MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPER

MAEDICA – a Journal of Clinical Medicine
2022; 17(2): 264-270
https://doi.org/10.26574/maedica.2022.17.2.264

In Vivo Sentinel Lymph Node Detection with Indocyanine Green in Colorectal Cancer

Daniel STANILOAIE[a, b], Constantin BUDIN[a, b], Alexandru ILCO[b], Danut VASILE[a, b], Amalia Loredana CALINOIU[c], Adina RUSU[c], George IANCU[a, d], Tarek AMMAR[b], Cristian Florin GEORGESCU[b], Maria-Daniela TANASESCU[a, e, f], Alexandru MINCA[a], Dragos Eugen GEORGESCU[a, g]

[a]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[b]First General Surgery Department, Emergency University Hospital, Bucharest, Romania

[c]"Prof. Dr. Agrippa Ionescu" Emergency Clinical Hospital, Bucharest, Romania

[d]"Filantropia" Clinical Hospital of Obstetrics and Gynecology, Bucharest, Romania

[e]Department of Semiology, Emergency University Hospital, Bucharest, Romania

[f]Department of Nefrology, Emergency University Hospital, Bucharest, Romania

[g]1st General Surgery Department, "Dr. Ion Cantacuzino" Clinical Hospital, Bucharest, Romania

ABSTRACT

Introduction: *The indocyanine green fluorescence imaging system allows the identification of lymphatic vessels, lymph nodes and blood flow during surgery. Colorectal cancer is the second commonest cancer in women, the third in men, being the fourth commonest cause of cancer death. One of the most important factors for staging and prognosis in colorectal cancer is the involvement of the regional lymph nodes. In the literature, there are several methods for identifying sentinel lymph nodes, including methylene blue, technetium (^{99m}Tc) and indocyanine green. The current article presents the use of indocyanate in the identification of sentinel node/nodes in malignant tumors of the colon, by a technique performed* in vivo, *before the primary ligation of the vascular pedicles.*

Material and methods: *The study was prospectively conducted on a group of 23 patients who had undergone a standard surgical resection – 21 of them for a malignant tumor of the colon and two patients for a malignant rectal tumor – in the 1st General Surgery Department, Emergency University Hospital, Bucharest, Romania, between January 2020-March 2022. During surgery, sentinel lymph node detection was performed using indocyanine green and the Karl Storz® Vitom ICG probe. Sentinel lymph nodes were separately excised and sent to the Department of Pathological Anatomy for analysis.*

Address for correspondence:
Dr. Maria Daniela Tănăsescu, MD, PhD
Nephrology Department, University Emergency Hospital, Bucharest, Splaiul Independentei, No. 169, District 6, 020021 Bucharest, Romania
Tel: +40745042785; email: maria.tanasescu@umfcd.ro

Article received on the 17th of May 2022 and accepted for publication on the 30th of May 2022

Results: *Sentinel nodes were successfully identified in 13 patients and the overall identification rate was 56.52% (13/23 cases). In seven cases, the number of invaded nodes was the same as that of identified and invaded sentinel nodes. Complete lymphadenectomy was performed in all cases regardless of the staining status of the sentinel lymph nodes.*

Conclusions: *The use of fluorescence imaging with indocyanine green in colorectal cancer remains controversial. Since no specific receptor target is used, the fluorescent signal is not specific for lymph node metastases. The learning curve is particularly important for increasing the accuracy of the technique and is responsible for the negative results in some cases. Cases in which lymph nodes have not been invaded require further evaluation through immunohistochemistry and chain polymerization reaction (RT-PCR).*

Keywords: indocyanine green, sentinel lymph node, colorectal cancer, oncology.

Abbreviations:
CCR=colorectal cancer
GI=gastrointestinal
ICG=indocyanine green
SLN=sentinel lymph node

INTRODUCTION

According to data quantified for 2020, colorectal cancer ranks third in terms of incidence of neoplasms in Romania, representing 10% of all cancers diagnosed in approximately 2.000.000 cases for both sexes (1). Lately, the aplications of indocyanine green (ICG) for fluorescent-guided surgery has been expanding. Here we assessed the use of fluorescence imaging in gastrointestinal (GI) tract surgery. The feasibility of sentinel lymph nodes (SLNs) for GI malignancies, gastric cancer and colon cancer has not yet been established. Various dye-guided (2) and radioisotopic radio-guided methods (3) have been used to detect SLN, but each method had some technical and cost-benefit disadvantages. Here, we evaluated the usefulness of ICG fluorescence imaging for the detection of SLN in colorectal cancer (4). The presence of the absorption maximum, confinement to the vascular compartment through binding with plasma proteins, the low toxicity and rapid excretion almost exclusively into the bile are the main advantages of using ICG. Indocyanine green is stable at room temperature and soluble in water (1 mg/mL) but not readily soluble in saline; therefore, ICG should be first dissolved in water and only afterwards diluted with saline if an isotonic solution is needed (5). The concept of sentinel node is not new – it was used in breast cancer and melanoma surgeries (6-9) since 1994, at John Wayne Cancer Institute, where researchers were able to correctly identify the nodal status in 95% of patients (10).

For colorectal cancer, the therapeutic management has substantially changed over the last decades, with the addition of neoadjuvant chemoradiotherapy, adjuvant therapies and improved surgical techniques (11). Although poorly understood (12, 13), pathologic complete response following neoadjuvant chemoradiotherapy is beneficial, as up to 75% of patients do not experience any re-growth of the primary tumor (14). Considering an increase in survival rates, each patient's quality of life should be taken into account. Therefore, considering the side effects of neoadjuvant treatment, the curative surgical step must be as precise as possible, aiming to perform a complete excision and precise lymphadenectomy, without remaining lymph nodes that could be invaded by the tumor. The sentinel node identification and biopsy technique represent a valuable oncological method, which aims to evaluate the regional lymphatic drainage, with the advantage of a correct staging of the disease, the prognosis of patients, and also the indication of neodjuvant therapy in cancer treatment. The sentinel node is the first lymph node that drains the lymph from the tumor, as such it has the highest risk to be invaded by malignant cells (15).

Colorectal cancer is a lymphophilic cancer and therefore, alongside individual (e.g., diabetes mellitus) prognostic factors, the status of lymph node metastases is the main tumor prognostic factor (16-18). The high mortality from colorectal cancer is caused by locoregional recurrences and metastases. These are due to unidentified micrometastases by classical histological examination of the lymph nodes. Indocyanine

green is an anionic water-soluble substance with multiple applications currently in medicine (19). Photodynamic therapy involves the administration of photosensitizing drugs, followed by tissue or cell exposure to light (20). Indocyanine green is able to penetrate cells without producing degenerative changes (21), and since 1995 it has been approved for use in human medicine for tests of cardiac and hepatic function (22). When ICG is injected into human tissues, it immediately binds tightly to blood plasma. The majority of injected ICG is accumulated by hepatic parenchymal cells and is then excreted from hepatic cells into bile juice without being metabolized. The typical medical applications of ICG include hepatic function and liver blood flow diagnostics, which use measurement of optical absorption functions (2, 22)

Discovering an adequate number of lymph nodes on the surgical piece depends not only on the extension of the colorectal resection, which should always meet the oncological principles, but also on the anatomopathological analysis of the resected specimen.

The fluorescence imaging system is one of the most popular imaging techniques in the biomedical field for visualizing tissues and cells both *in vivo* and *ex vivo*. Its benefits include a high contrast, high sensitivity and low concentrations being able to highlight the loco-regional lymphatic system efficiently (23).

The objective of this prospective study is to determine the identification rate and accuracy of the SLN procedure with ICG by a technique performed *in vivo* in patients with CCR and to analyze the learning curve for the SLN procedure in CCR patients. We would like to obtain a picture of how and to what extent ICG fluorescence-guided visualization is used in CCR. ❑

MATERIAL AND METHODS

We performed a prospective study on patients admitted in our surgical department between 06.01.2020 and 23.03.2022.

Data was collected from both patient observation sheets and histopathological reports of the postoperative excision piece. All patients offered their written consent for using the data only for scientific and statistical purposes, respecting patients' rights as well as ethics, law and medical ethics. The present study was conducted in accordance with the General Data Protection Regulation of the European Union and approved by the Ethics committee of Bucharest Emergency University Hospital (55321/15.10.2019).

After signing the informed consent, all patients with a histopathological diagnosis of CCR were considered potentially eligible for enrollment in the study. The following exclusion criteria were used: (a) age under 18 years, (b) another synchronous malignancy, (c) the presence of liver, lung, brain, peritoneal or other localization metastases, and (d) pregnancy or breastfeeding.

Injected ICG was used to identify the sentinel node is *in vivo*. The technique was performed intraoperatively immediately after laparotomy in all patients who met the criteria for enrollment in the study. After the primary tumor was identified, the indocyanate solution was prepared using a 25 mg vial containing ICG in powder form, which was diluted with 10 mL of sterile water. The resulting solution was extracted in a 10 mL syringe to which a 26 Gauge needle was mounted (24). Then, 2.5 mL of the indocyanate solution were injected into the peritumoral region in four cardinal points. After 10-15 minutes, the Vitom Karl Stortz probe for open surgery was used in order to visualize the sentinel nodes (Figure 1).

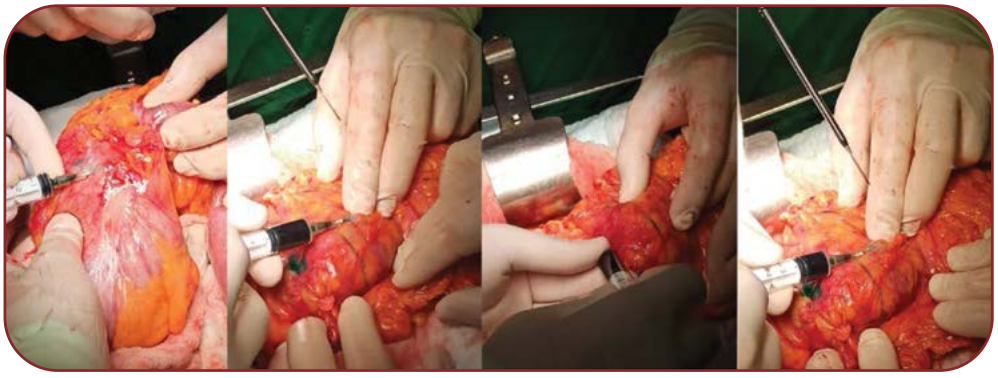

FIGURE 1. Injecting indocyanine green in four cardinal points

FIGURE 2. Sentinel lymph node

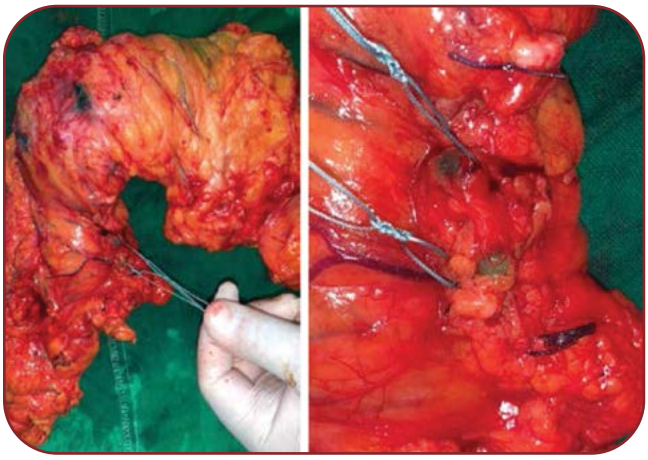

FIGURE 3. Separate marked sentinel nodes on the piece of colic resection

At the end of the intervention, the abdominal cavity was checked with the VITOM probe to check for any remaining ganglion stations. For tumor staging and case classification, we used the TNM system of the American Joint Committee on Cancer (AJCC) (25).

Patients presenting with malignancies usually have a high risk of developing acute kidney injury secondary to chemotherapy, exposure to contrast agents used in medical imaging, radiation therapy, tumor lysis syndrome, hypotension or as a direct effect of the malignancy. Since this technique does not involve intravenous administration of ICG, there is not risk for the development of acute kidney injury. ❑

RESULTS

In this prospective study, only 23 patients were enrolled and met the inclusion criteria. The novel COVID-19 pandemic has generated substantial disruptions worldwide and impaired the ability of hospitals to diagnose and treat cancer patients (26). Patients had a limited access to medical services and arrived at hospitals in more advanced stages. Sentinel nodes were successfully identified in 13 patients; the overall identification rate was 56,52% (13/23 cases). The characteristics of study group are shown in Table 1.

In seven cases, the number of invaded nodes was the same as that of identified and invaded sentinel nodes. We noticed that once the tumor was more advanced, the number of invaded nodes was higher and the number of detected sentinel nodes was increased. Also, nine of the 23 cases did not have any lymph nodes invaded. In none of the nine cases ICG was captured in the lymph nodes. Case no. 6 showed six lymph nodes with metastases, of which only three patients caught ICG and two tumor invaded, while one patient was false-positive. The same situation has also been encountered in the case no. 9 and 22. In each case, a false-positive sentinel node was found. ❑

DISCUSSION

Indocyanine green is a widely available dye of clinical importance that has been used for more than 50 years (27). When injected intravenously, indocyanine (800 nm fluorophore) binds to plasma proteins, thus being limited to the intravascular compartment with minimal clearance to the interstitium. Subserous or submucosal injection of indocyanate leads to absorption by the lymphatic vessels, binding again to plasma proteins and transporting the indocyanate to the Chyli sac, where it enters the circulation; ICG is exclusively excreted by the liver through bile without being metabolised (28).

It seems that cardiovascular disease, diabetes, diabetic arteriopathy and the degree of calcification of the arteries influence the blood perfusion of the colon and as a consequence, they might modify the dispersion of indocyanate through the lymphatic vessels and lymph nodes (29).

A literature review of articles published in PubMed in 2022, including 27 specialized studies comprising 8786 patients with colorectal anastomoses who had been assessed with (ICG) fluorescence angiography, were compared to patients who had only white light visual inspection

TABLE 1. Characteristics of study group

Case no.	Location	pT	pN	G	Stage	No. of examined lymph nodes	No. of invaded lymph nodes	Sentinel	Invaded sentinel
1	Ascending	pT2	pN0	G2	I	8	0	0	0
2	Descending	pT3	pN0	G1	IIA	18	0	0	0
3	Descending	pT3	pN0	G2	IIA	21	0	0	0
4	Left splenic flexure	pT3	pN2a	G2	IIIB	12	4	2	2
5	Left splenic flexure	pT4a	pN1a	G2	IIIB	13	1	0	0
6	Inferior rectum	pT3	pN2a	G3	IIIB	8	6	3	2
7	Descending	pT3	pN1a	G2	IIIB	23	1	1	1
8	Right hepatic flexure	pT3	pN0	G2	IIA	19	0	0	0
9	Sigmoid	pT4a	pN1b	G2	IIIB	24	2	2	1
10	Ascending	pT3	pN1b	G1	IIIB	36	2	2	2
11	Cecum	pT4b	pN2b	G2	IVC	23	8	3	3
12	Left splenic flexure	pT3	pN2a	G2	IIIB	28	3	3	3
13	Inferior rectum	pT2	pN0	G2	IIB	9	0	0	0
14	Recto-sigmoid	pT4a	pN0	G2	IIB	7	0	0	0
15	Cecum	pT3	pN1	G2	IIIB	34	0	0	0
16	Sigmoid	pT3	pN1a	G2	IIIB	11	1	1	1
17	Transverse colon	pT3	pN0	G2	IIA	11	0	0	0
18	Cecum	pT3	pN1a	G2	IIIB	22	1	1	1
19	Sigmoid	pT3	pN0	G2	IIA	18	0	0	0
20	Cecum	pT2	pN1c	G3	IIIA	21	1	1	1
21	Cecum	pT4b	pN1b	G3	IIIC	29	2	2	2
22	Left splenic flexure	pT4a	pN2b	G3	IIIC	24	21	3	2
23	Cecum	pT4b	pN2b	G2	IIIC	40	25	4	4

of their anastomosis. Assessment of colorectal anastomoses with ICG is likely to be associated with lower odds of anastomotic leak as compared to traditional white light assessment (30).

A comparison study between the use of methylene blue vs. ICG included a group of 132 patients with endometrial cancer, of which 46 underwent robotic surgery and 86 laparoscopic surgery. The injection of methylene blue was done on one side of the uterus and indocyanine on the contralateral side. The use of indocyanine instead of methylene blue resulted in a 26.5% increase in SLN detection rates in women with endometrial cancer (31).

The Chongqing China Department of Breast Surgery included in its study 471 breast cancer patients who were divided into two groups. The first group (271 patients) was given methylene blue + radioisotope 4-12 hours before surgery, and the second group (200 patients) received methylene blue + indocyanate 10 minutes before surgery. The result of the study did not identify any significant differences between the two groups, which showed that the use of indocyanine in combination with methylene blue might be a very good alternative in sentinel node identification as both were non-radioactive substances (32). Another study performed on 227 patients with uterine neoplasm, who were divided into two groups – in the first one (179 patients), only indocyanate was used, with a success rate of 79%, while in the second one (30 patients), indocyanine in combination with

methylene blue was used, with a success rate of 77% (33).

After procedure, in the presented case, the site of tumor excision and emergence of vascular pedicles were checked to ensure there were no remaining areas stained with indocyanine. The fluorescence imaging system is one of the most popular imaging techniques in the biomedical field for visualizing tissues and cells both *in vivo* and *ex vivo*. This technique has several benefits, including high contrast, high sensitivity, and low concentrations, which can highlight the loco-regional lymphatic system (34). Despite the major progress of chemotherapy, surgery remains the treatment of choice, and it implies the complete resection of the tumor (35). Early diagnosis of colon neoplasms should be made through screening programs. Most people who are diagnosed with a malignant tumor form experience a subsequently untreated anxiety-depressive syndrome, which can lead to suicide (36). ❑

CONCLUSIONS

The sentinel node identification technique using ICG is a feasible method to use in colorectal cancer. The learning curve is particularly important for increasing the accuracy of the technique and is responsible for the negative results in some cases. To increase the sentinel lymph node determination rate using indocyanine, a longer learning curve is needed. Early diagnosis is difficult to be settled but it is mandatory because any delay of the proper treatment could increase mortality (37). Cases in which lymph nodes have not been invaded require further evaluation to rule out the possibility of micrometastases and skip metastases using immunohistochemistry and chain polymerization reaction (RT-PCR) techniques. With further validation, this technique could become a valuable tool to guide personalized oncological colorectal resections (38). Unfortunately, colorectal cancer has an increasing incidence among the young population, and adopting a healthy diet correlated with regular medical analysis may decrease the incidence of this malignancy (39). ❑

Availability of data and materials: All data generated to analyzed during this study are available from the author on reasonable request.

Conflicts of interest: none declared.

Financial support: none declared.

Ethics approval: This article conforms to the ethical norms and standards in the Declaration of Helsinki. The present study was approved by the Ethical Committee of Emergency University Hospital, Bucharest, Romania, approval statement number 55321/15.10.2019. The patients freely-given written informed consent before the surgical intervention.

References

1. https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf. Accessed on 11.05.2022.
2. **Fattahi AS, Tavassoli A, Rohbakhshfar O, et al.** Can methylene blue dye be used as an alternative to patent blue dye to find the sentinel lymph node in breast cancer surgery? *J Res Med Sci* 2014;19:918-922.
3. **Ahmed M, Purushotham AD, Horgan K, et al.** Meta-analysis of superficial versus deep injection of radioactive tracer and blue dye for lymphatic mapping and detection of sentinel lymph nodes in breast cancer. *Br J Surg* 2015;102:169-181.
4. **Kusano M, Kokudo N, Toi M, Kaibori M.** ICG fluorescence imaging and navigation surgery. Japan: *Springer*, 2016.
5. **Alander JT, Kaartinen I, Laakso A, et al.** A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging* 2012;2012:940585.
6. **Saikawa Y, Otani Y, Kitagawa Y, et al.** Interim results of sentinel node biopsy during laparoscopic gastrectomy: possible role in function-preserving surgery for early cancer. *World J Surg* 2006;30:1962-1968.
7. **Kelder W, Nimura H, Takahashi N, et al.** Sentinel node mapping with indocyanine green (ICG) and infrared ray detection in early gastric cancer: an accurate method that enables a limited lymphadenectomy. *Eur J Surg Oncol* 2010;36:552-558.
8. **Tajima Y, Murakami M, Yamazaki K, et al.** Sentinel node mapping guided by indocyanine green fluorescence imaging during laparoscopic surgery in gastric cancer. *Ann Surg Oncol* 2010;17:1787-1793.
9. **Tajima Y, Yamazaki K, Masuda Y, et al.** Sentinel node mapping guided by indocyanine green fluorescence imaging in gastric cancer. *Ann Surg* 2009;249:58-62.
10. **Georgescu DE, Georgescu MT, Bobircă FT, et al.** Synchronous Locally Advanced Rectal Cancer with Clinical Complete Remission and Important Downstaging after Neoadjuvant Radiochemotherapy – Personalised Therapeutic Approach. *Chirurgia* (Bucur) 2017;112:726-733. doi: 10.21614/chirurgia.112.6.726.
11. **Georgescu MT, Patrascu T, Serbanescu LG, et al.** When Should We Expect Curative Results of Neoadjuvant

References

Treatment in Locally Advanced Rectal Cancer Patients?
Chirurgia (Bucur) 2021;116:16-23. doi: 10.21614/chirurgia.116.1.16.

12. **Giuliano AE, Kirgan DM, Guenther JM, et al.** Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391-398; discussion 398-401.
13. **Nagata K, Endo S, Hidaka E, et al.** Laparoscopic sentinel node mapping for colorectal cancer using infrared ray laparoscopy. *Anticancer Res* 2006;26:2307-2311.
14. **Georgescu DE, Patrascu T, Georgescu TF, et al.** Diabetes Mellitus as a Prognostic Factor for Locally Advanced Rectal Cancer. *In Vivo* 2021;35:2495-2501.
15. **Tovar JSD, Kassab G, Inada NM, Et al.** Photodegradation in the infrared region of indocyanine green in aqueous solution [Internet]. Proceedings. 2019. Available from: https://dx.doi.org/10.1109/SBFoton-IOPC.2019.8910207
16. **Ackroyd R, Kelty C, Brown N, et al.** The history of photodetection and photodynamic therapy. *Photochem Photobiol* 2001;74:656-669.
17. **Detty MR, Gibson SL, Wagner SJ.** Current clinical and preclinical photosensitizers for use in photodynamic therapy. *J Med Chem* 2004;47:3897-3915.
18. **Abels C, Fickweiler S, Weiderer P, et al.** Indocyanine green (ICG) and laser irradiation induce photooxidation. *Arch Dermatol Res* 2000;292:404-411.
19. **Wu PC, Guo LZ, Yu S, et al.** Noninvasive assessment of liver function reserve with fluorescent dosimetry of indocyanine green. *Biomed Opt Express* 2022;13:1995-2005.
20. **Van Manen L, Handgraaf HJM, Diana M, et al.** A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *J Surg Oncol* 2018;118:283-300.
21. **Tong GJ, Zhang GY, Liu J, et al.** Comparison of the eighth version of the American Joint Committee on Cancer manual to the seventh version for colorectal cancer: A retrospective review of our data. *World J Clin Oncol* 2018;9:148-161.
22. **Xu Y, Huang ZH, Zheng CZ, et al.** The impact of COVID-19 pandemic on colorectal cancer patients: a single-center retrospective study. *BMC Gastroenterol* 2021;21:185.
23. **Zelken JA, Tufaro AP.** Current Trends and Emerging Future of Indocyanine Green Usage in Surgery and Oncology: An Update. *Ann Surg Oncol* 2015;22 Suppl 3:S1271-S1283.
24. **Schols RM, Connell NJ, Stassen LP.** Near-infrared fluorescence imaging for real-time intraoperative anatomical guidance in minimally invasive surgery: a systematic review of the literature. *World J Surg* 2015;39:1069-1079.
25. **Chung BL, Toth MJ, Kamaly N, et al.** Nanomedicines for Endothelial Disorders. *Nano Today* 2015;10:759-776.
26. **Emile SH, Khan SM, Wexner SD.** Impact of change in the surgical plan based on indocyanine green fluorescence angiography on the rates of colorectal anastomotic leak: a systematic review and meta-analysis. *Surg Endosc* 2022;36:2245-2257.
27. **Rozenholc A, Samouelian V, Warkus T, et al.** Green versus blue: Randomized controlled trial comparing indocyanine green with methylene blue for sentinel lymph node detection in endometrial cancer. *Gynecol Oncol* 2019;153:500-504.
28. **Yuan L, Qi X, Zhang Y, et al.** Comparison of sentinel lymph node detection performances using blue dye in conjunction with indocyanine green or radioisotope in breast cancer patients: a prospective single-center randomized study. *Cancer Biol Med* 2018;15:452-460.
29. **Jewell EL, Huang JJ, Abu-Rustum NR, et al.** Detection of sentinel lymph nodes in minimally invasive surgery using indocyanine green and near-infrared fluorescence imaging for uterine and cervical malignancies. *Gynecol Oncol* 2014;133:274-277.
30. **Alander JT, Kaartinen I, Laakso A, et al.** A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging* 2012;2012:940585.
31. **Eklöv K, Nygren J, Bringman S, et al.** Trends in Treatment of Colorectal Cancer and Short-term Outcomes During the First Wave of the COVID-19 Pandemic in Sweden. *JAMA Netw Open* 2022;5:e2211065.
32. **Yang Y, Sun H, Luo X, et al.** Network connectivity between fear of cancer recurrence, anxiety, and depression in breast cancer patients. *J Affect Disord* 2022;309:358-367.
33. **Kørner H, Guren MG, Larsen IK, et al.** Characteristics and fate of patients with rectal cancer not entering a curative-intent treatment pathway: A complete nationwide registry cohort of 3,304 patients. *Eur J Surg Oncol* 2022:S0748-7983(22)00407-3.
34. **Ribero D, Mento F, Sega V, et al.** ICG-Guided Lymphadenectomy during Surgery for Colon and Rectal Cancer-Interim Analysis of the GREENLIGHT Trial. *Biomedicines* 2022;10:541.
35. **Stefani C, Miricescu D, Stanescu-Spinu II, et al.** Growth Factors, PI3K/AKT/mTOR and MAPK Signaling Pathways in Colorectal Cancer Pathogenesis: Where Are We Now? *Int J Mol Sci* 2021;22:10260.