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Sentinel node biospy using intravital blue dye

An useful technique for identification of skip metastases in gastric cancer

Tivadar Bara Jr[a], Simona Gurzu, PhD[b,c,*], Ioan Jung[b], Cristian Borz[a], Laura Banias[b], Tivadar Bara[a]

Abstract

As the lymph node status remains the main prognostic factor of gastric cancer (GC), several lymph node-based staging systems have been recently proposed for an appropriate postoperative therapy. The identification of sentinel lymph nodes (SLNs) might improve the postoperative protocols. The aim of this study was to present our experience in detecting SLNs in GC using methylene blue dye.

We have performed an observational study and retrospectively analyzed all of the consecutive cases of GC operated by the same surgical team and managed by the same pathologists during 2013 to 2015. In all of the cases SLN status was determined using the methylene blue that was intraoperatively administered in the peritumoral subserosal tissue. All blue colored nodes were histopathologically examined. In the node negative cases immunohistochemical stains using AE1/AE3 keratin were performed.

The blue SLNs were identified in 48 out of the 50 cases included in the study, with a 96% sensitivity and 87.50% specificity. From the 48 cases, 34 (70.83%) presented positive SLNs; in the other 14 cases the SLNs were negative (29.17%). False negativity was observed in 6 of the 14 cases. In 2 of the cases the false negativity of the group 20 was induced by the anthracotic pigment. In other 2 false negative cases, although no regional metastases were founded, sentinel skip metastases in the group 8 and 15, respectively, were identified.

Mapping of the SLNs is a simple and cheap method that might improve the accuracy of LN-based staging of patients with GC and favor identification of skip metastases.

Abbreviations: CT = Computed Tomography, GC = early gastric cancer, GC = gastric cancer, HE = Hematoxylin Eosin, IHC = immunohistochemical, MRI = Magnetic Resonance Imaging, pM = distant metastases-related stage, pN = lymph node-related stage, pT = tumor-related stage (staging based on depth of infiltration), R0 = free resection margins, SLN = sentinel lymph node.

Keywords: gastric cancer, lymph node metastases, sentinel lymph node, skip metastases

1. Introduction

Despite complex therapy and implementation of screening programs, gastric cancer (GC) remains in the top places in both the incidence and cancer mortality worldwide.[1–3] Although the overall incidence of GC is declining, in Eastern European countries it remains a public health problem and up to 55% of the cases are diagnosed in late stages.[2]

The number of lymph node metastases remains the more important prognostic factor but their preoperative accurate evaluation is difficult to be done.[1,3] The precise determination of the lymph node-related stage (pN) may only be made after histopathological assessment of several removed lymph nodes. The identification could be improved through sentinel lymph nodes (SLNs) mapping.

In 1960 Gould used the term of "sentinel lymph node" for the first time, and in 1977 Cabanas identified SLN in penile carcinoma by a contrast lymphangiography.[3,4] Nowadays, the SLN detection is extensively used in Europe in patients with melanomas and carcinomas of penile, breast, thyroid, vulva, prostate, but is not considered yet a gold standard technique for patients with GC.[3]

In GC, it was well-proved the prognostic value of SLN mapping in patients diagnosed in early stages (EGC)[3,5] but its identification in locally advanced GCs is not accepted by all of the surgeons. The aim of this study was to present the sensivity of blue dye-based SLN mapping, a method without any side effect, in patients with GC, and its possible role in detection of skip metastases.

2. Material and methods

All of the consecutive patients with GC that underwent open gastrectomy with D2 lymphadenectomy and SLN mapping during October 2013 to December 2015 were included in the study. Ethical approval from the Ethical Committee of University of Medicine, Pharmacy, Sciences and Technology, Tirgu Mures, Romania, and informed consent of the patients were obtained.

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Tivadar Bara Jr. and Laura Banias contributed equally to this work.

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[a] Department of Surgery, [b] Department of Pathology, [c] Advanced Medical and Pharmaceutical Research Center (CCAMF), University of Medicine, Pharmacy, Sciences and Technology, Tîrgu Mureş, Romania.

[*] Correspondence: Simona Gurzu, Head of Department of Pathology, University of Medicine, Pharmacy, Sciences and Technology, 38 Gheorghe Marinescu Street, Tîrgu Mureş 540139, Romania (e-mail: simonagurzu@yahoo.com).

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The patients gave their permission to perform surgery and be included in the research manuscript.

The following criteria of inclusion were used: patients with operable gastric carcinoma that was preoperatively diagnosed in a biopsy specimen and confer their informed consent, without synchronous tumors, without a previously gastric surgical intervention that could modify the lymphatic drainage. All of the patients that were hospitalized with recurrent GC or received preoperative radiochemotherapy or had pre-/intraoperatively detected systemic metastases (liver, lung, peritoneum, ovaries) and patients with R1 gastrectomy were excluded from this study.[3] The preoperative CT-scan was not performed in all of the cases. No preoperative clinical staging was done.

For detection of SLN, 2 ml of methylene blue solution were intraoperatively injected in the peritumoral subserosal layer. To avoid impairement of the lymphatic drainage, injection was done before the mobilization of the stomach. A tuberculin syringe was used to introduce the dye around the tumor, in a circumferential manner, in 4 points of the compass. After the injection, we have waited for 10 minutes and the first blue colored LNs were removed and considered as intraoperatively detected SLNs (Fig. 1). R0 partial or total gastrectomy and D1 or D2 lymphadenectomy was done (Table 1).

The surgical specimens were fixed in formalin and embedded in 10% neutral formalin. All of the blue nodes identified by the pathologist were counted as postoperatively identified SLNs. All of the lymph nodes (SLNs and non-sentinel nodes) were examined in Hematoxylin Eosin (HE). For each negative SLNs multiple sections were done and immunohistochemical (IHC) stains with AE1/AE3 Keratin (Dako, Glostrup, Denmark) were performed.

3. Results

Using the above mentioned criteria, 50 consecutive patients with GC have been included in the study with a median age of 67.70 ± 12.24 years. Most of the cases were adenocarcinomas with relatively equal distribution regarding the tumor location within anatomical segments of the stomach (Table 1).

From the 50 cases in which SLN mapping was performed, a total of 383 SLNs were examined. The average number of total lymph nodes examined per case was 40 (Table 1). The average number of colored nodes was 7.67 ± 1.35 (range 1–12 blue lymph nodes). Intraoperatively 1 to 2 SLNs were removed, the others being considered the blue nodes identified postoperatively by the pathologist.

Table 1

Clinicopathological characteristics of the patients.

Parameter	Number (n = 50)
Age	67.70 ± 12.24 years (range 38–88 years)
Male/female ratio	34/16 (2.12:1)
Tumor location	
Upper third	12
Middle third	18
Lower third	20
Tumor size – maximum diameter	48.04 ± 11.32 mm (range 5–100 mm)
Type of surgery	
Total gastrectomy	30
Subtotal gastrectomy	20
Type of lymphadenectomy	
D1	6
D2	22
D2+	22
Number of examined lymph nodes per case	40.52 ± 15.81 (range 11–90 nodes)
Number of sentinel lymph nodes per case	7.67 ± 1.35 (range 1–12 nodes)
pT stage	
pT1	5
pT2	5
pT3	12
pT4	28
pN stage	
pNo	8
pN1–3	32
Histological aspect	
Differentiated intestinal adenocarcinoma	21
Undifferentiated intestinal adenocarcinoma	11
Mucinous adenocarcinoma	3
Diffuse-type (poorly-cohesive) carcinoma	10
Neuroendocrine carcinoma	5
Neural invasion - present vs absent	29 vs 21 cases
Angiolymphatic invasion - present vs absent	32 vs 18 cases

Detection of the SLNs was possible in 48/50 patients (96% sensitivity). The 2 cases without identified SLNs were diagnosed in pT3 and pT4 stage, respectively (Table 2).

From the 48 cases with identified SLNs, 8 cases showed negative SLNs (no metastases) after classic and IHC assessment. False negativity (no metastases in the SLN with metastatic non-SLNs) was showed in 6 cases (87.50% specificity). SLN was positive (with metastases) in 34/48 cases of which 2 cases showed micrometastases only confirmed by immunohistochemistry.

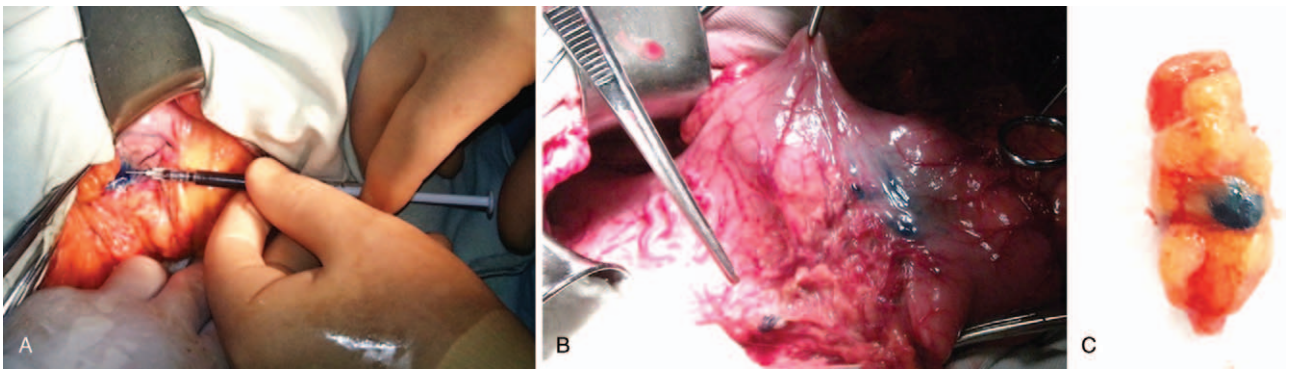

Figure 1. Blue dye injection in the subserosal peritumoral area (A) with intraoperatively identification of the sentinel node (B) that is then isolated and submitted for histopathological examination (C).

Table 2

The correlation between the number of identified sentinel lymph nodes (SLN) and the tumor depth of invasion (pT stage).

Stage	SLN not identified	SLN negative	SLN false negative	Micrometastases in SLN only	Metastases in SLN only	Metastases in SLNs and non-SLNs	Total
pT1a	–	2	–	–	–	–	2
pT1b	–	–	1	–	1	1	3
pT2	–	3	1	2	–	–	6
pT3	1	3	–	–	5	3	12
pT4	1	–	4	–	5	17	27
Total	2	8	6	2	11	21	50

Of the 34 positive SLN cases, metastases in the SLN only were seen in 13 cases (Fig.2).

In 4 of the 6 false negative cases we have postoperatively identified metastases in the regional non-SLNs. All of the cases were diagnosed in the pT4N3 stage. Two of them were localized in the pyloric area and 2 in the upper third stomach. In the 2 cases of the proximal stomach, the intraoperatively removed nodes, that were considered SLNs, were located on the esophageal hiatus (group 20). They had a diameter larger than 50 mm and showed at histological examination a rich amount of anthracotic pigment without tumor cells.

In the other 2 false negative cases, a particular aspect was noted. It refers to the identification of skip metastases in distant lymph nodes in both of these cases. These patients did not show metastases in the regional lymph nodes. In the first case, the $25 \times 20 \times 10$ mm ulcerated differentiated adenocarcinoma was localized in the pyloric antrum and involved the mucosa and deep submucosal layer (pT1b stage). The lymphatic follicles from mucosa showed tumor cells but no perineural or angiolymphatic invasion was observed. During surgery, the SLN removed from the lesser curvature (Group 3) was negative at microscopic examination. The other 35 regional lymph nodes were also negative. During surgery, the lymph nodes from the medium colic artery (Group 15) showed an incidentally blue color. They were removed and 1 of the 7 nodes from the group 15 showed metastatic cells.[7]

In the second case with skip metastases, the tumor was also located in the antrum. It was about a $25 \times 30 \times 7$ mm ulcero-infiltrative differentiated adenocarcinoma diagnosed in pT2 stage with multiple tumor emboli in the lymph vessels. During surgery, the subpyloric SLN (Group 6) was negative at microscopic examination. Other 16 regional lymph nodes were also negative. Skip metastases were identified in 2 lymph nodes from the group 8 (at the level of the common hepatic artery).

4. Discussion

The exact determination of the pN stage can only be made by extended lymphadenectomy and attentive histopathological examination. The complete tumor resection and regional lymph nodes dissection are the basic surgical principles for GC patients.[5]

Yet the main controversy is the usefulness of extensive lymphatic dissection (D2) in patients with EGC.[5,6] This type of dissection increases the duration of the operation and the complications rate and it is useless in the absence of angiolymphatic invasion, so that it should not be performed in all of the cases.[7] However, the 5-year survival rate for EGC is 90% or higher for intramucosal carcinoma but the recurrences are still frequent [8] and the reported metastaic rate is 9% to 20%.[9] This aspect makes difficult the therapeutic decision (surgery with lymphadenectomy vs minimal invasive treatment). Presence of skip metastases, such in 2 of our cases, increases the difficulty of the therapeutic decision that should be taken after a complete abdominal CT-scan of the patient, especially for patients with submucosal invasion.

For patients with advanced GC the most important prognostic factor also remains the lymph node metastases. The accurate preoperative determination of the pN stage is still a goal, but contrast CT-scan showed encouraging results.[10] However, intraoperatively identification of the SLNs might avoid unnecessarily extended lymphadenectomy and thus to avoid the complications associated.[10] Although the identification of SLNs in patients with GC was introduced in GC surgery in 2001 it is

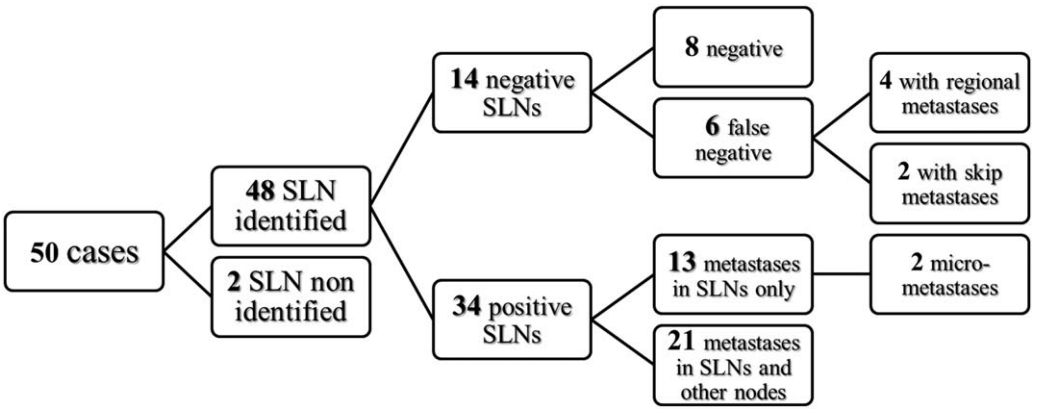

Figure 2. The sensivity and specificity of sentinel node mapping in patients with gastric cancer.

not used yet as a gold standard.[5,6] The controversy regarding the utility of SLN mapping is based on the multidirectional lymphatic drainage of the stomach and the presence of skip metastases that cannot be easily identified in the daily practice.[11,12]

SLN identification can be performed using vital dye (blue patent - isosuflan, methylene blue, fluorescein), radioactive isotope or combining the 2 methods.[7,13] Isosuflan blue dye was most commonly used in the past but due to the recent reports of anaphylactic reactions inLymphazurin solution it is trying to validate other dyes for the technique of mapping the SLN.[3,12] For this purpose the green fluorescein (green indocyanine) is commonly used in Japan.[13,14] Fluorescein seems to successfully cross the lymphatic route and the lymph nodes color turnes from blue to yellowish green with yellow fluorescent spots.[3,12,13] The double-tracer method uses the dye and radioisotope tracers and appears to be more effective than a single tracer method.[3,14,15] The administration may be intraoperatively done in the peritumor subserosa.[3,16] The newest SLNs detection technologies refer to infrared endoscopy, fluorescence imaging (*using Infrared Ray Electronic Endoscopes combined with Indocyanine Green*) and the near-infrared technology, carbon nanoparticles (*infrared ray endoscopy, florescence imaging and near-infrared technology, carbon nanoparticles*), etc.[3,13,16]

The ideal method for SLN mapping should allow accurate and secure SLN detection and real-time observation of lymph flow during surgery. In this study the methylene blue dye was found to be an useful agent presenting the following advantages: it can be done intraoperatively and is a simple, economical, and safe method, with a good sensitivity and specificity rate. Moreover, if lymphatic drainage is not interrupted, the dye progresses to lymph nodes intraoperatively (in vivo) as well as post-operatively (in vitro) and several nodes (from 1–12 in this study) might be identified as having risk for metastases.

The SLN biopsy is accepted in patients with EGC[12] but few studies showed its utility in advanced carcinomas that show a higher rate of false negativity.[3,5] In our material, 4 out of the 6 false negative cases were indeed diagnosed in the pT4N3 stage. In the advanced stage the obstruction of the lymph vessels by the tumor cells might produce retrograde metastases and the dye flux might be stopped or aberantly derived in the non-SLNs.[3]

In the present study the method totally changed the stage of the tumor, for the 2 patients with skip metastases, located in the groups 8 and 15 (from T1bN0 to T1bM1 and T2N0 to T2M1, respectively). We consider that larger studies should be performed to identify the cases with risk for aberrant metastases and any surgical intervention should be preceded by a complete contrast CT-scan of the abdominal cavity. The paraaortic group was also reported to be at risk for presenting skip metastases.[7]

A reason for the false negativity that was emphasized in this paper was the rich amount of the anthracotic pigment that could induce paracardiac/paraesophageal lymph node enlargement with capturing of dye. In the tumors localized on the small curvature and the lower third of the stomach the risk of aberrant metastases location is significantly higher than in other locations.[11]

The weak points of the study were the small number of cases and lack of the preoperative CT-scan. However, this study revealed that preoperative CT-scan or MRI should become the standard technique for patients with GC, to identify the depth of invasion and the possible skip metastases. In patients with pT1-pT3 cases intraoperative SLN mapping should complete the imaging.

Author contributions

Tivadar Jr. Bara – drafting the manuscript, participated at the surgical intervention; Simona Gurzu – interpretation of data from literature and study design; Ioan Jung - contributed to the histopathological evaluation and supervised the data interpretation; Cristian Borz - participated at the surgical intervention and performed the clinical evaluation; Laura Banias – participated at histopathological evaluation and interpretation of literature data; Tivadar Bara – performing the surgical interventions and allowed the final approval for publication.

Conceptualization: Simona Gurzu, Tivadar Bara.
Data curation: Cristian Borz, Laura Banias.
Formal analysis: Simona Gurzu, Ioan Jung, Laura Banias.
Funding acquisition: Tivadar Jr. Bara, Tivadar Bara.
Investigation: Cristian Borz, Laura Banias.
Methodology: Tivadar Jr. Bara, Simona Gurzu, Cristian Borz, Tivadar Bara.
Project administration: Simona Gurzu.
Software: Tivadar Bara.
Supervision: Tivadar Bara.
Validation: Ioan Jung.
Visualization: Ioan Jung.
Writing – original draft: Tivadar Jr. Bara.
Writing – review & editing: Ioan Jung, Tivadar Bara.
Simona Gurzu orcid: 0000-0003-3968-5118.

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