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*World Journal of Clinical Oncology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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REVIEW

# Relationship and interactions of curcumin with radiation therapy

Vivek Verma

Vivek Verma, Department of Radiation Oncology, University of Nebraska Medical Center, Omaha, NE 68198, United States

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Correspondence to: Vivek Verma, MD, Department of Radiation Oncology, University of Nebraska Medical Center, 987521 Nebraska Medical Center, Omaha, NE 68198, United States. vivek333@gmail.com
Telephone: +1-402-5523844
Fax: +1-402-5523926

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## Abstract

Curcumin is widely reported to have remarkable medicinal - and antineoplastic - properties. This review details curcumin's relationship with radiotherapy (RT), principally as a radiosensitizer for various malignancies and a radioprotector for normal tissues. First, examples of radiosensitization are provided for various cancers: Pediatric, lymphoma, sarcoma, prostate, gynecologic, pancreas, liver, colorectal, breast, lung, head/neck, and glioma. It is not the purpose of this article to comprehensively review all radiosensitization data; however, high-quality studies are discussed in relationship to currently-controversial RT questions for many cancers, and thus the importance of developing a natural radiosensitizer. Attention is then shifted to radioprotection, for which supporting research is discussed for the following RT toxicities: Dermatitis, pneumonitis, cataractogenesis, neurocognition, myelosuppression, secondary malignancies, and mucositis/enteritis. Though there is fewer data for radioprotection, the overall quality of clinical evidence is higher, and small clinical trials implicating the efficacy of curcumin for RT toxicities (*vs* placebo/current therapies) are also detailed. Though the overall level of evidence for curcumin as a radiosensitizer and radioprotector is low, it must be recognized that risks of adverse effects are exceedingly low, and clinicians may need to judge the yet-unproven rewards with low toxicity risks.

**Key words:** Curcumin; Turmeric; Radiation therapy; Cancer; Radioprotection; Radiosensitization



**Core tip:** The Indian spice curcumin (turmeric) has been widely reported, largely in the preclinical realm, to offer many health - including antineoplastic - benefits. Though this article is not meant as a summative review of all studies of curcumin and radiotherapy, selected studies will be discussed that demonstrate curcumin to be a radiosensitizer of many types of tumor cells. Furthermore, data illustrating curcumin as a radioprotector of normal organs - including clinical studies - are also described. It is a sincere hope that these promising results can lead to curcumin use in cancer patients, either on or off a clinical protocol.

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## INTRODUCTION

The Indian spice curcumin (also known as diferuloylmethane), extracted from the turmeric plant, has long held a role in Indian/Hindu rituals, traditions, customs, and cuisines. More recently, scientific evidence is mounting that curcumin offers innumerable health benefits (reviewed in multiple sources[1-4]), all stemming from the fundamental property of decreasing inflammatory mediators[5]. This leads hope to curb the unchecked progression of fundamentally inflammatory diseases[6], many of which are considered the scourge of medicine in the present day and age. Moreover, curcumin is a completely natural compound with essentially no side effects; tolerance in phase I clinical trials have shown no medically adverse effects for doses up to 8-12 g orally per day[7].

Cancer is a common conglomeration of diseases that can be termed as a "bane of healthcare" throughout the world, and affects hundreds of millions of persons throughout the world per year. Extensive work has been performed on curcumin's immense anti-cancer potential, which have been grossly underappreciated, largely owing to the notable roadblock of few clinical studies to date[8-13]. The phase I-II clinical trials that have been performed, however, have done nothing to dissuade further clinical study of this compound[14-16].

Primary management of cancer centers on various combinations of surgery, chemotherapy, and radiation therapy (RT). A comprehensive discussion of curcumin's effects on chemotherapy and surgical intervention is extremely broad and clearly beyond the scope of this article; rather, curcumin's interactions with RT will be evaluated. Additionally, though it is not the goal of this article to comprehensively and systematically detail all data of the curcumin-RT relationship[17,18], selected examples of curcumin's (1) radiosensitization ability and (2) radioprotective ability, will be enumerated in order to characterize the sheer breadth of curcumin's actions along with RT on cancer. The goal of this article, in turn, is to encourage clinicians to (1) commence clinical trials related to curcumin; and/or more importantly; (2) encourage their patients to routinely take curcumin for cancer therapy (despite a general dearth of solid data).

## RADIOSENSITIZATION BY CURCUMIN

There is a well-charted history of radiosensitizers, defined as molecular compounds that act to functionally amplify radiation-induced DNA and cellular damage, regardless of whether the compounds cause damage individually[19]. Though several radiosensitizers are used in cancer care today, such as platinum-based chemotherapeutic agents, the focus of this section is to describe many examples of curcumin as a radiosensitizer. The reader is first cautioned that nearly all evidence of radiosensitization comes from laboratory data, and clinically-apparent benefits of curcumin as a radiosensitizer are yet to be determined.

First, attention will be paid to pediatric, lymphoma, and musculoskeletal cancers. Why are these important? Clinically speaking, the fields of pediatric and lymphoma RT have undergone - and are undergoing - dramatic decreases in RT doses, so as to minimize secondary malignancy risk and ancillary procedures in the younger population[20-22]. The presence of a radiosensitizing agent, if proven clinically efficacious, would certainly aid the movement to de-escalate RT doses in this population. Sarcomas (many of which occur in children) are a logical extension for curcumin therapy, given its success in musculoskeletal inflammatory-based disorders[23]. As previously mentioned, inhibition of the transcription factor NF-κB is a primary mode of action of curcumin, which act to mediate various anti-inflammatory effects for various diseases[24]. However, what is often an overlooked fact between inflammatory diseases and cancer is that NF-κB has been widely implicated in both tumorigenesis and radioresistance[24]. Hence, results of pre-RT curcumin intake leading to radiosensitization in murine rhabdomyosarcoma models are not surprising in light of suppressing NF-κB[25]. These results have been echoed in neuroblastoma cells in a high-quality study by Aravindan *et al*[26]. However, the diverse pathways of curcumin's actions are not limited to this transcription factor; the same group studied mutant p53 Ewing's sarcoma cells, and radiosensitivity was found to be associated with other p53-response genes (despite the p53-mutated nature of the studied cells)[27]. There are also data to support the NF-κB suppression theory as means for radiosensitization in lymphomas, which are important in light of resistance to biologic therapies for some types of lymphomas[28]. Though RT is not the centerpiece of therapy for Burkitt's lymphoma, there are data supporting radiosensitization in this otherwise aggressive lymphoma[29]. The same group did demonstrate another interesting mechanism of radiosensitization in non-Hodgkin's lymphoma (which constitute large proportions of lymphomas treated with RT)[30]. The authors found that cell cycle arrest in the G2-M checkpoint was associated with curcumin administration, which is a normal effect of irradiating tumor cells and is hence presumably augmented by curcumin.

Shifting to genitourinary cancers, dose-escalation for prostate cancer (the most common genitourinary malignancy) is strongly proven to associate with improved outcomes[31], and hence great emphasis is placed on using high-fidelity imaging technology to guide RT planning/delivery[32,33]. Radiosensitization for these tumors could thus allow for "functional dose escalation", providing even greater tumor doses while

keeping a constant prescribed RT dose. Two convincing preclinical studies demonstrated the radiosensitizing effects of curcumin on the human prostate cancer cell line PC3. Chendil *et al*[34] postulated the mechanism to be related to NF-κB and found threefold fewer surviving PC3 cells when treated with both RT and curcumin. However, another report found another novel pathway of action, downregulation of the MDM2 oncogene (a p53-independent pathway), which provide encouragement that spontaneous mutagenesis in cancer cells could be less likely to cause multi-drug resistance affecting curcumin[35].

Next, data is not limited to the male genitourinary system, with one report demonstrating increased reactive oxygen species formation in cervical cancer cells with the addition of curcumin[36]. Similar to the aforementioned report on cell cycle arrest[30], this is a normal effect of RT that curcumin seems to augment. Lastly, though RT is not routinely utilized for ovarian neoplasms, a group at the University of South Dakota conjugated curcumin nanoparticles to an ovarian cancer-specific antibody and elicited both chemo- and radiosensitization phenomena[37]. Though the issue of curcumin delivery is beyond the scope of this review, it will briefly be addressed in the final section, and this study's use of nanoparticles is hence quite noteworthy.

Though gastrointestinal tumors are inherently very heterogeneous and diverse, brief examples for several tumor types are united by the overarching theme of NF-κB suppression by curcumin, despite any rises that could occur after a RT fraction. Again, this transcription factor is widely purported to relate to radioresistance, and the studies discussed hereafter in this paragraph will demonstrate sustained cellular killing, potentially as a result of decreased radioresistance. First, though pancreatic cancer is one of the deadliest known neoplasms, data have shown enhanced cell killing with five-fraction RT[38] especially as delivered by Veeraraghavan *et al*[39]. However, it is important to be skeptical of results insofar as questioning whether curcumin administration could be a panacea for a disease with dismal prognosis from aggressive tumor biology and high metastatic proclivity. The same criticism is true for similar results recently published on hepatocellular carcinoma, which also demonstrated NF-κB downregulation as a putative mechanism[40]. Lastly, curcumin may be a relatively good candidate to clinically sensitize colorectal cancer (the most common gastrointestinal malignancy) to RT. Two high-impact publications from M.D. Anderson Cancer Center also implicated NF-κB modulation - although its expression rises after RT - as an effector of curcumin[41,42]. There were several additional effects of note as measured by the authors. First, not only were proliferation markers downregulated, angiogenesis was decreased as well. Though this effect could result in decreased nutrients feeding the tumor (thus augmenting cell killing), potential decreases in tumor oxygenation could be problematic, as this is strongly related to tumor radioresistance. Importantly, this study also demonstrated decrease in matrix metalloproteinase (MMP) expression. This enzyme is thought to be a gateway for metastasis, by dissolving bonds to extracellular matrix (an "anchor" preventing dissemination) as well as promoting an overall microenvironment for growth and spread[43]. Hence, after RT the upregulation (presumably nonsustained) in NF-κB and MMP lead to some degree of increased risk for radioresistance (persistent growth) and spread[44]. Curcumin may hence act to decrease this risk, and it would certainly be helpful to examine tumor growth and metastasis from a clinical perspective to examine whether decreased NF-κB and MMP expression translate into "clinical gains".

Moving to neoplasms of the thorax, breast cancer is the most common noncutaneous cancer in the United States; RT is a major part of management, including several different techniques and RT modalities[45-49]. One example of breast cancer radiosensitization with curcumin was shown by Calaf *et al*[50]. The most important observation of this study was increased amounts of cleaved (inactive) PARP-1, a protein known to repair DNA after RT damage and thus attenuate RT damage[51]. There is an enormous amount of current research being done on PARP inhibitors, including multiple phase Ⅱ and Ⅲ clinical trials. If further results can corroborate the association between cleavage/inactivation of PARP by curcumin, these could have substantial implications on this burgeoning field.

Lung cancer, most commonly non-small cell lung cancer (NSCLC), is another common and deadly tumor[52,53] for which screening has recently been instituted[54-56]. A radiosensitizer would therefore be a welcome addition to recently-developed and cutting-edge RT technologies used for treatment of some NSCLCs[57]. Two important studies in NSCLC will be highlighted. A group from the University of Pennsylvania claimed a survival improvement with dietary curcumin administration along with RT, although there are several methodological flaws precluding reliability of these data[58]. More importantly, however, that dietary curcumin was able to cause clinical effect in the murine model is encouraging, because bioavailability remains a challenge of curcumin (further discussed in a subsequent section). In light of this fact, another research group utilizing liposomal curcumin was able to demonstrate potentiated NSCLC cell apoptosis with the presence of curcumin, and additionally found greater evidence of post-RT microvascular change, which (though uncorroborated) could be a surrogate marker for greater tumoral RT damage[59].

Regarding the diverse head and neck cancers, more common in Southern and Eastern Asia than the United States, treatment centers are on RT for the vast majority. Furthermore, cisplatin (administered concurrently with RT in select patients) has proven to be a radiosensitizer, increasing local tumor control in large randomized trials[60,61]. However, cisplatin's amplification of adverse RT toxicities beckons whether

the lack thereof with curcumin could prove to be a helpful utility[62]. Since an initial publication describing curcumin's ability to radiosensitize head and neck tumor cells *in vitro* and *in vivo*[63], another demonstrated the mechanism to be NF-κB - consistent with mechanistic relationships of curcumin on multiple aforementioned neoplasms[64]. Next, it is also worth mentioning another study of curcumin in nasopharyngeal carcinoma, in which greater amounts of cleaved PARP were discovered[65]. This is consistent with results for malignant breast carcinoma cells in a previously discussed study[50]. However, the most thought-provoking results were published by Tuttle *et al*[66] who illustrated that curcumin offers radiosensitization to head and neck malignancies that were human papillomavirus (HPV)-negative but not $HPV^+$. Ever since it was published in 2010 that $HPV^+$ oropharyngeal cancers had substantially better prognoses[67], a major focus of upcoming trials has been to determine whether de-escalation of therapy is feasible for $HPV^+$ tumors[68]. Though it is counterintuitive that curcumin did not radiosensitize $HPV^+$ tumors - they are vastly more sensitive to RT - it is in fact important that the HPV-negative neoplasms (worse prognosis) could be favorably addressed by curcumin and RT, if proven clinically efficacious.

Lastly, application of curcumin radiosensitization in gliomas will be briefly touched upon. Although a report posited G2-M cell cycle arrest as a mechanism[69], other data has displayed synergism of curcumin with an anti-glioblastoma antibody, including sustained NF-κB suppression[70]. This is noteworthy because biologics are at the forefront of oncologic therapy, and are already approved for relapsed glioblastoma[71]. Though it may be unlikely that simple administration of curcumin could curb the aggressive spread of glioblastoma, it rather provides hope that a clinical difference could be gleaned with curcumin for less aggressive neoplasms.

In summary, there is a great breadth of corroboratory data for many different tumor types available that demonstrate the radiosensitizing potential of curcumin. It is likely that other untested tumor types could likely show similar radiosensitization in laboratory models[72-77]. Though there has been no documentation to date in patients, encouragement does exist that there could be small observed differences in outcomes (with appropriate sample sizes), and even if there are no changes in survival parameters, recurrence rates and local control (a prime marker of radiosensitization) could be affected if eventually tested in the clinic.

## RADIOPROTECTION BY CURCUMIN

Though radiosensitization is important to enhance tumor death, equally important is toxicity minimization of normal tissues, the pursuit of which is one of the most prime goals of radiation oncology. Though the evidence for curcumin's radioprotection is less diverse/broad as compared to radiosensitization, the overall quality and applicability of data to human patients is noticeably greater. In this section, focus will be on curcumin's benefits against the following common RT toxicities: Dermatitis, pneumonitis, cataractogenesis, neurocognition, myelosuppression, secondary tumors, and mucositis/enteritis. Many of these toxicities are inflammatory in nature, so it intuitively follows that curcumin's potent anti-inflammatory effects[1,3,4,6] could lessen these inflammatory toxicities, likely through decreased inflammatory molecule production[5] as well as increasing the balance of antioxidants to oxidants[78].

RT dermatitis is one of the most common adverse effects of RT regardless of anatomic area, and two high-quality studies are as follows. Okunieff *et al*[79] documented reduction in both acute and chronic RT dermatitis in mice. This correlated with decreased levels of proinflammatory cytokines as well as subsequently-released fibrogenic cytokines such as TGF-β. Though a criticism of the study is the utilization of a single 50 Gray RT dose (extremely rare in humans), the radioprotection was consistent with another study that showed improved irradiated wound healing with curcumin[80]. The second major piece of evidence is a randomized and double-blinded trial of oral (2.0 g thrice daily) curcumin tablets ($n$ = 14) *vs* placebo ($n$ = 16) in breast cancer RT[81]. Patients were equal in terms of demographics, receipt of chemotherapy, surgery type, stage, RT dose, and baseline skin and pain assessment. A RT dermatitis standardized scale was the primary endpoint and favored curcumin ($P$ = 0.008) along with decreased moist desquamation in the curcumin group ($P$ = 0.002). There were largely no differences in patient-reported pain scores. This trial provides the highest level of evidence offering real hope that curcumin can have clinically significant impact on radiotoxicity, and it should secondarily not be discounted that the study was able to obtain statistically significant differences between groups despite randomizing only thirty patients.

Radiation pneumonitis has been extensively studied and well-validated to several RT dose-volume parameters; hence, it is a major focus of RT treatment planning especially because severe RT pneumonitis can be fatal[82-84]. Two studies demonstrating radioprotection against RT pneumonitis and its delayed sequela - pulmonary fibrosis - have already been discussed in the radiosensitization section[60,61] and corroborated by another report[85]. All three studies have shown, mechanistically, that curcumin's action is due to decreasing oxidative stress, proinflammatory cytokines, NF-κB expression, and fibrogenic cytokines - all of which tend to occur both simultaneously and sequentially. Undoubtedly, the presence of a lung radioprotector, if clinically proven, would be of great use to the ubiquitous NSCLC patients, many of which have risk factors for RT pneumonitis such as baseline lung disease and receipt of concurrent carboplatin-paclitaxel[86].

Two small studies examining central nervous system adverse effects of RT will now be addressed. Ozgen *et al*[87] examined cataractogenesis, a late toxicity that was hastened in the study by giving high single-

fraction doses to the lens (a relatively uncommon clinical scenario). Irradiation with curcumin lowered the cataract rate from 100% to 40%, correlating with lower levels of oxidative stress. Next, substantial ongoing research (and clinical trials) in radiation oncology relates to whether patients with primary or secondary brain tumors that undergo brain irradiation could be spared of its resulting memory/cognitive decline[88,89]. Curcumin is widely thought to be neuroprotective; its high consumption is associated with minimal rates of several neurodegenerative diseases in India, which is backed by convincing experimental evidence of such[90]. Pre-RT administration of curcumin was able to improve results in post-RT spatial/memory functional tests (Morris water maze) in mice administered carbon ion RT (high biologically effective dose owing to the heavy particle size)[91]. Furthermore, histologically-apparent neuropathological changes were also present between both groups. Hence, if other research can confirm these results, it will not be difficult to design clinical trials examining learning/memory tests in patients undergoing whole-brain RT with or without curcumin.

Curcumin can also protect lymphocytes, the most RT-susceptible blood cell, especially when radiating bony lesions (marrow) in patients[92]. The authors postulated that curcumin's actions could consist of radiosensitization or radioprotection, with the latter observed in non-cycling cells (in G0 phase) and the former in cycling cells (G2 transitioning to M phase), which is a theory that could sum up all the radioprotective and radiosensitizing data in this entire review.

Japanese researchers published an impactful article in 2002 demonstrating that rats undergoing whole body irradiation (dose of 9.6 Gray) - simulating a natural disaster such as Chernobyl - produced lower levels of an oxidant metabolite if fed curcumin pre- and post-RT, as compared to control rats[93]. Furthermore, post-exposure implantation of the carcinogen diethylstilbestrol led to significantly more secondary tumors in rats not having been administered curcumin. The results lead to query as to whether curcumin could directly prevent further mutations, but data for this is scant at best. Nevertheless, there is more evidence to support that curcumin lowers circulating reactive oxygen species (oxidative stress), which are normally known to cause DNA mutations (*i.e.,* how ionizing radiation causes DNA damage in cancer cells).

Lastly, owing to the relatively high proliferative index of mucosal cells, any mucosal surface is particularly sensitive and susceptible to acute and chronic RT-induced damage[94-96]. A Turkish study demonstrated that intestinal mucosa was protected to a greater degree in rats fed curcumin, as detected histopathologically[97]. These results, especially if validated, are important for three major reasons: (1) bowel toxicity is relatively common and may occur at any point of the RT course (even well-below the bowel tolerance dose); (2) parts of the bowel receive RT dose for several common (*e.g.,* prostate, gastrointestinal, gynecologic, and some palliative/pediatric) cancers; and (3) because curcumin is poorly absorbed in the gastrointestinal tract, it remains in direct contact with intestinal mucosa and hence could directly act on mucosal cells.

Next, another large area of morbidity in irradiated patients is mucositis of the soft tissues of the head and neck, some of which can be so severe that it necessitates feeding tube placement due to lack of oral feeding[98]. In 2004, a publication demonstrated a clinically evident decrease in oral mucosal ulceration in rats fed curcumin[99]. However, this issue remained untranslated into the clinical realm until Indian researchers published a study in 2013[100]. In this single-blinded and randomized trial, patients with mostly oral cavity/pharyngeal neoplasms undergoing RT (with or without surgery and chemotherapy) were given oral rinses of turmeric ($n$ = 40) or povidone-iodine ($n$ = 39) to take six and two times per day respectively. Tumor characteristics and treatment interventions (including RT dose and chemotherapy receipt) were balanced between groups. The group receiving curcumin was less likely to receive treatment breaks in the initial (< 4 wk) period ($P$ < 0.01) and displayed decreased weight loss ($P$ < 0.001). Though incidence of overall mucositis did not differ between groups, the curcumin group experienced lesser intolerable mucositis ($P$ < 0.0001) as well as decreased severity of overall mucositis as per Radiation Therapy Oncology Group criteria ($P$ < 0.003). Though povidone-iodine is uncommonly used for RT mucositis, similar agents (*e.g.,* lidocaine, chlorhexidine) used more often are likely no different because they are designed to treat symptoms rather than causative molecular inflammatory agents as curcumin does.

Another recent clinical protocol by Patil *et al*[101] will now be expounded upon. Twenty patients, mostly with oral cavity/pharyngeal cancer that received concurrent chemoradiation, received either chlorhexidine ($n$ = 10) or 0.004% curcumin oral rinse ($n$ = 10) thrice daily during RT. Patient and tumor characteristics, including chemotherapy receipt and RT dose, were underreported but equivalent between groups. Outcomes included a prespecified numerical pain score, oral mucositis assessment scales for erythema and ulceration, and the World Health Organization (WHO) mucositis scale. Most of these parameters were favorable for the curcumin group ($P$ < 0.001 for pain, $P$ = 0.05 for erythema, $P$ < 0.001 for ulceration, and $P$ = 0.003 for WHO mucositis). Though the methodology of this trial was less sound as compared to the aforementioned dermatitis trial[81], it should again be mentioned that a statistically significant difference was found despite the comparison of only ten patients in each group.

Taken together, there are greater clinical data available to support the use of curcumin as a radioprotector of normal tissues, especially epithelial tissues, potentially owing to direct contact with at-risk surfaces. Curcumin's mechanisms seem associated with decreased

oxidative stress in normal tissues.

## CONCLUSION

A substantial volume of evidence exists that curcumin is a radiosensitizer of multiple cancers as well as a radioprotector of several normal tissues. However, the overall quality of evidence is low; there is no clinical evidence of radiosensitization and a few low-volume clinical trials of radioprotection published thus far. However, there is certainly something to be said about the sheer volume of corroborative positive data, particularly in radioprotection.

What do the aforementioned litany of laboratory and clinical studies mean for the clinician? On one hand, clinical evidence is weak, and there is no guarantee curcumin would provide a clinical difference in outcomes (*e.g.,* survival, local/regional recurrence). On the other hand, as previously discussed, curcumin administration has exceedingly low chances to produce adverse effects; empiric administration without solid clinical evidence will likely not harm the patient whatsoever.

Further research is greatly needed to strengthen curcumin's major weakness - poor gastrointestinal absorption leading to low oral bioavailability. After absorption in the gastrointestinal tract *via* a liposomal mechanism, four double bonds are reduced, followed by glucuronidation/sulfation and excretion through bile[102-104]. Several discussed studies[37,59], as well as undiscussed studies in other diseases[23], have used special formulations (*e.g.,* liposomal, intravenous, molecular analogs, and conjugated forms such as Meriva® and Theracurmin®) which could become more mainstream in the future with more research.

A subsequent question, then, is whether to administer curcumin therapeutically as in these studies, or preventatively - long prior to any therapy - or even prophylactically prior to any disease onset. These questions should likely be addressed after basic clinical efficacy/utility issues, so as to provide more solid footing on curcumin use. Although not the purpose of this article to provide recommendations of curcumin administration to clinicians, it is certainly encouraged to consider that in light of immature but still broadly corroborative data (including clinical studies), curcumin is extremely safe and not harmful to the cancer patient undergoing radio(chemo)therapy.

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MINIREVIEWS

# Neoadjuvant therapy for gastroesophageal adenocarcinoma

Emmanuelle Samalin, Marc Ychou

Emmanuelle Samalin, Marc Ychou, Digestive Oncology Department, Institut régional du Cancer de Montpellier (ICM), 34298 Montpellier, France

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Correspondence to: Emmanuelle Samalin, MD, Digestive Oncology Department, Institut régional du Cancer de Montpellier (ICM), 208 avenue des Apothicaires, 34298 Montpellier, France. emmanuelle.samalin@icm.unicancer.fr
Telephone: +33-4-67613136
Fax: +33-4-67613022

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## Abstract

Gastric and esophageal adenocarcinomas are one of the main causes of cancer-related death worldwide. While the incidence of gastric adenocarcinoma is decreasing, the incidence of gastroesophageal junction adenocarcinoma is rising rapidly in Western countries. Considering that surgical resection is currently the major curative treatment, and that the 5-year survival rate highly depends on the pTNM stage at diagnosis, gastroesophageal adenocarcinoma management is very challenging for oncologists. Several treatment strategies are being evaluated, and among them systemic chemotherapy, to decrease recurrences and improve overall survival. The MAGIC and FNCLCC-FFCD trials showed a survival benefit of perioperative chemotherapy in patients with operable gastric and lower esophageal cancer, and these results had an impact on the European clinical practice. New strategies, including induction chemotherapy followed by preoperative chemoradiotherapy, targeted therapies in combination with perioperative chemotherapy and the new cytotoxic regimens, are currently assessed to improve current standards and help developing patient-tailored therapeutic interventions.

**Key words:** Gastric adenocarcinoma; Lower esophagus adenocarcinoma; Gastroesophageal junction adenocarcinoma; Preoperative treatment; Neoadjuvant treatment



**Core tip:** Gastric and esophageal adenocarcinomas are one of the main causes of cancer-related death worldwide. The incidence of gastroesophageal junction adenocarcinoma is rapidly rising in Western countries. Surgical resection is currently the major curative treatment. As the 5-year survival rate highly depends on the pTNM stage, the treatment strategy is very challenging for oncologists. Several treatments, including systemic chemotherapy, are being assessed to prevent recurrences and improve overall survival. New strategies, such as induction chemotherapy followed by preoperative chemoradiotherapy, targeted therapies and new cytotoxic regimens in perioperative chemotherapy, are currently assessed to improve current standards and develop more tailored therapeutic interventions.

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## INTRODUCTION

Survival of patients with esophageal, gastric or gastro-esophageal junction adenocarcinoma is poor because they are frequently locally-advanced or with distant metastases at diagnosis. Even if the incidence of gastric adenocarcinoma is decreasing, it stays the second most frequent cause of cancer-related death worldwide. In 2012, 952000 new cases of gastric cancer were diagnosed with 723000 estimated deaths worldwide[1]. In Western countries, a faster increase of the incidence of gastroesophageal junction adenocarcinoma compared to that of other gastrointestinal adenocarcinomas has been reported over the last 25 years[2,3]. Surgical resection of the primary tumor is the major curative treatment for these upper gastrointestinal cancers. Esophageal cancers can be treated with exclusive radiochemotherapy[4], but this review will focus on (neo)adjuvant therapies. The 5-year survival rate is correlated with the pTNM stage, lymph node metastases being the major poor prognostic factor. A 5-year survival of 20% to 30% is reported in localized tumors, which extend beyond the submucosa[5-7]. Also, gastroesophageal adenocarcinomas (GEA) are often detected at an already advanced stage, in Western countries, about 30% of resectable patients were not identified at an early stage. For this group, the rate of recurrence following resection of a gastric cancer is high. Currently, various strategies (including nutritional management) are developed and tested to reduce this risk and improve patient's survival[8].

## ADJUVANT THERAPIES FOR GASTROESOPHAGEAL ADENOCARCINOMA

### *Adjuvant chemotherapy*

Like in colorectal cancer, adjuvant systemic therapy in patients with GEA is used to treat post-resection occult residual micro-metastatic disease and to increase survival. Many clinical studies have investigated the possible positive impact of adjuvant therapy on the patients' outcomes. Between 1994 and 2002, meta-analyses of the data from these trials suggested that adjuvant chemotherapy slightly increases overall survival, with a reduction of the risk of death between 12% and 18%[9-12]. However, no recommendation for the treatment of resectable gastric cancer could be proposed because of the heterogeneity of the methodology used in these meta-analyses including studies with a small number of patients, and because old chemotherapy regimens were often followed in these trials. Several clinical studies have focused on chemotherapy drugs that are currently used in the clinical practice. For instance, in France, the randomized phase Ⅲ trial of the French Federation of Digestive Oncology [Fédération Française de Cancérologie Digestive (FFCD)] on adjuvant chemotherapy combined with 5-fluorouracil (5-FU) and cisplatin (CDDP) after curative resection of gastric cancer showed no benefit on survival compared with surgery alone[13]. In Japan, a randomized phase Ⅲ trial on adjuvant chemotherapy with the S-1 fluoropyrimidin derivative in patients with stage Ⅱ-Ⅲ GEA found that the 5-year overall survival (OS) rate was 71.7% in the S-1 arm compared with 61.1% in the surgery alone arm (HR = 0.68, 95%CI: 0.52-0.87, $P$ = 0.003)[14]. More recently, the CLASSIC study reported a higher 5-year OS in patients with stage Ⅱ or Ⅲ gastric cancer who received adjuvant chemotherapy treatment with capecitabine plus oxaliplatin compared to patients who underwent only D2 gastrectomy (5-year OS rate: 78% *vs* 69%; HR = 0.58, 95%CI: 0.47-0.72, $P$ < 0.0001)[15].

Finally, a meta-analysis by the Global Advanced/Adjuvant Stomach Tumor RESEARCH International Collaboration (GASTRIC) Group that combined data from 3838 patients (17 trials) showed a benefit of 5-FU-based adjuvant chemotherapy *vs* surgery alone on OS (55.3% *vs* 49.6%, respectively; HR = 0.82, 95%CI: 0.76-0.90, $P$ < 0.001) with stable results at 10 years (48% *vs* 40%, respectively)[16]. For this reason, 5-FU-based adjuvant chemotherapy is now considered as a therapeutic option and has been included in the French National Thesaurus of Digestive Oncology (www.snfge.asso.fr) for patients with resected GEA.

### *Adjuvant chemoradiotherapy*

The efficacy of adjuvant chemoradiotherapy after a R0 resection in GEA patients (stage Ⅰb to Ⅳ M0) was studied with the SWOG 9008/INT 0116 phase Ⅲ trial in 603 patients[17,18]. They were randomized in two therapeutic arms: Surgery alone *vs* surgery combined with adjuvant chemoradiotherapy. The patients' characteristics, including the tumor stage, were similar in the two groups: 65% of patients had pT3/T4 stage tumors and 85% $N^+$ stage tumors. Treatment started with chemotherapy (1 cycle of the FUFOL Mayo Clinic regimen) followed with chemoradiotherapy after 1 mo. Radiotherapy was delivered in 25 fractions (45 Gy each) and FUFOL was administered during the first 4 d and the last 3 d of irradiation. Two additional FUFOL cycles were then given 1 mo after the end of chemoradiotherapy. In this study, 64% of patients completed the therapeutic protocol. The digestive and hematological toxicity rates were respectively of 33% and 54%. The administration of adjuvant chemoradiotherapy resulted in an improvement of the 5-year OS rate compared with surgery alone (40% *vs* 26%; HR = 1.31, 95%CI: 1.09-1.39, $P$ = 0.005) and of the median disease-free survival (27 mo *vs* 19 mo; HR

= 1.52, 95%CI: 1.25-1.53, $P < 0.0001$), with a median follow-up of more than 10 years. The risk of death was reduced by 31%, and relapses were decreased by 52%. However, D2 lymph node dissection was reported in 10% of patients (36% had a D1 resection, and 54% a < D1 resection). In conclusion, adjuvant chemoradiotherapy appears to be a reasonable option for the treatment of resectable GEA, for patients with inadequate lymph node dissection and/or at high risk of recurrence (pT3/T4 and/or $N^+$ cancer).

Recently, the ARTIST study reported a benefit of adjuvant chemoradiotherapy with capecitabine and cisplatin on disease-free survival of patients with node-positive and intestinal-type gastric adenocarcinoma compared with patients treated with adjuvant chemotherapy alone[19].

In all cases, the nutritional status of patients should systematically and safely be evaluated before initiating adjuvant therapy at 6 wk post-surgery[20].

## NEOADJUVANT SYSTEMIC THERAPIES IN GEA

The choice of administering systemic chemotherapy to the patients before surgery of resectable GEA is mainly based on the possibility of improvement of the R0 resections and primary tumor downstaging/downsizing. Systemic neoadjuvant therapy may also remove occult micro-metastatic disease and facilitate the preoperative chemo-sensitivity assessment. Possible disadvantages of neoadjuvant systemic therapies include the risk of disease progression until surgery, the increase in secondary morbidity and chemotherapy-related toxicity and the difficulty of assessing the preoperative treatment response.

In all clinical trials, patients with gastric, gastro-esophageal junction and lower esophagus adenocarcinomas were considered together as a single population. In this review, we will distinguish the different tumor sites, and, concerning esophageal cancer, we will focus on the results of neoadjuvant chemoradiotherapy.

### *Neoadjuvant chemotherapy*

The feasibility of neoadjuvant chemotherapy as treatment of resectable gastric cancers was initially shown in phase Ⅱ studies[21-23], which reported an acceptable toxicity and no increase of the surgical mortality and morbidity rates using this therapeutic approach. The R0 resection rates following neoadjuvant chemotherapy were of 61% and 77%, with or without intra-peritoneal chemotherapy, respectively.

A randomized clinical trial coordinated by the Dutch Gastric Cancer Group, compared two groups of patients who underwent surgery alone ($n = 30$) or received neoadjuvant chemotherapy after surgery ($n = 29$), *i.e.*, 4 cycles of the FAMTX regimen (5-FU, doxorubicin and methotrexate)[24,25]. The R0 resection rates were 62% *vs* 56% in the surgery alone arm and in the neoadjuvant chemotherapy plus surgery arm, respectively. Although the trial was stopped early due to the small number of patients included ($n = 59$), the authors concluded to the poor efficacy of the FAMTX regimen in the treatment of resectable gastric cancer.

A randomized phase Ⅲ trial compared in 503 patients with resectable stomach, lower esophagus or esophageal-gastric junction adenocarcinomas, surgery alone ($n = 253$) with perioperative chemotherapy ($n = 250$)[26]. This Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial comprised the ECF regimen (50 mg/m² epirubicin on day 1, 60 mg/m² CDDP on day 1 and continuous venous infusion of 5-FU, 200 mg/m² per day, for 3 wk). Three cycles were administered pre- and post-surgery, which took place within the 6 wk after randomization for patients in the surgery group, or 3 to 6 wk after the third chemotherapy cycle for the perioperative chemotherapy group. The surgical procedure, which was left to the surgeon's discretion, included D1 or D2 lymphadenectomy. The patients' characteristics were similar in both arms; 26% of patients had a lower esophageal or gastroesophageal junction tumor. In the chemotherapy arm, 86% of patients completed the neoadjuvant treatment, 55% initiated postoperative treatment and 42% completed the chemotherapy protocol. Concerning surgery, 88% and 95% patients underwent the intervention in the chemotherapy and surgery alone arms. The pathological analysis of the resected specimens showed significant tumor downsizing in the chemotherapy group compared to the surgery alone arm (mean tumor size: 3 cm *vs* 5 cm; $P < 0.001$). An improvement of T stage ($P = 0.009$) and N stage ($P = 0.01$) was also reported in the chemotherapy group. The 79% R0 resection rate was significantly higher in the chemotherapy arm than in the surgery alone group (70%; $P = 0.03$). Grade 3-4 hematological toxicities during pre- and postoperative chemotherapy were not significantly different in the two groups, with 24% and 28% of neutropenia, respectively. With a median follow-up of 3 years, the median OS was 24 mo *vs* 20 mo in the chemotherapy and surgery alone groups, respectively (HR = 0.75, 95%CI: 0.60-0.93, $P = 0.009$) and the 5-year survival rates were 36% and 23%, respectively. Progression-free survival was significantly longer in the chemotherapy than in the surgery alone group (HR = 0.66, 95%CI: 0.53-0.81, $P = 0.0001$) (Table 1). The therapy efficacy was independent of the tumor site. The ECF chemotherapy regimen was chosen based on the results of a phase Ⅲ trial that compared the ECF and FAMTX regimens in patients with advanced GEA. In this study, ECF was associated with better response rate (45% *vs* 21%; $P < 0.001$), better median OS (8.7 mo *vs* 6.1 mo) and acceptable hematological toxicity (grade 3-4 neutropenia: 36% *vs* 58%) compared to FAMTX[27]. Recently, a meta-analysis showed that addition of an anthracycline to the CDDP and 5-FU regimens increased the OS of patients with advanced disease, but this

**Table 1 Two neoadjuvant chemotherapy schedules offered for the treatment of resectable gastroesophageal adenocarcinomas**

| | MAGIC[24] | | | FNCLCC-FFCD[27] | | |
|---|---|---|---|---|---|---|
| | ECF *n* = 250 | Surgery *n* = 253 | *P* | FP *n* = 113 | Surgery *n* = 111 | *P* |
| Median age, yr (range) | 62 (29-85) | 62 (23-81) | | 63 (36-75) | 63 (38-75) | |
| Sex, male (%) | 82% | 76% | | 85% | 82% | |
| Performance status, 0/1 | 68/32 | 68/32 | | 74/26 | 75/25 | |
| Gastric ADK (%) | 74% | 74% | | 25% | 24% | |
| GOJ ADK (%) | 26% | 26% | | 75% | 76% | |
| Downstaging/downsizing (%) | | | | | | |
| T1/T2 | 52% | 38% | 0.09 | 42% | 32% | 0.16 |
| T3/T4 | 48% | 62% | | 58% | 68% | |
| N0/N1 | 84% | 76% | 0.01 | (N0) 33% | 20% | 0.54 |
| N2/N3 | 16% | 29% | | (N+) 67% | 80% | |
| R0 resection rate (%) | 79% | 70% | 0.03 | 84% | 73% | 0.04 |
| 5-yr overall survival rate (%) | 36% | 23% | 0.009 (HR = 0.75) | 38% | 24% | 0.002 (HR = 0.69) |

ADK: Adenocarcinomas; GOJ: Gastro-esophageal junction; ECF: Epirubicin, cisplatin and 5-FU; FP: 5-fluorouracil and cisplatin; MAGIC: Medical research council; FNCLCC-FFCD: Fédération nationale des centres de lutte contre le cancer - fédération française de cancérologie digestive.

advantage was not reported in the GASTRIC meta-analysis that combined data from 3226 patients[16,28].

A recently published French trial (FNCLCC 94012-FFCD 9703) evaluated another perioperative (two neoadjuvant cycles and four postoperative cycles) chemotherapy regimen (continuous protracted intravenous infusion of 5-FU 800 mg/m² per day from days 1 to 4 and 100 mg/m² CDDP on day 1 or 2 every 4 wk)[29]. It included 224 patients who were randomized between perioperative chemotherapy (*n* = 113) and surgery alone (*n* = 111) Patients underwent surgery 4 to 6 wk after neoadjuvant chemotherapy, and postoperative chemotherapy started 4 to 6 wk after surgery. The patients' characteristics were similar in the two groups. The originality in this study was the high percentage (75%) of patients with tumor in the cardia and in the lower esophagus. Preoperative staging was evaluated by endoscopic ultrasound examination and CT scan. In the perioperative chemotherapy arm, 96% underwent surgery compared with 99% in the surgery alone arm, and 87% of patients completed neoadjuvant chemotherapy. The pathological assessment of the resected specimens was similar in the two groups in terms of pT, whereas slightly less tumors were classified as N+ in the chemotherapy arm than in the surgery alone arm (67% *vs* 80%; not significant difference). The R0 resection rate was of 87% in the chemotherapy group, higher than the 74% rate in the surgery alone group (*P* = 0.004). The 5-year disease-free survival rates were 34% (95%CI: 26%-44%) *vs* 19% (95%CI: 13%-28%), and the 5-year OS rates were 38% (95%CI: 29%-47%) *vs* 24% (95%CI: 17%-33%) (HR = 0.69, 95%CI: 0.50-0.95, *P* = 0.02).

The results of the EORTC study showed an improvement of the R0 resection rate in patients treated with neoadjuvant chemotherapy (two courses of 50 mg/m² CDDP, Ⅳ on days 1, 15 and 29 followed by 500 mg/m² folinic acid, IV and 2000 mg/m² 5-FU by continuous infusion for 24 h on days 1, 8, 15, 22, 29 and 36; day 1 = day 48) compared to patients treated with surgery alone (81.9% *vs* 66.7%; *P* = 0.036). OS was comparable between the two arms, but this study lacked statistical power due to recruitment failure (expected patients per arm = 180; patients included in each arm = 72)[30].

The MAGIC and the FNCLCC 94012-FFCD 9703 studies reported similar benefits as those of the MRC OE02 trial that assessed the effect of CDDP (80 mg/m² administered by intravenous infusion for 4 h on day 1, day 1 = day 21) and 5-FU (1000 mg/m² by continuous protracted venous infusion from days 1 to 4, day 1 = day 21) in patients with resectable esophageal cancer[31]. In this study, 802 patients (66% had tumors located in the lower portion of the esophagus or in the cardia) were randomized in two arms, surgery alone (*n* = 402) *vs* preoperative chemotherapy (*n* = 400). The median OS was significantly higher in the chemotherapy group (17 mo *vs* 13 mo; 95%CI: 30-196 d) as well as the 5-year OS (23% *vs* 17%; HR = 0.84, 95%CI: 0.72-0.98, *P* = 0.03) according to the updated results[32].

At the 2015 ASCO annual meeting, Alderson *et al*[33] reported the results of the OEO5 trial that compared the ECX (4 cycles, *n* = 446) and 5-FU with CDDP (2 cycles, *n* = 451) regimens as neoadjuvant chemotherapy in patients with resectable adenocarcinoma of the esophagus or of gastroesophageal junction (Siewert type Ⅰ and Ⅱ). No significantly survival difference was observed between treatments even when an anthracycline was added to the CDDP and 5-FU regimen [3-year OS: 42% (95%CI: 37-46) in the 5-FU/CDDP arm *vs* 39% (95%CI: 35-44) in the ECX arm; HR = 0.92, 95%CI: 0.79-1.08, *P* = 0.8]. However, grade 3-4 toxicities were more frequent in patients treated with ECX (diarrhea, neutropenia, hand-foot syndrome and mucitis).

These two trials (MAGIC and FNCLCC 94012-FFCD 9703) were the first studies to demonstrate better survival rates with a perioperative systemic approach for the treatment of localized GEA (Table 1). These results

**Table 2 Neoadjuvant chemoradiotherapy schedule offered in the treatment of resectable gastroesophageal junction and lower esophagus adenocarcinomas**

| Trials | Patients (*n*) | Pathology | CT-RT | 5-yr OS rate (%) |
|---|---|---|---|---|
| Walsh 1996 | CT-RT = 58 | ADK (100%) | CDDP-5FU | CT-RT 32% |
| | S = 55 | | 40 Gy | S 6% (3-yr OS) ($P$ = 0.01) |
| Urba 2001 | CT-RT = 50 | ADK (75%) | CDDP-VLB-5FU | CT-RT 30% |
| | S = 50 | SCC | 45 Gy | S 16% (3-yr S) ($P$ = 0.15) |
| Burmeister 2005 | CT-RT = 128 | ADK (62%) | CDDP-5FU | CT-RT 22 mo |
| | S = 128 | SCC | 35 Gy | S 19 mo ($P$ = 0.38) |
| Tepper 2008 | CT-RT = 30 | ADK (75%) | CDDP-5FU | CT-RT 39% |
| | S = 26 | SCC | 50.4 Gy | S 16% (P < 0.008) |
| CROSS 2010 | CT-RT = 180 | ADK (75%) | Paclitaxel-carboplatin | CT-RT 47% |
| | S = 188 | SCC | 41.4 Gy | S 34% ($P$ = 0.03) |

CT-RT: Chemoradiotherapy; OS: Overall survival; S: Surgery; ADK: Adenocarcinoma; SCC: Squamous cell carcinoma; VLB: Vinblastin.

have been confirmed in the meta-analysis by Li *et al*[34], and neoadjuvant chemotherapy is now considered as standard treatment for GEA in Europe. Moreover, based on the first OEO5 trial results, neoadjuvant chemotherapy with 5-FU and CDDP seems to be the best option in this setting.

### *Neoadjuvant chemoradiotherapy*

Considering the improvement brought by systemic neoadjuvant chemotherapy for the management of patients with gastroesophageal junction and lower esophagus adenocarcinoma, several randomized phase Ⅲ study assessed the benefit of neoadjuvant chemoradiotherapy compared to surgery alone. The clinical trials by Urba *et al*[35] and Burmeister *et al*[36] did not show any benefit of chemoradiotherapy compared with surgery concerning the 3-year OS rate (30% *vs* 16%; $P$ = 0.16) and the median OS (21.7 mo *vs* 18.5 mo; $P$ = 0.38), in a population of patients among whom 75% had a gastroesophageal junction or lower esophagus adenocarcinoma (Table 2).

Only one phase Ⅲ trial found that chemoradiotherapy before surgery improved survival[37]. In this study, 113 patients with gastroesophageal junction and lower esophagus adenocarcinoma were randomized in two groups, PReoperative chemoradiotherapy plus surgery or surgery alone. Two cycles of 5-FU and CDDP were administered during radiotherapy, followed by surgery (multiple procedures) 8 wk after the beginning of the combined treatment. After a median follow-up of 10 mo, the median OS (16 mo *vs* 11 mo, $P$ = 0.01) and the 3-year OS rates (32% *vs* 6%, $P$ = 0.01) were significantly higher in the combined treatment arm than in the surgery alone arm, and the pathologic complete response rate was 25% in the chemoradiotherapy plus surgery arm. However, this monocentric study was closed prematurely after an intermediate analysis and with an unusually low survival rate in the surgery alone arm. Therefore, it is unclear how its results could be generalized.

The meta-analysis of 10 randomized trials (1209 patients) by Gebski *et al*[38] found an OS benefit of 13% at two years in patients treated with neoadjuvant chemoradiotherapy plus surgery *vs* surgery alone (HR = 0.81, 95%CI: 0.70-0.93, $P$ = 0.002 and HR = 0.75, 95%CI: 0.59-0.95, $P$ = 0.05) for the adenocarcinoma type in esophageal carcinoma. More recently, the CALGB Group reported a benefit of neoadjuvant chemoradiotherapy with a 5-FU-CDDP regimen in 56 patients (75% with a gastroesophageal or lower esophagus cancer)[39]. The 5-year OS rate was 39% in patients treated with neoadjuvant chemoradiotherapy compared with 16% ($P$ < 0.008) in patients treated with surgery alone (median follow-up of 6 years). The complete pathologic response rate was 40%. These results are controversial due to the recruitment failure relative to the number of expected patients ($n$ = 500).

Finally, the CROSS trial ($n$ = 368 with esophageal or gastroesophageal junction cancer) clearly reported an improvement of the R0 resection rate (92% *vs* 69%; $P$ < 0.0010) in patients treated with neoadjuvant chemoradiotherapy (paclitaxel-carboplatin regimen) plus surgery ($n$ = 178) compared with patients who had only surgery ($n$ = 188), with a complete pathologic response of 29%. The median OS was also significantly higher in the combined treatment arm than in the surgery arm (49 mo *vs* 24 mo; HR = 0.66, 95%CI: 0.49, 0.87, $P$ = 0.003)[40].

## COMBINATION OF NEOADJUVANT INDUCTION CHEMOTHERAPY AND CHEMORADIOTHERAPY

Ajani *et al*[41] proposed a three-step strategy combining induction chemotherapy (two cycles of 5-FU, levofolinate and cisplatin) with preoperative chemoradiotherapy (45 Gy of radiation concomitantly with 5-FU) and surgery. Their phase Ⅱ trial assessed response and survival in 33 patients with resectable gastric cancer. They showed a R0 resection rate of 70% and a 54% pathological response rate and a complete pathologic response of 30%. The median OS was 34 mo with a median follow-up of 5 years. The same authors assessed in another phase Ⅱ trial the same three-step strategy adding paclitaxel to 5-FU during chemoradiotherapy[42]. The trial

included 49 patients with resectable gastric carcinoma and showed a 77% R0 resection rate and a complete pathologic response rate of 26%. A median OS of 23 mo was reported, with a median follow-up of 22 mo.

A similar strategy (neoadjuvant chemoradiotherapy after two or three cycles of 5-FU-cisplatin induction chemotherapy followed by surgery) was also evaluated in patients with T3/T4 GEA who were randomized in the three-step protocol group or in the neoadjuvant chemotherapy alone plus surgery group. Although the trial was stopped early due to poor accrual, there was a trend towards a higher efficacy in the three-step protocol arm than in the neoadjuvant chemotherapy arm (3-year disease-free survival rate: 47.7% *vs* 27.7%; HR = 0.67; 95%CI: 0.41-1.07; $P = 0.07$)[43]. An Australian randomized phase Ⅱ study reported a significant reduction of the R1 resection rate in a similar population of patients treated with neoadjuvant chemoradiotherapy *vs* neoadjuvant chemotherapy alone (0% *vs* 11%, $P = 0.04$; pathological complete response rates of 31% *vs* 8%, respectively, $P = 0.01$)[44].

## PERSPECTIVES IN THE GEA MANAGEMENT: UNANSWERED QUESTIONS

We described different treatment modalities and therapeutic strategies in the management of resectable GEA. However, some questions remain unresolved.

First, there is no strong evidence of the benefit of preoperative chemoradiotherapy over perioperative chemotherapy in patients with gastroesophageal junction adenocarcinoma. As surgical findings showed a higher incomplete tumor resection in locally-advanced T3/T4 gastroesophageal junction adenocarcinomas, the evaluation of the effects of preoperative chemoradiotherapy is urgently needed in this setting[45]. One ongoing phase Ⅲ clinical trial is focusing on this question. The all-Ireland Cooperative Oncology Research Group trial is currently comparing preoperative chemoradiotherapy (as it was done in the CROSS study) and perioperative chemotherapy with the epirubicin, cisplatin and 5-FU (ECF) regimen. The Dutch Colorectal Cancer Group is currently assessing postoperative chemoradiotherapy, comparing perioperative epirubicin, cisplatin, capecitabin (ECC) chemotherapy and preoperative ECC chemotherapy combined with postoperative chemoradiotherapy (the CRITICS study) in GEA.

Second, during chemotherapy/chemoradiotherapy, many patients experience life-threating effects and cannot complete the treatment. Conroy *et al*[46] showed that in patients with non-resectable esophageal carcinoma treated only with chemoradiotherapy, using oxaliplatin instead of CDDP (the FOLFOX4 regimen), reduced toxicities and toxic deaths compared with the standard 5-FU-CDDP regimen. A neoadjuvant FOLFOX6 chemotherapy regimen could be substituted to 5-FU-CDDP and proposed as ambulatory treatment.

In patients with metastatic GEA, the intensification of chemotherapy with docetaxel, 5-FU and cisplatin (TCF) is more effective than with 5-FU plus cisplatin alone, but with a significantly higher level of hematologic toxicities. The FLOT regimen (50 mg/m² docetaxel and infusion of 5-FU, leucovorin and oxaliplatin (TEF regimen) was compared with 5-FU plus oxaliplatin (the FLO regimen) in a randomized phase Ⅱ clinical trial. This study included patients older than 65 years and showed an improvement of the response rate and the progression-free survival in patients with locally-advanced cancer[47]. The combination of docetaxel, 5-FU, leucovorin and oxaliplatin as first-line treatment was effective with an intention-to-treat objective response rate of 66% (95%CI: 50.55-78.44) and two confirmed complete responses, progression-free survival of 6.3 mo (95%CI: 4.5-7.3) and OS of 12.1 mo (95%CI: 6.5-15.3)[48]. At the 2015 ASCO meeting, the authors presented preliminary results on the pathologic response in patients with resectable lower esophagus or gastroesophageal junction adenocarcinomas treated with FLOT or ECF. The complete pathologic response rate was significantly higher in patients who received FLOT than in those treated with ECF (15.6% *vs* 5.8%, $P = 0.015$)[49].

For T3/T4 and or N+ GEA, using hyperthermic intraperitoneal chemotherapy (HIPEC) could reduce the frequent peritoneal recurrences in this setting. The PRODIGE French scientific group study is currently assessing HIPEC as adjuvant treatment.

Concerning targeted therapies, the use of trastuzumab combined with neoadjuvant chemotherapy is not recommended in patients with resectable GEA that overexpress HER2. Also, the MAGIC group is currently assessing the association of bevacizumab and the ECC (capecitabine instead of 5-FU) chemotherapy regimen in a perioperative setting compared to chemotherapy with ECF alone.

Finally we need predictive markers of neoadjuvant treatment response as early PET-scan metabolic response or assessment of biomarkers using systematic pre-therapeutic or liquid biopsies.

## CONCLUSION

Currently, two main therapeutic options can be proposed for the treatment of resectable GEA: (1) adjuvant chemoradiotherapy (Macdonald *et al*[17]) and (2) perioperative chemotherapy with 5-FU and platin salts-based regimens (Cunningham *et al*[26] or Ychou *et al*[29]). But what is best for our patients? Comparing the two strategies is not possible because the patients' profiles are too different. In both the MAGIC and FNCLCC 94012-FFCD 9703 trials, patients were identified at diagnosis, and not after surgery as it was done in the INT-0116 trial regarding the pT3/T4 or pN stage, the OMS (0 or 1) and the nutritional status.

Therefore, the treatment choice could be summarized

between two unsatisfactory options: A preoperative approach in which patients with a good-prognosis tumor may be over-treated, or a postoperative approach in which patients with high risk of recurrence and poor nutritional status after surgery might be under-treated. We recommend deciding the therapeutic management of each individual patient in a multidisciplinary committee, before the primary tumor surgery. Future applications of cytotoxic therapies, *e.g.*, oxaliplatin, capecitabine or docetaxel, or targeted therapies may help improving resectable GEA management.

For patients with gastroesophageal junction or lower esophagus adenocarcinomas, neoadjuvant chemoradiotherapy could be a viable option, but needs to be compared with perioperative chemotherapy.

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ORIGINAL ARTICLE

Basic Study

# Association of colorectal cancer with pathogenic *Escherichia coli*: Focus on mechanisms using optical imaging

Julie Veziant, Johan Gagnière, Elodie Jouberton, Virginie Bonnin, Pierre Sauvanet, Denis Pezet, Nicolas Barnich, Elisabeth Miot-Noirault, Mathilde Bonnet

Julie Veziant, Johan Gagnière, Virginie Bonnin, Pierre Sauvanet, Denis Pezet, Nicolas Barnich, Mathilde Bonnet, UMR1071 Inserm/Université d'Auvergne and INRA USC2018, Clermont Université, 63000 Clermont-Ferrand, France

Julie Veziant, Johan Gagnière, Pierre Sauvanet, Denis Pezet, Service de Chirurgie Digestive, Centre Hospitalier Universitaire, 63000 Clermont Ferrand, France

Elodie Jouberton, Elisabeth Miot-Noirault, UMR990 Inserm/Université d'Auvergne, Clermont Université, 63000 Clermont-Ferrand, France

Elodie Jouberton, Service de Médecine Nucléaire, Centre Jean Perrin, 63000 Clermont Ferrand, France

Author contributions: Veziant J, Gagnière J, Bonnet M conceived and designed the study, analyzed data and drafted the manuscript; Veziant J, Jouberton E, Bonnin V, Bonnet M performed experiments and analyses; Miot-Noirault E contributed to imaging platform management; Sauvanet P performed histological technics; Gagnière J, Pezet D, Barnich N, Miot-Noirault E and Bonnet M contributed to critical comments and revised the manuscript; all authors read, contributed to, and approved the final manuscript.

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Correspondence to: Dr. Mathilde Bonnet, PhD, UMR1071 Inserm/Université d'Auvergne and INRA USC2018, Clermont Université, 28 Place Henri Dunant, 63000 Clermont-Ferrand, France. mathilde.bonnet@udamail.fr
Telephone: +33-4-73178381
Fax: +33-4-73178381

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## Abstract

**AIM:** To investigate the molecular or cellular mechanisms related to the infection of epithelial colonic mucosa by *pks*-positive *Escherichia coli* (*E. coli*) using optical imaging.

**METHODS:** We choose to evaluate the tumor metabolic activity using a fluorodeoxyglucose analogue as 2-deoxyglucosone fluorescent probes and to correlate it with tumoral volume ($mm^3$). Inflammation measuring myeloperoxidase (MPO) activity and reactive oxygen species production was monitored by a bioluminescent (BLI) inflammation probe and related to histological examination and MPO levels by enzyme-linked immunosorbent assay (ELISA) on tumor specimens. The detection and quantitation of these two signals were

validated on a xenograft model of human colon adenocarcinoma epithelial cells (HCT116) in nude mice infected with a *pks*-positive *E. coli*. The inflammatory BLI signal was validated intra-digestively in the colitis-CEABAC10 DSS models, which mimicked Crohn's disease.

**RESULTS:** Using a 2-deoxyglucosone fluorescent probe, we observed a high and specific HCT116 tumor uptake in correlation with tumoral volume ($P$ = 0.0036). Using the inflammation probe targeting MPO, we detected a rapid systemic elimination and a significant increase of the BLI signal in the *pks*-positive *E. coli*-infected HCT116 xenograft group ($P$ < 0.005). ELISA confirmed that MPO levels were significantly higher (1556 ± 313.6 *vs* 234.6 ± 121.6 ng/mL $P$ = 0.001) in xenografts infected with the pathogenic *E. coli* strain. Moreover, histological examination of tumor samples confirmed massive infiltration of *pks*-positive *E. coli*-infected HCT116 tumors by inflammatory cells compared to the uninfected group. These data showed that infection with the pathogenic *E. coli* strain enhanced inflammation and ROS production in tumors before tumor growth. Moreover, we demonstrated that the intra-digestive monitoring of inflammation is feasible in a reference colitis murine model (CEABAC10/DSS).

**CONCLUSION:** Using BLI and fluorescence optical imaging, we provided tools to better understand host-pathogen interactions at the early stage of disease, such as inflammatory bowel disease and colorectal cancer.

**Key words:** Colorectal carcinoma; *Escherichia coli*; Colibactin; Myeloperoxidase; *In vivo* optical imaging



**Core tip:** Approximately 15% of cancers are related to infectious agents. Colorectal cancer (CRC) is thus a complex association of non-neoplastic and tumoral cells and a large amount of microorganisms. Recent studies reported that *pks*-positive *Escherichia coli* (*E. coli*) strains are more frequently detected in CRC, suggesting their possible role in tumor development. Optical imaging has emerged as a powerful tool in translational cancer research, providing new possibilities for the spatiotemporal monitoring of carcinogenesis in mouse models. It may be particularly helpful in better understanding the *in vivo* host-pathogen-interactions in tumor development. This is the first study to use optical imaging to explore CRC carcinogenesis and associated pathogenic *E. coli*.

Veziant J, Gagnière J, Jouberton E, Bonnin V, Sauvanet P, Pezet D, Barnich N, Miot-Noirault E, Bonnet M. Association of colorectal cancer with pathogenic *Escherichia coli*: Focus on mechanisms using optical imaging. *World J Clin Oncol* 2016; 7(3): 293-301 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i3/293.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i3.293

## INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide[1]. Despite recent advances in therapeutic care, CRC remains the second cause of cancer-related death after lung neoplasia and is responsible for over 600000 deaths annually[1,2]. It is a multifactorial disease, strongly associated with genetic and environmental factors that favor tumor development[3]. Approximately 15% of cancers can be related to infectious agents[4,5], such as *Helicobacter pylori* (*H. pylori*) and gastric cancer[6]. Colorectal cancer thus involves a complex association of non-neoplastic and tumoral cells and a large amount of microorganisms. Gut microbiota, a bacterial community of over 100 trillion microbial cells, plays a major role in colorectal carcinogenesis. Indeed, high bacterial density in the colon ($10^{12}$ commensal bacteria/g of intestinal contents) compared to the small intestine ($10^{2}$ commensal bacteria/g of intestinal contents) is correlated with a higher risk of cancer development[7]. Gut microbiota dysbiosis has recently been linked to CRC[8-13], and several bacteria are involved in colorectal carcinogenesis, such as *Streptococcus bovis*[14,15], *Enterococcus* spp.[16], *H. pylori*[17-19], *Bacteroides fragilis*[20,21], *Clostridium septicum*[22], *Fusobacterium* spp.[23,24] and *Escherichia coli* (*E. coli*)[25,26].

*E. coli* is a commensal bacteria of the human gut microbiota that plays a major role in maintaining intestinal homeostasis[27]. Some strains became pathogenic, carrying virulence factors and producing toxins, such as cyclomodulins. These toxins can affect differentiation, apoptosis, and cell proliferation by interfering with the eukaryotic cell cycle and/or inducing DNA damage. Particularly, one of these toxins, the colibactin, is encoded by the *pks* genomic island and can lead to the creation of double-strand DNA breaks and thus induce the chromosomal instability involved in CRC[28,29]. Recent studies reported that *pks*-positive *E. coli* is more frequently detected in CRC patients, suggesting a possible role in tumor development[30-32]. Various independent studies showed that *pks*-positive-*E. coli* exhibit procarcinogenic properties in murine models, such as the multiple intestinal neoplasia (Min) mice model[33], azoxymethane (AOM)-treated *Il10*$^{-/-}$ mice[34] and AOM/DSS models[26]. Thereby, some pathogenic *E. coli* strains involved in colon carcinogenesis are now emerging. Nevertheless, mechanisms of action remain to be clarified, particularly in *in vivo* models.

The aim of the present study was to investigate *in vivo* the molecular or cellular mechanisms related to the infection of epithelial mucosa by *pks*-positive *E. coli* using 2D optical imaging. Indeed, optical imaging is emerging as a new powerful sensitive technology for the non-invasive spatiotemporal visualization of carcinogenesis in mice models, and it may help to better understand the host-pathogen interactions in colorectal tumor development[35-37]. Because chronic inflammation and reactive oxygen species (ROS) production are

key factors in bacteria and CRC interactions, we chose to evaluate *pks*-positive *E. coli* infection on these mechanisms using commercial, available and validated probes[31,38-40]. Indeed, inflammation could play a key role in the development of dysbiosis related to CRC[40]. *E. coli* is also the most characterized bacteria associated with inflammatory bowel disease, which is a known risk factor for CRC[41,42]. Moreover, Raisch *et al*[38] demonstrated that *E. coli* in colon cancer induces a significant increase in COX-2 expression in macrophages, the predominant type of immune cell that infiltrates tumors. Moreover, macrophages and other immune cells infiltrate the tumors, release myeloperoxidase (MPO), and produce ROS by several chemical reactions. Arthur *et al*[31,39] investigated *in vivo* the complex interplay between inflammation, bacteria and carcinogenesis and suggested that chronic inflammation is essential for tumor development by maintaining the expression of *pks* island genes. ROS production has also been reported in many suspected mechanisms related to CRC development. Neutrophils and macrophages, which are present in inflamed tissues such as colon tumors, are major providers of ROS. Maddocks *et al*[40,43] described a possible interaction between *E. coli* and the DNA repair system with elevated ROS levels. Because ROS oxidizes the luminescent probe and thus produces proportional light that is detectable *in vivo* with an optical imager[44,45], we choose to monitor the inflammatory pathway and ROS production using on a bioluminescent (BLI) approach. The monitoring of inflammation was first performed and validated on a colitis murine model (CEABAC10/DSS mice). Then, by this approach, we showed, on a xenograft murine model, that *pks*-positive-*E. coli* significantly induces oxidative stress and inflammation before stimulating HCT116-tumor growth. While monitoring longitudinal inflammation, we choose to assess tumor growth by determining tumor metabolic activity with a fluorescent tool based on the fluorodeoxyglucose analogue 2-deoxyglucosone.

## MATERIALS AND METHODS

### Animal models

Studies were performed in accordance with the French Regional Ethical Animal Use Committee (No. CEEA-02). All mouse models were housed in specific pathogen-free conditions (22 ± 2 ℃, 50% humidity, 12 h light/12 h dark) in the animal care facility of the Université d'Auvergne, Clermont-Ferrand, France.

### HCT116 xenograft models

The HCT116 colorectal cancer cells were maintained as monolayers in culture flasks using culture medium consisting of McCoy's 5a Medium (Modified) supplemented with 10% FCS (Biowest, Nuaillé, France), 2 mmol/L glutamine and 1% antibiotics. All the cells were grown at 37 ℃ in a humidified incubator containing 5% $CO_2$.

Xenografts of human CRC were induced in male nude mice (Swiss nu/nu), weighting 26-33 g at the time of injection (7 wk old, Charles Rivers). We excluded female nude mice in order to avoid a possible hormonal influence. A total of 10 male nude mice were divided into two groups: Non-infected control xenograft ($n$ = 5) and *pks*-positive *E. coli*-infected xenograft ($n$ = 5). According to the infected xenograft, HCT116 cells were mixed with *pks*-positive *E. coli* as previously described by Cougnoux *et al*[34]. Beforehand, bacteria were grown at 37 ℃ in Luria-Bertani medium. *Pks*-positive *E. coli* is an ampicillin- and kanamycin-resistant *E. coli* strain named 11G5, isolated from a patient presenting with colon cancer and previously presented by Bonnet *et al*[33]. We used human colon adenocarcinoma epithelial cells (HCT116) to establish the xenograft models.

Then, animals anesthetized by isoflurane inhalation were inoculated with $2 \times 10^6$ HCT116 cells embedded in growth factor-reduced Matrigel (Becton Dickinson) by dorsal subcutaneous injection at day 0 of the experiment.

Tumor size was assessed two times per week and tumor volume was obtained according to the following formula: $(\text{width}^2 \times \text{length})/2 = V\ (\text{mm}^3)$. The longest diameter (L) and maximum diameter (W) perpendicular to the direction of the longest diameter were determined using a caliper. Mice were sacrificed at 35 d post-injection, and the xenograft was collected from all animals and subjected to histologic examination and enzyme-linked immunosorbent assay (ELISA).

### Colitis-CEABAC10 DSS models

Six CEABAC10 transgenic mice in an *in vivo* model mimicking colitis and Crohn's disease[46] were used to monitor intra-digestive inflammation. They were divided into two groups. Mice from the same generation were used for experimentation. One group ($n$ = 3) received one cycle of dextran sodium sulfate (DSS) in drinking water for 6 d at 1% (DSS-treated mice group) as described previously in Denizot *et al*[46]. The other group received only drinking water ($n$ = 3; DSS-).

### Optical imaging

For both BLI and fluorescence imaging acquisition, all animals were imaged using a dedicated high-sensitivity peltier-cooled (-90 ℃) backlit charge-coupled device camera (IVIS Spectrum®, Perkin Elmer, United States). All acquisitions were performed under the same exposure conditions according to fluorescence or BLI imaging, with acquisition settings (binning and duration) set up depending upon the signal at the time of acquisition.

Prior to imaging, animals were anesthetized with 2%-3% isoflurane in an induction chamber; then, 2% isoflurane in air/$O_2$ was continuously delivered *via* a nose cone system in the dark box of the imaging system (delivered gas to up to 5 mice). To limit auto-

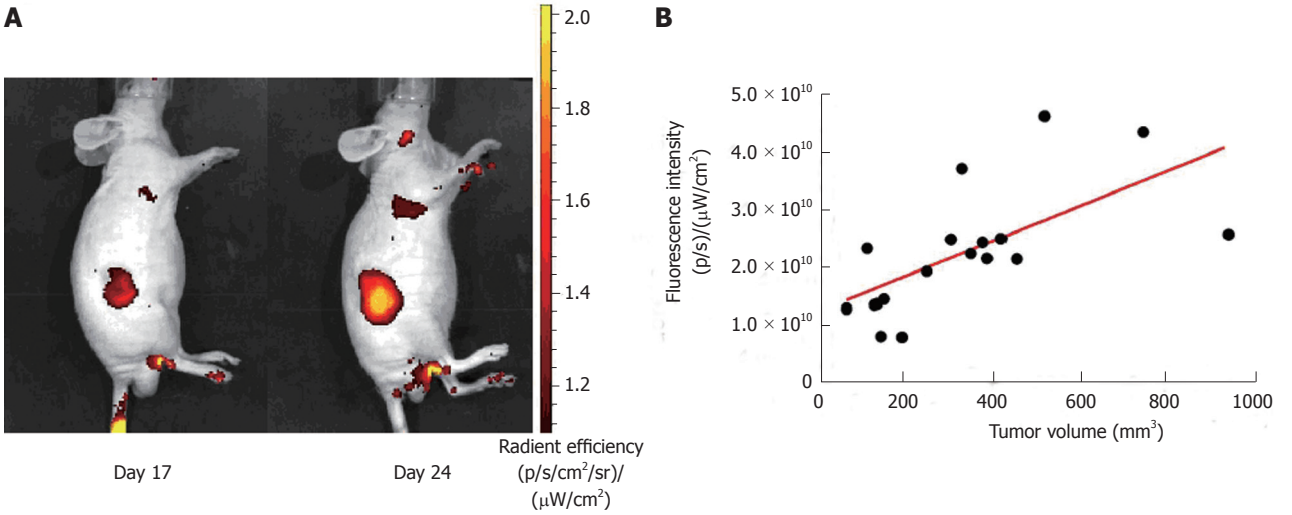


**Figure 1 Correlation of tumor volume (HCT116 cells xenograft) and 2-DG-750 fluorescence signal uptake.** A: Representative animal images showing the increase in fluorescence signal intensity with tumor development (day 17 and day 24 post-xenograft); B: Correlation between tumor development determined using a caliper and increase in fluorescence signal intensity (Pearson's correlation factor $r = 0.4197$; $P = 0.0036$).

fluorescence related to melanin, mice were shaved before all imaging procedures (except nude mice, which are hairless).

We used the XénoLight Rediject 2-DG-750 fluorescent probe or the XénoLight Rediject Inflammation chemiluminescent probe (Perkin Elmer, United States) to monitor metabolic activity or inflammation (MPO and ROS detection), respectively.

To monitor inflammation in HCT116-grafted nude mice at 20 d and 34 d post-xenograft, we administered by intraperitoneal (i.p.) injection of 150 μL of the XenoLight Rediject Inflammation probe per mouse. Mice were then imaged 10 min post-injection (exposure time of 5 min). In the colitis CEABAC10 model, imaging was performed 6 d after the DSS cycle. To monitor inflammation at depth and limit the decrease of the BLI signal intensity in this model, we administered by an intravenous (i.v.) injection of 150 μL/mouse. With i.v. injection, the best time to image the animal was immediately post-injection (exposure time of 5 min).

For tumor metabolic activity, we administered by an intravenous injection of 100 μL/mouse and imaged them 3 h after 2-DG-750 probe injection using one filter set (excitation: 745 nm, emission: 820 nm) and a high-throughput epi-illumination acquisition mode. All nude mice were imaged individually at 17 d and 24 d post-xenograft.

Quantitative analysis of imaging was performed using Living Image® Software (Caliper Life Science, United States) with the region of interest delineated manually over organs exhibiting probe accumulation. Every image series had the same scales, set manually, to facilitate the visual comparison of signal intensity at each time point. For the BLI signal, photon flux was expressed as the average radiance in p/s/cm²/sr. Fluorescence emission was also normalized to photons per second per centimeter squared per steradian (p/s/cm²/sr).

### *Histological analysis*

After mouse sacrifice, tumor pieces were fixed in formol solution. Paraffin-embedded sections were cut into 5-μm slices, and tissue sections were prepared for hematoxylin-eosin-safran staining and routine pathological analysis with focus on the mitotic index, infiltrating cells and tumor necrosis. Sample preparations and observations were made in the Centre Imagerie Cellulaire Santé platform (Clermont-Ferrand).

### *MPO activity determination*

After mouse sacrifice, tumor pieces were frozen in liquid nitrogen and stored at -80 ℃ until used. We performed an enzyme-linked immunosorbent assay to determine the levels of MPO (ng/mL) in all the tumors according to the manufacturer's instructions (R and D systems). Data were standardized on whole protein extracts stained with Coomassie blue.

### *Statistical analysis*

Graph Pad Prism 5 STATA (StataCorp) was used for all statistical analysis. Unpaired Student's *t* test was used for the comparisons of the 2 groups. We determined the Pearson's correlation coefficient *r* to assess the degree of correlation. We considered *P* values of $< 0.05$ to be statistically significant.

## RESULTS

### *Metabolic activity of the CRC xenograft model*

In HCT116-grafted nude models, we confirmed a rapid systemic elimination of the 2-deoxyglucosone fluorescent probe with a very weak whole body uptake 180 min after probe-injection (Figure 1A). Moreover, a high and specific HCT116 tumor uptake was evidenced for each tumor 17 and 34 d post-graft. We demonstrated an increase of signal intensity over time, reflecting tumor growth. The tumor uptake of the

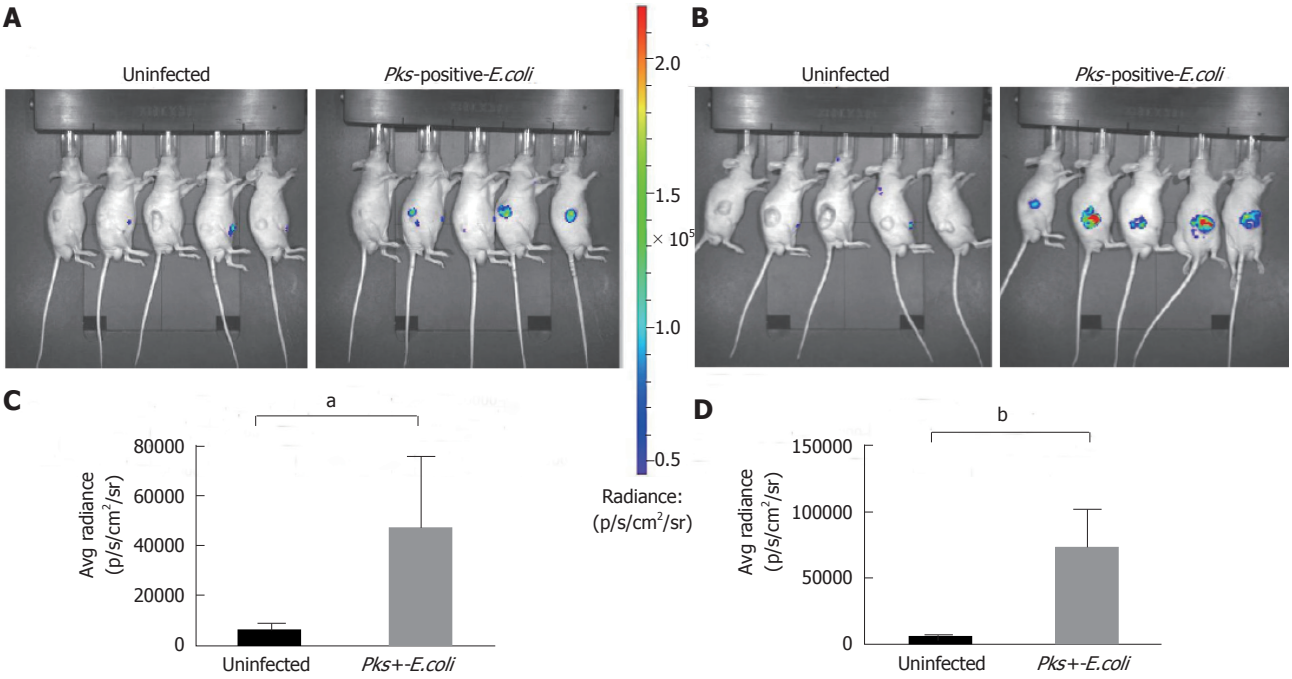


**Figure 2 Significant increase of inflammation in HCT116 tumors infected with *Escherichia coli* strains measured by optical imaging using the inflammation probe.** *In vivo* BLI imaging was performed at day 20 (A) and day 34 (B) post-HCT116 xenograft in nude mice ($n$ = 5 animals with uninfected xenograft; $n$ = 5 animals in *pks*-positive *E. coli*-infected xenograft). BLI images comparing uninfected to *pks*-positive *E. coli*-infected xenografts are shown on day 20 (A) and day 34 (B) post-xenograft. Intensity of emission is represented as the pseudocolor image. A sharp increase in the BLI signal was seen in the *pks*-positive *E. coli*-infected xenograft group at each time point. The BLI signal detected 10 min after i.p. probe injection was quantified from the ROI drawn manually. Photon flux was expressed as the average radiance in p/s/cm$^2$/sr. Graphs reveal a statistically significant increase in the BLI signal in the *pks*-positive *E. coli*-infected tumors: day 20, $^aP$ = 0.0132 (C) and day 34, $^bP$ = 0.0006 (D). BLI: Bioluminescent; *E. coli*: *Escherichia coli*; i.p.: Intraperitoneal.

2-deoxyglucosone fluorescent signal was significantly ($P$ = 0.0036) correlated with tumor volume, as determined using a caliper (Figure 1B). The *in vivo* monitoring of HCT116 tumor growth by the 2-deoxyglucosone fluorescent probe was efficient. No difference in tumor uptake was observed between uninfected and *pks*-positive *E. coli*-infected HCT116 cells (data not shown), as previously described with caliper determination by Cougnoux *et al*[34] at 34 d post-xenograft.

We tested 2-deoxyglucosone fluorescent imaging on Colitis-CEABAC10 DSS models. We did not observe any fluorescent signal *in vivo* in mice, reflecting that the targeting probe is specific for tumor cells (data not shown).

### *Pks*-positive *E. coli in vivo* induces inflammation in the CRC xenograft model

Using the inflammation probe, all nude mice were imaged 20 and 34 d post-xenograft. We detected a rapid systemic elimination in all mice and a strong BLI signal in HCT116 tumors in the infected group 10 min after probe injection (Figure 2A and B). Figure 2A and B clearly show that the intensity of the BLI signal (average radiance in p/s/cm$^2$) was stronger in xenografts infected with the pathogenic *pks*-positive *E. coli* strain compared to uninfected ones at each time point investigated (20 and 34 d post-xenograft). Quantitation confirmed a significant increase of the BLI signal in the infected tumors 20 d ($P$ = 0.0132, Figure 2C) and 34 d ($P$ = 0.0006, Figure 2D) after the xenograft.

### Monitoring intra-digestive BLI signals in Colitis-CEABAC10 DSS models

Then, we analyzed the monitoring of intra-digestive BLI signals using the inflammation probe in the CEABAC10 colitis mouse model. We induced intra-digestive inflammation using DSS in the first group, while control mice received only drinking water. To visualize BLI imaging in deep tissues *in vivo* in mice, the probe was injected intravenously. DSS group imaging showed a high BLI signal in DSS animals, reflecting severe intra-digestive inflammation (DSS+) relative to the untreated group (DSS-) (Figure 3). We demonstrated that monitoring intradigestive inflammation with the BLI signal is feasible and consistent.

### Histological characterization

The histologic analysis of tumor samples indicated and confirmed that tumor cells in *pks*-positive *E. coli*-infected xenografts were surrounded by a remarkable infiltration of activated phagocytes (Figure 4C and D) compared to the uninfected group (Figure 4A and B). In addition, tumor necrosis was observed, especially in the *pks*-positive *E. coli*-infected group (Figure 4C). Moreover, HCT116 are characterized by megalocytosis and the progressive enlargement of the cell body and nucleus in *pks*-positive *E. coli*-infected xenografts. Histological examination confirmed the data from BLI

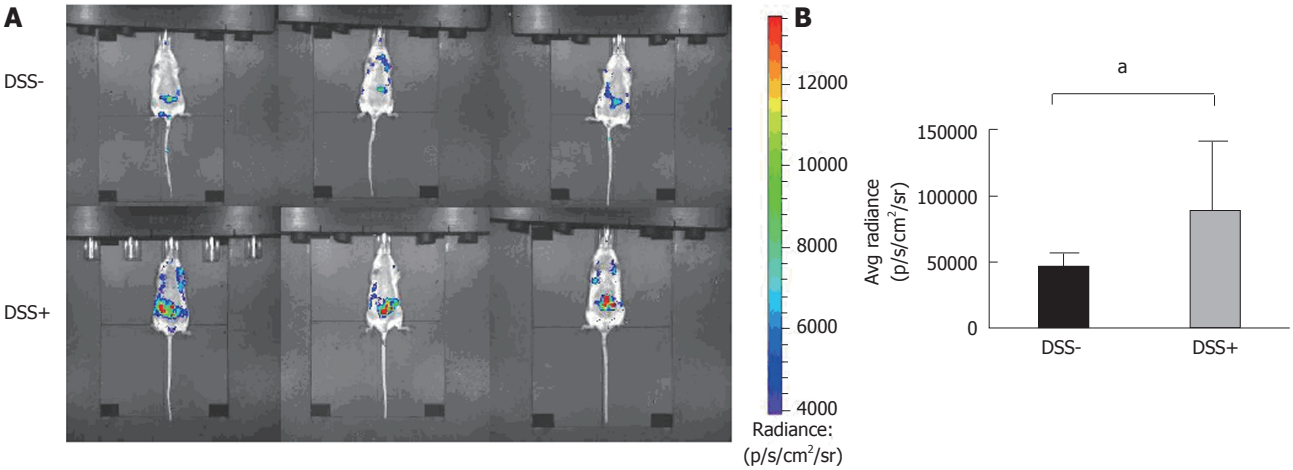


**Figure 3 Monitoring of intra-digestive bioluminescent signals using the inflammation probe in the CEABAC10 mouse model.** One mouse group was treated with 1% dextran sodium sulfate (DSS-treated mice group) in drinking water for 6 d ($n$ = 3), while the other group received only drinking water ($n$ = 3; DSS-). Mice were subjected to one cycle of DSS and then imaging with the inflammation probe before sacrifice. Mice were intravenously injected with the inflammation probe, and the BLI signal was detected immediately after injection. DSS-treated mouse group acquisition (A) showed severe intra-digestive inflammation was correlated with a significant increase in the BLI signal on graphs (B) compared to the DSS group ($^aP$ = 0.03). BLI: Bioluminescent; DSS: Dextran sodium sulfate.

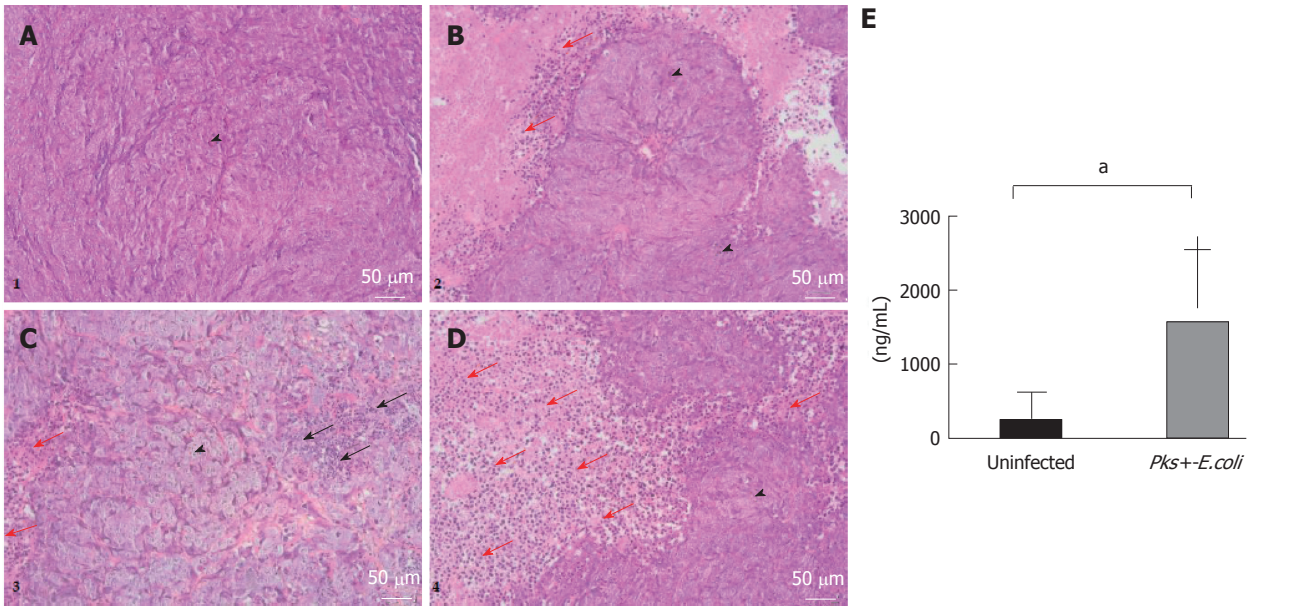


**Figure 4 Histological and molecular analyses of HCT116 tumor samples.** A-D: Histological examination of representative HCT116 tumor samples. Xenografts were harvested, paraffin embedded and processed for hematoxylin/eosin/safran. A and B are representative histological examinations from the uninfected xenograft group. C and D are representative histological examinations from the *pks*-positive-*E. coli* infected xenograft group. We noted that HCT116 tumor cells (arrowheads) infected with pathogenic *E. coli* strains are characterized by megalocytosis and progressive enlargement of the cell body and nucleus. Tumor cells in the *pks*-positive *E. coli*-infected xenograft group were surrounded by a remarkable infiltration of inflammatory cells (red arrow) compared to the uninfected xenograft group. Tumor necrosis was observed, especially in the infected xenograft group (black arrow). (Scale bars: 50 μm = × 20); E: MPO levels by enzyme-linked immunosorbent assay (ELISA) on HCT116 tumor specimens. An ELISA test was performed on tumor specimens after mouse sacrifice (day 34 post-xenograft). MPO standardized levels were significantly higher (1556 ± 313.6 *vs* 234.6 ± 121.6, $^aP$ = 0.001) in xenografts infected with pathogenic *pks*-positive *E. coli* strains. *E. coli*: *Escherichia coli*; MPO: Myeloperoxidase.

imaging (inflammation probe).

### *MPO levels by ELISA*

To confirm the quantitation of inflammation imaging, we assessed MPO activity on HCT116-xenograft-tumor specimens by performing an ELISA. MPO levels (ng/mL) were significantly higher (1556 ± 313.6 *vs* 234 ± 121.6, $P$ = 0.001) in xenografts infected with the *pks*-positive *E. coli* strain. These results showed that the pathogenic *E. coli* strain enhanced MPO release compared to uninfected xenografts and confirmed the data from BLI imaging.

## DISCUSSION

Optical imaging appears to be a powerful, highly sensitive tool in translational cancer research, providing new possibilities for *in vivo* molecular imaging and

allowing a better understanding of host-pathogen interactions in several tumor processes. Some studies have reported the pro-carcinogenic activities of *pks*-positive *E. coli* in murine models[26,33,34]. Most of these studies required animal sacrifice and did not allow longitudinal investigation. Ideally, it would be useful to non-invasively, longitudinally monitor these procarcinogenic processes. Here, we report the first study that utilized optical imaging in these settings. More precisely, we focused on CRC carcinogenesis and pathogenic *E. coli* association.

We described a specific accumulation of the 2-deoxyglucosone fluorescent probe to the tumor site (CRC xenograft model), thus establishing a correlation between tumor volume and fluorescent signal intensity. We showed that this method provides an effective tool to assess longitudinal data on CRC tumor growth *in vivo*. Moreover, in our experimental conditions, xenografts infected with *pks*-positive *E. coli* exhibited comparable development to uninfected ones, confirming results reported for the same experimental conditions by Cougnoux *et al*[34]. However, they observed a significant increase in tumor volume induced by colibactin, starting from day 44 after the xenograft. In the present study, we chose to evaluate inflammation and ROS production at an early stage, before the effect of colibactin on tumor cells proliferation. Indeed, Arthur *et al*[39] suggested that inflammation is necessary for *E. coli*'s cancer-promoting activity, probably through the enhancement of its resilience among gut microbiota in the intestine. Our results showed that the BLI signal significantly increases with bacterial infection. Pathogenic *E. coli* seemed to enhance inflammation and ROS production, which could participate in carcinogenesis. Using luminol-based BLI, we showed that *pks*-positive *E. coli* induced oxidative stress, which is involved in carcinogenesis process. We confirmed this observation on histological examination, which showed that inflammatory cells were mostly recruited in infected xenografts. Tumor necrosis also appeared in the *pks*-positive *E. coli* group. Moreover, a significant increase of MPO activity, which led to ROS production by infiltrating immune cells, was confirmed with an ELISA on HCT116-cells tumor specimens. Finally, these data showed that *pks*-positive-*E. coli* induced inflammation and ROS production at an early stage after infection, and could thus be an important mechanism involved in pro-carcinogenic activity.

To validate the use of luminol-based BLI imaging to monitor inflammation and oxidative stress, we used an *in vivo* colitis model (CEABAC10/DSS). We proved that monitoring inflammation in deep tissues is feasible and effective. This suggests that optical imaging should be tested on other murine models ($APC^{min/+}$, AOM-$IL10^{-/-}$, AOM-DSS) used to determine the pro-carcinogenic proprieties of *pks*-positive *E. coli* strains, and it may facilitate a better understanding of how pathogenic bacteria impact the carcinogenesis process by various mechanisms. Using CEABAC10 models mimicking Crohn's disease, we suggest that optical imaging is an effective method in inflammatory bowel disease research.

In conclusion, by using optical imaging, particularly the BLI approach, we provided additional tools to better understand host-pathogen interactions in digestive pathology, including CRC and inflammatory bowel disease.

## COMMENTS

### Background

Colorectal carcinoma (CRC) is a complex association of non-neoplastic and tumoral cells and a large amount of microorganisms. *Escherichia coli* (*E. coli*) is a consistent commensal of the human gut microbiota but some pathogenic strains have acquired the ability to produce toxins as cyclomodulins that can interfere with eukaryotic cell cycle or directly induce DNA damages. It was observed that cyclomudulin-producing-*E. coli* are more frequently detected on CRC patients and exhibit procarcinogenic properties on murine models.

### Research frontiers

Novel imaging techniques like optical imaging could be a powerful tool in translational cancer. Particularly, *in vivo* optical imaging is an innovative tool for non-invasive, spatiotemporal and quantitative monitoring of carcinogenesis process in murine models. It may help to better understand the host-pathogen interactions in colorectal tumor development.

### Innovations and breakthroughs

This study investigates the *in vivo* mechanisms of epithelial colonic mucosa infection by cyclomodulin-positive-*E. coli*. Because chronic inflammation and reactive oxygen species (ROS) production are key factors in bacteria and CRC interactions, the authors choose to evaluate cyclomodulin-positive-*E. coli* infection on these mechanisms using optical imaging and commercial, available and validated probes. By using this technique, the authors provided tools to better understand host-pathogen interactions on murine models at the early stage of disease, such as inflammatory bowel disease and CRC.

### Applications

By using optical imaging, particularly the bioluminescent approach, the authors provided additional tools to better understand host-pathogen interactions in digestive pathology, including CRC and inflammatory bowel disease. The data suggest that cyclomodulin-positive-*E. coli* induced inflammation and ROS production at an early stage after infection, and could thus be an important mechanism involved in pro-carcinogenic activity of these bacteria.

### Terminology

Oxidative stress reflects an imbalance between the systemic manifestation of ROS and the cellular biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. In humans, oxidative stress is thought to be involved in the development of several cancers.

### Peer-review

Good interesting novel study. The experiments were well designed.

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ORIGINAL ARTICLE

Retrospective Cohort Study

# Contralateral prophylactic mastectomy rate stable at major Canadian breast cancer center

Amanda Roberts, Lakhbir Sandhu, Tulin D Cil, Stefan O P Hofer, Toni Zhong

Amanda Roberts, Lakhbir Sandhu, Department of Surgery, University of Toronto, Toronto, ON M5G 2C4, Canada

Tulin D Cil, Division of General Surgery, University Health Network and Women's College Hospital, Toronto, ON M5G 2M9, Canada

Stefan O P Hofer, Toni Zhong, Division of Plastic and Reconstructive Surgery, University Health Network, Toronto General Hospital, Toronto, ON M5G 2C4, Canada

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Correspondence to: Toni Zhong, MD, MHS, FRCSC, Associate Professor, Division of Plastic and Reconstructive Surgery, University Health Network, Toronto General Hospital, 8N-871, Toronto, ON M5G 2C4, Canada. toni.zhong@uhn.ca

Telephone: +1-416-3403858

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## Abstract

**AIM:** To examine trends of contralateral prophylactic mastectomy (CPM) rates at a Canadian academic breast cancer center.

**METHODS:** A single-institution retrospective cohort study was completed. Women of any age who underwent at least a unilateral mastectomy (UM) for primary unilateral breast carcinoma between January 1, 2004 and December 31, 2010 were included. Patients who underwent CPM on the same day as UM were isolated to create two distinct cohorts. Patient and procedure characteristics were compared across groups using R software (version 3.1.0). The percentage of CPMs per year was determined. The Cochrane-Armitage test was used to assess the trend of CPMs over time. A $P$ value of $< 0.05$ was considered significant.

**RESULTS:** A total of 811 women met the inclusions/exclusion criteria; 759 (93.6%) underwent UM alone and 52 (6.4%) underwent UM with immediate CPM. The absolute number of procedures (UM and UM + CPM) increased over time, from 83 in 2004 to 147 in 2010 reflecting an increase in mastectomy volume. Annual CPM rates did not increase over time ($P = 0.7$) and varied between 2.6% to 10.7%. Family history of breast cancer [OR 3.6 (1.8-7.3)] and immediate reconstruction [10.0 (5.2-19.3)] were both significantly associated

with CPM. Women who underwent CPM were younger (median age CPM 49 years *vs* UM 52 years, $P < 0.0001$) but age less than 50 years was not statistically associated with increased rates of CPM.

**CONCLUSION:** CPM rates from 2004 to 2010 at a high-volume Canadian breast cancer center did not increase over time, in contrast to trends observed in the United States.

**Key words:** Breast; Oncology; Prophylactic; Mastectomy; Surgery



**Core tip:** Contralateral prophylactic mastectomy rates from 2004 to 2010 at a high-volume Canadian breast cancer center do not demonstrate the same rising trend observed in the United States.

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## INTRODUCTION

During the past decade, the percentage of women with unilateral breast carcinomas undergoing immediate contralateral prophylactic mastectomy (CPM) has steadily increased over time[1-3] despite minimal evidence supporting a survival benefit[4-7]. These increased CPM rates have mainly been described in literature from the United States. CPM trends of similar magnitude and scope have not been quantitatively documented in literature from countries outside of the United States. Switzerland and England have reported much lower CPM rates during a similar time period as the United States data[8,9] - with no significant increase over time noted in Switzerland. It is possible that in these countries, as well as in Canada, where the health care system is based on a socialized medicine model, and surgeons routinely practice under budgetary restraints; a nonlife-saving surgical service such as CPM may not be as readily available as in the United States.

In Canada, lower CPM rates have been noted on a national level compared to the United States[10], but these rates were obtained through administrative databases and no patient level, surgical procedure detail, or outcomes data were reported[10]. Since CPM rates are known to be influenced by resource factors, such as access to genetic testing and immediate breast reconstruction, and other patient factors such as age[1-3,7,8,11-14], examining CPM trends with patient and procedure level data will provide more detailed information on the CPM landscape in Canada.

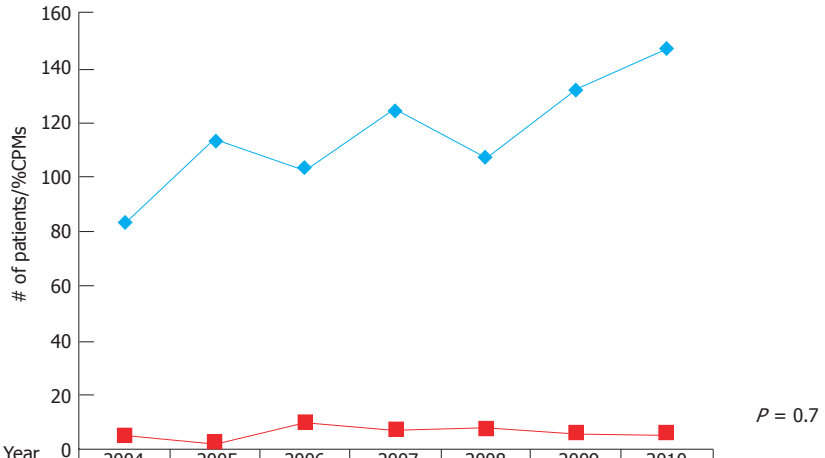
Therefore, our main objective was to examine the rates and trends of immediate CPM and to compare outcomes of patients undergoing CPM to patients having unilateral mastectomy (UM) alone at a Canadian academic breast cancer center.

## MATERIALS AND METHODS

We conducted a single institution retrospective cohort study using a prospectively maintained database. The Princess Margaret Breast Disease Database contains information on all patients who undergo any form of breast disease evaluation or treatment at the Princess Margaret Hospital in Toronto, Ontario, Canada. There is an opt-out policy for patients who do not wish to have their medical information collected and used for research purposes. Research Ethics Board approval was acquired and maintained throughout the study. The population of interest included women of any age who underwent at least a UM for primary unilateral invasive or *in situ* breast carcinoma between January 1, 2004 and December 31, 2010. This time period was chosen to be as contemporary as possible while still coinciding with the literature from the United States and Switzerland. Patients who underwent CPM on the same day as the UM were isolated to create two distinct cohorts: Women who underwent UM alone and women who underwent UM with immediate CPM. Patients with known bilateral disease at the time of their primary surgery were excluded. A diagnosis of atypical hyperplasia, lobular carcinoma *in situ*, ductal carcinoma *in situ* or invasive breast cancer in the contralateral breast was considered as bilateral disease. These exclusions were applied in order to isolate, as accurately as possible, CPMs of truly benign breasts.

Patient and procedure variables were extracted from the database and included: Age, history of benign disease, family history of breast cancer, pre-operative BRCA status, pre-operative diagnosis, pre-operative systemic therapy, receipt of radiation, year of surgery, axillary evaluation (sentinel lymph node biopsy or axillary lymph node dissection) and receipt of reconstruction. The incidence of ipsilateral locoregional recurrence and development of a new contralateral breast cancer were also extracted.

Patient and procedure characteristics were compared across groups using R software (version 3.1.0). Mean values and standard deviations were calculated for normally distributed continuous variables and median values with interquartile range (IQR) were calculated for variables with a non-normal distribution. For categorical variables, results were expressed as counts and percentages. The Cochrane-Armitage test was used to assess the trend of CPMs over time. A $P$ value of $< 0.05$ was considered significant. A multivariable logistic regression model was generated with receipt of CPM as the main outcome variable. Covariates used in this model included age, family history of breast cancer, personal history of benign breast disease and receipt




| Year | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|---|---|
| Total # patients | 83 | 114 | 103 | 125 | 107 | 132 | 147 |
| %CPM | 4.8 | 2.6 | 10.7 | 7.2 | 8.4 | 6.1 | 5.4 |

**Figure 1 Contralateral prophylactic mastectomy trends.** CPM: Contralateral prophylactic mastectomies.

of immediate reconstruction. Age was dichotomized to a binary variable (< 50 years or ⩾ 50 years old). The remaining covariates were also binary. Covariates were chosen based on clinical significance. Model fit took into consideration any interactions and assessments of collinearity. The statistical review of the study was performed by a biomedical statistician.

## RESULTS

A total of 811 women met the inclusions/exclusion criteria; 759 (93.6%) underwent UM alone and 52 (6.4%) underwent UM with immediate CPM. The characteristics of each group are summarized in Table 1. The absolute number of procedures (UM and UM + CPM) increased over time, from 83 in 2004 to 147 in 2010 reflecting an increase in mastectomy volume (Figure 1). CPMs per year did not increase over the study time period ($P$ = 0.7) and varied between 2.6% to 10.7% (Figure 1).

Women who had a CPM were more likely to be younger [median age CPM = 49 years (range 29-67); UM = 52 years (range 28-88)] and to have a breast cancer family history (CPM 67% *vs* UM 37%, $P$ < 0.001). Receipt of neoadjuvant chemotherapy was not significantly different between the two groups (CPM 31% *vs* UM 23%, $P$ = 0.23), yet there were significantly more patients in the CPM group who underwent an ALND at the time of mastectomy (CPM 81% *vs* UM 49%, $P$ < 0.001). Significantly more patients having CPM underwent immediate reconstruction (CPM 54% *vs* UM 8%, $P$ < 0.001).

On the multivariable analysis, CPM patients were almost four times as likely to have a family history of breast cancer compared to those patients who opted for UM alone [OR = 3.6, (1.8-7.3) $P$ < 0.001]. Patients who had a CPM were also 10 times more likely to receive immediate reconstruction when compared to patients who underwent UM alone [OR = 10.0 (5.2-19.3), $P$ < 0.001] (Table 2).

Locoregional recurrence rates were no different between the two groups (CPM 4% *vs* UM 3%, $P$ = 0.68). Over the same follow-up period, a new contralateral breast cancer was identified in 13 (2.0%) of patients who underwent UM alone. No new contralateral breast cancers were identified in the CPM group, although three (6.0%) of the patients who underwent CPM were found to have incidental invasive disease in the contralateral breast on final pathology.

## DISCUSSION

Between 2004 and 2010, CPM rates at our single Canadian institution did not show the same increasing trend observed in the United States. During this time period, our CPM rate was 6.4% (range: 2.6% to 10.7%) with no increase over time, while rates in the United States were noted to increase between 2003 and 2010 from 4.1% to 9.7% for all ages, and 9.3% to 24.1% for patients aged 45 years or less[1]. In contrast, during a similar time period in Switzerland, CPM rates remained stable at 7%[8]. In Canada, the only reported data examined national CPM rates between 2007 and 2010 and demonstrated an overall CPM rate of 6% (5% in 2007/2008 to 7% in 2009/2010) - consistent with our institutional results and the rates from Switzerland[8,10].

Interestingly, the factors associated with increased rates of immediate CPM at our institution are similar to those found in the United States literature - suggesting that the patient factors influencing the decision to undergo a CPM are possibly the same between the two countries. Within our population, younger patients and those with family history were more likely to undergo CPM, similar to patients in the numerous United States studies[1-3,7,8,12-14]. Furthermore, the use of immediate reconstruction has been associated with increased CPM rates[11,13], and our study results support this finding.

As noted, the lower CPM rates reported from

**Table 1 Patient characteristics**

| | UM | CPM | *P* value |
|---|---|---|---|
| | *n* = 759 (94%) | *n* = 52 (6%) | |
| Age (median years, range) | 52 (28-88) | 49 (29-67) | < 0.001 |
| Pre-operative diagnosis (%) | | | 0.74 |
| Invasive carcinoma only | 585 (77.1) | 38 (73.1) | |
| DCIS only | 144 (19.0) | 12 (23.1) | |
| Invasive + DCIS | 30 (4.0) | 2 (3.8) | |
| Previous benign disease (%) | | | 0.26 |
| No | 566 (74.6) | 34 (65.4) | |
| Yes | 164 (21.6) | 15 (28.8) | |
| Unknown | 29 (3.8) | 3 (5.8) | |
| Pre-op BRCA1/BRCA2 mutation status (%) | | | < 0.001 |
| Negative | 43 (6) | 9 (17) | |
| Positive | 6 (1) | 11 (21) | |
| Both unknown | 710 (94) | 32 (62) | |
| Family history (%) | | | < 0.001 |
| Positive | 283 (37.3) | 35 (67.3) | |
| Negative | 440 (58.0) | 13 (25.0) | |
| Unknown | 36 (4.7) | 4 (7.7) | |
| Pre-op systemic therapy (%) | | | 0.23 |
| No | 588 (77) | 36 (69) | |
| Yes | 171 (23) | 16 (31) | |
| Any radiation treatment (%) | | | 0.008 |
| No | 435 (57) | 40 (77) | |
| Yes | 324 (43) | 12 (23) | |
| SLNB (%) | | | 0.76 |
| No | 316 (42) | 20 (38) | |
| Yes | 443 (58) | 32 (62) | |
| ALND (%) | | | < 0.001 |
| No | 387 (51) | 10 (19) | |
| Yes | 372 (49) | 42 (81) | |
| Reconstruction (%) | | | < 0.001 |
| No | 695 (92) | 24 (46) | |
| Yes | 64 (8) | 28 (54) | |
| Local/regional recurrence (%) | | | 0.68 |
| No | 734 (97) | 50 (96) | |
| Yes | 25 (3) | 2 (4) | |
| New contralateral breast cancer (%) | | | 0.05 |
| No | 746 (98) | 49 (94) | |
| Yes | 13 (2) | 3 (6) | |
| Follow-up (median years, range) | 2.32 (0.04-7.15) | 2.54 (0.13-6.91) | 0.56 |

UM: Unilateral mastectomy; CPM: Contralateral prophylactic mastectomies; BRCA: Breast cancer; SLNB: Sentinel lymph node biopsy; ALND: Axillary lymph node dissection; DCIS: Ductal carcinoma *in situ*.

**Table 2 Multivariable analysis**

| Predictor | OR | 95%CI | *P* value |
|---|---|---|---|
| Age | | | 0.99 |
| < 50 | 1 | 0.52-1.90 | |
| ⩾ 50 | Ref | Ref | |
| Family history | | | < 0.001 |
| Yes | 3.56 | 1.83-7.30 | |
| No | Ref | Ref | |
| History of benign disease | | | 0.51 |
| Yes | 1.26 | 0.61-2.49 | |
| No | Ref | Ref | |
| Immediate reconstruction | | | < 0.001 |
| Yes | 10.01 | 5.22-19.32 | |
| No | Ref | Ref | |

our single institution are consistent with the national Canadian administrative data yet are considerably lower than those reported in the United States during a similar time period. Despite this lower rate, our data highlight similar patient factors influencing the receipt of CPM. Given these similar patient factors, it may be the health system, or practice environment, of Canadian breast cancer surgeons and patients that is contributing to the discrepancy in CPM rates between Canada and the United States.

To address the possible influence of the surgeon and their practice environment on mastectomy and CPM rates, Covelli *et al*[15] obtained qualitative data from surgeons in both Canada and the United States in 2012 and 2013. Anecdotally, surgeons in both countries endorsed an increasing CPM rate and highlighted incidental findings on pre-operative MRI, access to reconstruction and the surgeon's initial discussion of treatment options as possible factors influencing these trends. Differences between the surgeons' practices were noted mainly by the availability of immediate breast reconstruction - a product of the health system. The majority of United States surgeons had ready access to immediate breast reconstruction whereas this was less commonly available to Canadian breast surgeons[15-17]. Barriers to access immediate breast reconstruction may contribute to more women in Canada seeking out a delayed CPM with bilateral reconstruction years after their initial unilateral therapeutic mastectomy. This model of care has several advantages in our healthcare system, as it obviates any cancer surgery wait-time constraints, delay in adjuvant cancer therapies and allows patients time to seek out an institution where specialized breast reconstruction surgeons are available. In addition to immediate breast reconstruction availability, legislation and guidelines within each country were also felt to influence surgical decisions. Legislation in various United States require the discussion of all treatment options available (including reconstruction) at initial consultation whereas, in Canada, no such legislation exists. This examination at the surgeon level provides evidence that the medico-social context of the patient's treatment environment, or health system, influences the decision to undergo mastectomy with CPM. These results also lead one to consider if the stable CPM rates in Canada are actually lower overall than in the United States due to these possible practice or health system differences, or if the trends in Canada will eventually mimic those of the United States over time given the surgeons' anecdotal observations, as a similar "lag" was observed regarding IBR trends in Canada[16,17].

Within our single institution study, we were able to evaluate CPM trends among a large sample of patients undergoing mastectomy for unilateral breast carcinoma at a high-volume academic breast center. The prospectively-collected patient level data provided important information regarding factors that may influence a patient's decision to undergo a CPM. This patient level data allowed us to not only evaluate the CPM rates, but to also investigate possible influencing factors for CPM and to provide insight on the demogra-

phics of CPM patients in Canada.

While our study provides patient level data related to CPM trends, the overall low number of patients who underwent CPM ($n$ = 52) should be considered a limitation when interpreting the results as we did not observe a trend over time in regards to CPM rates. While our overall rates are consistent with the national level data, at this point in time it is challenging to draw accurate conclusions related to the influence of the health system on Canadian CPM rates. Our results suggest that health system factors such as access to immediate breast reconstruction and patient factors such as a family history of breast cancer, both influence Canadian CPM rates. In addition, it should be noted that this study only evaluated immediate CPM and did not identify the number of delayed CPMs that occurred at our institution.

Overall, the immediate CPM rates between 2004 and 2010 at a high-volume Canadian breast cancer center did not demonstrate the same increasing trend documented in the United States. However, in keeping with the United States findings, our analysis showed that Canadian patients who had CPM were generally younger, more often had a breast cancer family history and also had immediate breast reconstruction.

## COMMENTS

### Background

Rates of contralateral prophylactic mastectomies (CPM) in women with early stage unilateral invasive breast cancer have been increasing, despite a lack of survival advantage. These rates have mainly been described in literature from the United States. Rates of CPM, and the factors influencing these rates, may be different in the Canadian healthcare system.

### Research frontiers

CPM is often performed for younger patients and associated with immediate breast reconstruction. With increasing interest in healthcare costs, plus patient satisfaction and outcomes, CPM is an important topic of investigation.

### Innovations and breakthroughs

CPM rates at a single Canadian institution do not show the same increasing trend as reported in the United States. Similar to United States literature, CPM is associated with immediate reconstruction and patients with a family history.

### Applications

By identifying findings in contrast to those reported in the United States, further investigation into potential reasons (*i.e*., lack of hospital resources, lack of patient desire, *etc*.) for the difference in CPM rates should be undertaken.

### Terminology

CPM is the removal of a healthy breast for breast cancer prevention or for cosmetic balancing requirements.

### Peer-review

The proffered paper is thoughtful, timely and publishable.

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# Extrapulmonary small cell carcinoma of lymph node: Pooled analysis of all reported cases

Inderpaul Singh Sehgal, Harpreet Kaur, Sahajal Dhooria, Amanjit Bal, Nalini Gupta, Digambar Behera, Navneet Singh

Inderpaul Singh Sehgal, Harpreet Kaur, Sahajal Dhooria, Digambar Behera, Navneet Singh, Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Amanjit Bal, Department of Histopathology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Nalini Gupta, Department of Cytology and Gynecological Pathology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Author contributions: Sehgal IS collected patient data, systematic review, drafted and revised the manuscript; Kaur H collected patient data, drafted and revised the manuscript; Dhooria S drafted and revised the manuscript; Bal A and Gupta N interpreted and reported pathological specimens of index cases, drafted and revised the manuscript; Behera D managed index cases, revised the manuscript; Singh N managed index cases, conceived the idea, systematic review, drafted and revised the manuscript.

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Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at navneetchd@yahoo.com.



Correspondence to: Dr. Navneet Singh, MD, DM, Associate Professor, Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Sector-12, Chandigarh 160012, India. navneetchd@yahoo.com
Telephone: +91-172-2756826
Fax: +91-172-2748215

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## Abstract

**AIM:** To study clinical outcomes and management of lymph nodes extrapulmonary small cell carcinoma (LNEPSCC).

**METHODS:** Herein, we perform a systematic search of published literature in the PubMed and EMBASE databases for studies describing LNEPSCC. For uniformity of reporting, LNEPSCC was staged as limited if it involved either single lymph node station or if surgery with curative intent had been undertaken. The disease was staged extensive if it involved two or more lymph node regions.

**RESULTS:** The systematic literature review yielded eight descriptions ($n$ = 14) involving cervical, submandibular and inguinal lymph nodes. Eleven (64.7%) patients had limited disease (LD) and six (35.3%) had extensive disease (ED) at presentation. Chemotherapy ($n$ = 6, 35.3%) or surgery ($n$ = 4, 23.5%) were the most common form of treatment given to these patients. Complete response was achieved in 12 (70.6%) of the patients. Median (interquartile range) progression free survival and overall survival was 15 (7-42) mo and 22 (12.75-42) mo respectively. Of the three illustrative cases, two patients each had ED at presentation and achieved complete remission with platinum based combination chemotherapy.

**CONCLUSION:** LNEPSCC is a rare disease with less than 15 reported cases in world literature. Surgical resection with curative intent is feasible in those with LD while platinum based combination chemoradiation is associated with favorable outcomes in patients with ED. Prognosis of LNEPSCC is better than that of small cell lung cancer in general.

**Key words:** Extrapulmonary; Small cell; Carcinoma; Lymph node; Small cell lung cancer



**Core tip:** Extrapulmonary small cell cancer confined to lymph nodes (LNEPSCC) is extremely rare. A systematic literature review yielded 3 index and 14 previous case descriptions. Chemotherapy or surgery was most common treatments given with complete response achieved in 70% of the cases. Surgical resection with curative intent is feasible in those with limited disease. Prognosis of LNEPSCC is better than that of small cell lung cancer in general.

Sehgal IS, Kaur H, Dhooria S, Bal A, Gupta N, Behera D, Singh N. Extrapulmonary small cell carcinoma of lymph node: Pooled analysis of all reported cases. *World J Clin Oncol* 2016; 7(3): 308-320 Available from: URL: http://www.wjgnet.com/2218-4333/full/v7/i3/308.htm DOI: http://dx.doi.org/10.5306/wjco.v7.i3.308

## INTRODUCTION

Extrapulmonary small cell carcinoma (EPSCC) is a rare disorder that is characterized by histological evidence of small cell carcinoma (SCC) from body organs other than the lungs[1]. First described in 1930[2], much needs to be determined about natural history and clinical behaviour of EPSCC. EPSCC is known to involve gastrointestinal tract (esophagus, stomach, liver, bile ducts, intestines and pancreas)[3-5], genitourinary tract (kidney, ureter, pelvis, bladder)[6-10], head and neck region (tonsils, larynx, nasopharynx, and paranasal sinuses)[11-14], bones[15] and lymph nodes (locoregional or distant)[16-23]. Although, EPSCC has histological similarity with small cell lung cancer, it has a different biological behaviour[1,24,25]. Also, the biological behaviour varies with the site of origin and the extent of the cancer. The disease limited to the site of origin and female genital tract is associated with a better survival[26,27]. Amongst the various types of EPSCC, patients with EPSCC of the lymph nodes have an even better overall survival and is considered to be a separate subgroup amongst patients with EPSCC[28]. Due to the paucity of evidence not much is known regarding the optimal schema for staging and classification of lymph node EPSCC (LNEPSCC). Further, the best treatment modality and the diagnostic approach remains to be determined. Herein, we describe three cases of LNEPSCC involving the cervical lymph nodes. We also perform a systematic review of the literature describing LNEPSCC.

## MATERIALS AND METHODS

### *Search strategy*

We searched the PubMed and EMBASE databases for articles published until August 15, 2015 using the free text terms: ("extra pulmonary small cell cancer" or "extra pulmonary small cell carcinoma" or "extra pulmonary small cell malignancy" or "extra pulmonary small cell tumor" or "extra thoracic small cell cancer" or "extra thoracic small cell carcinoma" or "extra thoracic small cell tumor" or "extra thoracic small cell malignancy"). We reviewed the reference list of all the included articles and previous review articles.

### *Inclusion criteria*

We included full-text, peer-reviewed, cross-sectional studies, cohort studies and case-reports that described SCC of the lymph node (LN). We excluded the following studies: (1) abstracts, comments, editorials, and reviews; (2) studies published in non-English language; (3) studies done in pediatric age group; and (4) animal studies. We also excluded the studies describing LNSCC involving the mediastinal LNs and studies or case reports that did not provide details about the site of LN station involved or follow up.

### *Initial review of studies*

The database thus created from the electronic searches was assimilated in the reference manager package Endnote (version X7.4; Thomson Reuters) and all duplicate citations were discarded. Two authors (Sehgal IS and Singh N) screened these citations by review of the title and abstract to identify the relevant studies. Any disagreement was resolved by discussion between the authors. The full text of each of these studies was obtained and reviewed in detail.

### *Study selection and data abstraction*

Two authors (Sehgal IS and Singh N) independently assessed all the articles for inclusion in the systematic review and extracted the data; the data was entered into a standard data extraction form. The following items were extracted: (1) publication details (authors, year of publication); (2) study design (prospective, retrospective or case-report); (3) number of patients (including the demographic profile) and inclusion criteria; (4) details such as LN region involved, size of the LN, number of LNs involved; (5) stage of the disease; (6) details of the treatment given (surgical or chemotherapy); (7) response to treatment (complication of chemotherapy progression free survival, overall survival, site of relapse, second line treatment given); and (8) final outcome. Any differences in the study selection and data extraction process between the two authors were resolved by discussion. For uniformity of

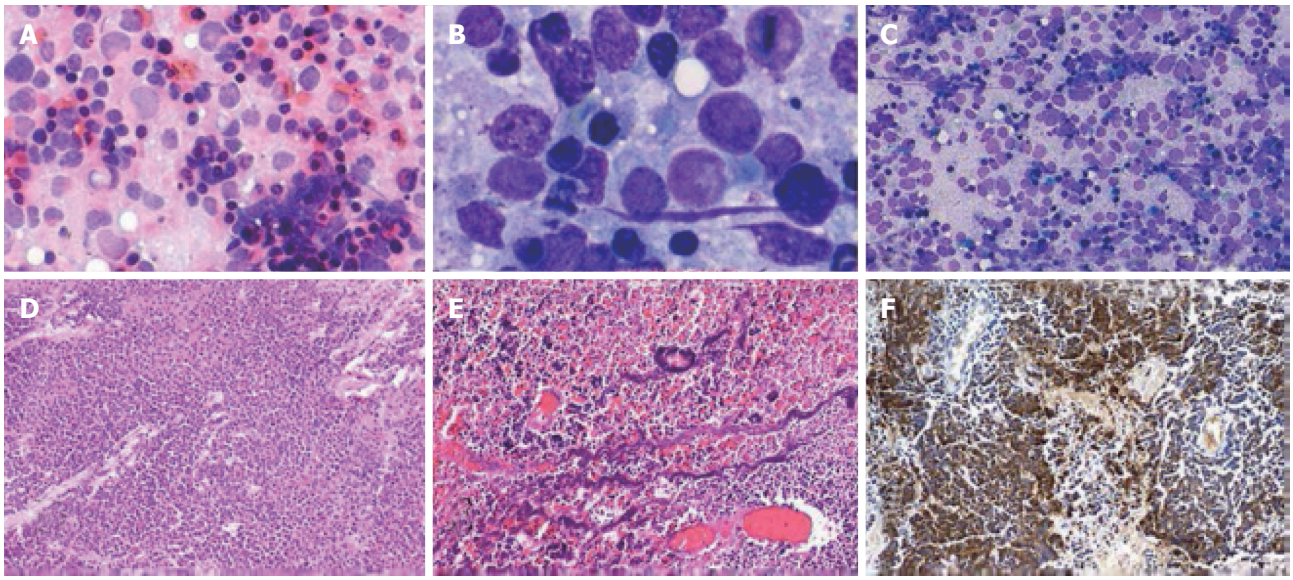


**Figure 1 Histopathology and cytology of lymph node samples of illustrative case 1.** A: Microphotograph showing predominantly dispersed population of tumor cells (MGG × 20 ×); B: Microphotograph showing tumor cells with very high nuclear cytoplasmic ratio, scant cytoplasm, round nuclei and fine granular chromatin (MGG × 100 ×); C: Microphotograph showing tumor cells with many apoptotic bodies (HE × 40 ×); D: Photomicrographs showing clusters of tumour cells with small hyperchromatic nuclei, scanty cytoplasm and apoptosis; E: Photomicrographs showing Azzopardi phenomena (basophilic nuclear chromatin spreading to wall of blood vessels); F: Photomicrographs showing synaptophysin immunostain showing intense cytoplasmic positivity.

reporting, LNEPSCC was staged as limited if it involved either single LN station or if surgical resection with curative intent had been undertaken. The disease was staged extensive if it involved two or more LN regions and/or other body organs.

### *Statistical analysis*

Data from all individual patients (case reports or case series) were entered into a spreadsheet (Microsoft Excel 2016). Data was analyzed using the commercial statistical package SPSS (version 22, IBM Inc.) and is presented in a descriptive fashion as proportions, mean (95%CI) or median (IQR). $\chi^2$ and Mann Whitney *U* tests were used to compare the categorical and numerical data, respectively.

## RESULTS

### *Illustrative cases*

**Case 1**: A 59-year-male, a known case of chronic obstructive pulmonary disease, presented with progressively increasing swelling in the left cervical region of 9-mo duration. He denied any history of fever or night sweats. On examination a 3 cm × 3 cm hard mass was identified in the left cervical region. FNAC and LN biopsy revealed clusters of tumour cells with hyperchromatic nuclei, nuclear molding and scanty cytoplasm (Figure 1). The pancytokeratin staining showed patchy dot like positivity and synaptophysin immunostain had an intense cytoplasmic positivity with an overall morphology suggestive of small cell carcinoma. Contrast enhanced computed tomography of the neck revealed a conglomerate mass of left cervical LN of size 2 cm × 1.2 cm abutting the left sternocleidomastoid muscle (Figure 2). Further evaluation with CECT thorax and abdomen did not show a primary anywhere and a diagnosis of limited disease (LD) LNEPSCC involving the left cervical LN was considered. Patient was unwilling for radical neck dissection and hence was started on platinum based doublet chemotherapy regimen. He was started on a combination of irinotecan (100 mg/m$^2$) and cisplatin (60 mg/m$^2$) each on $D_1$ of three weekly cycle for six cycles. After third cycle of chemotherapy patient developed grade Ⅱ hematological toxicity. A repeat CT of the neck revealed complete resolution of the LN mass. He achieved complete remission and was kept on follow up. Nine months after chemotherapy he again had a locoregional relapse of his disease and presented with a LN swelling of 5 cm × 4 cm. He was reinitiated on the same chemotherapy regimen (sensitive disease) to which he had responded and is currently doing well on follow up with no evidence of metastasis elsewhere in the body.

**Case 2**: A 65-year-old female with no previous comorbid illness presented with a progressively increasing mass over the right side of the neck. She also complained of loss of weight and appetite. She denied any history of cough, hemoptysis, hoarseness of voice, and fever. On examination there was a 6.8 cm × 4.2 cm hard mass in the right cervical and submandibular region that was fixed to the underlying structures. FNAC and a subsequent biopsy from the LN mass was suggestive of SCC morphology (Figure 3). Further evaluation with CECT thorax, paranasal sinuses, and abdomen did not reveal any primary in the lung, sinuses, or the abdomen. CECT of the neck revealed a right LN

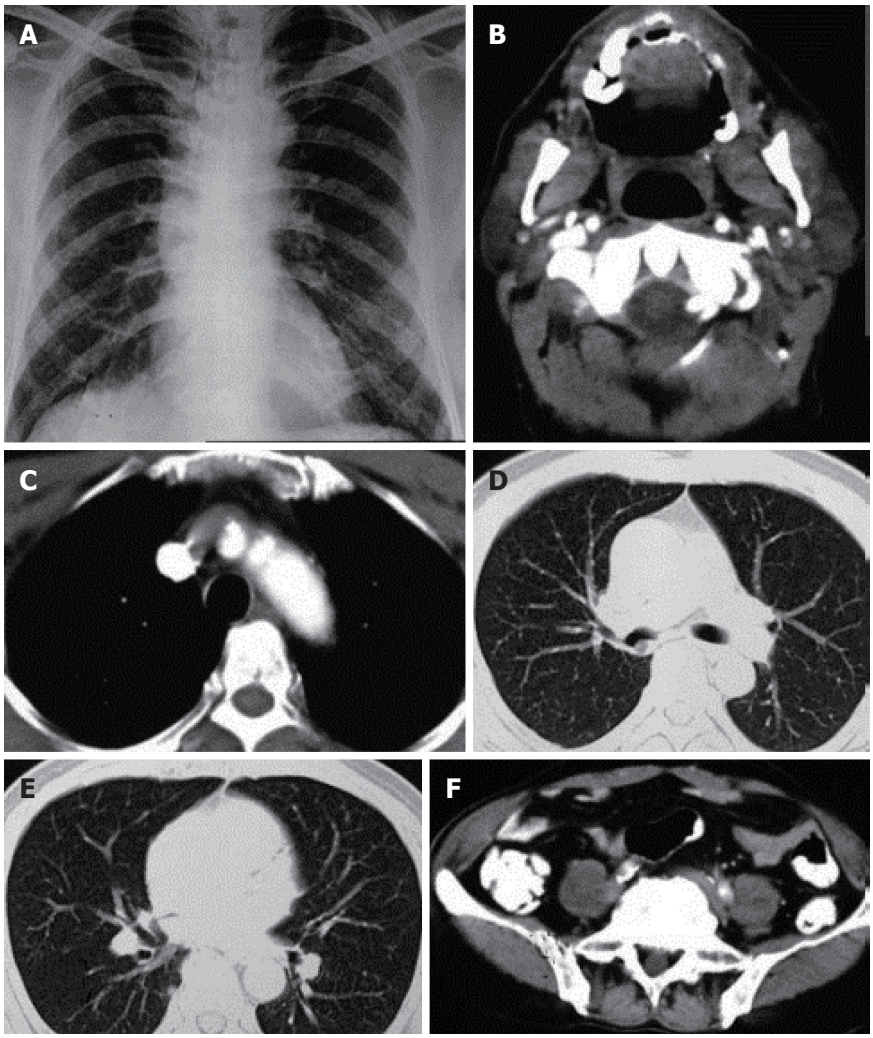


**Figure 2 Thoracic imaging at baseline and after treatment of illustrative case 1.** A: Chest radiograph revealing hyperinflated lung fields with no evidence of any parenchymal abnormality; B: Contrast enhanced computed tomography (CECT) of the neck revealing enlarged right sided cervical group of lymph nodes; C: Mediastinal window of CECT of the thorax with no evidence of mediastinal lymph node enlargement; D and E: Lung window of CECT thorax with no evidence of primary in the lung; F: CECT of the abdomen with no evidence of any abnormality in the abdomen.

mass extending from the submandibular region to the supra sternal region (Figure 4). Patient was diagnosed with extensive disease (ED) EPSCC of lymph node (submandibular and cervical) and was initiated on platinum based combination chemotherapy regimen [intravenous irinotecan (65 mg/m$^2$) and intravenous cisplatin (30 mg/m$^2$) each on $D_1$ and $D_8$ of three weekly cycle for six cycles]. The patient developed grade Ⅱ constipation, and hematological complication (anaemia and leukopenia) that responded to conservative treatment with hematinics and stool softening agent. A partial response was achieved with the chemotherapy. Patient was advised radiotherapy but was unwilling for any further treatment. Three months after the last cycle of chemotherapy she presented with an increase in the size of submandibular lymph node mass and was restarted on same regimen (sensitive disease) to which she had initially responded. The submandibular mass reduced in size clinically. After third cycle of chemotherapy she presented in the emergency department with generalized tonic-clonic seizures and altered sensorium. CECT head revealed bony metastasis to the skull bone invading the underlying brain parenchyma. She was given the option of cranial irradiation but she denied the same and succumbed to her illness 13 mo after her initial presentation.

**Case 3**: A 38-year-old previously healthy male presented with history of gradually increasing swelling in the neck region and loss of weight of nine-month duration. He also complained of fever of three-month duration. He denied any history of cough, hemoptysis, night sweats or hoarseness of voice. On physical examination he had bilateral enlarged cervical LNs (3 cm × 3 cm; 3 cm × 2 cm) that were firm to feel and were freely mobile. Fine-needle cytological examination was performed and he was diagnosed as non-Hodgkin's lymphoma and was referred to our center for further management. At presentation to our center (six months after initial presentation), he had jaundice and the LN size had increased (5 cm × 4 cm; 4 cm × 4 cm). A repeat FNAC was performed from the

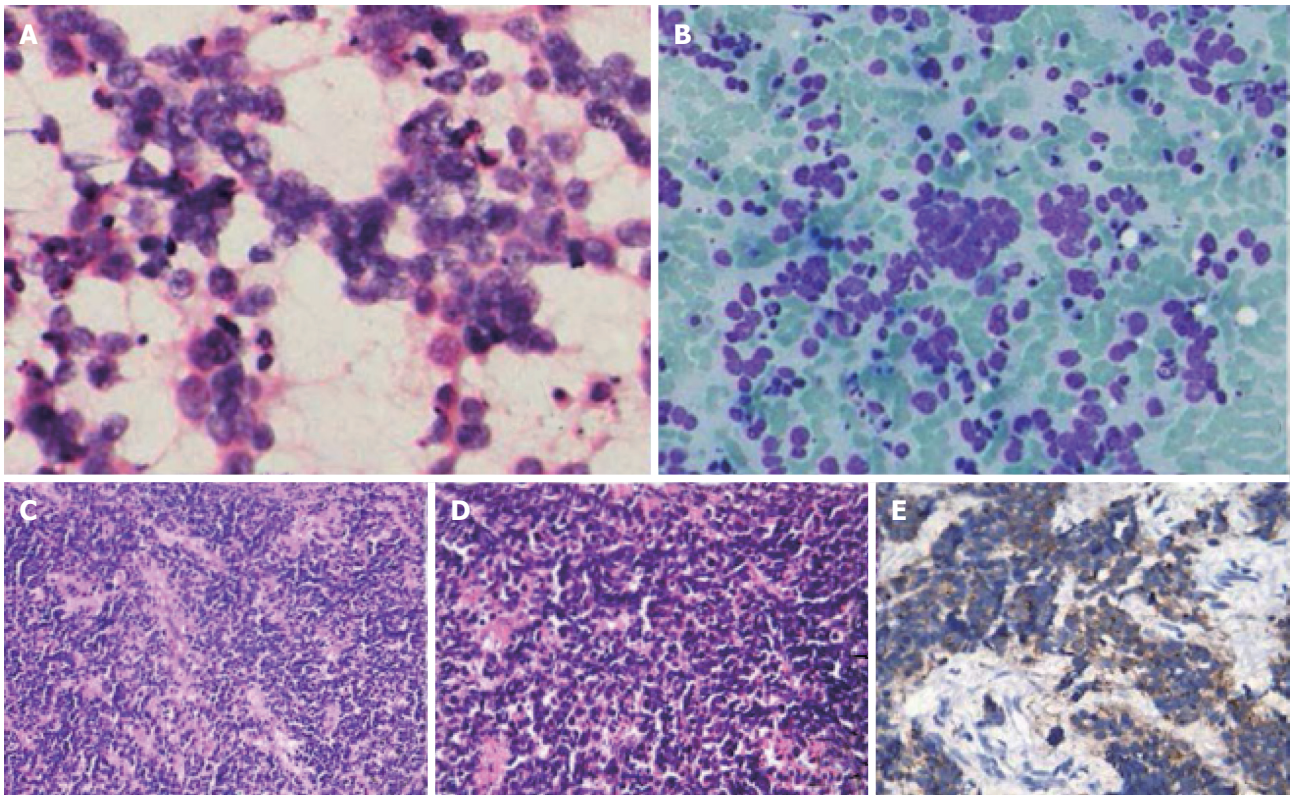


**Figure 3 Histopathology and cytology of lymph node samples of illustrative case 2.** A: Microphotograph showing dispersed population of tumor cells along with few loose clusters. The tumor cells with high nuclear cytoplasmic ratio and showing nuclear moulding (MGG × 20 ×); B: Microphotograph showing small tumor cells with high N:C ratio, salt and pepper type chromatin and inconspicuous nucleoli (HE × 40 ×); C: Photomicrographs showing tumour with extensive crushing artefact; D: Tumour cells having hyperchromatic nuclei, scanty cytoplasm and apoptosis; E: Synaptophysin immunostain showing cytoplasmic positivity.

right cervical LN that revealed small sized tumor cells that were positive for cytokeratin, and synaptophysin and negative for CD-3, CD-20 suggestive of SCC (Figure 5). A whole body 18F-fluorodeoxyglucose positron emission tomography (FDG-PET CT) was performed that revealed FDG avid LNs in bilateral cervical region with diffuse FDG uptake over background uptake in liver and entire skeleton. A diagnosis of ED LNEPSCC (primary in cervical LN with metastasis to liver and skeletal system) was made. The patient was initiated on palliative platinum based chemotherapy [irinotecan (65 mg/m$^2$) and cisplatin (30 mg/m$^2$) on $D_1$ and $D_8$ of 3 weekly cycle]. He developed grade Ⅳ hematological toxicity (neutropenia and anemia) and deranged liver function test (more than four times the baseline) due to which further chemotherapy was deferred. He succumbed to his illness four week after his presentation at our centre due to disease progression.

### *Systematic review*

Our initial search of the PubMed and EMBASE databases yielded 1189 citations of which 954 were excluded after initial review. Eight studies ($n$ = 14) were included in the current analysis (Figure 6)[16-23]. Studies that did not provide separate information for patients with LNEPSCC[29-41], and the treatment given or follow up were not included in the current review[42-50]. A total of 17 patients including the three index cases (mean ± SD, 59.5 ± 10.8 years; 81.8% males) with LNEPSCC were included in the current analysis (Table 1). There was no difference in the age and gender distribution based on the stage of the disease at presentation (61 ± 10.3 years in LD *vs* 57.6 ± 12.3 years in ED; $P$ = 0.931). Cervical group (9, 52.9%) of LN region followed by submandibular LNs (4, 23.5%) was the most common site of primary disease. Two patients had inguinal LN enlargement while in two patients both submandibular and cervical LNs were involved. Eleven (64.7%) patients had LD as the disease involved only single LN region whereas 6 (35.3%) had ED at presentation. One patient had evidence of involvement of liver and skeletal system, and in two patients' central nervous system was involved at presentation, while three patients were labelled ED as they involved two LN regions (bilateral cervical LN; submandibular and cervical LNs). To rule out primary at other sites, chest radiograph, flexible bronchoscopy, CECT thorax and abdomen was performed in all patients. Whole body PET-CT was done in only one patient (index case 2). Chemotherapy ($n$ = 6, 35.3%) or surgery ($n$ = 4, 23.5%) were the most common form of treatment given to the patients. A combination of chemotherapy with radiation ($n$ = 2, 11.8%) and surgery with radiation ($n$ = 2, 11.8%) were the other forms of treatment. Five patients received radiation (one only radiotherapy, two each combined radiotherapy and chemotherapy, and combined

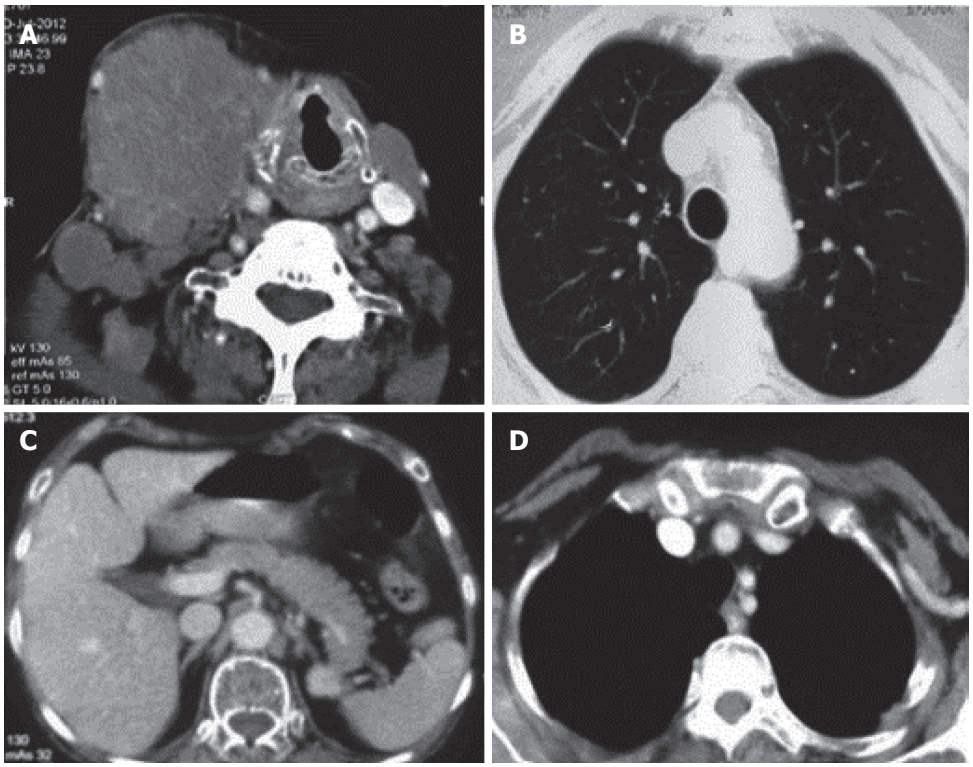


**Figure 4 Thoracic imaging at baseline and after treatment of case 2.** A: Contrast enhanced computed tomography of the neck revealing a conglomerate lymph node mass of size 7 cm × 4 cm involving the right submandibular region. The mass is pushing the larynx towards the left side; B: Contrast enhanced computed tomography of the thorax (lung window) with no evidence of primary in the lung; C: Contrast enhanced computed tomography of the abdomen with normal abdominal organs and no evidence of any primary in the abdomen; D: Mediastinal window of CECT thorax revealing no enlarged lymph node stations in the mediastinum. CECT: Contrast enhanced computed tomography.

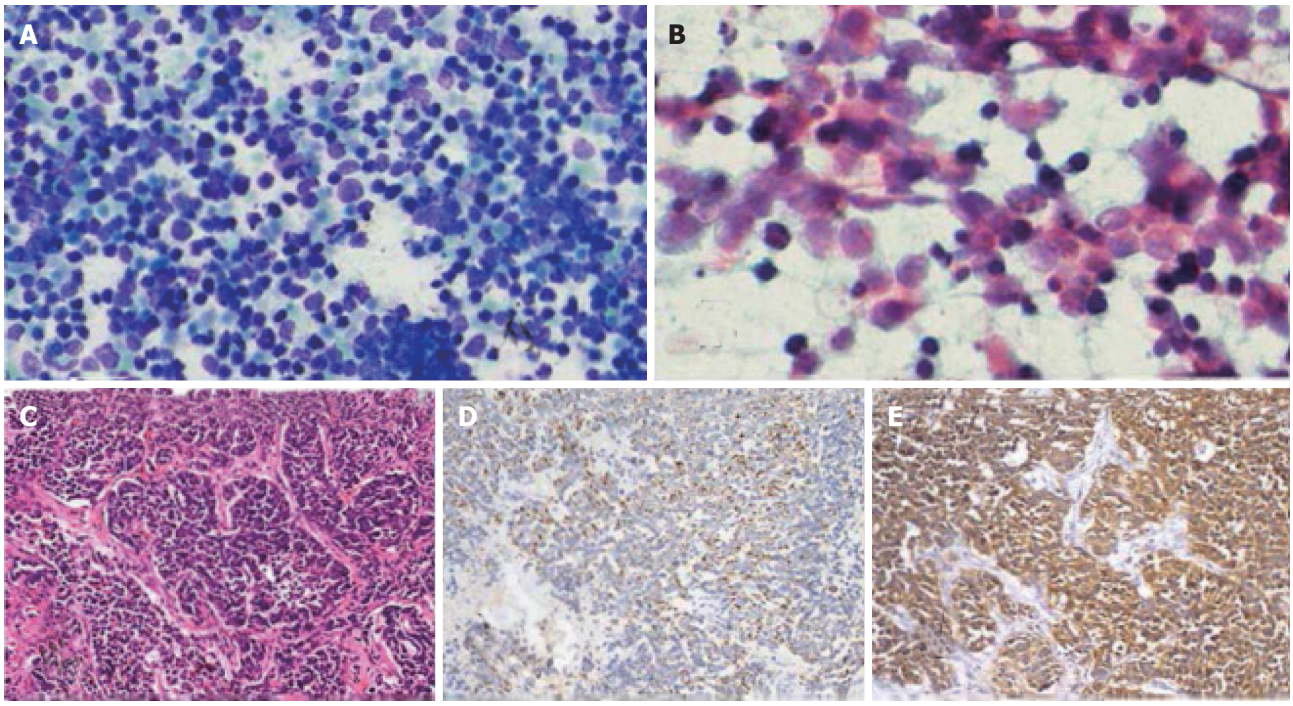


**Figure 5 Histopathology and cytology of lymph node samples of case 3.** A: Microphotograph showing dispersed population of small sized tumor cells with high nuclear cytoplasmic ratio along with many degenerated cells (MGG × 20 ×); B: Microphotograph showing nuclear threading and crushing along with tumor cells with round nuclei, high N:C ratio, salt and pepper type chromatin and inconspicuous nucleoli (Pap × 40 ×); C: Photomicrographs showing clusters of tumour cells with hyperchromatic nuclei, nuclear molding and scanty cytoplasm; D: Pancytokeratin staining showing patchy dot like positivity; E: Synaptophysin immunostain showing intense cytoplasmic positivity.

**Table 1 Details of studies included in the review**

| Ref. | No. of patients | Gender | Age | Stage | Lymph node area involved | Investigations performed to rule out primary elsewhere | Treatment regimen used | No. of cycles received | Response | Complication of treatment | PFS, in months | Overall survival, in months | Site of relapse | Final outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Levenson *et al*[23] | 1 | Male | 49 | LD | Cervical | CXR, FB, radionuclide scan of liver, bone, spleen and brain | CMC-VAP + local RT | NA | CR | NA | 15+ | 15+ | None | Alive with disease |
| Kasimis *et al*[22] | 2 | Male | 60 | ED | Right submandibular | CXR, panendoscopy of larynx, pharynx, bronchial tree and esophagus | Surgery (radical neck dissection) + RT (5860 rads); cyclophosphamide and doxorubicin + cranial irradiation | NA | SD | None | 24 | 36 | Left cervical LN, left scapular region, left parotid gland, left submandibular LN, right tonsil, right testis | Died of disease |
| | | Male | 70 | ED | Right submandibular and posterior cervical | CXR, sputum cytology, bone and liver scan, panendoscopy of larynx, pharynx, bronchial tree and esophagus | None (patient refused treatment) | - | SD | None | 18+ | 40+ | Locoregional enlargement of submandibular LN at 18 mo | Alive with disease |
| Remick *et al*[21] | 1 | Male | 52 | LD | Left cervical LN | CXR, CECT thorax and abdomen, bone scan | RT (46 Gray) | NA | CR | None | NA | 84+ | None | Alive with disease |
| Hainsworth *et al*[20] | 2 | Female | 77 | LD | Inguinal | CXR, CECT thorax and abdomen | Surgery | - | CR | None | 12+ | 12+ | None | Alive with disease |
| | | Male | 61 | LD | Cervical | CXR, CECT thorax and abdomen, bone scan | CAV + RT | NA | CR | NA | 100+ | 100+ | None | Alive with disease |
| Van Der Gaast *et al*[19] | 2 | NA | 55 (26-72) | LD | Cervical LN | CXR, CECT thorax and abdomen, bone scan | CDE (*iv* cyclophosphamide 1 g/sq.m on day 1, *iv* doxorubicin 45 mg/sq.m on day 1 and *iv* etoposide 100 mg/sq.m on days 1, 3 and 5) | Five | CR | Hematological (grade 3-4 leukopenia, neutropenia, thrombocytopenia), nausea and vomiting, alopecia | NA | 22+ | Locoregional | Alive with disease |
| | | NA | 55 (26-72) | LD | Cervical LN | CXR, CECT thorax and abdomen, bone scan | CDE (*iv* cyclophosphamide 1 g/sq.m on day 1, *iv* doxorubicin 45 mg/sq.m on day 1 and *iv* etoposide 100 mg/sq.m on days 1, 3 and 5) + RT (6000 cGy) | Five | CR | Hematological (grade 3-4 leukopenia, neutropenia, thrombocytopenia), nausea and vomiting, alopecia | 12+ | 12+ | None | Alive with disease |
| Galanis *et al*[18] | 4 | NA | NA | LD | Submandibular LN | CXR, CECT thorax and abdomen, bone scan | Surgery | Nil | CR | None | 42+ | 42+ | None | Alive at with disease |
| | | NA | NA | LD | Submandibular LN | CXR, CECT thorax and abdomen, bone scan | Surgery | Nil | CR | None | 42+ | 42+ | None | Alive with disease |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NA | NA | LD | Inguinal | CXR, CECT thorax and abdomen, bone scan | Surgery | Nil | CR | None | 42+ | 42+ | None | Alive with disease |
| | | NA | NA | ED | Submandibular | CXR, CECT thorax and abdomen, bone scan | NA | NA | NA | None | NA | 42+ | None | Alive with disease |
| Orhan *et al*[17] | 1 | Male | 55 | ED | Right cervical LN and multiple cranial metastasis | CXR, CT (thorax and abdomen), FB | Etoposide (100 mg/sq.m on days 1, 3, 5) and cisplatin (80 mg/sq.m on day 1) | Six | CR | None | 7 | 7+ | None | Alive with disease |
| Ochsenreither *et al*[16] | 1 | Male | 68 | LD | Cervical LN | CECT thorax and FB | Surgery and RT | - | CR | None | 5 | 22 | NA | Died of disease |
| Current report | 3 | Male | 59 | LD | Unilateral LN (left cervical LN) | CXR, CECT, neck, thorax and abdomen, FB | Irinotecan (100 mg/sq.m) and cisplatin (60 mg/sq.m) each on D1 of 3 weekly cycle | 6 (3 weekly) | CR | None | 9 | 16+ | Locoregional | Alive with disease |
| | | Male | 38 | ED | Bilateral cervical LNs | CXR, CECT thorax and abdomen and whole body PET CT | Irinotecan (65 mg/sq.m) and cisplatin (30 mg/sq.m) each on D1 and D8 of 3 weekly cycle | 1 (C1D1) | NR | Grade IV hematological (pancytopenia), deranged liver enzymes | Not assessable | 1 | Systemic | Died of disease |
| | | Female | 65 | ED | Right sided submandibular and cervical LNs | CXR, CECT neck, thorax, abdomen and; CT head | Irinotecan (65 mg/sq.m) and cisplatin (30 mg/sq.m) each on D1 and D8 of 3 weekly cycle | 4 (twice weekly; C1D1 and C1D8) | PR | Nausea and vomiting, constipation (grade II) and grade II hematological (anaemia and leucopenia) | 7 | 13 | Increase in cervical LN size and skull bone metastasis with invading the bone | Died of disease |

CAV: Cyclophosphamide, doxorubicin and vincristine; CDE: Cyclophosphamide, doxorubicin, etoposide; CMC-VAP: Cytoxan, methotrexate, CCNU [1-(2-chlorethyl)-3-cyclohexyl-1-nitrosourea] alternating with vincristine, adriamycin, and procarbazine; CECT: Contrast enhanced computed tomography; CR: Complete response; CXR: Chest radiograph; FB: Flexible bronchoscopy; ED: Extensive disease; LD: Limited disease; LN: Lymph node; NA: Not available; PR: Partial response; RT: Radiotherapy; SD: Stable disease.

surgery and radiotherapy). All patients who had ED at presentation received platinum based chemotherapy except one who refused any form of treatment and was kept on follow up. A complete response (CR) was achieved in 12 (70.6%) of the patients whereas one had partial response, and in two patients the disease remained stable. Eleven patients with LD achieved CR whereas only one with ED had CR ($P$ = 0.008). Median (IQR) PFS and overall survival (OS) was 15 (7-42) mo and 22 (12.25-42) mo respectively. In patients with LD the median (IQR) overall and PFS was not significantly different from those with ED LNEPSCC at presentation [22 (15-42) and 28.5 (11.3-52.5) mo *vs* 13 (4-38) and 7 (3.5-21) mo respectively; $P$ value 0.145 and 0.129]. Overall 4 (23.5%) patient died while 13 (76.5%) subjects were labelled as alive with disease at the time of reporting of their individual publications. One patient with LD and three patients in ED died ($P$ = 0.057). Adverse events due to chemotherapy were reported in 4 (23.5%) patients and included grade 2 hematological complications, alopecia, constipation, nausea and vomiting.

## DISCUSSION

The results of the systematic review and the illustrative cases highlight that LNEPSCC is a distinct clinical entity. Patients with disease limited to only one LN region responded well to surgical management consisting of radical neck dissection or regional LN dissection. Patients with ED at presentation responded favorably to

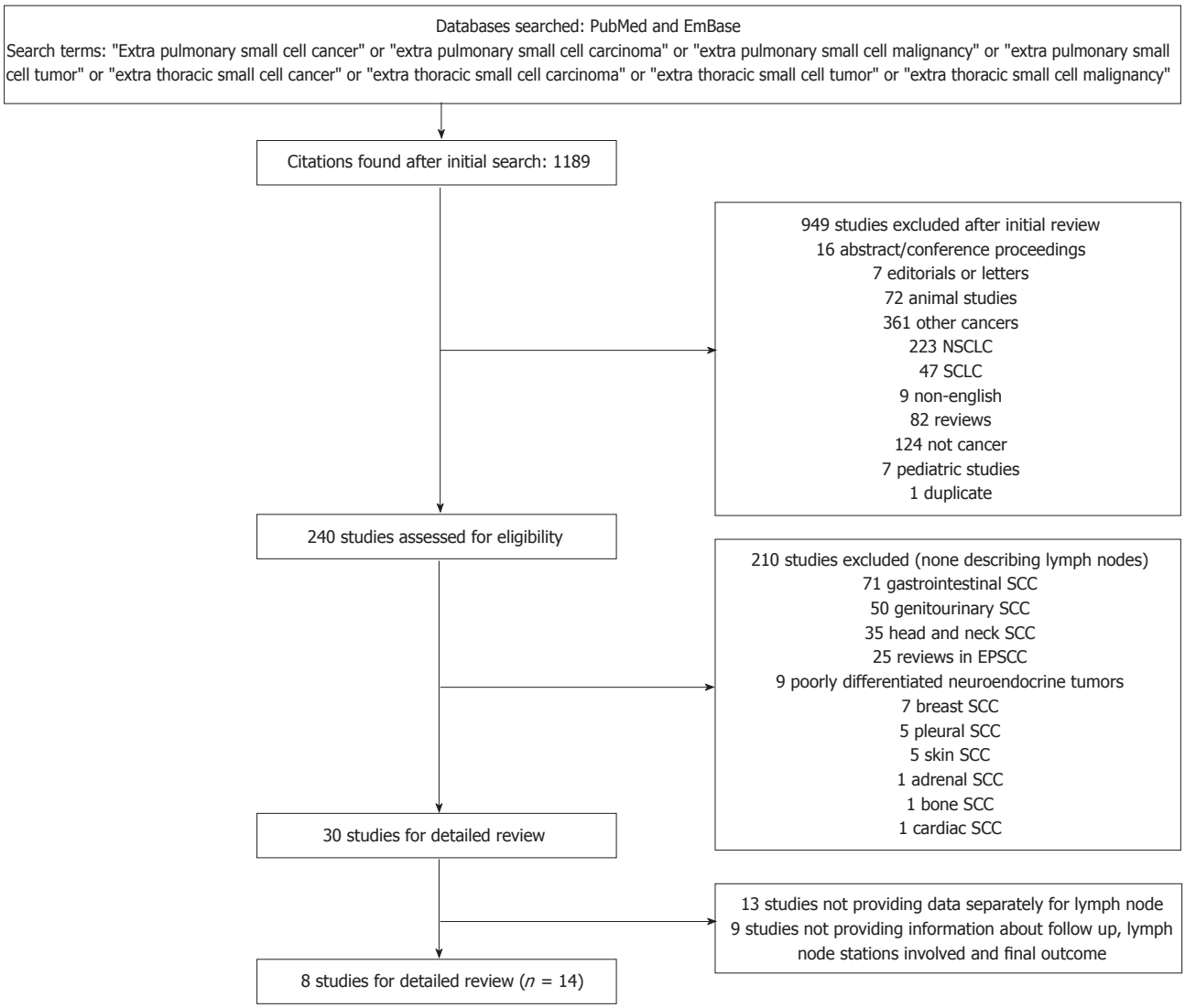


**Figure 6 Study selection process for systematic review.** NSCLC: Non small cell lung cancer; SCLC: Small cell lung cancer; SCC: Small cell cancer; EPSCC: Extrapulmonary small cell cancer.

chemotherapy with or without radiation.

Small cell carcinoma of the lung (SCLC) accounts for 18%-20% of all lung cancers and is invariably associated with a dismal prognosis[51,52]. Median overall survival is 9-10 mo despite treatment in extensive SCLC and 18-24 mo in case of LD SCLC[53,54]. Role of surgical resection in SCLC is limited with treatment being primarily combined chemoradiation[54]. However, SCC has also been described in various other parts of the body including the gastro-intestinal tract, male and female genital tract, musculoskeletal system and others[5,15,55-57]. Extrapulmonary SCLC, especially in the lymph nodes, is an extremely rare entity. Infact, in our more than two decade of experience with lung cancer, we have only seen three patients. However, this is an important condition as if identified then the treatment can result in good response and clinical outcomes, in contrast to SCLC. This is likely to benefit in the patient care and management.

The exact pathogenesis of LNEPSCC is controversial, although several hypotheses exist[28,58,59]. It is believed to arise from multipotent stem cells in the LN and hence a slow growing nature of the LNEPSCC. It can also be due to a primary elsewhere in the body with secondary metastasis to the LNs and spontaneous regression of the primary tumor. However, the fact that patients with LD LNEPSCC had a prolonged survival (median survival 22 mo) makes this theory unlikely. Further, LNEPSCC is cytogenetically different from SCLC as the loss of chromosome 3p, 10q and deletion of chromosome 13 are not seen in EPSCC[60]. It may also be plausible that EPSCC is derived from neuroendocrine amine precursors uptake and decarboxylation cells as neurosecretory granules are frequently seen in the tumor[60].

Apart from pathogenesis, the schema for the staging of LNEPSCC is also uncertain. In the systematic review we could only identify 14 patients with LNEPSCC suggesting it to be a rare disease. An attempt was made to stage LNEPSCC in the current analysis on the basis of ability to perform curative surgery and the number of LN regions involved, and involvement of other organs. If TMN staging process was to be followed, then the stage

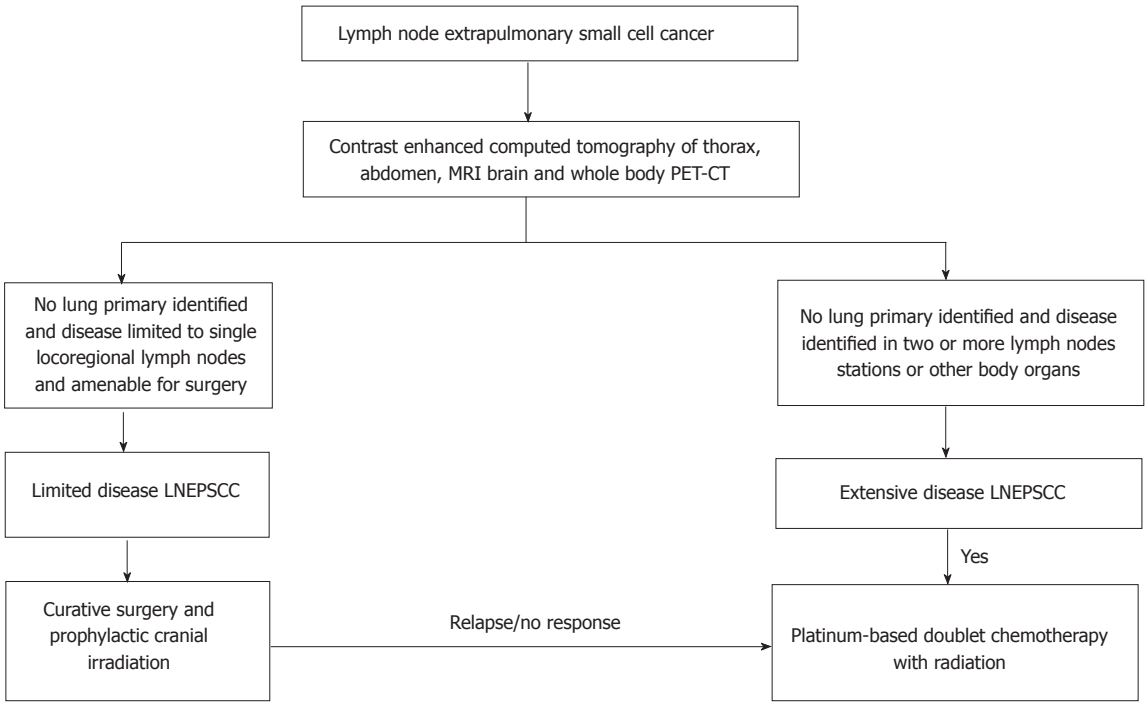


**Figure 7 Algorithm for the diagnostic work-up and treatment of lymph node extrapulmonary small cell carcinoma.** LNEPSCC: Lymph nodes extrapulmonary small cell carcinoma; PET: Positron emission tomography; CT: Computed tomography; MRI: Magnetic resonance imaging.

of all the LNPSCC would have been stage Ⅳ disease or ED that is associated with a poor prognosis and survival outcome[54]. However, this was in contrast to our index cases and the results of the systematic review, where patients with LD LNEPSCC had a longer survival. The PFS and OS in both the LD LNEPSCC and in ED LNEPSCC was higher than that of the SCLC. Thus the staging schema adopted in the current analysis seems appropriate. This has important clinical implications as contrary to LD SCLC where surgery has no role, surgery has a definite role in patients with LD LNEPSCC[18,20,22].

SCC limited to LNs usually develops slowly as highlighted by a prolonged stable disease despite no treatment[22]. However, at an unpredictable time there may be rapid dissemination of the disease and the tumor enters its aggressive phase with an outcome similar to progressive SCLC[20,22]. This was highlighted in one of the index cases that had a fulminant course after diagnosis. Hence, patients presenting with LD LNEPSCC should be treated early with curative surgery (regional LN dissection)[18]. Patients with ED LNEPSCC should also be treated aggressively and early in contrast to SCLC where despite treatment the results are dismal[54]. Treatment with combination chemotherapy, concurrent chemoradiation resulted disease stabilization in five of the six patients with ED LNEPSCC. However, due to the paucity of data and various chemotherapy regimen used with or without radiotherapy, the best treatment option for EDLNSCC remains uncertain. Thus, patients with ED LNEPSCC may be treated similar to patients with ED SCLC until further good quality evidence is available.

Finally, the role of FDG-PET CT (18F-fluorodeoxyglucose positron emission tomography) in evaluating the cases of LNEPSCC needs to be discussed. The studies included in the current systematic review do not clarify this issue. The authors in the various studies have used a combination of chest radiograph, CECT thorax and abdomen and bone scan along with blind mucosal biopsies to investigate the patients with LNEPSCC. This seems reasonable as most patients who were still alive in the studies did not demonstrate primary in the lung or at any other site. However, the advent of PET CT and its inclusion in the diagnostic algorithm will further clarify and enable a better staging process[61,62]. This was highlighted in one of the index cases where performance of PET CT upstaged the tumor to ED with FDG uptake in liver and the entire skeletal system. Hence, future studies should include PET-CT in the diagnostic evaluation of patients with presumed LNEPSCC.

Our systematic review has several limitations. Most of the studies included were either case reports or retrospective data and included only a small number of patients. Further, most studies did not have a complete follow-up data and hence the interpretation of overall survival may not be correct with most patients still alive with disease at the time of publication of studies. However, in rare diseases it is not possible to conduct randomized trials and generate good quality evidence. Also, most reports did not utilize PET-CT in the diagnostic evaluation of the cases. In current era, the authors believe that PET-CT may enable a better understanding and staging of disease and may also pick up small primaries in the lung and should be incorporated in the diagnostic algorithm (Figure 7). Although, Cisplatin and Etoposide is now the current standard chemotherapy regimen, at our center a

combination of irinotecan and cisplatin is preferred and was used in the three illustrative cases[52]. This is because this combination is cost effective and is better tolerated by our patients as the patients. The use of this combination might have resulted in unfavorable outcomes in the illustrative cases.

In conclusion, LNEPSCC is a rare disease and seems to be distinct from SCLC and other EPSCCs. LNEPSCC that remains confined to single group of LN region should be considered for surgical resection with curative intent combined with chemotherapy. Those patients who present late or with an ED should be offered treatment with a combination of concurrent chemoradiation. Prognosis of LNEPSCC seems to be better than that of SCLC in general emphasizing the need for recognition of this unusual entity.

## COMMENTS

### Background

Lymph node extrapulmonary small cell carcinoma (LNEPSCC) is a rare disorder that is characterized by histological evidence of small cell carcinoma in lymph nodes without a primary in the lungs.

### Research frontiers

Contrary to small cell carcinoma of the lungs, LNEPSCC is associated with better overall survival. However, due to the rarity of this disease much needs to be ascertained regarding the staging schema and the appropriate management of patients affected with this entity.

### Innovations and breakthroughs

The current study provides a pooled analysis of all the reported cases in literature and provides a schema for management of subjects with LNEPSCC. The study highlights that subjects who are affected with LNEPSCC have a better overall survival than those with extensive disease (ED) small cell lung carcinoma. For staging purpose, a primary in the lungs should be ruled out with the help of contrast enhanced computed tomography of thorax and a whole body 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) to detect the overall extent of the disease. LNEPSCC should be staged as limited if it involves either a single LN station or if surgical resection with curative intent can be undertaken. The disease is staged extensive if it involves two or more LN regions and/or other body organs. Subjects who have disease limited to only one LN region should be offered surgical management consisting of radical neck dissection or regional LN dissection with a curative intent. Subjects with ED at presentation respond favorably to chemotherapy with or without radiation.

### Applications

This review suggests that LNEPSCC is a rare disease and seems to be distinct from small cell lung cancer (SCLC) and other EPSCCs. The diagnostic algorithm for future studies should include whole body PET-CT. LNEPSCC that remains confined to single group of LN region should be considered for surgical resection with curative intent combined with chemotherapy. Those patients who present late or with an ED should be offered treatment with a combination of concurrent chemoradiation.

### Terminology

Prognosis of LNEPSCC seems to be better than that of SCLC in general emphasizing the need for recognition of this unusual entity.

### Peer-review

This review is well-written.

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# Awareness that early cancer lump is painless could decrease breast cancer mortality in developing countries

Pankaj Garg

Pankaj Garg, Department of Surgery, Indus Super Specialty Hospital, Punjab 141001, India

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Correspondence to: Dr. Pankaj Garg, MBBS, MS, FIAGES, Department of Surgery, Indus Super Specialty Hospital, Phase 1, Sector 55, Mohali, Punjab 141001, India. drgargpankaj@yahoo.com
Telephone: +91-950-1011000
Fax: +91-172-2594556

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## Abstract

There are several factors which contribute to patients' reporting late to healthcare facility even after detecting the breast lump (patient delay). Amongst these, one of the important factors in low- and middle-income countries is lack of awareness that early cancer lump is painless (ECLIPs). Pain is often taken as a danger sign and absence of pain is often not taken seriously. The studies have shown that up to 98% of women in low-income countries are unaware that a painless lump could be a warning sign of early breast cancer. This fact is significant because this could be one of the prime reasons for the women having discovered a painless lump in the breast, accidentally or by breast self-examination, presume it to be harmless and don't report early to health care facility. Therefore, creating awareness about ECLIPs could be an effective strategy to reduce mortality due to breast cancer in low- and middle-income countries. Moreover, unlike modifying risk factors which requires long term behavior modification, creating awareness about ECLIPs is easy and cost effective.

**Key words:** Breast; Cancer; Screening; Lump; Pain; Painless



**Core tip:** Breast cancer mortality is quite high in low- and middle-income countries (LMICs) despite low incidence levels in these countries. One of the major reasons for this is late presentation of patients to clinicians in LMICs. The late presentation can be due to either inability to detect lump early or late realization that the detected lump can be cancerous. For the latter, lack of awareness that early cancer lump is painless (ECLIPs) is one major reason. Moreover creating awareness about ECLIPs is easy and cost effective. Therefore ECLIPs awareness should be part of breast cancer national programs in LMICs.

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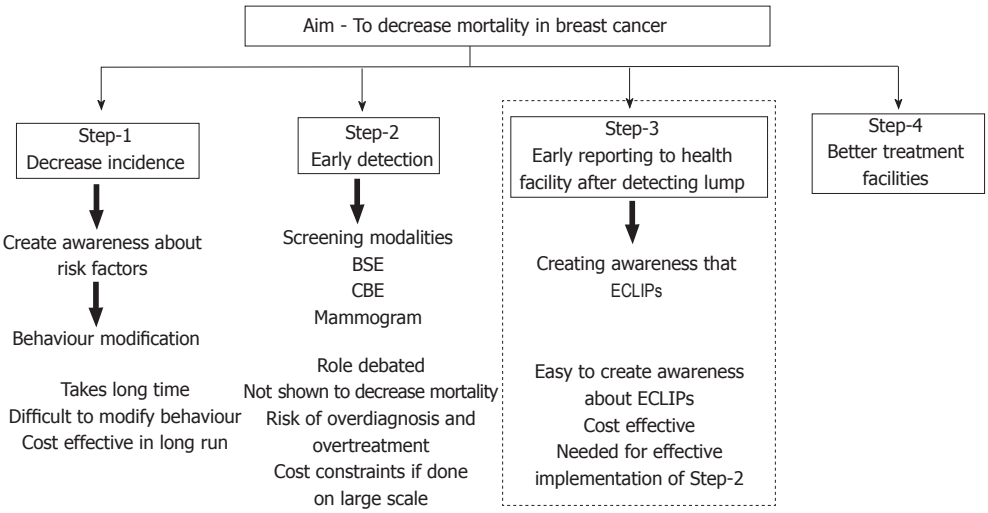


| | |
|---|---|
| Step-3 | More relevant in low-income countries (high levels of illiteracy) |
| Step-1 | More relevant in developed countries (high incidence) |
| Step-2 and 4 | Important in developed and low-income countries |

**Figure 1 Role of awareness about early cancer lump is painless in overall management of breast cancer.** BSE: Breast self-examination; ECLIPs: Early breast cancer lump is painless; CBE: Clinical breast examination.

## TO THE EDITOR

I read with great interest the article titled "Lay perceptions of breast cancer in Western Kenya". The article highlights the dismal level of awareness about breast cancer causes and symptoms in low- and middle-income countries (LMICs)[1]. Primary as well as secondary prevention can play an important part to decrease mortality due to breast cancer. In this context, an important aspect of secondary prevention [awareness about the fact that early breast cancer lump is painless (ECLIPs)] assumes quite importance in LMICs where the level of education is quite low.

The incidence of breast cancer in low-income countries is three to four times lower as compared to the developed countries (25.8 *vs* 95 per 100000)[2]. But the pressing issue in low-income countries is high mortality despite low incidence (12.7 *vs* 17.1 per 100000)[2]. The main reasons for this are delay in detecting a lump, delay in availing treatment even after detecting a breast lump (patient delay), and inadequate specialized healthcare facilities (hospital delay and inadequate treatment).

The strategies to tackle this are modifying risk factors (to reduce incidence), regular screening (to detect early), decrease patient delay, and provide better treatment facilities (Figure 1).

Modifying risk factors require behavior modification which is difficult and take much longer time. Regular screening by mammography, and clinical breast examination and provision of better healthcare facilities is hindered by logistical constraints[3]. Breast self-examination (BSE), though cheap, has sensitivity of only 12%-14%, has no positive effect compared to those who are not performing it and it also leads to false positives[4].

Amongst the patient delay factors, one of the important factors is lack of awareness about ECLIPs[5]. In low-income countries, due to high prevalence of illiteracy, pain is usually taken as a danger sign and presence of a painless lump is often not taken seriously and is ignored[5]. In these countries, up to 75% of women perceived breast pain as a symptom of breast cancer[6] and up to 98% of women were unaware that a painless lump could be the first warning sign of a breast cancer[7]. This fact is significant because this could be one of the prime reasons for the women having discovered a painless lump in the breast, accidentally or by any other screening method, presume it to be harmless and don't report early to health care facility. Therefore, creating awareness about ECLIPs could be an effective strategy to reduce mortality due to breast cancer in LMICs. Moreover, creating awareness about ECLIPs is easier. It is a single small fact which can easily reach large populations if highlighted properly at various platforms including media. This step (by decreasing patient delay) would also increase the efficacy of screening. Therefore ECLIPs awareness is a logical, easy, and cost effective strategy to decrease breast cancer mortality in LMICs and should be a part of national cancer management strategies in these countries.

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