Sentinel node biopsy using intravital blue dye
An useful technique for identification of skip metastases in gastric cancer

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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 60 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 arthritic 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.[1,4] 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 GGCs 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, Tîrgu Mures, Romania, and informed consent of the patients were obtained.
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. 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 impairment 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.

| 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 | pN0: 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.
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 50mm 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 × 20 × 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. 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.

Yet the main controversy is the usefulness of extensive lymphatic dissection (D2) in patients with EGC. 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. However, the 5-year survival rate for EGC is 90% or higher for intramucosal carcinoma but the recurrences are still frequent and the reported metastatic rate is 9% to 20%. 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. However, intraoperatively identification of the SLNs might avoid unnecessarily extended lymphadenectomy and thus to avoid the complications associated. Although the identification of SLNs in patients with GC was introduced in GC surgery in 2001 it is
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.

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