は，Japan Clinical Oncology Group(JCOG)胃癌外科グループにおいて，大規模多施設共同臨床試験としてその妥当性が検討されている．本法の合理性および有用性が証明されれば，乳癌における縮小手術のようにただちに臨床応用につながると考えられるため，その成績が期待されている．

2. 大腸癌

前述のごとく，結腸癌を中心に色素法が行われている．わが国では，結腸癌に対してsentinel node conceptを利用した縮小手術を行っても，術後のquality of lifeに影響を与えないと考えられていることから，あまり行われていないのが現状である．これに対して欧米では，sentinel nodeにおける微小転移を詳細に調べることによって，ultrastagingを行ったり，術後化学療法の適応を決定したりする目的で，積極的に行われている[18]．

結腸癌における色素の注入部位は，筋層以深に浸潤する進行癌が多いことや，術中内視鏡を行うことが非常に負担であることから，ほとんど漿膜側アプローチで行われている．漿膜側アプローチはSahaら[19]のグループにより，確立されている．すなわち，腸間膜のリンパ系を破壊しないように，腫瘍のある結腸を遊離した後に，mappingを行う．1%リンファズリンを腫瘍を取り囲むように漿膜下に注入する．全体で1～2 m*l*注入するが，この際，30 G針を用いることと，薄い結腸壁を突き抜けて色素を内腔に漏らさないようにすることに注意しなければならない．いったん腸内に色素が漏れると粘膜から再吸収が起こり，偽のsentinel nodeを染め出してしまうとされている．この方法により，mapping成功率は100%で，感度は93%ときわめて良好な成績を報告した．彼らは同時にサルファコロイド(RI)との成績を比較しているが，それぞれ89%，92%とまったく遜色はなかったとしている．

Codignolaら[20]は，パテントブルーを用いて56例の大腸癌で検討しているが，mapping成功率は100%で，感度は89%とやはり良好な成績を示している．これまでの報告をみると，胃癌とほぼ同様で単一施設からの成績は，同定率90%以上，正診率80%以上と，おおむね良好である．しかし2004年に発表された多施設共同研究の成績は，惨憺たるものであった[21]．それによるとsentinel node同定率は92%に対して，偽陰性率が54%ときわめて高く，sentinel node conceptに疑問を投げかけている．しかし，この試験では外科医一人当りのmapping施行例が少ないことが問題とされており，このことは色素法によるmappingにおけるlearning curveの必要性を示唆しているといえよう．

おわりに

この項のポイント

- 臨床応用のためには，多施設共同臨床試験の結果が期待されるが，色素法の成績の向上のためには色素の観察法の工夫や新しい色素の開発が重要である．

そのほかにも色素法によるmappingは膵癌などでも行われているが[22]，トライアル的なものである．今後，早期胃癌においてはevidenceに基づいた縮小手術を行うためにはsentinel node mappingは欠かせないものであり，二つの多施設共同臨床試験の結果が重要な意味をもっている．この二つの試験については別稿を参照していただきたい．一方，結腸癌においては，わが国ではすべての郭清リンパ節の転移の有無を調べるのが常になっているが，欧米のようにsentinel nodeを詳細に検討してstagingを行うという考え方にshiftするよう

なことがあれば，mappingが臨床に取り入れられていく可能性もあると思われる．さらなる同定率，正診率の向上のために，前述のICGの観察方法における工夫や，新しい蛍光色素の開発などが行われている．

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Summary

Detection of Sentinel Node — Dye-directed Method

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Tetsuo Ohta* and Koichi Miwa**

Dyes, such as Lymphazurin, patent blue V, and indocyanine green, are frequently used as tracers for sentinel node mapping. This is because dye-directed mapping is less expensive and does not require any special equipment other than that used with the radioisotope-guided method. Stained lymph nodes and lymphatics can be observed with the naked eye. However, the performance of the dye-directed mapping requires training to learn how to inject dye and how to search for stained lymph nodes, requiring experience with at least 30 patients.

Sentinel node mapping, using these dyes, is applied for gastric and colonic cancer in the gastrointestinal field. Identification and accuracy rates are over 90% and over 85%, respectively. To improve outcomes in the dye-directed mapping it is necessary to utilize some devices to detect indocyanine green staining and to develop new fluorescein dyes.

Key words : dye-directed sentinel node mapping, gastrointestinal cancers, Lymphazurin, patent blue V, indocyanine green

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Ⅱ．各　論

2. 消化器癌

a) 食道癌

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2. 消化器癌
a) 食道癌*

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〔要旨〕食道癌は頸部から腹部まで3領域に広範にリンパ節転移しやすく跳躍転移も多く，また手術侵襲も大きい．近年，乳癌などではセンチネルリンパ節理論を応用した縮小手術が行われている．食道癌においてセンチネルリンパ節理論はRI法でcT1N0，腫瘍径5cm以内と症例を限定すれば成立する可能性が示唆された．今後，センチネルリンパ節理論を応用した個別化治療により，食道癌治療後のQOL改善が期待される．

はじめに

わが国では，胸部進行食道癌のリンパ節郭清は1980年中ごろよりそれまで主流であった胸部・腹部の2領域リンパ節郭清から頸部・胸部・腹部の3領域リンパ節郭清が行われ，予後の向上が得られるようになり標準術式とされている[1,2]．手術の侵襲などによる合併症は術中・術後管理の進歩により少なくなったが，難渋する症例も依然として存在する．近年，食道表在癌は診断および技術の進歩により，精度の高い進行度診断ができるようになり，内視鏡的粘膜切除術の適応の拡大が行われている．しかし，手術においては進行度に応じた術式が選択されてはいない．乳癌[3]や悪性黒色腫[4]においてはセンチネルリンパ節(SN)生検が行われ，SNに転移のない症例にはリンパ節郭清を省略する縮小手術が行われている．侵襲が大きく，郭清範囲の広い食道癌にこのSN理論を応用した縮小手術の妥当性が立証できれば，侵襲の軽減と根治性を両立させ，標準的治療から個別化治療に発展することができる．

本稿では，食道癌におけるSN理論の検証と臨床応用について概説する．

I. 食道癌のリンパ節転移

第44回食道疾患研究会のアンケート調査[5]によれば占居部位別のリンパ節転移は胸部上部食道(Ut)では頸部から上縦隔に多いが，腹部にも10%の頻度でみられた．胸部中部食道(Mt)では頸部，縦隔，腹部にわたる広範囲にみられた．胸部下部食道(Lt)では中下縦隔から腹部に多くみられたが頸部にも数%みられた．癌の占居部位によりある程度リンパ節転移の多い部位は推察することはできるが，跳躍転移も多く，症例ごとに正確に診断することは困難である．

キーワード：食道癌，センチネルリンパ節，縮小手術

* Sentinel node navigation for esophageal cancer

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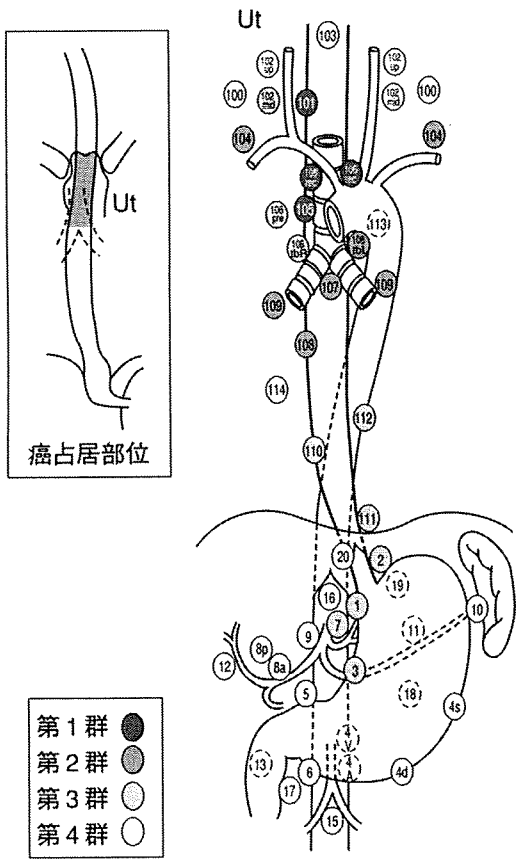


	転移リンパ節（単独転移群）	SN
頸　部	1例（33%）	2例（100%）
上縦隔	2例（67%）	2例（100%）
中下縦隔	—	2例（100%）
腹　部	—	—
106rec（＋）	1例（33%）	1例（50%）
症例数	3例	2例

図1．転移リンパ節，SNの分布（Ut）

II．リンパ節転移1個症例の検討

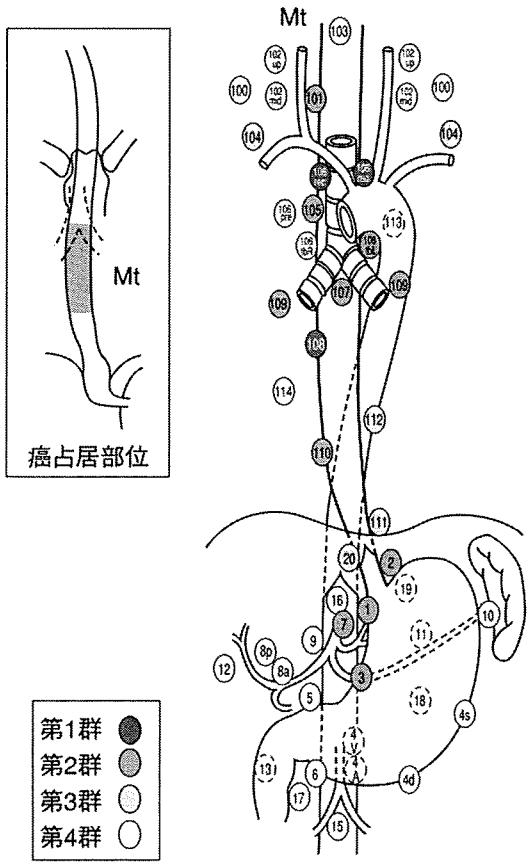
胸部食道癌314例中，右開胸で切除されたリンパ節転移が1個の51例（16.2%）を対象にした．転移リンパ節を占居部位別に頸部，上縦隔，中下縦隔，腹部に分け分布を検討すると，転移1個のみの症例は上縦隔と腹部に多い結果であった．これは梶山ら[6]の報告と同様な結果であった．占居部位別にみると，Utでは頸部と上縦隔に多く（図1），Mtでは上縦隔と腹部に多かったがすべての領域に認められ（図2），Ltでも上縦隔と腹部に多く認められたが頸部にも認められ予測は困難であった（図3）．

もし，SN理論が成立するならば，転移1個症例のその転移リンパ節はSNのはずである．この結果からは，SNはどの部位にも存在しえることが示唆された．

III．食道癌におけるSN同定方法

1．トレーサーの選択

SNを同定する方法はisosulfan blue，indocyanine greenを用いる色素法とradioisotope（RI）法がある．粒子径の小さい色素は注入直後よりリンパ管に流入するため視認できるという利点がある．しかし，食道癌の手術では頸，胸，腹部と手術部位が3領域となり，さらに食道を剥離授動することにより正常なリンパ流が破壊され，色素が漏れて同定困難になったり，色素のリンパ節滞留時間が短いために視認できなくなり不向きである．一方，RI法では半減期が6時間と比較的短い^{99m}Tcで標識したスズコロイドかフチン酸が用いられている．RI法は色素法に比べて滞留時間が長く，深部のリンパ節同定にも有用である．また，術前にリンパシンチグラフィによりSNの位置が



	転移リンパ節（単独転移群）	SN
頸　部	2例（7%）	1例（7%）
上縦隔	6例（21%）	8例（53%）
中下縦隔	5例（18%）	12例（80%）
腹　部	15例（54%）	6例（40%）
106rec（＋）	4例（14%）	7例（46%）
症例数	28例	15例

図2．転移リンパ節，SNの分布（Mt）

ある程度確認できるという利点からも，現時点では食道癌においてはRI法が有用と考えている．

2．RIの注入法と注入量

RIは診療用放射性同位元素使用室で取扱わなければならない．トレーサーは術前日，内視鏡下に23 G食道静脈瘤穿刺針を用いて粘膜下層に0.5 m*l*ずつ4ヵ所，腫瘍を取り囲むように注入している．投与量は，手術時のアイソトープの活性を0.3 mCiとするためには^{99m}Tcの半減期が6時間であるので同定12時間前1.2 mCi，18時間前2.4 mCi，24時間前4.8 mCiのトレーサーの注入が必要となる[7]．

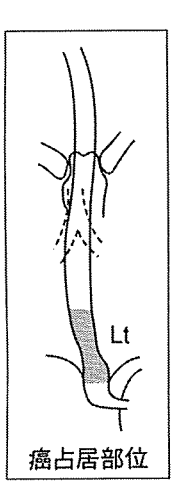
3．センチネルリンパ節同定基準

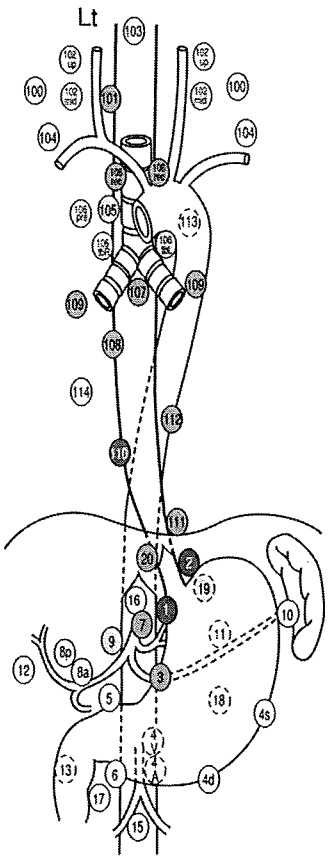
多くの施設では10秒間の積算カウントがバックグランドの10倍以上をSNとしている[8]．このバックグランドの10倍とは抽象的でわかりにくいが，バックグランドのカウントを1として10秒間の積算カウントが10 count/10秒以上にしているのが現状である．SNの多くは100 count/10秒以上になるので同定に難渋することは少ない．主病巣周囲では注入部位からの強い散乱線などの影響があるため，必ず*ex vivo*でカウントを確認する必要がある．

4．SNの同定法

RI法の利点として，術前にリンパシンチグラフィによりSNの部位をある程度特定できることがあげられるが，トレーサー局注部位周囲は放射活性が高いため（shine through現象）SNを描出することが困難な症例もある．そのためにいろいろな画像処理をすることにより，トレーサー局注部位周囲のSNも描出できるようになってきている．

手術中はリンパシンチグラフィを参考に丹念に検索する必要がある．SNは，リンパ節内に流入





	転移リンパ節（単独転移群）	SN
頸　部	1例（5%）	—
上縦隔	3例（15%）	2例（40%）
中下縦隔	2例（10%）	5例（100%）
腹　部	14例（70%）	5例（100%）
106rec（＋）	2例（10%）	2例（40%）
症例数	20例	5例

図3．転移リンパ節，SNの分布（Lt）

したトレーサーから放出されるγ線を検出するガンマプローブで検索し同定される．トレーサー局注部位周囲は放射活性が高いため，この部位を避ける方向からのガンマプローブの操作が重要となる．また，より正確に同定するためにガンマプローブの先端にコリメータを装着したり[9]，局注部位を鉛板で遮蔽しshine through現象を減少させる工夫をしている．

IV．SN同定の成績

適応は比較的リンパ節転移の少ないcT1N0にしている．SNの同定率は95.7%（22/23例），転移陽性検出率は75%（6/8例），リンパ節転移正診率は91%（20/22例）であった．偽陰性2例は5 cm以上の表層拡大型であり，腫瘍径5 cm以下のリンパ節転移正診率は100%（20/20例）であった．腫瘍径5 cm以上では，粘膜下層に0.5 mlずつ4ヵ所の注入では局注したトレーサーが腫瘍全体に及ばず，適応外と考えられた．SNの個数は平均5.3個で，リンパシンチグラフィによるSNは平均1.4個であった．

術中のSN個数とリンパシンチグラフィによるSNの個数に乖離がみられたのは，主病巣周囲では強い散乱線によりリンパ節への集積が隠されてしまうためと考えられた．また，リンパシンチグラフィのプラナー像では，SNの正確な位置を確認することは困難でありSPECT画像を追加したりCTなど他のモダリティとfusionさせることにより，より解剖学的に正確にSNを描出できるように検討中である．

食道癌におけるSNの分布を検討してみると，頸部には3例（14%），上縦隔12例（55%），中下縦隔19例（86%），腹部11例（50%）にみられた．占居部位別にみると，Utでは頸部，上縦隔，中

下縦隔に認められたが腹部には認めなかった(図1)．Mtでは上縦隔と中下縦隔に多かったが，すべての領域に認められた(図2)．Ltでも中下縦隔と腹部に多く認められたが，頸部には認められなかった(図3)．

リンパ節106recがSNであったのは10例(45%)で，頸部にSNが同定できた3例中2例は106recにSNを認めなかった．SN理論からは，106recリンパ節を頸部郭清の指標にするのは危険であると推察された．食道癌取扱い規約によるNカテゴリーにおける検討では，SNが1群のみに分布する症例は2例(9%)にすぎなかった．また，跳躍転移といわれる症例が5例(23%)あり，跳躍転移の中にはSNがたまたま2群以遠に存在した症例が含まれる可能性が示唆された．

今回の検討では適応症例をcT1N0，腫瘍径5cm以下に限定すればSN理論が成立する可能性が示唆された．

V. 臨床応用と今後の課題

食道癌では胃癌のように腫瘍の局所切除はむずかしい．Mtで頸部郭清の必要のない症例やLtで頸部郭清の必要な症例の見極めに有用と考える．また，思わぬ部位にSNが存在したり，郭清後の取り残しのリンパ節の検索にも有用である．さらに適応拡大されている内視鏡的粘膜切除術/内視鏡的粘膜下層剥離術(EMR/ESD)の追加治療の決定においての一つの手段になりうると考える．

術中迅速診断のあり方については，現時点で一定の合意は得られていない．現在の最大割面1切片のHE染色のみで転移の有無を決定するには不安が残る．さらに，サイトケラチン抗体などを用いた免疫組織染色やRT-PCR法などによる微小転移の検索までの必要性の有無など迅速診断の精度の向上は，臨床応用に向けてデータの集積が必要である．

現在使用されているトレーサーはSNを同定するために開発された製剤ではない．SNをより特異的に同定できるような特殊なトレーサーの開発が望まれる．

おわりに

SN理論が成立することにより，個別の必要に応じたテーラーメードの治療が可能になり食道癌の治療後のQOLは改善されると思われる．

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Detection of sentinel and non-sentinel lymph node micrometastases by complete serial sectioning and immunohistochemical analysis for gastric cancer

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Abstract

Background: We investigated the presence and distribution of the sentinel and the non-sentinel node micrometastases using complete serial sectioning and immunohistochemical staining (IHC), to inspect whether lymph node micrometastases spread to the sentinel lymph nodes first.

Methods: A total of 35 patients, who underwent gastrectomy with a sentinel lymph node biopsy for gastric cancer, were enrolled in this study. Total of 1028 lymph nodes of 35 patients having gastric cancer without metastasis of lymph node by permanent section with hematoxylin and eosin staining (H&E) were selected. There were 252 sentinel nodes and the other 776 were non-sentinel nodes. All nodes were sectioned serially and stained alternately with H&E and IHC. Lymph node micrometastases was defined as proving to be positive first either the IHC or the complete serial sectioning.

Results: Micrometastases were detected in 4 (11%) of the 35 patients, 6 (0.58%) of 1028 nodes. Of these 4 patients, 3 had micrometastases exclusively in sentinel nodes, and the other had micrometastasis in both sentinel and non-sentinel nodes. There was no patient who had the micrometasitases only in non-sentinel nodes.

Conclusion: These results support the concept that lymph node micrometastasis of gastric cancer spreads first to sentinel nodes.

Background

The prognosis of patients with gastric cancer is influenced primarily by the presence of lymph node metastases. Lymph node metastases in gastric cancer patients with submucosal invasion occur in 15 to 20% of patients; therefore, a lymph node dissection may be unnecessary

for the remaining 80 to 85% of patients [1]. An accurate and reliable indicator to predict the absence of lymph node metastases would eliminate many unnecessary lymphadenectomies [1]. Therefore, a preoperative and accurate diagnosis of lymph node metastases remains important [2-4]. A sentinel node biopsy for gastric cancer is an intraoperative diagnostic method to detect lymph node metastases [5-7]. In 1992, Morton et al. [8] introduced the technique of intraoperative dye injection at the site of melanoma to identify the "sentinel" node, which is the first node that the afferent lymphatics enter from the tumor site. Miwa et al. [7,9] employed this type of dye mapping technique to identify the sentinel nodes of gastric cancer, and reported a high positive predictive value and accuracy for the sentinel node biopsy of early gastric cancer. On the other hand, the presence of a micrometastasis in a lymph node is a serious issue for the clinical application of sentinel node biopsy for early gastric cancer. Lymph node micrometastases have been found in patients determined to be node-negative by routine histological examination. Previous investigators have reported that lymph node micrometastases could be detected using step sectioning, immunohistochemical staining and the reverse transcriptase-polymerase chain reaction [10-12]. However, there have been a few reports about the distribution of micrometastases in both the sentinel and non-sentinel nodes in node-negative gastric cancer patients by routine histologic examination [13-15].

In this study, we retrospectively investigated the presence and distribution of sentinel and non-sentinel node micrometastases using complete serial sectioning and immunohistochemical staining. These technique are the most accurate methods to detect micrometastases in nodes so that we could determine whether lymph node micrometastases had spread to the sentinel lymph nodes first.

Methods

A sentinel lymph node (SLNs) biopsy for gastric cancer was performed on 243 patients at the Department of Gastroenterologic Surgery, Kanazawa University Hospital from 1993 to 2002. Before the sentinel node biopsy was performed, written informed consent was obtained in accordance with the ethical standards of the Committee on Human Experimentation of Kanazawa University Hospital. Of these patients, we enrolled 35 who had a cancer that had invaded to the submucosa or muscularis propria and had no lymph node metastasis by routine histologic examination for this study. None of the patients had received preoperative chemotherapy or radiotherapy. Based on the Japanese Classification of Gastric Carcinoma, all 35 patients underwent a sentinel node biopsy followed by conventional lymphadenectomy for back-up dissection [16]. A total of 1028 lymph nodes were removed from the 35 patients. Of these, 252 lymph nodes were SLNs and the other 776 were non-SLNs. All 252 SLNs were negative for metastases both on intraoperative frozen-section examination and permanent section with hematoxylin and eosin staining (H&E). The other 776 nodes were negative for metastases on histological examination by H&E of multiple step sectioning at 0.2 cm intervals. The clinicopathologic data were evaluated according to the Japanese Classification of Gastric Cancer [16]. The patients characteristics are listed in Table 1. For detecting the SLNs, we used intraoperative endoscopic lymphatic mapping (IELM), which consisted of an intraoperative injection of 0.2 ml of 2% patent blue into the submucosal layer at four sites around the gastric carcinoma through a gastroscope [9]. The dye immediately appeared at the serosal surface and stained the lymphatic vessels and nodes [5,9]. In this study, the SLN was defined as the lymph node that stained blue 20 minutes after the injection. The lymphatic basins were defined as the area containing the stained lymphatic vessels, and which were able to be divided into five categories according to the directions of the arteries surrounded the stomach, as follows: the left gastric artery area, the right gastric artery area, the right gastroepiploic artery area, the right gastroepiploic artery area and the posterior gastric artery area. The excised SLNs were sent for frozen-section examination. The lymph nodes stained with H&E on representative sections were cut along the plane with the largest diameter that included the node hilus, and examined intraoperatively for metastases.

The remaining frozen tissues were thawed, and the tissues and non-SLNs were routinely cut at 0.2 cm intervals. Subsequently, the multiple sectioned lymph nodes and

Table 1: Patients characteristics

Median age (range)	62 (37–85)
Sex	
Male	23
Female	12
Depth of invasion	
SM	24
MP	11
Histological type	
Differentiated *	18
Undifferentiated **	17
Lymphatics invasion	
Negative	16
positive	19
Vascular invasion	
Negative	30
positive	5

SM, submucosa; MP, muscularis propuria
*The differentiated type;papillary, well and moderately differentiated tubular adenocarcinomas
**The undifferentiated type;poorly differentiated adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma.

resected specimens were fixed in 10% formalin, processed through graded ethanol, and embedded in paraffin for permanent sections. The lymph nodes were stained with H&E and were examined by two pathologists.

In this study, all resected lymph nodes were sectioned serially at 25-μm intervals of 4-μm thickness in addition and either alternately stained with H&E and immunohistochemical staining (IHC) using an anti-cytokeratin antibody. The ENVISION technique was used (DAKO, Carpinteria, CA) for IHC and we used the monoclonal anti-human cytokeratin 8/18 antibody (Santa Cruz Biotechonology, California, USA) [17-19]. All main tumor specimens from 35 patients were subjected to cytokeratin staining and were used as a positive control. A lymph node micrometastasis was defined as a node negative for metastasis by our routine histologic examination, but positive by either the IHC or the complete serial sectioning methods.

Results

The total number of sections examined was 24,094. Of these, 5986 were SLNs and 18,108 were non-SLNs. Of the 35 patients, 4 (11%) had micrometastases. A micrometastasis was found in 6 of 1028 nodes (0.6%) and 60 sections (0.3%) of 24,094 (Figs. 1, 2). Of these 6 nodes involving a micrometastasis, 4 were SLNs and the other 2 were non-SLNs in the lymphatic basin. No micrometastases were detected outside the basin (Table 2). The details of the distribution pattern, location and size of the micrometastases are shown in Tables 3, 4 and 5. Of the 4 patients who had a lymph node micrometastasis, 3 patients had micrometastases exclusively in the SLNs. The other patient had a micrometastasis in both the SLN and non-SLNs in the lymphatic basin. No patient had a micrometastasis only in non-SLNs. No patient has yet suffered a recurrence or has died as of December, 2007.

Discussion

Miwa et al. introduced the concept of sentinel node biopsy for gastric cancer [7]. The clinical use of the sentinel node biopsy to determine the surgical approach for gastric cancer requires the verification of this concept at the level of lymph node micrometastases. In this study, we investigated the presence and distribution of lymph node micrometastases in patients with gastric cancer who had a sentinel node biopsy. Recently, the presence of lymph node micrometastases undetectable by routine histological examination has been reported in breast, lung, esophagus, stomach, colon and gallbladder cancers. It has been reported that a lymph node micrometastasis was a poor prognostic indicator in breast, lung, and colon cancers [20-22]. A few authors have reported that a lymph node micrometastasis was a poor prognostic factor in gastric cancer patients [23,24]. Thus, the importance of the detecting a lymph node micrometastasis has been emphasized for various neoplastic diseases.

A variety of methods to detect lymph node micrometastases exist, including IHC and polymerase chain reaction assays. Matsumoto et al. demonstrated that the reverse transcriptase-polymerase chain reaction (RT-PCR) is more sensitive than IHC for the detection of micrometastases [2]. However, Yamamoto et al. [25] suggested that positive results with a molecular assay such as RT-PCR may not be indicative of the presence of viable tumor cells, but rather the presence of tumor DNA and thus, may be associated with a greatly increased false positive rate despite the higher sensitivity of the molecular assay. On the other hand, it has been reported that the serially sectioning increased the identification of tumor cells in the peripheral sinuses of lymph nodes [26]. It is thought that serial sectioning with IHC is the most accurate method for the detection of lymph node micrometastases. Therefore, we subjected the entire specimen to serial sectioning and IHC. The antibody used for IHC was a monoclonal anti-human cytokeratin 8/18 antibody which is more sensitive, specific, simple, accurate, and economic than other antibodies for IHC.

Tumor deposits within lymph nodes were classified and staged according to the revised guidelines set by the International Union Against Cancer (UICC) 6th Edition. According to this classification system, metastases less

Table 2: Number and location of lymph nodes with micrometastasis

Location of nodes	Number of nodes	
	with micrometastasis	without micrometastasis
SLNs	4	248
non-SLNs in lymphatic basin	2	653
non-SLNs out of basin	0	121
All nodes	6	1022

SLNs, sentinel lymph nodes

Table 3: Distribution pattern of lymph node micrometastasis

Distribution pattern of micrometastasis	Number of cases	
	Routine histological examination	complete serial sectioning and IHC
SLNs (-), non-SLNs (-)	35	31
SLNs (+), non-SLNs (-)	0	3
SLNs (+), non-SLNs (+)	0	1
SLNs (-), non-SLNs (+)	0	0

SLNs, sentinel lymph nodes; IHC, immunohistochemic staining

than 0.2 cm were considered micrometastases (MMs), and isolated tumor cells (ITCs) were single tumor cells or small clusters of cells that measured no greater than 0.2 mm and were usually detected by IHC or molecular methods, but may be verified with H&E. ITCs do not typically show evidence of metastatic activity by proliferation, induction of a stromal reaction, or penetration of vascular or lymphatic sinus wall invasion [27,28]. Nakajo et al. [23] and Siewert et al. [29] reported that lymph node involvement is classified into cluster formation or single cell forms, according to the results of IHC for cytokeratin. Their results suggested that single cells cannot proliferate in lymph nodes because they were already killed by local and general immunocytes. A cluster of cells with a stromal reaction may easily proliferate and therefore have metastatic potential. In our department, resected lymph nodes are routinely cut at 0.2 cm intervals and the lymph nodes are examined for metastases. So, in this study, the definition of lymph node micrometastasis differed from the UICC classification. For this study it was defined as a node, negative for metastasis by our routine histological examination of sections cut at 0.2 cm intervals, but positive by complete serial sectioning with H&E and IHC. In addition, all single cell types and small cluster types without a stromal reaction by cytokeratin positive staining were not recognized as cancer cells in the next H&E stained slide. Thus, we excluded all single cells and small clusters without a stromal reaction, which are classified as ITCs in the UICC classification system, from the positive lymph node micrometastasis group [27,28].

We excluded the gastric cancer patients whose tumors had invaded to the mucosa in this study because doing complete serial sectioning and immunohistochemical staining was a lot of work; in addition, the rate of a lymph node micrometastasis was low. Accordingly we enrolled 35 who had a gastric cancer that had invaded to the submucosa or muscularis propria and had no evidence of a lymph node metastasis by histologic examination for this study.

In this study, we observed a lymph node micrometastasis in 4 patients (11%) with a gastric cancer that had invaded to the submucosa or muscularis propria. In gastric cancer, Isozaki et al. [30] and Natsugoe et al. [31] reported that lymph node micrometastases were identified in 10 to 30% of specimens by step-sectioning or IHC. Our results were the most accurate of all the past studies and proved the actual circumstances of lymph node micrometastasis of gastric cancers that had invaded to the submucosa or muscularis propria.

In this study, we examined the lymph node micrometastases of SLNs and non-SLNs. We found lymph node micrometastases in the SLNs of 4 patients. One patient also had a micrometastasis in a non-SLN of the lymphatic basin, though no micrometastases of non-SLNs were identified outside the basin. Furthermore, no patient had a lymph node micrometastasis only in a non-SLN. Our results revealed that the patients who didn't have a lymph node micrometastasis in the SLNs also didn't have a micrometastasis in the non-SLNs. These results may support the concept that lymph node micrometastases spread

Table 4: Location of lymph node micrometastasis

case	Location of tumor	Stained lymphatic basins	Number of SLNs	Number of micrometastsis	Station of micrometastasis of SLNs	Station of micrometastasis of non-SLNs
1	M, Less	Left GA	8	1	No.3 LN	-
2	M, Post.	Left GA Right GEA	5	1	No.3 LN	-
3	M, Ant.	Left GA	2	1	No.3 LN	-
4	M, Less	Left GA Right GEA	10	3	No.3 LN	No.3 LN

SLNs, sentinel lymph nodes; M, Middle third of the stomach; Less, lesser curvature; Post.; posterior wall; Ant. anterior wall; GA, gastric artery; GEA, gastroepiploic artery; No.3 LN, LN along the lesser curvature

Table 5: Site and size of lymph node micrometastasis

case	Type of lymph node	Site in lymph node	Size of micrometastasis(mm)
1	SLN	peripheral sinus	1.2
2	SLN	peripheral sinus	0.3
3	SLN	peripheral sinus	0.2
4	SLN	peripheral sinus	0.6
	non-SLN	peripheral sinus	1.0
	non-SLN	peripheral sinus	1.0

SLN, sentinel lymph node

first to the SLNs, then to the non-SLNs in the lymphatic basin and finally to non-SLNs outside the basin. Therefore, based upon this concept, it is sufficient to examine only the SLNs to determine whether or not there are lymph node micrometastases in patients with gastric cancer.

It is still unclear whether a lymph node micrometastasis is a prognostic factor in gastric cancer. However, a lymph node micrometastasis was found in gastric cancer patients who had no evidence of a lymph node metastasis by routine staining. This result suggests that we should cautiously reduce the extent of lymph node dissections. The intraoperative absence of a SLN micrometastasis suggests that the extent of lymph node dissection may be safely reduced, because it is unlikely for non-SLNs to have micrometastases without a SLN micrometastasis. In the case of breast cancer, the need for the intraoperative diagnosis of lymph node micrometastases is not essential, because additional dissection of the axillary lymph nodes can be performed easily. However, the subsequent dissection of lymph nodes is difficult in gastric cancer; therefore, the intraoperative diagnosis of lymph node micrometastases is crucial. We believed that when a lymph node micrometastasis was present, we should perform a lymph node dissection at the present. Our study utilized the most accurate methods, but we could not obtain the results rapidly enough for an itraoperative diagnosis. Therefore, we need to establish an accurate method for rapid intraoperative identification. Matsumoto et al. claimed that intraoperative rapid immunostaining was a simple and useful technique for detecting lymph node micrometastases [32]. An ultra-rapid RT-PCR system, which can complete the detection of cancer cells within approximately 70 minutes, has been developed. In the near future, these methods will be applied to detect lymph node micrometastases in SLNs during surgery [33].

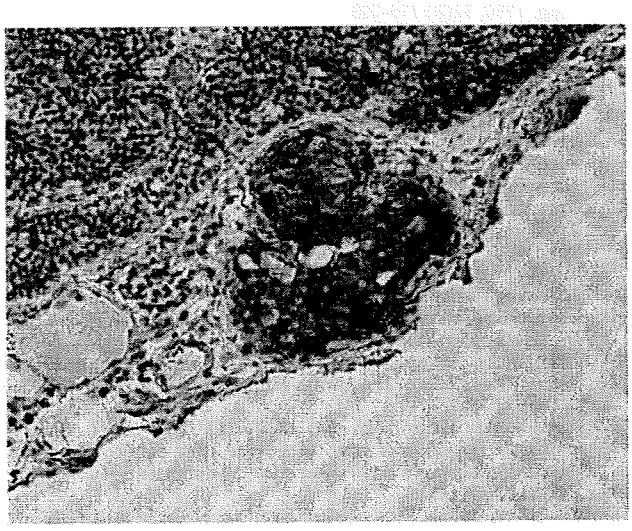

Figure 1
Lymph node mircrometastasis as detected by immunohistochemical staining with a cytokeratin antibody. (×400).

Conclusion

we have demonstrated the ability to detect lymph node micrometastases by subjecting the entire specimen to complete serial sectioning and IHC for node-negative gastric cancer patients who have had a sentinel node biopsy. These results support the concept that lymph node micrometastases spreads first to the SLNs. In addition, the intraoperative and rapid diagnosis of lymph node micrometastases in SLNs may help guide the appropriate lymph node dissection in gastric cancer patients. Therefore, a rapid and accurate intraoperative diagnosis of lymph node micrometastases in SLNs will be necessary and should be the focus of future studies.

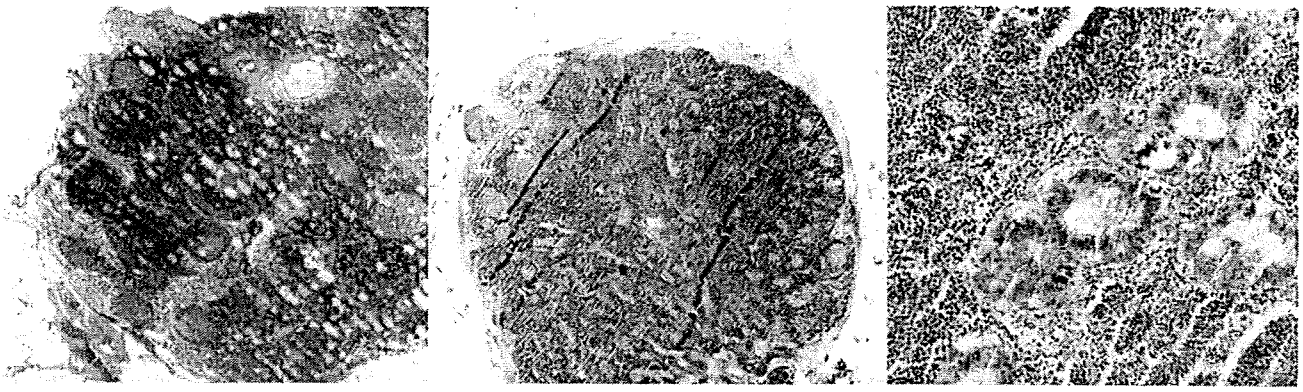

Figure 2
No cancer cells were identified in this section by routine histologic examination. (hematoxylin & eosin staining, ×40). (b) Lymph node micrometastasis in the representative section by the entire serial sectioning method. (Immunohistochemical staining, ×40). (c) Micrometastasis lymph node in the representative section by the entire serial sectioning method. (Immunohistochemical staining, ×400)

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ORIGINAL ARTICLE

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PTD classification: proposal for a new classification of gastric cancer location based on physiological lymphatic flow

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Abstract

Background. We propose a new classification for the location of gastric cancer – the PTD classification (i.e., zones P, T, and D; see below), with the zones classified according to the physiological lymphatic flow.

Methods. Three hundred and thirty-six patients with T1 or small T2 gastric cancer who underwent sentinel node mapping at our hospital were enrolled. The relationship between the location of the gastric cancer and the physiological lymphatic flow derived from sentinel node mapping was investigated. Lymphatic basins were defined as lymphatic zones divided by the stream of stained lymphatic canals.

Results. One hundred and forty-six patients underwent standard gastrectomy with more than D2 dissection and the other 190 patients underwent function-preserving gastrectomy with the omission of lymph node dissection outside the lymphatic basin. In the former group, the progression pattern of lymph node metastasis was observed; nodal metastasis occurred in sentinel nodes first, and rarely extended outside the lymphatic basin. In the latter group, none of the patients have had a recurrence. The PTD classification we propose is as follows: the dividing line between the proximal region (zone P) and the transitional region (zone T) is the line that links the point of the watershed between the left gastroepiploic artery and right gastroepiploic artery, to the point that is the inflow point of the first descending branch of the left gastric artery; and the dividing line between zone T and the distal region (zone D) is an arc at a radius of 8 cm from the pylorus. There were no lymphatic basins within the right gastric artery area for tumors located in zone T.

Conclusion. The advantage of the PTD classification is that if the PTD classification were to be used as a guide for gastric resection procedures, preservation of the pylorus would become possible without diminishing the prognosis in patients with cT1N0 cancer located in zone T.

Key words Gastric cancer · Gastrectomy · Sentinel node · Pylorus-preserving gastrectomy

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Introduction

In the *Japanese classification of gastric carcinoma*,[1] the location of gastric cancer is classified according to the three portions and cross-sectional circumference of the stomach. The three portions of the stomach are upper (U), middle (M), and lower (L), and they are delimited by dividing the lesser and greater curvature at two equidistant points, then joining these corresponding points. Therefore, this UML classification merely divides the stomach into three equal areas, and the areas are not defined according to anatomical specificity. However, in the *Japanese classification of gastric carcinoma*, the regional lymph nodes of the stomach are strictly classified into stations according to anatomical structure. Thus, the lymph node groups for dissection, classified according to the UML classification, are complicated, and in part, the classification is not clear for denoting the frequency of lymph node metastasis. Consequently, the UML classification is simpler, and remains useful regardless of the various stomach shapes encountered, though it is unsuitable as a guide for strict lymph node dissection and function-preserving limited gastrectomy for early gastric cancer. In order to perform function-preserving gastrectomy radically and lymph node dissection appropriately, a new classification for the location of gastric cancer, along the long axis of the stomach, based on the physiological lymphatic flow, seems to be needed to replace the UML classification.

Sentinel node biopsy for early gastric cancer is now widely used to predict nodal status and to enable the

employment of function-preserving gastrectomy with the omission of the standard lymph node dissection.[2-10] A sentinel node is defined as a lymph node that afferent lymphatics directly enter. We developed a method for sentinel node mapping named IELM (intraoperative endoscopic lymphatic mapping) and we have performed IELM for patients with early-stage gastric cancer.[2] IELM is a method for obtaining the vital dyeing of afferent lymphatics in early gastric cancer, and we have demonstrated the proper pattern of lymphatic flow in early gastric cancer using IELM.

In this article, we propose a new classification for the location of gastric cancer, based on the physiological lymphatic flow, derived from the proper pattern of afferent lymphatics in sentinel node mapping.

Patients and methods

Patients

Between February 1993 and March 2007, 348 patients with gastric cancer underwent sentinel node mapping at our hospital. The eligibility criteria for mapping were T1 cancers, or T2 cancers less than 5 cm in diameter, diagnosed by preoperative gastrofiberscopy. The methods used for mapping at our department were dye mapping, radioisotope (RI) mapping, and combination mapping. Our dye mapping was named the IELM technique,[2] and consisted of injecting a vital blue dye into the submucosa intraoperatively, using a gastrofiberscope. RI mapping is a technique in which a radio-labeled colloid is injected into the submucosa the day before surgery, using a gastrofiberscope. Combination mapping is a method of performing both dye mapping and RI mapping. We began with dye mapping in 1993, then adopted combination mapping from November 2000. Of the 348 patients in this series, 208 underwent dye mapping, 4 underwent RI mapping, and the remaining 136 underwent combination mapping. Detection of the sentinel nodes by dye mapping failed in 8 of these patients during the learning phase. During dye mapping and combination mapping, the dye immediately appeared on the gastric serosal surface and gradually stained the lymphatic basins and the sentinel nodes in the lymphatic basins, while it was not possible to detect the lymphatic basins by RI mapping only. Eight patients in whom we failed to detect the sentinel node and 4 patients who underwent RI mapping only were excluded from the study. Finally, 336 patients who underwent either dye mapping or combination mapping were enrolled. The characteristics and clinicopathological features of the patients are listed in Table 1. In this series, 146 patients underwent standard gastrectomy with lymph node dissection to the extent of D2 or D3, and the other 190 underwent function-preserving gastrectomy with limited lymph node dissection of D0 or D1. The surgical and histological findings were assessed in accordance with the guidelines of the Japanese Research Society for Gastric Cancer.[11] Before sentinel node mapping was performed, written informed consent was obtained from each patient; all procedures were conducted in accordance with the ethical standards of the institution's Committee on Human Experimentation.

Table 1. Patients' characteristics

Median age, years (range)	62 (26–86)
Male:female	226:110
Depth of invasion	
T1	275
T2	51
T3	10
Tumor size, cm (range)	2.3 (0.2–9.8)
Pathological typing	
Differentiated type	193
Undifferentiated type	143
Surgical procedure	
Standard gastrectomy D2–3	146
Limited gastrectomy D0–1	190

Mapping technique

For dye mapping, we applied the IELM technique developed by Miwa et al.[2] First, at the time of preoperative gastrofiberscopy for close examination, a sniping biopsy was obtained from four points surrounding the tumor, 1 cm from the tumor edges, to confirm the cancer-free region, and four metallic marking clips were attached to the sampling sites. These clips served as a guide for tracer injection and for gastrectomy with a safe margin. After laparotomy, the stomach was mobilized from the lesser and greater omenta, and 0.2 ml of 2% patent blue dye (Wako Pure Chemical Industries, Osaka, Japan), or 0.5 ml 1% Lymphazulin (Ben Venue Laboratories, Bedford, OH, USA) was injected into the submucosal layer at each of the four points marked by the clips, using a gastrofiberscope inserted through the mouth. The dye immediately appeared as four blue spots on the serosal surface bordering the carcinoma, and gradually stained the lymphatic basins (Fig. 1).

Between November 2000 and March 2002, for RI mapping, we injected 4 mCi of 99m-Tc-phytate into the submucosal layer at the four points marked by the clips (as described above), using a gastrofiberscope, the day before surgery. In April 2002, we changed to 4 mCi of 99m-Tc-Tin colloid.

Sentinel node biopsy

Sentinel node biopsy was performed under close observation of the spread of staining to the lymph nodes and lymphatic canals during a 10- to 15-min period after injection. In dye mapping, the nodes stained blue during this period were regarded as sentinel node candidates. Blue nodes were defined as those nodes that were stained blue within 20 min after injection. Lymphatic basins were defined as the lymphatic zones divided by the stream of the stained lymphatic canals; the proximal border was the fatty tissue attached to the stomach wall, and the distal border was the front of the blue node most distal from the carcinoma 20 min after dye injection. The lymphatic basins were thought to be the primary lymphatic drainage area in each patient, and

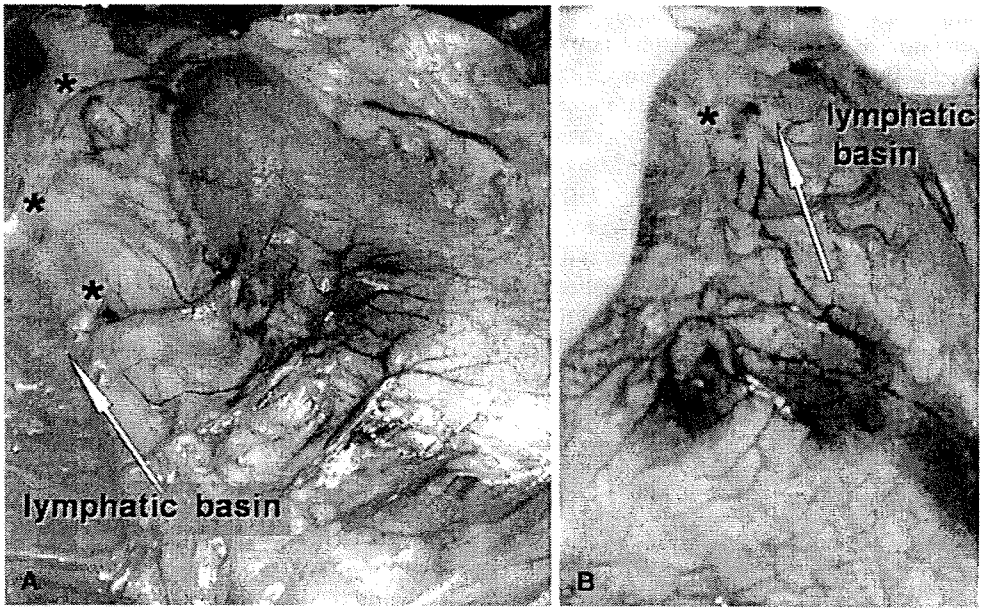

Fig. 1A, B. Intraoperative pictures of sentinel node mapping for early gastric cancer. The dye gradually stained the lymphatic basins, the most important lymphatic area in which lymph node metastasis tends to appear first and in which lymph node dissection should be performed. **A** Mapping in a patient who had an early gastric cancer located in the greater curvature of the lower body. In this patient, the blue nodes belonged to the lymphatic basin within the lymphatic compartment of the right gastroepiploic artery area. **B** In another patient, the tumors were located in the lesser curvature of the middle body, and the blue node was in the lymphatic basin within the lymphatic compartment of the left gastric artery area. *Asterisks*, blue nodes

patients with gastric cancer often had two or three basins. The blue nodes were found at the end of stained lymphatic canals within the lymphatic basins. We adopted two procedures for sentinel node biopsy, the pick-up method and lymphatic basin dissection. The pick-up method, the same method as an ordinary lymph-node biopsy procedure, was performed from February 1993 to March 2000, during the early phase of our series when dye mapping alone was performed. Lymphatic basin dissection is a procedure for sentinel node biopsy in gastric cancer developed by Miwa et al.[2] This method consists of en-bloc dissection of the lymphatic basin and ex vivo examination of the sentinel nodes. The procedure was performed as follows: first, the principal gastric blood vessels at the site proximal to the blue node most distal from the carcinoma were ligated and divided. Next, the omental fatty tissue, including the lymphatic basins, was removed from the gastric wall by careful division of the individual vessels supplying the anterior and posterior gastric walls. Finally, the resected tissues were immediately dissected on a back table by a surgeon familiar with gastrointestinal anatomy, and blue nodes and "hot" nodes were detected. Hot nodes, also regarded as candidate sentinel nodes, were defined as nodes having radioactivity above 10 counts per 10 s, measured with a gamma-probe (Navigator GPS; Tyco Healthcare Japan, Tokyo, Japan). We adopted this technique beginning in April 2000. The blue nodes and hot nodes were sent immediately for histological examination as frozen sections.

Surgical procedures

At our department, the surgical procedures for sentinel node navigation of gastric cancer progressed in five phases. The early phase was a feasibility study of dye mapping, done between February 1993 and March 1996. At first, we adopted the pick-up method and then we performed standard D2 gastrectomy regardless of the findings on frozen section diagnosis. After the accuracy of sentinel node biopsy was confirmed, we shifted to the second phase, i.e., the clinical application of sentinel node navigation to decision-making regarding limited surgery in selected patients with mucosal cancer, starting in April 1996. The patients underwent sentinel node biopsy using the pick-up method, and function-preserving limited surgery associated with back-up lymph node dissection of the lymphatic basin was performed when the sentinel nodes were negative for metastasis on frozen section. If the sentinel nodes demonstrated metastasis on frozen section, the patient underwent the standard D2 gastrectomy. From April 2000, we changed the method of sentinel node biopsy from the pick-up method to lymphatic basin dissection, and expanded the eligibility for limited surgery, according to the sentinel node biopsy, to all patients with early-stage gastric cancer. The patients underwent lymphatic basin dissection after mapping, and function-preserving gastrectomy without additional lymphadenectomy was performed in patients whose samples were node-negative on frozen section. For patients whose samples were node-positive, standard D2 gastrectomy was performed. The fourth phase was the introduction of combination mapping, in November 2000. From April 2005, the fifth phase involved laparoscopic sentinel node navigation surgery, which consisted of combination mapping, lymphatic basin dissection, and function-preserving gastrectomy for patients whose samples were node-negative on frozen section. If the sample was node-positive on frozen section, the operation was converted to open surgery, and D2 gastrectomy was performed.

Pathological examination

Each blue or hot node was bisected at the maximal dimension, and a frozen section was obtained. The slice was

Table 2. Results of sentinel node biopsy for gastric cancer in patients who underwent standard gastrectomy with D2 or D3

Frozen section diagnosis of blue or hot nodes	Permanent diagnosis of all dissected nodes	
	Node-positive	Node-negative
Metastasis	44	0
No cancer cells	5	97

Table 3. Locations of metastatic lymph nodes in patients with metastasis

Blue node or hot node	Non-blue and cold node within lymphatic basin	Non-blue and cold node outside lymphatic basin	No. of patients
O	–	–	19
O	O	–	24
O	O	O	1
–	O	–	5[a]
–	O	O	0
–	–	O	0

O, presence of metastasis; –, no metastasis
[a]These five patients had clinically apparent metastasis

stained with hematoxylin and eosin. The remaining node tissues were processed separately in individual blocks for permanent sectioning. The non-blue nodes, harvested in back-up dissection, were cut into 2-mm sections parallel to the maximal dimension, and embedded in paraffin. Permanent slices were cut into 5-μm sections and stained with hematoxylin and eosin.

The pathological diagnosis was carried out by the pathologists at the Division of Pathology, Kanazawa University Hospital.

Analysis of physiological lymphatic flow

In this study, we analyzed the relationship between the location of the gastric cancer and the physiological lymphatic flow, derived from sentinel node mapping. The relationship between the lymphatic flow and the proper pattern of lymph node metastasis was also investigated. The location of the tumor was expressed according to both the UML classification and the distance between the pylorus and the distal margin. From these findings, the outlines of a new longitudinal classification for the location of gastric cancer, based on the physiological lymphatic flow, were proposed.

Results

Results of sentinel node biopsy for gastric cancer in patients who underwent standard gastrectomy

Of the 146 patients who underwent standard gastrectomy with lymph node dissection, 49 demonstrated nodal metastasis on permanent histological examination. Forty-four of these 49 patients were intraoperatively diagnosed as having nodal metastasis by blue or hot node biopsy. Accordingly, the sensitivity of the sentinel node biopsy was 89.8% (44/49), the specificity was 100% (97/97), and the accuracy was 96.6% (141/146; Table 2). All 5 patients with false-negative findings had a large clinically apparent metastatic node, and it was easy to diagnose metastasis intraoperatively. The stained vessels ran from the tumor directly to the nodes in the lymphatic basins, but the metastatic node was not stained. Therefore, in this series, all patients who had pathological metastasis were diagnosed intraoperatively by sentinel node biopsy, except for those with macroscopic metastasis.

Nodal status in patients who underwent standard gastrectomy

In patients who underwent standard gastrectomy, regional lymph nodes could be divided into three categories: blue or hot nodes, non-blue and cold nodes within lymphatic basins, and nodes outside the lymphatic basins. All blue nodes and all hot nodes were within the lymphatic basin. Table 3 shows the locations of the metastatic nodes in the 49 node-positive patients. In 19 patients, metastatic nodes were confined to only blue or hot nodes; 24 patients showed metastasis in both blue/hot nodes and non-blue and cold nodes, though all metastatic nodes were within the lymphatic basin. In 5 patients demonstrating macroscopic metastasis, all metastatic nodes were also restricted to within the lymphatic basins. In this series, there was only 1 patient who had nodal metastasis beyond the lymphatic basin. This patient had advanced cancer with a large number of metastatic nodes; M, Gre, Φ3 cm, small Borrmann 3 type, pT2 (SS), pN1 (10/44), H0, P0, CYX, M0, por2, scirrhous, INFγ, ly2, v0. The lymphatic basin of this patient was near the right gastroepiploic artery. The numbers and stations of the metastatic nodes were: 6 number 4d nodes (lymph nodes along the right gastroepiploic artery) within the basin and 4 number 3 nodes (lymph nodes along the lesser curvature) outside the basin. There was no patient with metastasis outside the lymphatic basin only.

Nodal status and prognosis in patients who underwent function-preserving gastrectomy

One hundred and ninety patients underwent function-preserving gastrectomy with limited D0 or D1 lymph node dissection. They were diagnosed as node-negative intraoperatively by sentinel node biopsy, and underwent function-preserving gastrectomy, which consisted of a reduction in the excised area of stomach and omission of lymph node dissection outside the lymphatic basins. Therefore, all these patients underwent prophylactic back-up dissection of the lymphatic basins.

Two of these 190 patients were finally diagnosed with lymph node metastasis. One patient had one metastatic node that was stained blue, and the other patient had three