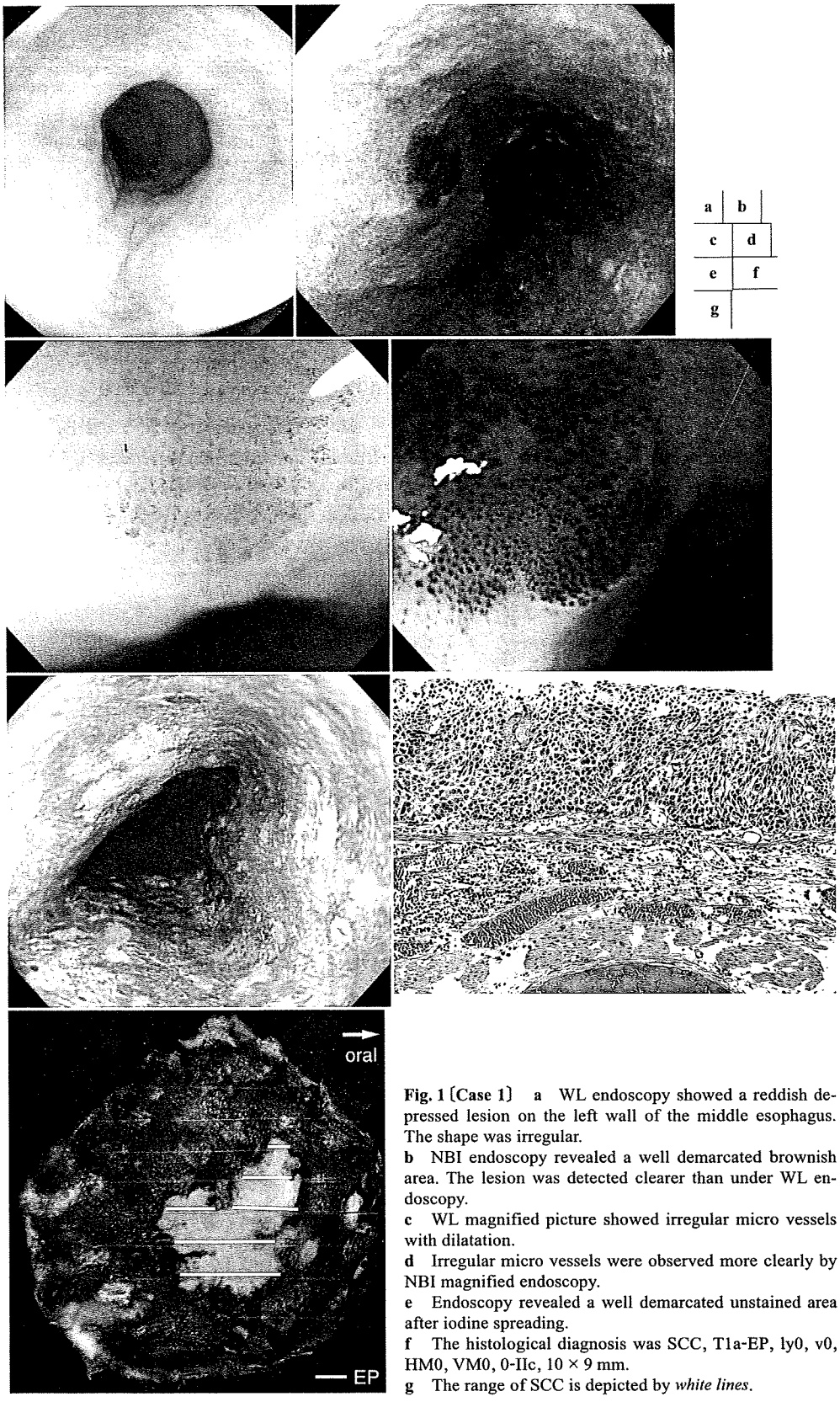


**Fig. 1**〔**Case 1**〕 **a** WL endoscopy showed a reddish depressed lesion on the left wall of the middle esophagus. The shape was irregular.
**b** NBI endoscopy revealed a well demarcated brownish area. The lesion was detected clearer than under WL endoscopy.
**c** WL magnified picture showed irregular micro vessels with dilatation.
**d** Irregular micro vessels were observed more clearly by NBI magnified endoscopy.
**e** Endoscopy revealed a well demarcated unstained area after iodine spreading.
**f** The histological diagnosis was SCC, T1a-EP, ly0, v0, HM0, VM0, 0-IIc, 10 × 9 mm.
**g** The range of SCC is depicted by *white lines*.

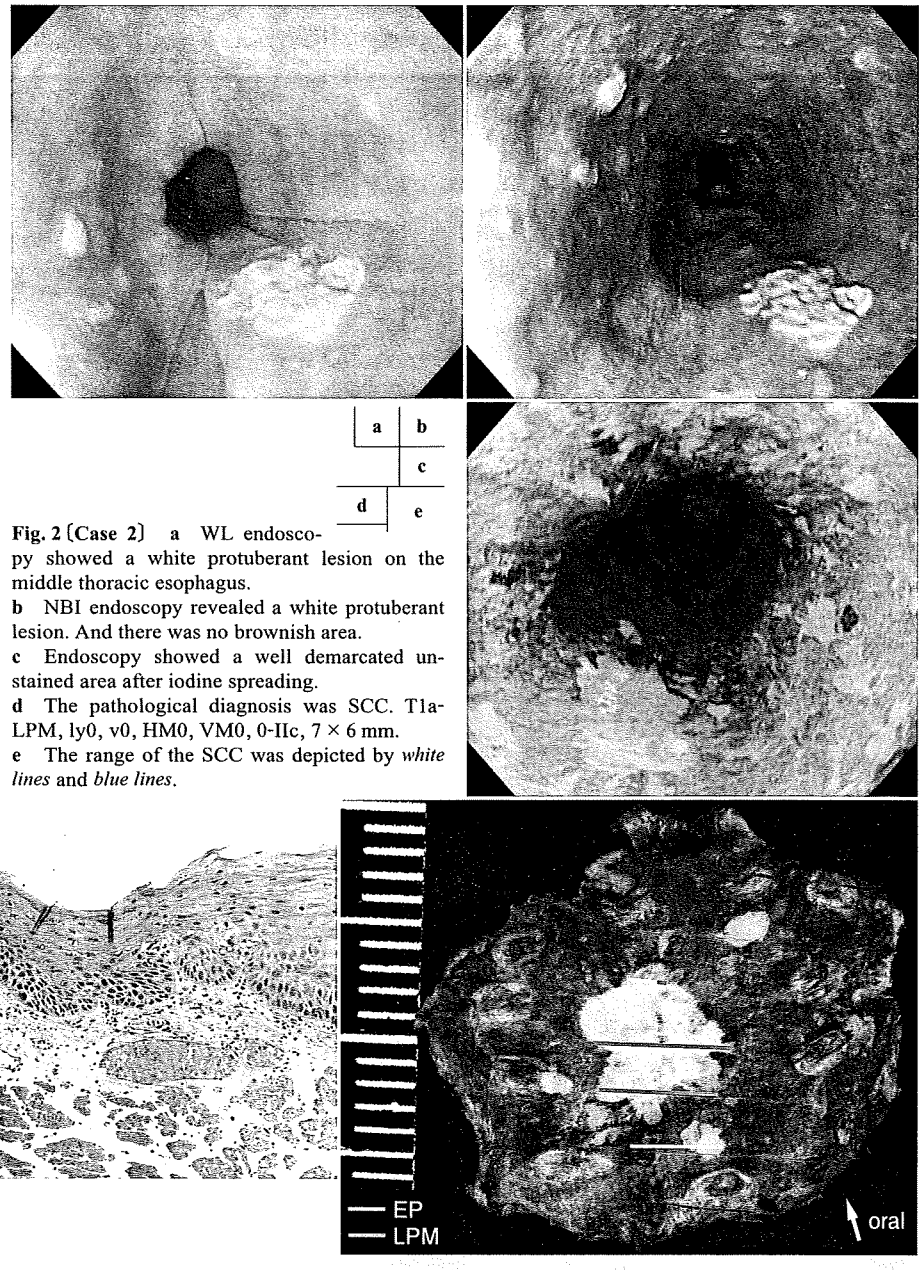


**Fig. 2〔Case 2〕** **a** WL endoscopy showed a white protuberant lesion on the middle thoracic esophagus.
**b** NBI endoscopy revealed a white protuberant lesion. And there was no brownish area.
**c** Endoscopy showed a well demarcated unstained area after iodine spreading.
**d** The pathological diagnosis was SCC. T1a-LPM, ly0, v0, HM0, VM0, 0-IIc, 7 × 6 mm.
**e** The range of the SCC was depicted by *white lines* and *blue lines*.

ヨード染色にてMt左壁に不整形な不染を認めた(**Fig. 4 c, d**). ヨード染色の所見を重視してSCCと診断しESDにて一括切除を施行した. 病理組織学的所見では基底層型のSCCであった(**Fig. 4 e**). 最終診断は, SCC, T1a-EP, ly0, v0, HM0, VM0, 0-IIc, 7×6 mmであった(**Fig. 4 f**).

## 考　察

Mutoら[3]は食道表在癌の発見能に関して, 多施設共同無作為前向き研究で, WLよりもNBIが有用であると報告している. 筆者らの検討では食道小扁平上皮癌のみを対象としたが, WLの感度66％に対しNBIの感度は84％と良好であった. 一方, 北村ら[10]は内視鏡の熟練者が食道小扁平上皮癌を発見する際にはNBIが有用であったが, 初心者では有用性が証明されず, NBIによる食道小扁平上皮癌発見にはある程度の習熟が必要であると報告している.

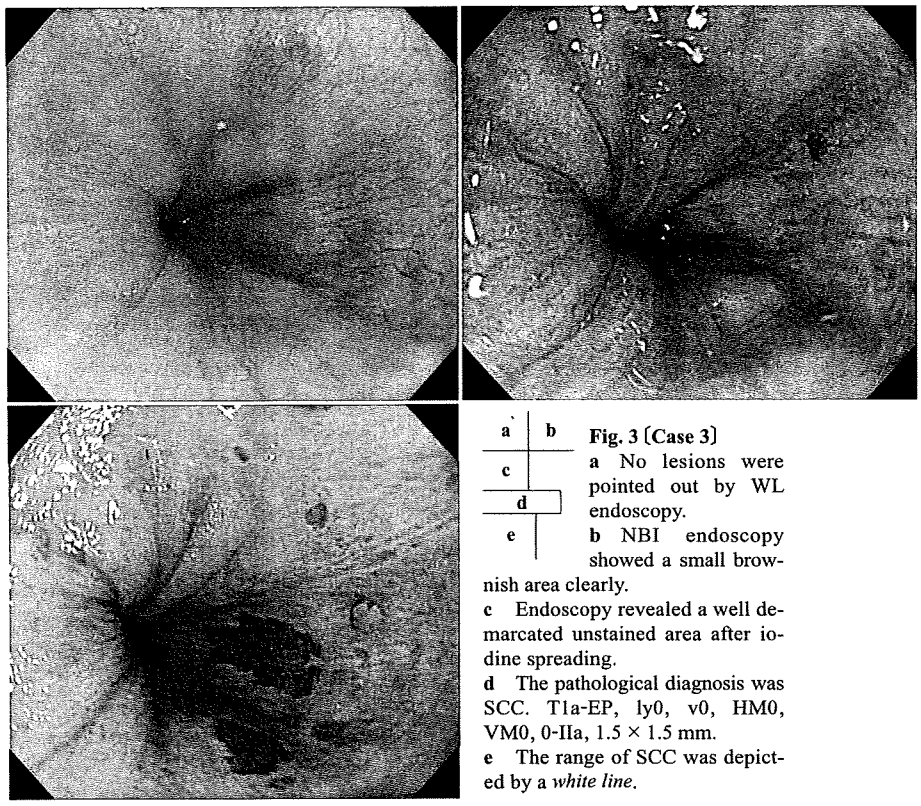


**Fig. 3 [Case 3]**
**a** No lesions were pointed out by WL endoscopy.
**b** NBI endoscopy showed a small brownish area clearly.
**c** Endoscopy revealed a well demarcated unstained area after iodine spreading.
**d** The pathological diagnosis was SCC. T1a-EP, ly0, v0, HM0, VM0, 0-IIa, 1.5 × 1.5 mm.
**e** The range of SCC was depicted by a *white line*.

Kuraoka ら[11]は食道癌のハイリスク患者49人を対象とし，NBI観察後にヨード染色を行い，最後に不染領域を生検し病理学的検討を行った．118か所の不染のうち5か所が食道扁平上皮癌で，そのすべてがNBIにて検出されていた．癌に対するヨードのsensitivity，PPV (positive predictive value) はそれぞれ100％，4.4％で，NBIでは100％，9.8％であり，PPVはNBIのほうが高かったと報告している．

今回の筆者らの検討では食道小扁平上皮癌の発見感度はWL 66％，NBI 84％，ヨード染色100％であり，NBIの感度はヨード染色には及ば

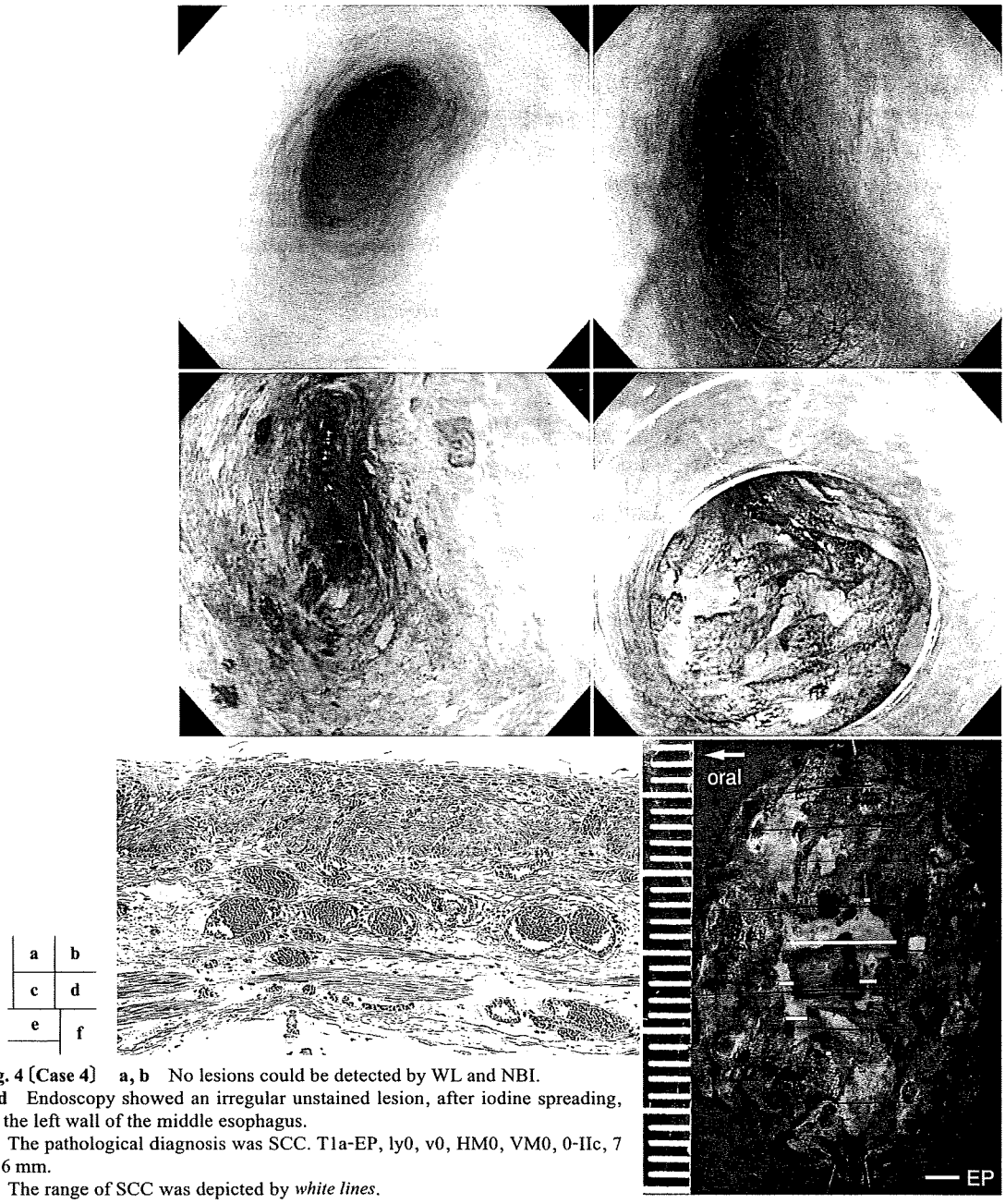


**Fig. 4〔Case 4〕** **a, b** No lesions could be detected by WL and NBI.
**c, d** Endoscopy showed an irregular unstained lesion, after iodine spreading, on the left wall of the middle esophagus.
**e** The pathological diagnosis was SCC. T1a-EP, ly0, v0, HM0, VM0, 0-IIc, 7 × 6 mm.
**f** The range of SCC was depicted by *white lines*.

なかった．また〔症例2〕(Fig. 2)のように，WLでは存在診断が可能だが，NBIでは診断が困難な症例が3％に認められ，NBI単独ではなくWLでの観察も不可欠であることが示唆された．

したがって，咽頭癌・食道癌治療後の患者など，異時多発癌のハイリスクグループに関しては，WLとNBIに加え，ヨード染色も併用したサーベイランスを行うことが必要と思われた．

## おわりに

食道小扁平上皮癌の発見感度はWL 66％，NBI 84％であり，NBIは食道小扁平上皮癌の発見に有用であった．しかし，WLでは存在診断が可能だが，NBIでは不可能であった症例もあり，WLとNBIを併用した診断が重要と思われた．

また，ヨード染色の感度は100％であったことから，ハイリスク群のサーベイランスでは依然としてヨード撒布が必須であると思われた．

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***Summary***

### Detection of Small Esophageal Squamous Cell Carcinoma by NBI



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Usefulness of NBI for the early detection of superficial esophageal SCC has been reported. However, the detection sensitivity for small esophageal SCC by WL, NBI and iodine staining has never been reported.

Patients and Methods : 32 lesions of small SCC (29 lesions of EP and 3 lesions of LPM, tumor median size : 3 mm) treated by EMRC or ESD from May, 2005 to December, 2008 were enrolled in this study. The endoscopic pictures of WL, NBI and iodine were investigated retrospectively. The SCC was detected from the endoscopic findings such as protuberant, depression or color change by WL, well demarcated brown area by NBI and irregular unstained area by iodine, respectively. Small SCC was defined as a lesion 10 mm or less in size.

Results : Sensitivity of WL, NBI and iodine was 66％, 84％ and 100％, respectively.

Conclusion : NBI was useful for the detection of small esophageal SCC, however the sensitivity of NBI was lower than that of iodine staining. Therefore, iodine staining is still necessary for the surveillance in the high risk group for esophageal SCC.



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# Circulating tumor cells as a surrogate marker for determining response to chemotherapy in patients with advanced gastric cancer

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**The purpose of this study was to quantify circulating tumor cells (CTCs) in advanced gastric cancer (AGC) patients, and to demonstrate the role of CTCs in cancer therapy. This study investigates the hypothesis that CTCs can predict clinical outcomes in patients with AGC. From November 2007 to June 2009, 52 patients with AGC were enrolled into a prospective study. The chemotherapy regimen was an S-1-based regimen (S-1 with or without cisplatin) or paclitaxel. CTCs of whole blood at baseline, 2 weeks, and 4 weeks after initiation of chemotherapy, were isolated and enumerated using immunomagnetics. Patients with ≥4 CTCs at 2-week points and 4-week points had a shorter median progression-free survival (PFS) (1.4, 1.4 months, respectively) than those with the median PFS of <4 CTCs (4.9, 5.0 months, respectively) (log-rank test; $P < 0.001$, $P < 0.001$, respectively). Patients with ≥4 CTCs at 2-week points and 4-week points had shorter median overall survival (OS) (3.5, 4.0 months, respectively) than those with the median PFS of <4 CTCs (11.7, 11.4 months, respectively) (log-rank test; $P < 0.001$, $P = 0.001$, respectively). In conclusion, this study demonstrates that CTC measurement may be useful as a surrogate marker for determining response to S-1-based or paclitaxel regimens in AGC. (*Cancer Sci* 2010)**

Gastric cancer is more prevalent in Asia, Eastern Europe, and Central and South America than in other areas. In Japan, this cancer is one of the most common causes of cancer-related mortality, despite dramatic advances in diagnosis and treatment. Outcomes are extremely poor in patients with unresectable gastric cancer, with the median survival ranging from 3 to 5 months with the best supportive care.[1–3] The ability to identify patients with the worst prognoses or those destined to progress quickly could have broad clinical applications.

Circulating tumor cells (CTCs) or disseminated tumor cells (DTCs) in bone marrow and peripheral blood from patients with cancers have been documented.[4–6] Braun *et al.*[7,8] reported that ~30% of women with primary breast cancer have DTCs in bone marrow, and a 10-year follow-up of these patients revealed a significantly decreased disease-free survival and overall survival (OS) when compared with patients without DTCs. However, aspiration of bone marrow is time consuming and, in many cases, uncomfortable for the patients precluding multiple samplings for therapy monitoring studies. Therefore, recent efforts have concentrated on the detection of CTCs in the peripheral blood of cancer patients. Cristofanilli *et al.*[9,10] showed in a prospective study that CTC detection provided significant prognostic information for patients with metastatic breast cancer. Cohen *et al.*[11] showed that the number of CTCs before and during treatment was an independent predictor of PFS and OS in patients with metastatic colorectal cancer. It is not clear whether CTC detection using this system provides prognostic information for patients with advanced gastric cancer. We initiated this study to evaluate whether CTCs could serve as a prognostic and/or predictive marker in patients with AGC.

## Materials and Methods

**Patients.** All patients were enrolled using institutional review board-approved protocols at the Cancer Institute Hospital at the Japanese Foundation for Cancer Research and provided informed consent. The study population consisted of patients aged 18 years or older with histologically proven AGC. Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 2; adequate organ function; and S-1-based (S-1 with or without cisplatin) or paclitaxel chemotherapy regimen. The subjects were five patients treated with S-1 (40 mg/m$^2$, twice daily, days 1–28, repeated every 6 weeks), 26 patients treated with S-1 plus CDDP (S-1 40 mg/m$^2$, twice daily, days 1–21, CDDP 60 mg/m$^2$, day 8, repeated every 5 weeks), and 21 patients treated with paclitaxel (80 mg/m$^2$, weekly).

**Sample preparation for isolation of CTCs from blood.** Blood was drawn from advanced gastric cancer patients into 10 mL of evacuated blood for CTC in a Cell Save Preservative Tube (Veridex, Raritan, NJ, USA). Blood was always drawn from cancer patients before treatment initiation (baseline), 2 weeks, and 4 weeks after the administration of an S-1-based or paclitaxel regimen. The CellSearch system (Veridex) consists of the CellPrep system, the CellSearch Epithelial Cell Kit (for the measurement of CTC), and the CellSpotter Analyzer. The CellPrep system is a semi-automated sample preparation system, and the CellSearch Epithelial Cell Kit consists of ferrofluids coated with epithelial cell-specific EpCAM antibodies to immunomagnetically enrich epithelial cells; a mixture of two phycoerythrin-conjugated antibodies that bind to cytokeratin 8, 18, and 19; an antibody to CD45 conjugated to allophycocyanin; nuclear dye 4′,6- diamidino-2-phenylindole (DAPI) to fluorescently label the cell; and buffers to wash, permeabilize, and resuspend the cells. Sample processing and evaluation were done as described by Allard *et al.* Briefly, 7.5 mL of blood for CTCs were mixed with 6 mL of buffer, centrifuged at 800*g* for 10 min, and then placed on the CellPrep system. After aspiration of the plasma and buffer layer by instrument, ferrofluids were added. After incubation and subsequent magnetic separation, unbound cells and remaining plasma were aspirated. The staining reagents were then added in conjunction with a permeabilization buffer to fluorescently label the immunomagnetically labeled cells. After incubation in the system, the magnetic separation was repeated, and

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excess staining reagents were aspirated. In the final processing step, the cells were resuspended in the MagNest Cell Presentation Device (Veridex). This device consists of a chamber and two magnets that orient the immunomagnetically labeled cells for analysis using the CellSpotter Analyzer.

**Sample analysis.** The MagNest was placed on the CellSpotter Analyzer, a four-color semi-automated fluorescence microscope. Image frames covering the entire surface of the cartridge for each of the four fluorescent filter cubes were captured. The captured images containing objects that met predetermined criteria were automatically presented in a web-enabled browser from which final selection of cells was made by the operator. The criteria for an object to be defined as a CTC include round to oval morphology, a visible nucleus (DAPI positive), positive staining for cytokeratin, and negative staining for CD45. Results of cell enumeration are always expressed as the number of cells per 7.5 mL of blood for CTCs.

**Statistical analysis.** Progression-free survival (PFS) was defined as the elapsed time from blood collection to progression. Kaplan–Meier survival plots were generated based on CTC levels each time blood was collected, and the curves were compared using a log-rank testing. A $P$-value <0.05 was considered significant. Cox proportional hazards regression was used to determine univariate and multivariate hazard ratios for selected potential predictors of PFS and OS. The distribution of patients above and below the CTC threshold and clinical response was compared using Fisher's exact test.

## Results

**Patient characteristics.** A total of 52 patients were enrolled. Patients' characteristics at baseline are summarized in Table 1. Patients' characteristics were as follows: median age, 62 years (range, 24–78 years); PS 0/1/2, 39/12/1; primary tumor +/−, 33/19; and regimen S-1/S-1 with cisplatin/paclitaxel, 5/26/21. Thirty-five patients had diffuse-type histology (67.3%). Seventeen patients (32.7%) had intestinal type. Among 52 patients, the best response rates were 28.8% (complete response [CR]/partial response [PR]/stable disease [SD]/progressive disease [PD]: 0/15/19/18). Of 31 patients treated with the S-1-based regimen (S-1 alone or S-1/cisplatin [CDDP]) assessable for response, we observed 14 PR (45.2%), 11 patients (35.5%) with SD, and six patients (19.4%) with PD during treatment. The overall response rate was 45.2%. On the other hand, of 21 patients treated with the weekly paclitaxel regimen assessable for response, we observed one PR (4.8%), eight patients (38.1%) with SD, and 12 patients (57.1%) with progression of disease during treatment, for an overall response rate (RR) of 4.8% (Table 2).

**Table 1. Patient demographics**

| Demographic | Number or median (range) |
|---|---|
| Median age (range) | 62 (24–78) |
| Male/female | 44/8 |
| PS: 0/1/2 | 39/12/1 |
| S1-based/PAC regimen | 31/21 |
| Line: 1st/2nd | 34/18 |
| Histopathology: diffuse/intestinal type | 35/17 |
| Primary tumor: +/− | 33/19 |
| Sites of metastasis: +/− | |
| Liver | 24/28 |
| Lung | 3/49 |
| Bone | 1/51 |
| Peritoneum | 22/30 |
| Lymph node | 37/15 |

**Table 2. Objective response**

| | S1-based regimen (31)<br>S1 alone (5), S1/CDDP (26)<br>1st line (31) | PAC (21)<br>Weekly PAC (21)<br>1st line (3), 2nd line (18) |
|---|---|---|
| CR | 0 | 0 |
| PR | 14 | 1 |
| SD | 11 | 8 |
| PD | 6 | 12 |

CDDP, cisplatin; CR, complete response; PAC, paclitaxel; PD, progressive disease; PR, partial response; SD, stable disease.

**Stratification according to CTC levels.** To select a level of circulating tumor cells that most clearly distinguished patients with a response of chemotherapy, thresholds of 1 to 88 cells for 2-week point were systematically correlated with PFS for 26 of the 30 patients in the training set. The median PFS among patients with levels above or below each threshold differed at the level of one circulating tumor cell per 7.5 mL of blood, and reached a plateau at approximately four cells per 7.5 mL of blood. At the latter level, the Cox proportional hazards ratio signifying the difference between slow and rapid progression of disease also reached a plateau. Thus, a cut-off of four circulating tumor cells per 7.5 mL of blood was chosen to distinguish patients.[12] The Kaplan–Meier circulating tumor-cell counts were available at a 2-week point for 26 of the thirty patients in the training set and for 21 of the 22 patients in the validation set. Neither PFS nor OS was significantly different in the two sets (data not shown). Because the two sets of data were nearly identical, they were combined for the estimation of PFS and OS for the entire population.

**CTCs and imaging to assess response to therapy.** Thirty-four (65.4%) of 52 patients were classified as having non-progressive disease (non-PD), with 24 of these patients (46.2%) having <4 CTCs and 10 patients (19.2%) having ≥4 CTCs before the initiation of therapy. Ten (19.2%) of 52 patients were classified as having PD, with 11 of these patients (21.2%) having <4 CTCs and seven patients (13.4%) having ≥4 CTCs before the initiation of therapy. The difference between the clinical responses and CTC levels were not significant. In contrast, 33 (64.7%) of 51 patients were classified as having non-PD, with 33 of these patients (64.7%) having <4 CTCs and no patients (0%) having ≥4 CTCs at 2 weeks. Eighteen (35.3%) of 51 patients were classified as having PD, with 11 of these patients (21.6%) having <4 CTCs and seven patients (13.7%) having ≥4 CTCs at 2 weeks. The difference between the clinical responses and CTC levels was highly significant. ($P$ = 0.001, Fisher's exact test). Thirty-two (64%) of 48 patients were classified as having non-PD, with 31 of these patients (64.6%) having <4 CTCs and one patient (2.0%) having ≥4 CTCs at 4 weeks. Sixteen (33.3%) of 48 patients were classified as having PD, with eight of these patients (16.7%) having <4 CTCs and eight patients (16.7%) having ≥4 CTCs at 4 weeks. The difference between the clinical responses and CTC levels were highly significant ($P$ < 0.001, Fisher's exact test) (Table 3).

**Analysis of PFS according to CTC level.** Figure 1 shows the Kaplan–Meier plots for prediction of PFS using the baseline CTC counts (Fig. 1a), at 2 weeks (Fig. 1b), and at 4 weeks (Fig. 1c). Seventeen of the patients (32.7%) had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with that of patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.4 months; 95% confidence interval [CI], 1.2–1.6) than the median PFS of <4 CTCs at 2 weeks (4.9 months; 95% CI, 4.0–5.8) ($P$ < 0.001) (Fig. 1b). Patients with ≥4 CTCs at the 4-week point had a shorter median



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Table 3. CTCs and correlation with response assessment by imaging

| | Non-PD | | | PD | | | Fisher's exact P-values |
|---|---|---|---|---|---|---|---|
| | No. of patients | CTCs <4 (%) | CTCs ≥4 (%) | No. of patients | CTCs <4 (%) | CTCs ≥4 (%) | |
| Baseline | 34 | 24 (46.2) | 10 (19.2) | 18 | 11 (21.2) | 7 (13.4) | 0.544 |
| 2 week | 33 | 33 (64.7) | 0 (0) | 18 | 11 (21.6) | 7 (13.7) | 0.001 |
| 4 week | 32 | 31 (64.6) | 1 (2.0) | 16 | 8 (16.7) | 8 (16.7) | <0.001 |

CTCs, circulating tumor cells; PD, progressive disease.

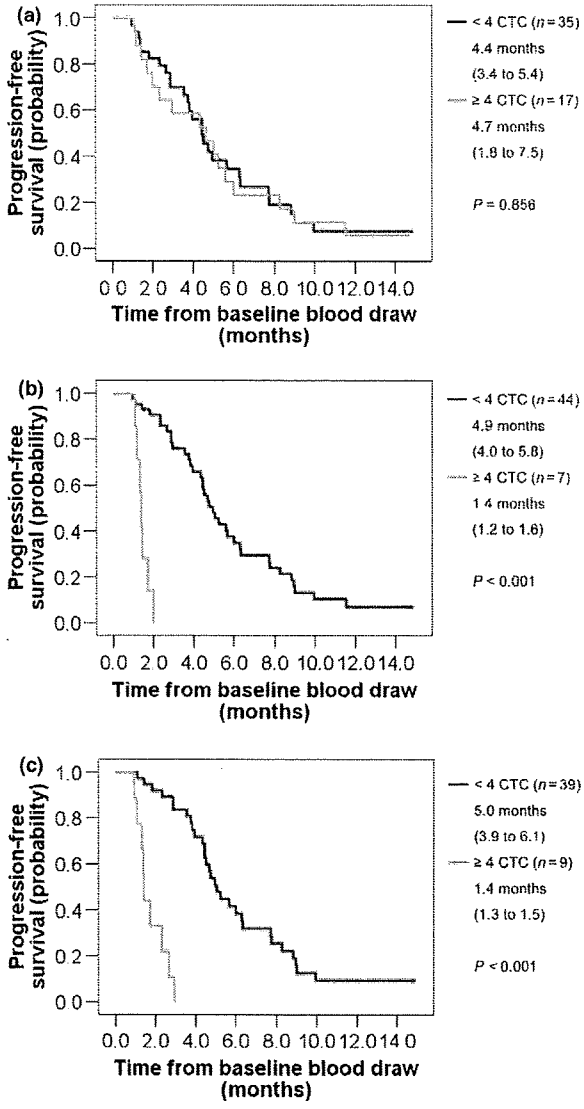


Fig. 1. Kaplan–Meier plots of progression-free survival (PFS) in advanced gastric cancer patients with less than four circulating tumor cells (CTCs) or ≥4 CTCs at baseline (a), 2 weeks (b), and 4 weeks (c).

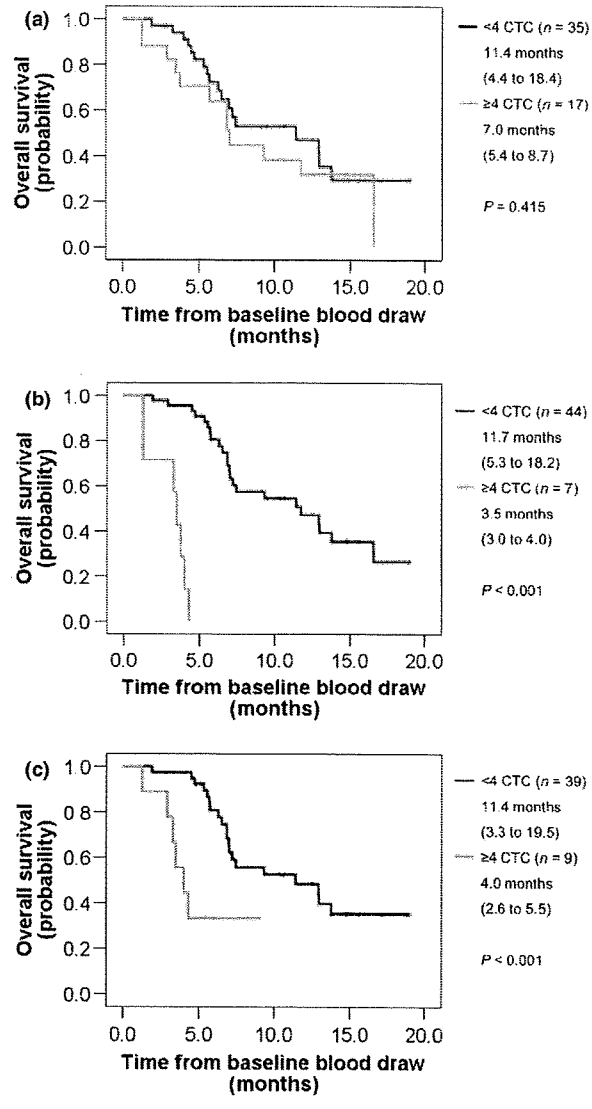


Fig. 2. Kaplan–Meier plots of overall survival (OS) in advanced gastric cancer patients with less than four circulating tumor cells (CTCs) or ≥4 CTCs at baseline (a), 2 weeks (b), and 4 weeks (c).

PFS (1.4 months; 95% CI, 1.3–1.5) than the median PFS of <4 CTCs at 4 weeks (5.0 months; 95% CI, 3.9–6.1) ($P < 0.001$) (Fig. 1c). With the S-1-based regimen, 10 patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with 21 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.2 months) than the median PFS of <4 CTCs at 2 weeks (6.0 months; 95% CI, 4.3–7.7) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median PFS (2.3 months; 95% CI, 0.7–3.9) than the median PFS of <4 CTCs at 4 weeks (6.3 months; 95% CI, 3.0–9.7) ($P < 0.001$). With the paclitaxel regimen, seven patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with 14

patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.4 months; 95% CI, 1.4–1.5) than the median PFS of <4 CTCs at 2 weeks (4.3 months; 95% CI, 3.5–5.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median PFS (1.4 months; 95% CI, 1.0–1.8) than the median PFS of <4 CTCs at 4 weeks (4.4 months; 95% CI, 3.6–5.3) ($P < 0.001$).

**Analysis of OS according to CTC level.** Figure 2 shows the Kaplan–Meier plots for prediction of OS using baseline CTC counts (Fig. 2a), at 2 weeks (Fig. 2b), and at 4 weeks (Fig. 2c). Seventeen of the patients (32.7%) with ≥4 CTCs per 7.5 mL of blood at baseline had no significant different OS compared with patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (3.5 months; 95% CI, 3.0–4.0) than the median OS of <4 CTCs at 2 weeks (11.7 months; 95% CI, 5.3–18.2) ($P < 0.001$) (Fig. 2b). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (4.0 months; 95% CI, 2.6–5.5) than the median OS of <4 CTCs at 4 weeks (11.4 months; 95% CI, 3.3–19.5) ($P = 0.001$) (Fig. 2c). With the S-1 based regimen, 10 patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significant different OS compared with 21 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (1.3 months) than the median OS of <4 CTCs at 2 weeks (13.8 months; 95% CI, 9.4–18.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (4.0 months; 95% CI, 2.3–5.7) than the median OS of <4 CTCs at 4 weeks (>11.7 months) ($P = 0.031$). With the paclitaxel regimen, seven patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significant different OS compared with 14 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (3.5 months; 95% CI, 3.1–4.0) than the median OS of <4 CTCs at 2 weeks (6.5 months; 95% CI, 5.9–7.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (3.5 months; 95% CI, 2.3–4.7) than the median OS of <4 CTCs at 4 weeks (6.5 months; 95% CI, 5.5–7.5) ($P = 0.013$).

**Univariate and multivariate analysis of predictors of PFS and OS.** Univariate and multivariate Cox proportional hazards regression was performed to assess the association between factors of interest and PFS or OS. In univariate analysis, PS, treatment regimen, line of chemotherapy, and CTC levels (cut-off, 4) at 2 and 4 weeks predicted PFS and OS (Table 4). In order to evaluate the independent predictive effect of chemotherapy, multivariate Cox regression analysis was carried out (Table 5). CTC levels at 2 and 4 weeks were the strongest predictors.

## Discussion

The CellSearch system is designed to enrich and enumerate CTCs from peripheral blood. Furthermore, it is the first system to validate the clinical use of CTCs in patients with advanced gastric cancer. Our results show that the system is a suitable tool for assessment of CTCs in these patients, enabling reliable detection of CTCs in whole blood.

Approaches for isolation of CTCs in a research setting range from enrichment of tumor cells using density-gradient centrifugation[13–15] and flow cytometry.[16,17] CTC number as quantified by the CellSearch methodology[18–21] has been shown to have prognostic significance, and post-therapy decreases and increases in CTC number are associated with a superior and inferior survival, respectively, in patients with breast cancer, prostate cancer, and colorectal cancer. In this study, a finding of <4 CTCs in 7.5 mL of peripheral blood at 2 and 4 weeks after initiation of chemotherapy was associated with significantly longer PFS and OS as compared with these patients with ≥4 CTCs in 7.5 mL of peripheral blood. The results of this analysis demonstrated that the presence of four or more CTCs in 7.5 mL of blood before initiation of chemotherapy is not associated with PFS and OS. But the levels of CTCs at 2 and 4 weeks after initiation of chemotherapy are predictive of treatment efficacy, PFS,

**Table 4. Univariate Cox regression analysis of independent parameters for prediction of PFS and OS**

| Parameter | No. of patients | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | *P*-values | $\chi^2$ | HR | 95% CI | *P*-values | $\chi^2$ |
| ECOG, 2 *vs* 1 *vs* 0 | 52 | 1.817 | 1.010–3.268 | 0.046 | 0.042 | 2.795 | 1.416–5.516 | 0.003 | 0.002 |
| Treatment regimen | 52 | 0.422 | 0.225–0.792 | 0.007 | 0.006 | 0.239 | 0.106–0.538 | 0.001 | <0.001 |
| Line of therapy | 52 | 3.155 | 1.577–6.311 | 0.001 | 0.001 | 4.527 | 2.031–10.088 | <0.001 | <0.001 |
| CTCs at the 2nd week | 51 | 22.633 | 6.214–82.429 | <0.001 | <0.001 | 42.796 | 8.382–218.515 | <0.001 | <0.001 |
| CTCs at the 4th week | 48 | 15.947 | 5.380–47.271 | <0.001 | <0.001 | 4.699 | 1.751–12.609 | 0.002 | 0.001 |

CI, confidence interval; CTCs, circulating tumor cells; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

**Table 5. Multivariate Cox regression analysis for prediction of PFS and OS**

| Parameter | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of patients | HR | 95% CI | *P*-values | No. of patients | HR | 95% CI | *P*-values |
| No. of patients | 51 | | | | 51 | | | |
| Line of therapy, 1st *vs* 2nd | | 0.463 | 0.219–0.977 | 0.043 | | 0.307 | 0.129–0.731 | 0.008 |
| Lymph node metastasis | | 0.458 | 0.214–0.980 | 0.044 | | | | |
| CTCs at the 2nd week | | 0.049 | 0.012–0.199 | <0.001 | | 0.037 | 0.007–0.191 | <0.001 |
| Model $\chi^2$ | | | <0.001 | | | | <0.001 | |
| No. of patients | 48 | | | | 48 | | | |
| Line of therapy, 1st *vs* 2nd | | 0.412 | 0.192–0.880 | 0.022 | | 0.217 | 0.089–0.504 | <0.001 |
| CTCs at the 4th week | | 0.082 | 0.027–0.224 | <0.001 | | 0.216 | 0.077–0.607 | 0.004 |
| Model $\chi^2$ | | | <0.001 | | | | <0.001 | |

CI, confidence interval; CTCs, circulating tumor cells; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.



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and OS. The presence of at least four CTCs at 2 and 4 weeks is a strong independent prognostic factor for inferior PFS and OS. These data demonstrate that CTC measurement may be a useful biomarker for monitoring response to therapy in AGC.

Outcomes are extremely poor in patients with ≥4 CTCs at 2 and 4 weeks, with the median OS ranging from 2 to 5 months. These data suggest the value of this technology in the identification of chemotherapy-resistant patients who could benefit from early treatment change and/or more investigational. Further study should prospectively address whether a change of treatment based on ≥4 CTCs at 2 or 4 weeks after initiation of chemotherapy early in the course of treatment will result in improvement in OS. CTC levels drawn at 2 and 4 weeks, before typical imaging intervals, may have the potential to suggest treatment choices and spare unnecessary toxicity by suggesting that an early change in therapy is warranted. Because the CellSearch system has not been approved in Japan, the price of one sample costs about ¥80 000 as in the case of the extra laboratory in the clinical trial. Several prospective trials led to the FDA approval of CTC counts for monitoring of patients with breast, colorectal, and prostate cancer. We expect CTC counts for monitoring of patients with gastric, breast, colorectal, and prostate cancer to be approved in Japan.

In conclusion, this study demonstrates the independent predictive value of CTCs for patients initiating chemotherapy for AGC. The data obtained in this clinical trial of the CellSearch system were for enumeration of CTCs in AGC. Our study was not designed to assess whether a change in therapy based on ≥4 CTCs is beneficial. However, clinical trials to explore this hypothesis are warranted.

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## Disclosure Statement

The authors have no conflict of interest.

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ORIGINAL PAPER

# Management of venous thromboembolism in colorectal cancer patients treated with bevacizumab

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**Abstract** Venous thromboembolism associated with use of a central venous access system is an urgent problem in patients treated with bevacizumab (bev). We investigated the effectiveness of Doppler ultrasound imaging (DUS) in the early detection of catheter-related thrombosis for avoidance of severe venous thromboembolism. Patients with metastatic colorectal cancer received either FOLFOX-4 + bev or FOLFIRI + bev. DUS was performed on the deep venous system for detection of thrombus formation during the initial cycle of treatment, followed by re-evaluation after the third cycle in patients with asymptomatic thrombus formation. All patients were followed up until treatment was interrupted. Median duration of follow-up was 484 days (range 72–574). Among 41 enrolled patients, curable symptomatic thrombosis occurred in one, and asymptomatic thrombosis in 21 (51.2%). Of 21 patients undergoing re-evaluation, thrombi remained without progression in 17 patients, and enlargement in 4 patients. In two of the patients in whom there was progression, pulmonary embolism occurred after the sixth cycle. In the asymptomatic group, no thrombi developed as far as the superior vena cava in any patient. In the cases of progression, thrombotic enlargement was observed in all the 4 patients, with decreased vascular flow in 2. Using DUS, we were able to detect asymptomatic thrombosis in the early cycles of treatment, indicating its potential in the monitoring of venous thrombi. In the event of an enlarging asymptomatic thrombosis developing into the superior vena cava along with decreased vascular flow, careful follow-up and appropriate anticoagulant therapy may be recommended without increased risk of bleeding.

**Keywords** Venous thromboembolism · Bevacizumab · Colorectal cancer

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## Introduction

Bevacizumab (bev) is a recombinant, humanized monoclonal antibody against vascular endothelial growth factor (VEGF). The combination of bev and chemotherapy for first- and second-line treatment of metastatic colorectal cancer has been shown to improve survival [1–4]. Furthermore, a large observational study indicated that use of bev beyond first progression correlated with improved survival [5]. However, use of bev in conjunction with chemotherapy is associated with an increased risk of arterial thromboembolism, and there is also some controversy as to whether bev contributes to the development of venous thromboembolism (VTE) [6]. Pulmonary embolism (PE) occurs in 2–5% of cases where bev and chemotherapy are

used together [1, 3]. A recent meta-analysis of 15 randomized controlled trials [7] found that bev significantly increased risk of VTE in cancer patients and anticoagulant therapy is indicated in the event of VTE.

An implantable central venous access system (CVAS) is a risk factor for VTE [8]. Many VTEs, although asymptomatic, can be as serious as PEs in terms of morbidity [9, 10]. Based on the results of studies on the prevention of catheter-related thrombosis, anticoagulant prophylaxis is not generally recommended with a CVAS [11–13].

In our hospital, symptomatic venous thrombosis associated with a CVAS occurred in patients treated with bev plus chemotherapy during the initial cycle. Appropriate screening and management of patients after detection of either symptomatic or asymptomatic VTE remain to be clarified.

We evaluated the effectiveness of Doppler ultrasound imaging (DUS) in the early detection of CVAS-associated venous thrombosis to determine its potential in the prevention of further development into severe VTE.

## Patients and methods

### Study design

This was a prospective cohort study conducted at a single institute. Patients were enrolled from July 2007 onward after approval of bev in June 2007 in Japan. The study protocol, including the use of DUS, was approved by the institutional review board of our institute. All the patients provided written informed consent before treatment.

The study design is shown in Fig. 1. DUS was performed on the deep venous system in the upper extremities where catheterization had been carried out to detect thrombus formation during the early cycles of chemotherapy. The first DUS was performed after the initial cycle of bev. Only patients with asymptomatic thrombus formation underwent follow-up evaluation by DUS after the third cycle of bev. During DUS, location and dimension of thrombus, vascular flow, and collateral vascular flow were evaluated and diagnosed by a radiologist at our institute. In addition, dimension of thrombus, location, whether it extended as far as the junction of the external jugular vein (EJV), suprascapular vein (SSV) or subclavian vein (SCV), collateral vascular flow, and retention of peripheral vascular flow were evaluated as important factors directly affecting vascular flow.

At our institute, time to treatment from implantation of a CVAS is usually just 2 days. This made it difficult to perform DUS prior to initiation of treatment and we could not delay treatment for that purpose in the patients enrolled in this study. Therefore, as a subordinate examination, we performed additional pre-treatment DUS between implantation of the CVAS and induction of bev in those patients who consented to the procedure.

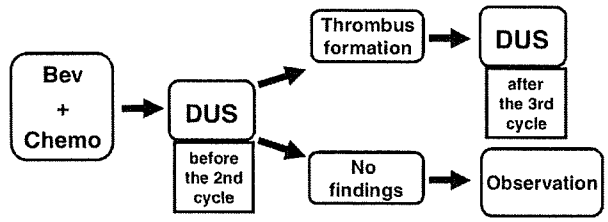



**Fig. 1** Timing of DUS: study schema

### Patients

All the patients conformed to the following criteria: histologically confirmed colorectal cancer; advanced metastatic colorectal cancer; age $\leq$ 70 years; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; no history of thromboembolic events; no prior use of bev; no increased risk factors for bleeding; hypertension, if present, controlled with a single agent.

All the patients received the initial cycle of treatment when they were in the hospital, and additional treatment cycles at an ambulatory center. Complete blood count, international normalized ratio (INR), and D-dimer were measured biweekly or before treatment in all the patients. Deficiencies of protein C, protein S, and antithrombin III as congenital risk factors for thrombosis were examined, as well as the presence of acquired risk factors before initial bev administration.

### Chemotherapy treatment

Treatment regimens were as follows: FOLFOX-4 + bev (biweekly cycles of 85-mg/m$^2$ intravenous oxaliplatin for 2 h on day 1 plus 100-mg/m$^2$ L-leucovorin (L-LV) for 2 h and 400-mg/m$^2$ bolus 5-FU, followed by a 22-h infusion of 600 mg/m$^2$ 5-FU on days 1 and 2, plus 5–10-mg/kg bev on day 1 every 2 weeks); or 5-mg/kg FOLFIRI + bev (biweekly cycles of 150-mg/m$^2$ intravenous irinotecan for 1.5 h on day 1 plus 200-mg/m$^2$ L-LV for 2 h and 400-mg/m$^2$ bolus 5-FU, followed by a 46-h infusion of 1200 mg/m$^2$ 5-FU on days 1 and 2 plus 5-mg/kg bev on day 1 every 2 weeks). Treatment continued until progression, unmanageable toxic effects, or patient refusal. Antiemetic agents were provided at the discretion of the treating physician. No prophylactic use of colony-stimulating factor was permitted. No anticoagulant therapy before initial evaluation by DUS was permitted. If an asymptomatic thrombus that could potentially cause a PE was identified on DUS, anticoagulant therapy was permitted. The anticoagulant treatment regimen was at the discretion of the physician.

### Evaluation of toxicity and efficacy

Patient data were recorded and reviewed on electronic clinical records. Adverse effects were graded in all the patients biweekly or before treatment using the National Cancer Institute Common Toxicity Criteria, version 3.0. Cancer response was assessed every 12 weeks using computed tomography according to the response evaluation criteria for solid tumors. Data on toxicity and tumor evaluation were analyzed using electronic medical records and by examination of films of each patient. A radiologist examined the films and made an assessment of status, and the evaluations were recorded in electronic medical records.

Differences in baseline characteristics and clinical features between patients with and without catheter-related thrombosis were analyzed. We used the Chi-square test (without the Yates correction) and Fisher's exact probability test for categorical comparisons of data. Differences in the means of continuous measurements were tested by the Student's $t$ test and checked by Mann–Whitney $U$ test. Quantitative variables such as time between two points were summarized using medians. A two-way repeated-measures analysis of variance was used to evaluate differences between sequential continuous variables. A $P$ value of $<0.05$ was considered significant.

## Results

### Characteristics of patients

Forty-one patients were enrolled in the study. The baseline characteristics of the patients are shown in Table 1. Median follow-up time from the date of initial bev administration was 484 days (range 72–574 days). Eight patients (19.5%) received an antihypertensive agent at baseline. In addition, no congenital factors for thrombosis were seen, but anticardiolipin antibody IgG and lupus anticoagulant were observed in 5 (12.2%) and 1 (2.4%) patients, respectively.

**Table 1** Baseline characteristics of patients ($N = 41$)

| Characteristics | $N$ (%) |
|---|---|
| Sex: male/female | 17/24 |
| Mean age (range), years | 58.4 (16–69) |
| Chemotherapy regimen | |
| FOLFOX4 + bev 5 mg/kg | 28 (68.3) |
| FOLFOX4 + bev 10 mg/kg | 1 (2.4) |
| FOLFIRI + bev 5 mg/kg | 12 (29.3) |
| ECOG performance status | |
| 0 | 39 (95.1) |
| 1 | 2 (4.9) |
| Treatment set as systemic chemotherapy for metastatic disease | |
| First-line | 28 (68.3) |
| Second-line | 13 (31.7) |
| Prior adjuvant fluorouracil | 5 (12.2) |
| No. of involved organs | |
| 1 | 16 (39) |
| 2 | 19 (46.3) |
| 3 | 5 (12.2) |
| 4 | 1 (2.4) |
| Sites of metastases | |
| Liver | 19 (46.3) |
| Lung | 20 (48.8) |
| Peritoneum | 8 (19.5) |
| Nodes | 17 (41.5) |
| Local recurrence | 7 (17.1) |
| Bone | 1 (2.4) |
| Previous history/complication | |
| Thromboembolic events | 0 |
| Hypertension | 8 (19.5) |
| Diabetes | 2 (4.9) |
| Hyperlipidemia | 1 (2.4) |
| Hyperuricemia | 1 (2.4) |
| Liver function failure | 1 (2.4) |
| Congenital risk factors | |
| Protein C deficiency | 0 |
| Protein S deficiency | 0 |
| Antithrombin III deficiency | 0 |
| Acquired risk factors | |
| Anticardiolipin antibody IgG | 5 (12.2) |
| Anticardiolipin antibody β2-glycoprotein 1 | 0 |
| Lupus anticoagulants | 1 (2.4) |

*Bev* bevacizumab, *ECOG* Eastern Cooperative Oncology Group, *INR* international normalized ratio, *CRP* C-reactive protein, *IgG* immunoglobulin G

### Effectiveness of DUS

The results of DUS are shown in Table 2. Catheter-related thrombosis was observed on initial DUS in 22 patients (53.7%), including both asymptomatic ($n = 21$ [51.2%]) and symptomatic ($n = 1$ [2.4%]) thrombi. No thrombus formation was detected in 19 patients (46.3%). Twenty-two patients received a follow-up DUS, one of whom received anticoagulant therapy after initial DUS. Thrombi disappeared completely in 3 (13.6%) of these 22 patients without anticoagulant therapy.

Comparisons of initial and follow-up DUS findings in asymptomatic thrombi are shown in Table 3. In 21 patients with asymptomatic thrombi, none of the thrombi extended to the superior vena cava, and complete disappearance was seen in 3. Thrombus size was <20 mm in 16 patients (76.2%) on initial DUS, compared to in 16 patients (76.2%) on follow-up DUS. Decreased vascular flow was observed

**Table 2** Results of DUS ($N = 41$)

| | |
|---|---|
| Median length, days (range) | |
| IP-CVAS—induction of bev | 7 (2–695) |
| IP-CVAS—initial DUS | 18 (7–700) |
| Induction of bev—initial DUS | 7 (4–14) |
| Induction of bev—follow-up DUS | 35 (14–49) |
| Results of initial DUS, *n* (%) | |
| Thrombus formation | 22 (53.7) |
| Symptomatic thrombosis | 1 (2.4) |
| Asymptomatic thrombosis | 21 (51.2) |
| No thrombus formation | 19 (46.3) |
| Results of follow-up DUS ($n = 22$), *n* (%) | |
| Thrombus formation | 19 (86.4) |
| No thrombus formation (disappeared) | 3 (13.6) |

*IP-CVAS* implantation of central venous access system, *bev* bevacizumab

in 3 patients (14.3%) on initial DUS that showed no progression on follow-up DUS; however, another 2 patients in whom adequate vascular flow was detected on initial DUS showed decreased vascular flow on follow-up DUS. We defined overall improvement as at least one improved finding without progression in location, maximum size, or (collateral) vascular flow and progression as at least one progressed finding; those fitting neither category were defined as the remainder. Overall, thrombi improved or remained stable in 17 patients (81%), and progressed without symptoms in 4 patients (19%). Correlations between vascular flow and other findings are shown in Table 4. Incidence of thrombi extending into the junction of the SCV, ECV, and SSV was significantly higher in patients with inadequate vascular flow than in patients with adequate vascular flow on initial DUS (66.7 vs. 5.6%, respectively; $P = 0.0414$) and on follow-up DUS (80 vs. 0%, respectively; $P = 0.0016$).

Symptomatic thrombosis was revealed on DUS in 1 patient (a DUS image with a diagram is shown in Fig. 2). This thrombus extended into the superior vena cava, was >40 mm in diameter, and resulted in decreased vascular flow. This patient received anticoagulant therapy after initial DUS and re-started bev after a follow-up DUS revealed that the thrombus had not progressed.

Characteristics of patients with and without thrombi are shown in Table 5. Median follow-up time from date of initial bev administration was 518 days (range 232–574 days) and 487 days (72–559), respectively, in these patients. No significant difference was observed in performance status or age. Presence of acquired risk factors showed no effect on thrombus formation or outcome in patients with asymptomatic thrombus. Incidence of thrombus formation was significantly higher in the FOLFOX + bev treatment group than in the FOLFIRI + bev group ($P = 0.0047$). Median length of time between implantation of CVAS and induction of bev was significantly shorter in patients with thrombus formation than in patients without thrombus formation (5 vs. 107.5 days, respectively; $P = 0.0048$). Similarly, median length of time between implantation of CVAS and initial DUS was significantly shorter in patients with thrombus formation than in patients without thrombus formation (13.5 vs. 116 days, respectively; $P = 0.0059$). In further follow-up after completion of the protocol, 1 patient in whom no thrombus was detected in the planned DUS experienced asymptomatic thrombosis of the inferior vena cava during the 12th cycle. However, we were able to continue FOLFOX in this patient without bev using warfarin.

A comparison of patients with improved thrombus findings on follow-up DUS ($n = 5$) with those showing thrombus progression ($n = 4$) revealed that median follow-up time from date of initial bev administration was 505 days (range 446–563 days) and 395.5 days (range 256–484 days), respectively. No significant differences were observed in findings on initial DUS, median time to induction of bev from implantation of CVAS (5 vs. 6 days; $P = 0.9004$), or laboratory data between the two groups. The results of a two-way repeated-measures analysis of variance to evaluate differences between sequential continuous variables such as platelet count, INR, and D-dimer showed no significant differences. Changes in thrombus size, as well as decreased vascular flow, were mainly related to outcomes of thrombus on initial DUS. However, two patients showing thrombus progression developed pulmonary embolism requiring urgent treatment with a thrombolytic agent followed by warfarin, after which, they were able to continue with FOLFOX without bev until progression of disease. None of the patients experienced sequelae, including post-thrombotic syndrome, and there were no deaths related to thromboembolic events or anticoagulant therapy.

## Discussion

In this study, we assessed the effectiveness of DUS in the early identification of catheter-related thrombosis and variations in asymptomatic venous thrombosis under use of bev.

According to the American Society of Clinical Oncology [14], the presence of a central venous catheter is a risk factor for VTE in cancer patients. Active chemotherapy [15, 16] and antiangiogenic therapy [2, 3] also carry risk of VTE. With the newer antiangiogenic agents, the use of a prophylaxis for VTE is controversial [2, 3, 17, 18].

Although a CVAS makes it easier to administer chemotherapy in ambulatory patients, its use is associated with

**Table 3** Findings associated with asymptomatic thrombosis ($n = 21$)

| Findings | Initial DUS | Follow-up DUS |
|---|---|---|
| Location, *n* (%) | | |
| Distal (not extended to SVC) | 21 (100) | 18 (85.7) |
| Central (extended to SVC) | 0 | 0 |
| Comparison | Improved (disappeared) in 3 patients (14.3) | |
| Maximum size, *n* (%) | | |
| 0–<10 mm | 14 (66.7) | 12 (57.1) |
| 10–<20 mm | 2 (9.5) | 4 (19) |
| 20–<30 mm | 3 (14.3) | 3 (14.3) |
| >30 mm | 2 (9.5) | 2 (9.5) |
| Comparison | Improved in 5 patients (23.8) (disappeared in 3 and reduced in 2) | |
| | Progressed in 4 patients (19) | |
| Vascular flow, *n* (%) | | |
| Adequate | 18 (85.7) | 13 (61.9) |
| Inadequate | 3 (14.3) | 5 (23.8) |
| Comparison | Improved in 3 patients (14.3) | |
| | Progressed in 2 patients (9.5) | |
| Collateral vascular flow, n (%) | | |
| Adequately increased | 2 (9.5) | 2 (9.5) |
| Inadequately increased | 1 (4.8) | 3 (14.3) |
| Comparison | Progressed in 2 patients (9.5) | |
| Overall evaluation[a] | Improved in 5 patients (23.8) | |
| | Stable in 12 patients (57.1) | |
| | Progressed in 4 patients (19) | |

[a] Overall improvement was defined as at least one improved finding without progression in location, maximum size, or (collateral) vascular flow and progression as at least one progressed finding; those fitting neither category were defined as the remainder. One patient receiving anticoagulant therapy after initial DUS showed a thrombus 45 mm in diameter that developed into the brachiocephalic vein; no progression was noted. One patient with thrombus progression experienced a symptomatic pulmonary embolism after the sixth cycle, and one progressed patient experienced an asymptomatic pulmonary embolism after the sixth cycle

*SVC* superior vena cava

**Table 4** Correlation between vascular flow and other findings of asymptomatic thrombosis ($n = 21$)

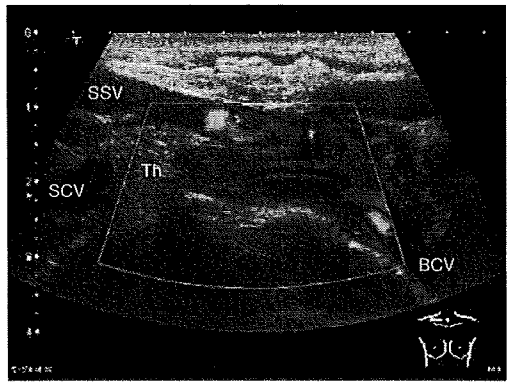
| Findings on DUS | Initial DUS ($n = 21$) | | Follow-up DUS ($n = 18$) | |
|---|---|---|---|---|
| | Adequate ($n = 18$) | Inadequate ($n = 3$) | Adequate ($n = 13$) | Inadequate ($n = 5$) |
| Location, *n* (%) | | | | |
| SCV–ECV–SSV junction[a] | 1 (5.6) | 2 (66.7) | 0 | 4 (80) |
| *P* value | 0.0414 | | 0.0016 | |
| Maximum size, *n* (%) | | | | |
| <30 mm | 18(100) | 1 (33.3) | 13 (100) | 3 (60) |
| ≥30 mm | 0 | 2 (66.7) | 0 | 2 (40) |
| *P* value | 0.0143 | | 0.0654 | |

[a] Thrombi extended into junction of SCV, ECV, and SSV in two inadequate patients at initial DUS; both thrombi sizes were ≥30 mm

*DUS* Doppler ultrasound imaging, *SCV* subclavian vein, *ECV* external jugular vein, *SSV* suprascapular vein, *N.A* not applicable

an increased risk for VTE and PE [8–10]. According to a review by Vescia et al. [19], the incidence of catheter-related thrombosis varied from 12 to 64% in four retrospective studies [20–24]. In a recent prospective trial using phlebography in patients with a CVAS, Verso et al. [25] found that the incidence of thrombosis in two groups receiving low molecular weight heparin (LMWH) or placebo for 6 weeks was 14.1 and 18%, respectively (95% CI 0.47–1.31; $P = 0.35$), with symptomatic upper limb thrombosis seen only in 1.0% of the LMWH group and 3.1% of the placebo group (hazard ratio 0.32; 95% CI 0.07–1.66). In another trial by Couban et al. [12], the rate of symptomatic thrombosis in a group received 1-mg warfarin for 9 weeks was 4.6% when compared with 4.0% in the placebo group (hazard ratio 1.20; 95% CI 0.37–3.94).

We summarized thromboembolic events reported in nine pivotal studies of bev plus chemotherapy [1–4, 17, 18, 26–28]. According to the results, the incidence of thromboembolism ranged from 3 to 26% in these studies, and PE was reported in 1–4% of cases. Prophylactic anticoagulant treatment was not permitted in any study, except for maintenance of CVAS in four studies [1, 2, 4, 18].



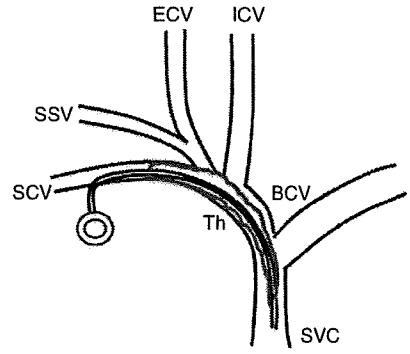




**Fig. 2** Findings of DUS image and illustration in symptomatic case. This thrombus (Th) extended into superior vena cava (SVC) through brachiocephalic vein (BCV), was >40 mm in diameter, and resulted in clearly decreased vascular flow

**Table 5** Comparison between patients with and without thrombus formation

| | With thrombus ($n$ = 22) | Without thrombus ($n$ = 19) | $P$ value |
|---|---|---|---|
| Sex: male/female | 9/13 | 8/11 | >0.9999 |
| Mean age (range), years | 62 (16–69) | 60.1 (47–69) | 0.9896 |
| ECOG performance status, $n$ (%) | | | |
| 0 | 22 (100) | 17 (89.5) | 0.2308 |
| 1 | 0 | 2 (10.5) | |
| Chemotherapy regimen, $n$ (%) | | | |
| FOLFOX4 + bev | 20 (90.9) | 9 (47.4) | 0.0047 |
| FOLFIRI + bev | 2 (9.1) | 10 (52.6) | |
| Prior treatment, $n$ (%) | | | |
| FOLFOX | 2 (9.1) | 10 (52.6) | 0.0047 |
| Hepatic arterial infusion | 3 (13.6) | 4 (21.1) | 0.6847 |
| Radiation | 3 (13.6) | 0 | 0.2354 |
| No. of involved organs, $n$ (%) | | | |
| 1/2/3/4 | 10/10/2/0 | 6/9/3/1 | 0.3899 |
| ≥3 | 2 (9.1) | 4 (21.1) | |
| Baseline laboratory data, mean ± SD | | | |
| Platelets ($\times 10^4$ μl) | 22.6 ± 5.68 | 18.89 ± 6.84 | 0.0652 |
| INR | 1.05 ± 0.49 | 1.05 ± 0.12 | 0.9811 |
| D-dimer | 1.15 ± 1.52 | 1.21 ± 0.83 | – |
| Acquired risk factors, $n$ (%) | | | |
| Anticardiolipin antibody IgG | 3 (13.6) | 2 (10.5) | >0.9999 |
| Lupus anticoagulants | 1 (4.5) | 0 | >0.9999 |
| Median length (range), $n$ (%) | | | |
| IP-CVAS—induction of bev | 5 days (2–252) | 107.5 days (2–695) | 0.0048 |
| IP-CVAS—initial DUS | 13.5 days (7–259) | 116 days (7–700) | 0.0059 |

*ECOG* Eastern Cooperative Oncology Group, *bev* bevacizumab, *INR* international normalized ratio, *IP-CVAS* implantation of central venous access system, *DUS* Doppler ultrasound imaging, *SD* standard deviation

According to these studies, the incidence of thromboembolic events was not high and routine prophylactic anticoagulant treatment for thromboembolism did not appear necessary.

Patient characteristics in our study were similar to those in previous reports, and no specific characteristics related to thrombus formation were seen. However, we observed a higher rate of thrombi than expected using DUS and almost all of them were asymptomatic. Indeed, this study was designed to detect diagnostic findings, not clinical findings.

This study had a number of limitations. First, there was no control population (with no administration of bev). In other words, thromboembolic events may have been due to prior chemotherapy rather than bev, as only small doses of bev had been given at first screening. Second, the study protocol did not provide true baseline DUS at pre-treatment, as the time to treatment from implantation of the CVAS was usually just 2 days or more. Therefore, it was difficult to establish whether there was a correlation between the treatment drugs and catheter-related thrombosis, or when