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Examined lymph node count for gastric cancer patients after curative surgery

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Abstract

Lymph node metastasis is the most common form of metastasis in gastric cancer (GC). The status and stage of lymph node metastasis are important indicators that reflect the progress of GC. The number of lymph node metastasis is still the most effective index to evaluate the prognosis of patients in all stages of lymph node metastasis. Examined lymph node (ELN) count refers to the number of lymph nodes harvested from specimens by curative gastrectomy for pathological examination. This review summarises the factors that influence ELN count, including individual and tumour factors, intraoperative dissection factors, postoperative sorting factors and pathological examination factors. Different ELN counts will lead to prognosis-related stage migration. Fine lymph node sorting and regional lymph node sorting are the two most important lymph node sorting technologies. The most direct and effective way to harvest a large number of lymph nodes is for surgeons to perform *in vitro* fine lymph node sorting.

INTRODUCTION

Metastasis is one of the lethal biological characteristics of malignant tumour cells. Lymph node metastasis is the most common form of metastasis in gastric cancer (GC). The proportion of lymph node metastasis can reach more than 50% in GC with deep submucosal invasion[1]. The status and stage of lymph node metastasis are important

indicators that reflect the progress of GC. Lymph node metastasis can remarkably affect therapeutic effect and clinical prognosis[2]. The lymph node metastasis staging methods that are used to evaluate the prognosis of patients with GC after radical resection include the range of lymph node metastasis, the number of lymph node metastasis, lymph node ratio, the maximum diameter of lymph node metastasis and the log odds of positive lymph nodes[3-6]. A number of clinical analysis and research results for prognosis evaluation show that the number of lymph node metastasis is still the most effective index for the evaluation of the prognosis of patients in all stages of lymph node metastasis[7-9]. The criteria of the lymph node metastasis (pN) staging of GC in the Union for International Cancer Control and American Joint Commission for Cancer (AJCC) are constantly changing and updating. Therefore, how to accurately evaluate the number of lymph node metastasis is still the focus of clinical attention. The examined lymph nodes (ELNs) used to determine the number of lymph node metastasis are from dissected lymph nodes (DLNs). ELN count refers to the number of lymph nodes harvested from specimens by curative gastrectomy for pathological examination. This article summarises the implication of ELN count for patients with GC after curative surgery.

INFLUENCING FACTORS OF ELN COUNT AFTER CURATIVE GASTRECTOMY

The accuracy of the evaluation of lymph node metastasis depends on standardised lymph node harvesting and subsequent detailed pathological examination. Therefore, ELN count is affected by the following factors(Table 1).

Individual and tumour factors

Individual differences in immune status, disease stage and the biological behaviour of tumour cells in different patients can lead to a certain difference in the number of perigastric lymph nodes. Lymph nodes are derived from the differentiation and development of endothelial cells in lymphatic vessels or lymphatic sac and their surrounding mesenchymal cells in the embryonic period; that is, in theory, lymph

nodes can be formed in areas where lymphatic vessels are located. This localization can also be considered a potential reason for the recurrence of local lymph node metastasis after standardised lymph node dissection for GC. A variety of tumour-derived driving factors, including multiple antigens, cytokine growth factors and exosomes, can be drained to tumour regional lymph nodes through the lymphatic duct system and then regulate the immune response, remodel lymphatic vessels and induce microenvironment adaptation and the metastasis and colonisation of cancer cells[10]. Dikken *et al*[11] showed that ELN count in female patients with GC after surgery is higher than that in male patients. The difference between the two sexes may be related to the difference in their immune system status. They also showed that ELN count in young patients is higher than that in elderly patients because elderly patients have weak immune response to tumour; thus, the elderly may be subjected to a more conservative strategy of intraoperative dissection. Kodera *et al*[12] showed that obesity can affect ELN count in patients with GC. ELN count was considerably reduced in surgical patients with BMI ≥ 27 kg/m^2 compared with male patients with BMI < 25 kg/m^2 and female patients with BMI < 22 kg/m^2. Obesity may have a negative impact on ELN count by increasing the difficulty of surgical dissection and lymph node identification. Tumour stage is also one of the factors that affect the detection of lymph nodes. The T stage of a tumour affects ELN count after surgery[13]. A higher T stage is related to more harvested lymph nodes. Although preoperative chemotherapy can inhibit tumour cells in perigastric lymph nodes to a certain extent and even achieve N-stage down-regulation, no evidence shows that it can remarkably affect ELN count[12].

Intraoperative dissection factors

DLN refers to the number of lymph nodes included in the surgical specimens removed from the abdominal cavity of a patient according to the radical range determined by the GC staging of the patient. DLN count is determined by the extent of lymph node dissection and the number of lymph nodes around the stomach during surgery. D1+ lymph node dissection is currently the main choice for early GC, and D2 Lymph node

dissection should be necessary for advanced resectable GC. The number of lymph nodes around the stomach in total gastrectomy is more than that in subtotal gastrectomy. Therefore, more lymph nodes can be dissected for postoperative pathological examination. Lu *et al*[14] reported that the average number of lymph nodes removed by subtotal gastrectomy and total gastrectomy are 26 ± 9.6 and 29 ± 10.7 ($p<0.01$), respectively. In the same way, the total number of lymph nodes dissected in patients with early GC who underwent partial gastrectomy and with preserved function may be decreased because parts of the perigastric lymph nodes do not need to be dissected. With the development of minimally invasive technology, laparoscopic gastrectomy can reduce intraoperative blood loss, accelerate postoperative recovery and shorten hospital stay. Bouras *et al*[15] showed that the number of lymph nodes detected in laparoscopic surgery is less than that in open surgery (26.7 *vs* 31.4, $p<0.05$) at the same TNM stage possibly because the extent of lymph node dissection in laparoscopic surgery is often less than that in open surgery. However, a meta-analysis of 12 studies comparing minimally invasive surgery with open surgery showed that laparoscopic surgery does not reduce the number of lymph nodes detected compared with open surgery[16]. Therefore, the effect of laparoscopic surgery on DLN count need to be further studied. In addition, the qualification of the surgeon has a direct impact on DLN count[17-19].

Postoperative sorting factors

Theoretically, ELN count should not exceed DLN count. A trained person needs to sort the lymph nodes from each group in the perigastric region one by one from the surgical specimens of GC and make corresponding records before sending the harvested lymph nodes for examination. Different sorting methods may lead to different lymph node counts. Almost all oncologists agree that postoperative factors can directly affect the follow-up diagnosis and treatment of cancer[20]. In the postoperative sample processing, the omission of small lymph nodes will likely cause an error in metastatic lymph node count, which will directly lead to the downgrading of TNM staging based on the

number of metastatic lymph nodes and cannot objectively reflect the actual situation. Noda *et al*[21] showed that 37.9% of lymph nodes with metastasis have a maximum diameter of less than 5 mm; hence, 37.9% of metastatic lymph nodes in GC specimens may be missed if 5 mm lymph nodes are not found. Downstaging will occur in 14.9% and 4.2% of the cases if all nodes less than 6 and 4 mm respectively are ignored. Hanna *et al*[22] pointed out that the proportion of smaller lymph nodes in ELNs showed an upward trend with the increase in ELN count. Different countries have differences regarding whether surgeons or pathologists carry out the sorting work after surgery. This work is done by pathologists in most European and American countries, whereas the procedure is done by surgeons in Japan. Bunt *et al*[23] compared the differences of lymph node detection in Europe, America and Japan and suggested that the sorting of lymph nodes should be done by surgeons immediately after surgery. The average number of lymph nodes harvested by surgeons after D2 gastrectomy is 60 ± 24.1, which is significantly higher than that (31 ± 16.4) harvested by pathologists *($p<0.001$)*. In Japan, the lymph nodes of different groups in the perigastric region are sorted by experienced surgeons immediately after curative resection; therefore, the number of lymph nodes harvested for GC surgery in Japan has always been in the leading position in the world with an average of 39.4[24]. By contrast, some Western pathologists object to post-operative lymph node sorting because it will destroy the edge of the tumour[22].

Pathological examination factors

Lymph nodes are fixed with neutral formaldehyde solution, embedded in paraffin and sectioned in the pathology department prior to the assessment of lymph node metastasis. This routine postoperative procedure can directly affect ELN count. The discovery of extranodal soft tissue and skip metastases has led to some controversy on the pathological diagnosis of lymph node metastasis. Some studies suggest that extranodal soft tissue nodule is a risk factor for the prognosis of patients with GC, and the postoperative survival rate of patients decreases considerably with the increase in the number of extranodal positive soft tissue nodule[25, 26]. Several extranodal soft tissue

nodules can be found microscopically. In fact, the structure of lymph nodes is partially or completely destroyed by the proliferation of metastatic GC cells, which makes it impossible to identify them correctly. Therefore, pathologists can only judge them as soft tissue nodules. A similar situation can also be seen in the destruction of lymph node structure after multiple preoperative radiotherapy. Although the impact of skip metastasis on the prognosis of GC remains controversial, it is still a negative factor affecting the survival of patients. The occurrence of skip metastasis is related to low DLN count; hence, the number of lymph nodes in a pathological section is difficult to determine[27]. In theory, lymph nodes have occult tumour cells (including micrometastases and isolated tumour cells), but serial sections of lymph nodes are difficult to carried out[28]. Many clinical reports still support that lymph node micrometastasis should be considered an unfavourable factor affecting the prognosis of patients[29].

In addition, fat clearance technology can also improve the detection rate of pathological lymph nodes[30-33]. Candela *et al*[34] reported a fat clearance technique applied to the treatment of GC specimens after operation. The average ELN count was increased from 20 to 36 by using different concentrations of alcohol and coniferous oil as pretreatment before staining, which improved the accuracy of staging. The ELN count by this method is higher than that reported by Japanese scholars in the same period, and this method has obvious advantages in detecting smaller lymph nodes. Aoyama *et al*[35] treated samples with 10% formaldehyde aqueous solution containing methylene blue for 48 h. The lymph nodes and lymphatic network were clearly displayed; therefore, the ELN count was increased (43.4 *vs* 33.6, $p=0.005$), and the efficiency of lymph node detection was improved (1.49/min *vs* 1.12/min, $p=0.010$). A meta-analysis included 27 studies on the application of fat clearance and methylene blue staining in the detection of lymph nodes in gastrointestinal tumour samples[36]. The results showed that compared with the traditional manual method, the two techniques could increase ELN count, harvest more metastatic positive lymph nodes and improve the identification of small lymph nodes. Carbon nanoparticles can be selectively absorbed by lymphatic vessels. Li

et al[37] applied nanocarbon to the surgery of advanced GC, which could increase ELN count (38.33 in the nanocarbon group and 28.27 in the control group, *p=0.041*) and identify smaller lymph nodes (the maximum diameters of lymph nodes in the nanocarbon and control groups were 3.32 and 4.30 mm respectively [*p=0.023*]). In addition, indocyanine green can be used as a tracer for lymph nodes in GC[38]. However, indocyanine green depends on special laparoscopic equipment during the surgery and cannot develop colour in pathological sorting.

ELN COUNT AND LYMPH NODE STAGE MIGRATION

The depth of primary invasion (pT) and distant metastasis (M) can be directly determined by pathologists under high-power microscope in the current AJCC postoperative pathological staging (pTNM) system. The final pathological report of lymph node metastasis stage may have errors, such as the Will–Roger phenomenon, due to the existence of lymph node dissection range, ELN count, disease stage and patient individuality and other factors[39]. Will–Roger phenomenon refers to the positive correlation between the number of lymph node metastasis and the range of lymph node dissection. Lymph node stage migration can be gradually reduced or avoided through the increase in lymph node dissection range. Therefore, ELN count for curative gastrectomy is closely related to lymph node stage migration. The clinical data of a large sample of patients undergoing radical gastrectomy in a single centre in China showed that the number of metastatic lymph nodes is positively correlated with the increase in ELN count[40]. The survival data of 7620 patients with GC from three centres in China suggest a substantial migration of postoperative lymph node stage (pN stage), especially in early stage patients with less than 15 Lymph nodes (pT1NanyM0 stage) and advanced stage patients with less than 35 Lymph nodes (pT2–4NanyM0 stage); hence, the 5-year survival rate of patients with different stages in China is obviously low than that in Japan, South Korea and other medical centres[7]. Sano *et al*[24] found that the proportion of patients with pN3b (8.7%) from East Asian countries except Japan and South Korea (including 979 patients in China) with a low number of lymph nodes (24.8

per case) is almost twice as high as those in Japan and South Korea. Some studies have shown that the survival rate of patients with positive lymph node metastasis whose ELN count is more than 30 is the highest in the same subgroup of patients with pN stage; this prognosis-related stage migration is also caused by difference in ELN count[41].

In 2005, Smith used the Surveillance, Epidemiology and End Results database to analyse 3814 patients with GC with equal staging of T1–2N0, T1–2N1, T3N0 and T3N1[42]. The survival time of patients with more than 15 Lymph nodes detected was better than that of patients with less than 15 Lymph nodes detected at the same stage. The 5-year survival rate increased by 5.7%–10.9% for every 10 additional lymph nodes. Volpe *et al*[43] analysed 114 patients who underwent proximal gastrectomy (including D1, D1+, D2 and D2+) and found no remarkable relationship between ELN count and overall survival. However, for patients who underwent extended radical gastrectomy (D2 or D2+), the median survival time of patients with more than 15 Lymph nodes detected increased from 25 mo to 42 mo. In 2009, the authors also found that according to the 6th edition of TNM staging of GC, patients with no less than 15 Lymph nodes have remarkably longer postoperative overall survival time, disease-free survival time or survival time than patients with less than 15 Lymph nodes after recurrence[44]. We also found that increased ELN count is an independent factor affecting the survival time of patients with GC who only have perigastric lymph node metastasis (only lymph node metastasis on the greater curvature and lesser curvature side)[45]. Therefore, in the 7th edition of the TNM staging of GC, the recommended number of lymph nodes was changed to no less than 16. The reason is that patients with pN3b stage need at least 16 Lymph node metastases confirmed by pathology.

However, 16 Lymph nodes are not the ultimate limit. Kim *et al*[46] pointed out that for patients with advanced differentiated GC, the prognosis when 25 and 40 Lymph nodes were used as the cut-off values of ELN count was also different. The study group with more ELN count had a longer average survival time compared with patients with less than 25 and 40 Lymph nodes detected. Chen *et al*[47] analysed 1363 patients with

curative gastrectomy and found that ELN count and N stage are independent prognostic factors. The 5-year survival rates of N2 and N3 patients with more than 25 Lymph nodes detected are 58.59% and 32.77%, respectively, which are remarkably better than 52.48% and 21.67% of patients with 15–24 Lymph nodes detected in the same period, respectively. The clinical data of 7620 patients with GC undergoing curative gastrectomy in three medical centres in China showed that for the same pN stage (except pN0 stage), the 5-year survival rate of patients with GC who have more than 30 Lymph nodes is 8%–15% higher than that of patients with less than 30 Lymph nodes[7].

For patients with negative lymph nodes, we demonstrated that insufficient ELN count may be a potential risk factor for the postoperative recurrence of GC[48]. An ELN count less than 16 means higher local recurrence rate and peritoneal metastasis rate[49]. Several studies have confirmed that ELN count can affect the prognosis of patients with pN0[49-51]. ELN count is an independent prognostic factor particularly for patients with stage III pN0 GC[52, 53]. In 2017, authors compared the clinicopathological data of pN0 patients in Tianjin Medical University Cancer Institute and Hospital (TJMUCH) and Tokyo Medical University Hospital (TMUH) in the past 10 years and found that ELN count in patients with pN0 GC in TMUH reached 34.84, which was much higher than that in TJMUCH, and the postoperative survival rate of patients was also significantly higher than that in TJMUCH ($p<0.001$)[8]. Further analysis showed that the postoperative survival rate of patients with pN0 GC in TMUH, also increased by 57% with the increase of ELN count. In addition, we also confirmed that increased ELN count can reduce or prevent stage migration in pN0 patients[41].

LYMPH NODE SORTING TECHNOLOGY

Lymph nodes need to be sorted out from the whole specimen obtained by radical gastrectomy according to the location of lymph node regions in each group for postoperative pathological examination, which can provide fine information for the number and location of lymph node metastasis. The most important factor that can

reflect ELN count is the operation of lymph node sorting. Sorting lymph nodes from fresh specimens during or immediately after surgery requires a detailed understanding and affirmation of the whole scope of surgical dissection. Detailed records of lymph nodes after sorting can provide clear information for postoperative pathology to detect the location of lymph node metastasis. In addition, perigastric lymph nodes are easier to harvest from fresh samples, especially in fat and soft tissue specimens, than lymph nodes isolated after neutral formaldehyde immersion.

The two main sorting methods are fine lymph node sorting and regional lymph node sorting. ELN count in the fine lymph node sorting group is much higher than that in the regional lymph node sorting group with the same pT, pN or pTNM stage ($p<0.001$). The number of metastatic lymph nodes in the fine lymph node sorting group was significantly higher than that in the regional lymph node sorting group ($p<0.001$)[54].

Fine lymph node sorting

Japanese scholars have been following "fine sorting," that is, lymph nodes are separated from GC samples and then separated from the soft tissue one by one according to the location of each group of lymph nodes around the stomach and the surrounding soft tissue. This method can harvest a larger number of lymph nodes, which helps in judging the extent of the local invasion of the disease and also provides objective evidence for postoperative pathology report to evaluate the quality of surgery. Schmidt *et al*[55] pointed out that on the basis of D2 Lymph node dissection, the fine sorting of lymph nodes from postoperative *ex vivo* specimens can make the average ELN count in each GC patient reach 40; thus, an accurate lymph node staging can be obtained for the prognosis evaluation of patients. According to the latest research results of the Sloan Caitlin Memorial Cancer Centre, ELN count by the fine lymph node sorting of *ex vivo* specimens by surgeons can be significantly increased (30 *vs* 21, $p<0.0001$) compared with regional sorting or sorting by pathologists[56]. Many scholars have explored a series of methods, including fat clearance and intraoperative marker staining, to achieve a more precise lymph node sorting[34, 57-61]. These methods provide more comprehensive information for the postoperative evaluation of patients with lymph node metastasis;

however, they take a long time, may require toxic reagents and need to be completed in the fume hood and could be difficult for beginners. In general, the most direct and effective way to harvest a large number of lymph nodes is for surgeons to perform *in vitro* fine lymph node sorting.

Regional lymph node sorting

Some scholars in other countries directly separate and label each group of lymph nodes around the stomach from the GC samples together with the surrounding soft tissues instead of separating each group of lymph nodes from the soft tissues one by one. This method saves time. However, the pathologist needs to separate the lymph nodes one by one. Theoretically, a certain stage migration occurs in postoperative lymph node metastasis. Hanna *et al*[22] reported a systematic fat blocking and microscopic search method for regional lymph node sorting to improve the number of regional lymph nodes. In this method, the pathologist obtains all the perigastric, periesophageal and periduodenal fat from the specimen (the fat in the greater omentum is not treated); removes the larger lymph nodes; divides the remaining fat into blocks and obtains single stained hematoxylin and eosin slices from each block to examine the lymph nodes under a light microscope. This method can detect more lymph nodes compared with the ordinary manual lymph node sorting method (66 ± 21 *vs* 50 ± 20, $p<0.05$), but it also has obvious disadvantages. It increases the cost of pathological examination and cannot determine the number of lymph nodes in each group; therefore, its popularisation and application are limited.

CONCLUSION

At present, the number of lymph node metastasis is still the most effective index to evaluate the prognosis of patients. The accuracy of the evaluation of lymph node metastasis depends on the standardised harvesting of lymph nodes and subsequent detailed pathological analysis. Different ELN counts will lead to prognosis-related stage migration. Fine lymph node sorting and regional lymph node sorting are the two most

important lymph node sorting technologies. Although ELN count is affected by many factors, the most direct and effective way to harvest a large number of lymph nodes is for surgeons to perform *in vitro* fine lymph node sorting.

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