

Short thesis for the degree of doctor of philosophy (PhD)

**MODERN METHODS IN THE ASSESSMENT OF THE
PROGNOSIS OF GASTRIC ADENOCARCINOMA AND
BREAST CANCER**

Zsolt Varga

Supervisor: Dezső Tóth



**UNIVERSITY OF DEBRECEN
Doctoral School of Clinical Medicine**

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By Zsolt Varga, MD

Supervisor: Dezső Tóth, MD, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Defense Committee:**

Reviewers:

Prof. Norbert Németh, PhD, DSc

András Papp, PhD

Andrea Furka, PhD

Members of the Defense Committee:

Aurél Ottlakán, PhD

Ákos Szűcs, PhD

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1. INTRODUCTION

The lymphatic system, an intricate network of lymph vessels and lymphoid organs, plays an indispensable role in maintaining the body's fluid balance and, through its immunological role, the body's defense capability. Paradoxically, however, due to its filtering function, it can also serve as a harbour for the dissemination of cancer cells. The presence of lymph node metastases in solid tumors has profound significance. On the one hand, it has a prognostic role: it signals that the malignant lesion - even if it has not yet given distant metastasis, has violated the boundaries of the given organ, becoming a locoregional disease. In this stage, the expected survival outcomes drastically decrease, and the risk of recurrence rises. On the other hand, it also has therapeutic implications: the treatment of the disease demands more aggressive, potentially more burdening therapy. Surgical interventions, to this day, form the cornerstone of solid tumor treatment, its invasiveness and extent are also influenced by the presence of lymphogenic metastases. One of the main focuses of current research is the examination of the role of surgeons and surgery in the prognosis of solid tumors.

In the first half of the dissertation, I examined a novel method for detecting lymph node metastases in gastric cancer. According to the latest GLOBOCAN 2020 estimate, gastric cancer is the fifth most common type of cancer worldwide. The number of new cases is estimated at 1,089,103 annually, which is associated with 768,793 deaths. The high mortality rate is due to the fact that the diagnosis of gastric cancer is often made only in advanced stages. It has been more than 140 years since Theodor Billroth (1829-1894) first performed a successful gastric resection for gastric cancer in 1881. Despite scientific and technological progress, the foundation for treating gastric cancer remains a multidisciplinary approach that relies on resection (be it surgical or endoscopic). Stage-adapted, or individualized treatment, is essential to achieve the most favorable oncological results. The fundamental goal of surgery is to achieve local control by ensuring tumor-free resection margins and removing regional, potentially involved lymph nodes. However, a significant proportion of patients do not have lymph node involvement after R0 resection. Over the past decades, there has been much debate about the optimal extent of lymph node removal, as both postoperative morbidity and the accuracy of staging are significantly influenced by the extent of lymph node removal. If lymph node removal is insufficient, the patient's stage may be underestimated and treated inadequately. The opposite is also true, with the frequency of postoperative complications increasing as a result of unnecessarily extended lymph node dissection.

The sentinel lymph node (SLN) is, by definition, the first (sentinel) station of lymph flow from the tumor, which theoretically represents the status of the other regional

lymph nodes as well. Sentinel node navigation surgery (SNNS) is a surgical technique in which the SLN is removed and examined histologically to determine if it contains cancer cells. During SNNS, a colloidal tracer is injected into the environment of the tumor, which is absorbed into the lymphatic vessel and migrates to the sentinel lymph node(s), where it accumulates due to its size. During gastric cancer surgery, various tracers have been tried: vital blue dye, indocyanine green (ICG) dye, radiocolloids, and their combinations. In some types of tumors, the extent of lymph node dissection needed during SNNS can be determined based on the status of the sentinel lymph node. In the case of gastric cancer, this therapeutic step is still in the clinical trial stage. Such trials aim to answer the question of whether, if the removed sentinel lymph node is free from metastases, gastric resection and D2 lymph node dissection are necessary. This promising approach can lead to less surgical stress, a higher rate of organ-preserving interventions, thus faster postoperative recovery, and better quality of life without compromising patient safety. At the same time, it draws the operating surgeon's attention to the critical lymph node stations, thus improving the efficiency of lymph node removal in each patient. Unfortunately, metastatic lymph nodes are not always marked by the tracer, and vice versa, marked lymph nodes are not necessarily metastatic. This already complex situation is complicated by the fact that the lymphatic drainage pattern of individual tumors can significantly vary not only between patients but even between different areas of the same tumor. This variability can often lead to unpredictably located sentinel lymph nodes, which the surgeon may not notice during surgery. If cancer cells accumulating in metastatic lymph nodes block the draining lymphatic path, the tracer used to identify the sentinel lymph node may also be diverted into another lymph node, leading to false-negative results.

The Maruyama Computer Program (MCP) was developed by Keiichi Maruyama in 1989. The program uses a database of parameters from 4302 patients with gastric cancer, treated at the National Cancer Center Hospital in Tokyo between 1968 and 1989. The software is capable of estimating the probability of lymph node involvement by tumor at lymph node stations 1-16 based on various prognostic factors. After manual data input, the software calculates a probability value for each station.

We focused on the potential complementary use of two modern techniques, sentinel lymph node biopsy and the Maruyama Computer Program. We aimed to determine the likelihood of lymph node metastases in gastric adenocarcinoma and the localization of the affected lymph nodes. Our goal was to measure the accuracy of predicting the location of lymph node metastases using sentinel lymph node biopsy, the Maruyama Computer Program, and their combination. With the latter combined technique, we attempted to establish an algorithm that would allow for the identification of all affected lymph nodes.

In the second part of the dissertation, I analyze the impact of the breast cancer screening program introduced in 2003 on surgical treatment, short-term, and 10-year survival outcomes. In Hungary, these parameters have not been studied yet, and no publication has been made in this direction, even though they are one of the most important indicators for every oncology screening program. The absence of these missing data makes it difficult to evaluate the effectiveness of the screening program, as without them, it is not possible to form an accurate picture of the program's functioning or make informed decisions about its development. Without them, we have limited opportunities to learn from screening programs and transfer this knowledge to other similar public health programs. The insights drawn from the results could contribute to the improvement of healthcare delivery. Breast cancer is the most common type of cancer among women, with approximately 2.3 million new cases diagnosed worldwide each year. Although breast cancer affects a large number of women, mortality rates have been continuously declining since the early 2000s. Unfortunately, in 2020 alone, breast cancer was responsible for about 685,000 deaths. The decline in mortality rates is largely due to early detection and advancements in treatment options. A prime example of the former was the introduction of organized, population-based mammography screening programs, which, according to estimates from the World Health Organization, reduced breast cancer-related mortality by approximately 20%. In Hungary, a breast screening program consisting of physical examination and mammography was launched as a public health program in 2002, targeting women in the 45-65 age group. The program is still ongoing, with every woman in this age group receiving a personalized letter inviting them to the screening every two years. The aim of the screenings is to detect tumors (and pre-cancerous conditions) that are not yet causing symptoms or complaints, in order to prevent more serious consequences in the future. There is ongoing debate as to whether breast cancer screening really improves patient survival alongside modern oncological treatments. Those who question the efficiency of the screening program have several arguments: the appearance of symptoms is just the tip of the iceberg, as the preclinical stage from biological onset to symptom onset can be extremely variable. Even in this preclinical stage, the presence of the tumor can only be detected with clinical examinations for an even shorter interval. However, this does not mean that the tumor could not have been capable of forming distant metastases even earlier. A 1 mm tumor consists of about 10^6 cells, which is already capable of angiogenesis.

We wanted to analyze the clinical and pathological characteristics of patients undergoing surgery after the introduction of the population-based breast cancer screening program, as well as the distribution of modes of detection. Beyond this, we aimed to assess the impact of the breast cancer screening program on short-term outcomes that significantly affect quality of life after surgery, such as the frequency of

breast-conserving surgeries and the need for oncological treatments. Our goal was to investigate whether there is a difference in 10-year survival data depending on the mode of breast cancer detection and to identify the factors that influence the 10-year disease-specific survival of breast cancer patients.

2. OBJECTIVES

1. To determine the probability of lymph node metastasis formation and the localization of affected lymph nodes in the case of gastric adenocarcinoma.
2. To determine how accurately the location of lymph node metastases in gastric adenocarcinoma can be estimated using sentinel lymph node biopsy, the Maruyama Computer Program, and a combination of these two techniques.
3. To investigate whether a combined use of these techniques can establish an algorithm for identifying all affected lymph nodes.
4. To analyze the clinical and pathological characteristics of patients who underwent surgery after the introduction of the population-based breast cancer screening program, and the distribution of modes of detection.
5. To assess the impact of the population-based breast cancer screening program on short-term outcomes that significantly affect the quality of life after surgery, such as the frequency of breast-conserving surgeries and the need for oncological treatments.
6. To be the first in Hungary to examine whether there is a difference in the 10-year survival data based on the method of breast cancer detection.
7. To identify the factors that influence the 10-year disease-specific survival of breast cancer patients.

3. PATIENTS ÉS METHODS

3.1. Investigation of Lymph Node Metastasis in Gastric Adenocarcinoma

Between 2008 and 2018, our research group collected data prospectively on patients who underwent surgery for curative purposes due to gastric adenocarcinoma, following ethical approval. This was a single-institution study, with all operations carried out at the Kenézy Gyula University Hospital (currently known as the University of Debrecen Clinical Center, Surgical Clinic, Kenézy Campus). We only included adult patients (over 18 years old) who were willing to participate in the study and provided their written consent. Another requirement was that the patient had histologically confirmed gastric adenocarcinoma, from which a biopsy was taken during gastro-duodenoscopy prior to the operation. The inclusion was carried out following the full oncological staging (CT examination of the chest, abdomen, and pelvis) and the assessment of suitability for surgery. Exclusion criteria included macroscopic infiltration of the peritoneum or any adjacent organ, a history of stomach surgery, a tumor located in the cardia (<5 cm from the gastroesophageal junction), and the presence of distant metastases.

Our primary and secondary endpoints were to measure the proportion of lymph node-negative patients and the accuracy of our combined technique. To this end, we collected data from 100 consecutively operated patients. We recorded the patients' age, gender, comorbidities, tumor location, histological type, and the clinical and pathological stage of the tumor. Before the surgical intervention, we used the Maruyama Computer Program to determine the probability of lymph node metastasis at each lymph node station. We recorded the percentage Maruyama Index (MI) generated by the software for each lymph node station in every patient.

During the operation, we injected 2 ml of blue dye (Bleu Patente V 2.5/100 mg, Guerbet, France) around the tumor using a subserosal or submucosal technique for sentinel lymph node mapping (Figure 6). After administering the dye, we waited 10 minutes for the lymphatic flow to begin. We then performed gastrectomy, subtotal or distal gastric resection with a standard D2 lymph node dissection, using an open or laparoscopic technique. The blue-dyed sentinel lymph nodes were also sent for intraoperative frozen section examination. Lymph nodes from the D3 compartment (stations 13-16) and the 10th (splenic hilum) station were only removed if we strongly suspected clinically that we would find a metastatic lymph node there.

Following the completion of the operation, the operating surgeon performed the ex vivo dissection of the specimen and the station-by-station separation of the lymph nodes. All removed lymph nodes were sent for a definitive (paraffin-embedded) histopathological examination. Lymph nodes from the blue-dyed stations were examined in 0.2 mm slices for the presence of micrometastases. Otherwise, sections were made parallel to the

longitudinal axis of the lymph nodes at 2 mm intervals. From the paraffin blocks, conventional histological examinations were performed with hematoxylin and eosin (HE) staining, and immunohistochemical examinations were performed with pancytokeratin staining.

We recorded all patient data collected during the examinations in an offline Microsoft Excel database. The normality of continuous variables was tested with the Shapiro-Wilk test. We report normally distributed data in the form of mean $\pm$ standard deviation and non-normally distributed data in the form of median and range. The former were compared using the Student's t-test and ANOVA, and the latter using the appropriate non-parametric method (Mann-Whitney U test or Kruskal-Wallis test). We present categorical variables in numbers and percentages and compared them using the Fisher's test. The level of statistical significance was set at $p < 0.05$ for all analyses.

We determined the detection rate of sentinel lymph nodes by dividing the number of patients with successfully marked sentinel lymph nodes by the total number of patients. We calculated the sensitivity and specificity of sentinel lymph node marking based on the number of patients with metastatic lymph nodes in the station containing the sentinel lymph node and the number of patients without metastatic lymph nodes.

All statistical analyses were performed using IBM SPSS Statistics for Macintosh, version 28.0.

3.2. Effects of early detection of breast cancer

We included female patients with breast cancer who underwent surgery at the Kenézy Gyula University Hospital (currently: University of Debrecen Clinical Centre, Department of Surgery, Kenézy Campus) between January 1, 2002 and December 31, 2007 in the study. For this purpose, we used a prospectively maintained database of patients diagnosed with breast cancer. The inclusion criteria were as follows: (1) histologically confirmed breast cancer and (2) availability of follow-up data after surgery. The exclusion criteria included: (1) diagnosis of recurrent breast cancer, (2) presence of distant metastases at the time of diagnosis, and (3) simultaneous presence of other malignant tumours. Patients were classified according to how the breast cancer was detected. Group A included those patients in whom breast cancer was diagnosed during a population screening or within 2 years thereafter. The latter aimed to more accurately identify interval carcinomas, which are part of the quality assurance process for screening. Group B consisted of patients between the ages of 45 and 65, in whom the diagnosis of breast cancer was made following self-examination or medical examination due to symptoms.

Following the surgical intervention, we recorded the following clinical data: patient's age at the time of surgery, histological and immunohistochemical subtype of the tumour, tumour size, as well as the pT and pN stage. We documented the type of surgery

performed (including the proportion of mastectomy and axillary lymphadenectomy), and the necessity for adjuvant and neoadjuvant oncological treatments. The long-term oncological parameters examined included 10-year overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS).

In addition to comparing groups A and B, we examined prognostic factors affecting disease-specific survival using both univariate and multivariate analysis. Statistical analyses were performed using IBM SPSS Statistics version 24.0. Clinical and pathological categorical variables are presented in case numbers and their percentage distribution, while continuous variables are represented by medians with their associated minimum and maximum values. Pearson's chi-square test and Fisher's test were used in the analysis of categorical variables, while the distribution of continuous variables was examined using the Shapiro-Wilk test. Depending on this, Mann-Whitney tests were used for non-normally distributed data sets. We used the Mantel-Cox log-rank test to compare the survival outcomes (OS, DSS, DFS) of groups A and B, and calculated Kaplan-Meier survival functions for 120 months to determine the 10-year survival rate. Cox's proportional hazard model was used in multivariate analysis, in which we included relevant factors that showed significant differences in univariate tests. The statistical significance threshold for all analyses was set at $p < 0.05$. The follow-up time was determined in the interval from the diagnosis of breast cancer to the last event-free visit, the occurrence of local and/or distant recurrence, or death.

4. RESULTS

4.1. Our results in identifying lymph node metastases of gastric adenocarcinoma

Using the synergistic combination of two modern techniques for identifying lymph node metastases of gastric adenocarcinoma, sentinel lymph node biopsy and the Maruyama Computer Program, we successfully identified an algorithm that can predict metastatic lymph node stations with 100% certainty in both early and advanced gastric cancer.

The sensitivity of sentinel lymph node marking in successfully marked patients ($n=97$) was 94.23% (95%CI=84.36%-98.43%), with 100% specificity (95%CI = 92.73%-100%), $p<0.001$. This sensitivity is considered excellent, however, if we were to rely solely on this technique, we would not have recognised the presence of lymph node metastases in three patients.

By examining the predictions of the MCP and the final histological findings, we determined that an $MI \geq 28$ threshold could ensure the desired 100% sensitivity level. With the help of the MCP alone, with an $MI \geq 28$ threshold, we could have identified 44 out of 52 patients with lymph node disease. Thus, the standalone sensitivity of the MCP was 84.62% (95%CI=72.48%-91.99%), and its specificity was 52.08% (95%CI=38.33%-65.53%). The positive predictive value was 65.57% (95%CI=53.73%-75.81%), and the negative predictive value was 75.00% (95%CI=57.89%-86.75%).

As an effective integration of the above two techniques, we examined a clinically applicable algorithm. In patients with negative sentinel lymph node examination, all metastatic lymph nodes can be identified by removing lymph node stations with $MI \geq 28$ in the MCP. With this approach, we can achieve 100% sensitivity and negative predictive value (95%CI=93.12%-100% and 88.30%-100%), and 60.42% specificity (95%CI=46.31%-72.98%). The positive predictive value was 73.42% (95%CI=61.95%-82.15%). The median number of additional lymph node stations to be removed (not dyed but $MI \geq 28$) was 1, within a range of 1-5.

With the study presented, we demonstrated that the shortcomings of these two modern techniques can be overcome by combining them, and they can be effectively applied together. We do not recommend neglecting the use of preoperative lymph node staging in clinical practice or abandoning standard D2 lymph node dissection. Our goal is rather to draw attention to a potentially useful tool that, by indicating critical lymph node stations to the surgeon, improves the efficiency of lymph node removal. Nevertheless, our results show that the extent of lymph node removal can be safely reduced with this method in selected cases.

4.2. Results in the early detection of breast cancer

We demonstrated that the proportion of tumor patients identified by screening who underwent breast-conserving surgeries increased significantly already in the early stages of the population-wide breast cancer screening program introduced in 2003 (68.8% vs. 59.4%; $p=0.032$). We were unable to show a statistically significant advantage in terms of 10-year overall survival ($p=0.427$), disease-specific survival ($p=0.245$), or disease-free survival ($p=0.074$) in patients with breast cancer detected by screening. We conducted a univariate analysis by examining the factors affecting 10-year DSS. We observed significant differences in patients' age ($p=0.004$), immunohistochemical subgroups ($p<0.001$), tumor size ($p<0.001$), pathological T-stage ($p<0.001$) and N-stage ($p<0.001$), type of breast surgery ($p<0.001$), and the use of adjuvant ($p<0.001$) or neoadjuvant chemotherapy ($p<0.001$). In the multivariate Cox regression analysis, we found that only the immunohistochemical subgroups, pT-, pN-stage, and neoadjuvant therapy remained independent prognostic factors for 10-year disease-specific survival (DSS). During our observational study, we found that although we can perform breast-conserving surgeries at a higher rate through breast cancer screening, early detection was not associated with better survival outcomes in the patient population we examined.

5. KEY FINDINGS AND CONCLUSIONS

1. We have successfully established, for the first time in the world, an algorithm for gastric adenocarcinoma that combines sentinel lymph node mapping and the previously unused Maruyama computer program. This algorithm achieves a sensitivity and negative predictive value of 100% (95% CI = 93.12% - 100%), along with a specificity of 60.42% (95% CI = 46.31% - 72.98%) and a positive predictive value of 73.42% (95% CI = 61.95% - 82.15%) in identifying lymph nodes affected by tumors.
2. In patients with breast cancer detected by the breast cancer screening program, we were able to perform breast-conserving surgeries, which are more favorable from a quality of life perspective, at a higher rate (68.75% vs. 59.41%, $p=0.032$).
3. We have been the first in Hungary to demonstrate that in breast cancer there was no statistically significant advantage in terms of overall survival ($p=0.427$), disease-specific survival ($p=0.245$), or disease-free survival ($p=0.074$) among breast cancer patients detected through screening. However, due to the clearly observable trend, further investigation of long-term outcomes is justified.
4. In examining the factors affecting 10-year disease-specific survival, we observed significant differences in age ($p<0.001$), immunohistochemical subgroups ($p<0.001$), tumor size ($p<0.001$), pathological T-stage ($p<0.001$) and N-stage ($p<0.001$), type of breast surgery ($p<0.001$), and the application of adjuvant ($p<0.001$) and neoadjuvant chemotherapy ($p<0.001$).
5. Multivariate Cox regression analysis revealed that the immunohistochemical subgroups, pT-, pN-stage, and the application of neoadjuvant therapy were independent prognostic factors for 10-year disease-specific survival in breast cancer.

CERTIFIED LIST OF PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION



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Registry number: DEENK/308/2023.PL
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Candidate: Zsolt József Varga
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Varga, Z. J.**, Biró, A., Török, M., Tóth, D.: A combined approach for individualized lymphadenectomy in gastric cancer patients.
Pathol. Oncol. Res. 29, 1-10, 2023.
DOI: <http://dx.doi.org/10.3389/pore.2023.1611270>
IF: 2.874 (2021)
2. Tóth, D., **Varga, Z. J.**, Tóth, J., Árkosy, P., Sebő, É.: Short- and Long-Term (10-year) Results of an Organized, Population-Based Breast Cancer Screening Program: comparative, Observational Study from Hungary.
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List of other publications

3. Kolozsi, P., **Varga, Z. J.**, Tóth, D.: Indications and technical aspects of proximal gastrectomy.
Front. Surg. 10, 1-8, 2023.
DOI: <http://dx.doi.org/10.3389/fsurg.2023.1115139>
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4. **Varga, Z. J.**, Nagy, K., Tóth, D.: Gyomorsebészet 100 év tükrében a debreceni Sebészeti Klinikán.
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Pathol. Oncol. Res. 22 (1), 209-215, 2016.
DOI: <http://dx.doi.org/10.1007/s12253-015-9999-3>
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