

**UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA”, BUCHAREST
DOCTORAL SCHOOL
MEDICINE**



Sentinel lymph node in colorectal cancer

PHD THESIS SUMMARY

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2022

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List of abbreviations and symbols:

99mTc	technetiu 99 metastabil
AJCC	american joint committee for cancer
AL	fistula anastomotică
APC	adenomatosis polyposis coli
APTT	timp de tromboplastină parțial activat
AUC	area under the curve
BC	boala Crohn
CA 19-9	carbohydrate antigen 19-9
CCR	cancerul colorectal
CEA	antigenul carcinoembrionar
CI	intervalul de confidență
DALM	displazia asociată unei leziuni sau mase proliferative
EMA	agenția europeană a medicamentului
FAP	polipoza adenomatoasă familială
FOBT	fecal occult blood tests - teste pentru depistarea hemoragiilor oculte din scaun
FDA	food and drug administration
FIT	testele imunohistochimice fecale
GLOBOCAN	global cancer observatory
HR	hazard ratio
ICG	verde de indocianat
IBD	boala inflamatorie intestinală

ICI	inhibitorii punctului de control imun
IHC	imunohistochimie
IMC	indicele de masă corporală
INR	international normalized ratio (Raportul internațional normalizat)
ITC	celule tumorale izolate
JSCCR	societatea japoneză pentru cancerul de colon și rect
LN	limfonodul
LNSM	lymph node skip metastasis (metastaze oculte)
MSI	instabilitate microsatelitară
MSI-H	instabilitate microsatelitară ridicată
MPV	volumul mediu al trombocitelor
M2-PK	piruvat kinaza M2 fecală
NAFL	ficatul gras non-alcoolic
NBI	narrow band imaging (Imagistica în bandă îngustă)
OS	supraviețuirea globală
PLT	trombocite
RDW	red cell distribution width(lățimea distribuției celulelor roșii)
RMST	restricted mean survival time
ROC	receiver operating characteristics
ROS	speciile reactive ale oxigenului
SD	deviația standard
SEPT9	gena Septin 9
SL	sindromul lynch

SLN	sentinel lymph node (ganglion santinelă)
SLNB	sentinel lymph node biopsy (biopsia ganglionului santinelă)
TMB	încărcătura mutațională tumorală

Introduction

In oncological surgery, the importance of the sentinel node concept is increasing, because it is important to reduce the extent of surgical interventions and the morbidity associated with it, while maintaining adequate oncological safety. Recently, lymph node mapping techniques have been developed, where the most promising method appears to be immunofluorescence using indocyanine green dye. This technique offers a high sensitivity in the detection of the sentinel node compared to other existing methods that use another dye (methylene blue) in combination with a radiotracer (technetium 99m) [1].

Knowledge of regional lymph node status is essential for establishing staging and prognostic outcomes in breast cancer and malignant melanoma. Axillary lymph node staging by sentinel lymph node biopsy (SLNB) is a widely used method and is now considered a standard of care in patients without clinical evidence of axillary lymph node metastasis in breast cancer

The sentinel node identification technique has proven to be a particularly effective method in breast cancer and malignant melanoma by estimating the status of regional lymphatic relays. In these cases, the positive/negative status of the sentinel node modifies the surgical procedure with the possibility of minimizing the intervention. In the case of colorectal cancer, the presence or absence of sentinel lymph node invasion does not change the surgical technique but can increase its accuracy, by identifying certain situations when the surgeon has to extend the lymphadenectomy.

Ernest A. Gould first introduced the term sentinel node in 1960 during a parotidectomy [2]. The lymph node was sent to the pathologist who issued the result: "lymph node invaded by tumor". In 1977 Ramon M. Cabanas identified a sentinel node in a patient with penile cancer [3].

Joosten et al, in 1999 conducted a study on 50 patients with colon cancer, in which they injected methylene blue 1% peritumorally in 4 cardinal points. Lymphatic mapping was possible in 35 of 50 patients (70%). Histopathological examination with hematoxylin-eosin staining identified invaded lymph nodes in 20 of the 35 patients [4].

Lymph node status is the best predictive marker for recurrence and survival in patients with colorectal cancer [5]. The lymph nodes that first drain lymph from the tumor area are known as sentinel nodes and are thought to be the first site for metastasis [6].

In colorectal cancer, lymphadenectomy is not influenced by positive or negative sentinel node status. The identification of lymph nodes with possible tumor invasion, by injecting a dye (methylene blue 1% or indocyanine green) at the level of the primary tumor can lead to improved staging and postoperative management. In other words, subsequent administration of neoadjuvant therapy (chemotherapy) to patients may result in lower recurrence rates.

Indocyanine green (ICG) is a contrast agent that becomes fluorescent when excited by light with a wavelength of 800-900 nm. The fluorescence imaging system using indocyanine green enables the identification of lymphatic vessels and blood flow during surgery. One of the most important factors for staging and prognosis in colorectal cancer is regional lymph node status. In recent years, the intraoperative use of fluorescence for angiographic purposes has been expanded by surgeons and centers worldwide.

The surgical stage of the multimodal oncological treatment must be as precise as possible and achieve a complete excision of both the tumor formation and a precise lymphadenectomy, without remaining nodes that can be invaded by the tumor. The technique of identifying and biopsying the sentinel lymph node is a valuable oncological method, which aims to evaluate the regional lymphatic basin, with the advantage of a correct staging of the disease, the prognosis of the patients, but also the indication of non-adjuvant therapy in the post-operative oncological treatment.

The identification of the sentinel node and the lymph nodes involved in the tumor drainage, leads to the realization of a radical oncological surgical intervention by excision of all the nodes with possible neoplastic invasion.

The thesis is structured in two parts:

The first part, the "general part" includes two chapters that present the current state of the researched topic, presenting a review of the specialized literature, based on a laborious documentation, in relation to the addressed research topic.

Chapter 1 includes a brief review of the current considerations of colorectal cancer regarding epidemiology, etiopathogenesis, screening methods, particularities of diagnostic exploration, AJCC and JSCCR staging, anatomopathological aspects, therapeutic strategy in colorectal cancer and postoperative complications according to the Clavien Dindo classification.

Chapter 2 presents the sentinel lymph node concept, the importance and usefulness of the detection technique, which is today also used in other pathologies as a "gold standard" technique.

The second part of the doctoral thesis includes the contributions of individuals

I established from the beginning the objectives of the study: ***the first objective*** was a descriptive analysis of the variables tracked in the study, ***the second objective*** represented the determination of the performances of the two ganglion identification methods; the main endpoints being: the number of nodes identified, the percentage of invaded nodes from those identified, the number of sentinel nodes identified and the percentage of invaded sentinel nodes from those identified, ***the third objective*** was to identify associations between tumor characteristics (stage, grading, histopathological type) and a series of humoral parameters (CEA, CA 19-9, fibrinogen), the main endpoints being the value of these parameters, related to tumor characteristics; ***the fourth objective*** was a survival analysis of all patients in the study, with the identification of some demographic-clinical factors that can influence survival, the main endpoint being OS (overall survival); ***the last objective*** was to identify the role of sentinel node detection and regional lymph basin mapping in colorectal cancer.

The general methodology of scientific research

To achieve these objectives, we used the results of two techniques, carried out during the scientific research within the doctoral school. Both were unicentric, carried out in the Surgery Clinic I of the Bucharest University Emergency Hospital (detection of the sentinel node using methylene blue in colorectal cancer: the in vivo and ex vivo technique took place between January 2018 and February 2020 and included a number of 26 patients) and (detection of the sentinel node using indocyanine green in colorectal cancer by the in vivo technique carried out between January 2020 and May 2022 and included a number of 34 patients).

The studies were carried out prospectively, experimentally, randomized but without a randomization algorithm before the study, on a total sample of 60 patients diagnosed with colon or rectal neoplasm, with indication for radical surgery, who underwent one of the procedures of identification of lymph nodes with the help of tracer substances, representative sample for a population of patients diagnosed and treated for colorectal neoplasms in a tertiary center.

The patients were divided into two groups: group A consisted of 26 patients in whom the tracer was methylene blue 1%, and group B consisted of 34 patients in whom the tracer was indocyanate green.

The inclusion criterion was represented by the existence of a malignant colorectal tumor with confirmed histopathological diagnosis and curative surgical indication.

The exclusion criteria were:

- the coexistence of another malignant tumor (synchronous tumor);
- the presence of metastatic tumors;
- pregnancy or breastfeeding
- preoperative chemotherapy.

The R program[7] was used for the statistical analysis, with the following packages installed: survival[8,9], survminer[10], gtsummary[11], table1[12], lattice[13], effects[14-16].

The level of significance α in the study was 0.05, so p values lower than 0.05 were considered statistically significant.

Materials and methods

Sentinel node detection using methylene blue in colorectal cancer: in vivo and ex vivo technique

In this group, 26 patients were enrolled (14 women, 12 men) aged between 35 and 83 years old. The patients were hospitalized in the General Surgery and Emergency I Department of the Bucharest University Emergency Hospital between January 2018 and February 2020. The present study was approved by the Ethics Committee of the Bucharest University Emergency Hospital no. 55321/24.09.2019. Written informed consent was obtained from the patients before surgery.

Methylene blue 1% was used to identify lymph nodes with possible neoplastic invasion. Two techniques, in vivo and ex vivo, were used to identify the sentinel node and lymph territory.

Sentinel node detection using indocyanine green in colorectal cancer by the in vivo technique

The fluorescence imaging system using indocyanine green enables the identification of lymphatic vessels, lymph nodes and blood flow during surgery.

The group of patients included in this study is 34. All patients had written informed consent before the injection of indocyanine green with the approval of the local bioethics committee. The technique we choose is in-vivo. This is carried out intraoperatively, immediately after the exploratory laparotomy and the verification of exclusion conditions. After identifying the primary tumor, the indocyanine solution is prepared. I used a 25 mg vial containing indocyanine green in powder form, which I diluted with 10 mL of sterile serum. The solution thus obtained is extracted into a 10 mL syringe to which a 26 Gauge needle is mounted. 2.5 mL of indocyanate solution is injected peritumorally, at the level of the 4 cardinal points, and wait approximately 30 minutes, other studies have imposed a waiting time of 10-20 minutes. In order to visualize the sentinel nodes, we used the KARL STORZ VITOM ICG probe for open surgery, in ICG mode [17-19].

Conclusions and personal contributions

Conclusions

The main conclusions that emerge from the results obtained from the scientific research of this doctoral thesis are:

1. The sentinel node identification technique using indocyanate green has the best performance in detecting invaded sentinel lymph nodes in cases of CCR.
2. Carbohydrate antigens (CEA and CA 19-9), although they have a role in monitoring patients after surgical resection and establishing prognosis, in my study elevated preoperative values were associated with a more aggressive histopathological type and with a more advanced stage of neoplasia.
3. The histopathological type NOS has the lowest preoperative values of CEA and CA 19-9 having a very good prognosis.
4. Tumors that invade the visceral peritoneum, invade or adhere to adjacent organs in T4 stage, have the highest CA 19-9 values.
5. The cases that had 4 or more positive lymph nodes had a CA19-9 value 36 times higher than the cases without tumor invasion of the lymph nodes and a mortality 5 times higher than the cases with negative lymph nodes. Thus, the value of preoperative carbohydrate antigens has a particularly important value on the severity of neoplasia but also on survival.
6. The "signet ring" histopathological type had a 25-fold higher mortality than mucinous tumors reflecting a clearly superior aggressiveness of "signet ring" tumors .
7. Perineural invasion is associated with a lower serum calcium value than cases without invasion. Preoperative hypocalcemia may represent a predictive factor for the severity of perineural tumor invasion, but studies on larger groups of patients are needed to establish this aspect.
8. In the studied group, lymphovascular invasion was associated with a 5 times higher mortality compared to cases without invasion. Thus, the presence of lymphovascular invasion detected after the histopathological examination guides the survival of patients postoperatively.

9. Increased preoperative fibrinogen is associated with 6 times higher mortality compared to those with normal fibrinogen. Patients with normal preoperative fibrinogen had a 1-year longer survival than those with hyperfibrinogenemia.
10. An extra invaded lymph node increases the risk of death by 31%, so it is imperative to have a lymphadenectomy as complete as possible with as many lymph nodes excised per patient as possible.
11. An extra invaded sentinel node increases the risk of death by 2.25 times.
12. The risk of death was 8 times higher in patients with category N2 compared to cases without tumor-invaded nodes.
13. An accurate N-category staging becomes essential and classic histopathological examination sometimes fails to identify lymph nodes with micrometastases or tumor deposits, which could explain local or distant recurrence in stage II patients.
14. The methods of mapping the lymphatic drainage with the identification of the sentinel nodes and the regional lymphatic basin, increase the lymph node excision rate by 2.16 times compared to the classic excision. The number of lymph nodes identified was significantly improved in the study group compared to the control group, with a double lymph node excision rate compared to the classic technique, thus increasing the accuracy of the lymph node examination.
15. Cases in which the lymph nodes did not present metastases require additional evaluations to eliminate the possibility of the presence of micrometastases (skip metastases) using immunohistochemistry and polymerase chain reaction (RT-PCR) techniques, which may reveal micrometastases that would have been missed during routine examination with hematoxylin eosin (H&E), thus achieving a more precise "upstaging" staging and implicitly the administration of chemotherapy as a postoperative treatment, thus increasing the patients' prognosis.
16. Radiotherapy destroys the normal conformation of the lymphatic ducts and implicitly the ganglion anatomy constituting an exclusion criterion in future studies for rectal cancer cases.
17. The number of invaded lymph nodes is greater the more advanced the tumor is and the more certain the lymphatic dissemination of the tumor cells is.

Personal contributions

During the doctoral school, I carried out two prospective, unicentric, experimental studies that aimed to identify the sentinel nodes in patients with colorectal cancer, using two different techniques in patients with a curative surgical indication, pursuing 5, main objectives:

Objective 1 performs an extensive descriptive analysis of the variables monitored in the study (age, sex, environment of origin, type of method used in the study, fibrinogen value, RDW, MPV, PLT, Ca, CEA, CA 19-9, tumor location, histopathological type, degree of tumor differentiation, lymphovascular and perineural invasion, tumor stage according to TNM classification, postoperative complications according to Clavien-Dindo classification, death, number of examined nodes, number of invaded nodes, number of identified sentinel nodes and number of invaded sentinel nodes for each batch separately, the total sample being 60 patients, a significant number for this type of study.

Objective 2 quantified the performance of the two lymph node identification methods by detecting an increased performance of the sentinel node identification technique using indocyanate green and the Karl Storz Vitom ICG probe compared to the method that used methylene blue; in patients from group B, the percentage of invaded sentinel nodes is higher (86.20%) than in patients from group A (37.50%), demonstrating a superior efficiency in favor of the use of indocyanate green.

Objective 3 sought to identify some associations between certain tumor characteristics (histopathological type, degree of tumor differentiation, lympho-vascular or perineural invasion, T and N category value, TNM stage) and a series of humoral parameters (CEA, CA 19-9, fibrinogenemia, PLT, RDW, MPV, Calcium).

Objective 4 analyzed the overall survival (OS) in the patients in the sample with the identification of factors with a prognostic role for it.

Objective 5 investigated the predictive value of sentinel node detection and regional lymph node mapping in colorectal cancer, comparing the results of personal research with a control group in which neither of the two methods of node identification was performed.

The proposed objectives were met and the main results are:

1. The comparative analysis of the number of invaded sentinel nodes from the total number of sentinel nodes examined, highlights the fact that in group B (where indocyanine green was used as a tracer), the percentage of invaded sentinel nodes is higher (86.20%) than in the group A (where 1% methylene blue tracer was used) (37.50%), $p = 0.0004$.

2. The histopathological type of the neoplasm, with the NOS type is associated with lower values of 12 ng/mL of CEA, in relation to the neoplasm with "signet ring" cells.

3. The CEA value for the mucinous type is close to the CEA value in the case of "signet ring" cell neoplasm.

4. Tumors with "signet ring" cells having the highest CA 19-9 values associated, mucinous tumors had CA 19-9 values 70U/mL lower than the "signet ring" cell form, the form NOS had 114U/mL lower values than the "signet ring" cell form.

5. Tumors in the T4 category had CA 19-9 values 75U/mL higher than tumors in the T2 stage, tumors in the T3 category had CA 19-9 values similar to those in the T2 stage.

6. An effect for which there was insufficient evidence (marginally non-significant p-value, $p = 0.09$) was related to tumor category N, with tumors in the N2 category having CA 19-9 values 36 higher than those in the N0 category (tumors in stage N1 had CA 19-9 values similar to those in N0).

7. Patients who had perineural invasion, the serum Ca value is 1 mg lower than patients without perineural invasion.

8. Mortality was more than 5 times higher in patients with lymphovascular invasion than in patients without lymphovascular invasion.

9. We identified an association at the limit of statistical significance, between the histopathological type of the neoplasm and the serum Ca value. In patients with NOS tumors, the Ca value was 1.6 mg higher than in patients with "signet ring" tumors.

10. The mortality of the patients in the sample was 10%, and the median survival (RMST) was 46.35 months.

11. Mortality was almost 6 times higher in patients with fibrinogen greater than or equal to 514.50 ($p = 0.01$).

12. There was a 1-year survival difference in favor of patients with fibrinogen less than 514.5.

13. The risk of death was 6.60 times higher in patients with fibrinogen greater than or equal to 514.5.

14. An elevated preoperative CEA value is a risk factor for a more serious evolution of the disease.

15. The lowest mortality and highest survival was observed for right colon cancer, followed by left colon and rectal cancers.

16. The most severe evolution was for signet ring cell neoplasm (highest mortality and lowest RMST value), while the best evolution was for NOS tumors (lowest mortality and highest value high RMST).

17. In the examined group (there were only 4 cases of "signet ring" cell neoplasms), the risk of death in "signet ring" cell tumors was 25 times higher than in mucinous tumors (upper limit of CI indicates a 2 times higher risk of death) and almost 100 times higher (the CI upper limit was 10 times higher).

18. The difference was statistically significant, patients with lympho-vascular invasion present, have an RMST almost 8 months lower than patients without lympho-vascular invasion.

19. Mortality was approximately 5 times higher in patients in category N2, compared to patients who presented the value of category N earlier.

20. The risk of death was approximately 8 times higher for the N2 category compared to the N0 stage, this effect being statistically significant, the patients in the N1 category not having a different risk of death compared to the patients in the N0 stage.

21. Patients in stage I according to the AJCC classification had 0 mortality and the highest RMST.

22. Tumor excision and lymphadenectomy were performed in all cases, regardless of the status of the lymph nodes. After the intervention was completed, the site of tumor

excision and the emergence of the ligated vascular pedicles were checked to ensure that there were no remaining stained areas.

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1. **Stăniloae D**, Budin C, Ilco A et al. In vivo sentinel lymph node detection with indocyanine green in colorectal cancer. *Maedica – A Journal of Clinical Medicine*. Mai 2022. doi: 10.26574/maedica.2022.17.2.264
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