The presence of lymph node metastases (LNM) in colorectal cancer is well recognized as one of the most important prognostic factors for long-term outcome (1, 2, 3). It may also determine patients most likely to benefit from adjuvant chemotherapy (4, 5). There is overwhelming evidence to suggest that increased LNH improves staging. More recently it has become evident that the total number of dissected lymph nodes, regardless of the status (positive or negative), can impact on outcome (6-14). It seems logical, therefore, to optimise methodologies of LNH.

This article is designed to study the factors that affect LNH and its impact on quality of pathological examination and surgery.

DOES THE NUMBER OF LNH RELATE TO SURVIVAL?

There is a large amount of literature suggesting that patients with low LNH, specially in stage II disease, have a worse outcome impacting both disease free (DFS) and overall survival (OS) (6 -14).

Several studies have shown a positive correlation between the total number of lymph nodes retrieved and survival. One of the earliest reports recording this association was published in 2003 by Le Voyer et al. (9). In this study that originated from the Intergroup trial INT-0089 with 3,411 patients, multivariate analysis demonstrated that even controlling for the number of metastatic lymph nodes, survival increased as more lymph nodes were assessed.

Subsequently, in a systematic analysis of 17 studies from 9 countries with a total of 61371 patients, Chang et al. (14) showed that in 16/17 reports, extended survival was achieved with an increased number of lymph nodes examined. More recently, Budde et al. (15), analysed 147,076 colon cancer cases extracted from the SEER database were able to certify a small but significant correlation between the number of lymph nodes found in the surgical specimens and survival.

The explanation for the influence of lymph node harvest on outcome remains undefined. The phenomenon of “upstaging” that results from examining a large number of LNS can, in part, explain the prognostic impact of LNH. In a study evaluating 196 subjects, Wong et al. (16) found that node-negative patients had fewer nodes harvested on average (mean, 14 nodes) than patients with metastatic lymph nodes (mean, 20) concluding that stage migration was indeed the result of an increased number of dissected lymph nodes. A similar finding was found by Namm et al. (17) working with the California Cancer Registry. In this study of 7,192 patients the risk of understaging significantly increased according to the number of nodes dissected: for cases with 0 to 3 nodes, 78.3% of patients were pN0; for 4 to 7 nodes, 67.6%; 8 to 9 nodes, 62.1%; 10 to 12 nodes, 59.5% and more than 12 nodes 57.2%. However, more recent reports have questioned this hypothesis. In one of the largest cohorts of patients studied to date with 86,394 cases extracted from SEER, Parsons et al. (18) concluded, using Cochan-Armitage
tests and multivariate logistic regression, that although the lymph node yield increased over time, no significant change was found in the number of metastatic lymph nodes. As in the previously cited studies (6-14) this article described a positive correlation between outcome and lymph node harvest, this benefit was more pronounced in patients with stage II disease. The latter fact coupled with the failure to correlate the total number of dissected lymph nodes with the number of metastatic nodes led to the conclusion that upstaging is likely not the only mechanism by which lymph node yield influences prognosis.

Equitable to this study another group led by Baxter in Canada (19) arrived to a similar conclusion. In a cohort of 11,044 patients they found that upstaging of colon cancer correlated with lymph node number only when the count was low (<5-7 nodes), instead in cases with 7 nodes dissected the likelihood of finding metastatic nodes was the same as if 30 or more nodes were evaluated.

Based on the mentioned studies it is only fair to assume that other mechanism besides accurate staging play a role in the prognostic influence of LNH. In fact several other factors have been shown to influence LN retrieval and likely have a role in the link between LN counts and survival.

FACTORS THAT GOVERN LNH

Tumor factors

Several studies have shown that poorly differentiated tumours result in a higher LNH. The reason for this finding is unclear but it is possible that poor differentiation stimulates a stronger immune response. Similarly, advanced T stage also correlates with a higher lymph node yield (20, 21, 22). It is counterintuitive that histopathological factors associated with adverse prognosis such as poor differentiation and T stage could actually be linked to increased lymph node numbers, however, it is possible that the more potent immunological response elicited by these factors leads to larger nodes that are easier to identify by pathologists and not necessarily to an actual increase in the total number of mesenteric/mesorectal LN.

Anatomical location

Several studies have shown that the number of nodes found in surgical specimens of colorectal cancer decreases with progression from the proximal to the distal colon/rectum (13, 21-24), and significantly less in the sigmoid colon (p<0.001) and the rectum (p=0.001) when compared to the cecum/ascending colon. More nodes have been identified in specimens with tumours arising within the distribution of the superior mesenteric artery compared to the inferior mesenteric vessels (25). It is well recognized that the rectum contains less and smaller lymph nodes compared to other sites of the bowel (26, 27), recently Miscusi et al. (28) described in detail the number and distribution of nodes in the mesorectum, however similar studies addressing this topic in other parts of the bowel are lacking.

Patient factors

Age

Several studies have documented an inverse association between patient’s age and number of LN retrieved from surgical specimens of colorectal cancer. Shen et al. (20) demonstrated that in 434 consecutive colorectal patients at a single tertiary referral centre the mean number of LNs harvested was significantly more in patients aged ≤60 vs >60 (p=0.002). More recently, in a study that collected 128,071 patients, those patients 65 and older had significantly fewer LN harvested compared with younger individuals (11.8 vs 13.6; p=0.001) (29). Interestingly, the difference in LN yield continues with increasing age. The group from John Hopkins demonstrated on data extracted from the SEER that even after the age of 65 this difference can be noted. The pathophysiological mechanism for this phenomenon remains obscure, however it is likely that older individuals have a weaker immune response compared to younger patients leading to a decreased in LN dissected (30, 31) It is also conceivable that lymphoid tissue may undergo involution with increasing age. An important point to consider is that some surgeons could be performing less radical resection in elderly patients with comorbidities to decrease postoperative complications.
Body mass index

There is no evidence based data to support that obesity is linked to a decreased number of LN retrieved from colorectal cancer specimens. Linebarger et al. (32) concluded that, although surgery was prolonged in obese individuals, LN yield was not affected by BMI. In our experience with a 416 patients (unpublished results) with rectal cancer study, 20% of whom had a BMI ≥30 Kg/m², no difference was noted between the obese and non-obese population (median number of LNs 16 vs 17.9 p=0.146) nor in the number of positive lymph nodes (0.9 vs. 1.23 p: 0.066). Furthermore, the proportion of patients with more than 12 lymph nodes in the specimens was similar in both groups (58% vs. 68% p: 0.079).

Tumor-host interactions

In the recent years a tremendous amount of research has concentrated on the interaction between tumor and host and its impact on patient prognosis. For example, it is now recognized that microsatellite unstable colorectal tumours (MSI-H), either those arising in the setting of HNPCC, or those sporadic lesions, often result in an increased number of LN retrieved (33). This discovery could, in some way, be linked to the well-known histological finding of an intense intra and peritumoral lymphocytic infiltrate, suggesting some kind of interaction between the neoplastic cells and the immune system of the host. Some authors have postulated that the molecular mechanisms that lead to abnormalities in the MMR proteins could unleash an immune response that results in an increased in the number and size of mesenteric/mesorectal lymph nodes. A few cutting edge experiments addressing the differential expression of peritumoral T-cell subtypes in colorectal cancer have shed light into the critical role of the local immune response in the overall outcome of patients with colorectal cancer irrespective of the TN stage (30, 31). The relationship between the immunological phenotype within the wall of colon and rectum and the number of LN retrieved has not yet been satisfactorily determined.

Surgeon and pathologist factors

We have listed several patients and tumor factors that clearly impact LNH, all of these variables are, certainly, non-modifiable, however, there is ample evidence that the number of LN found in colorectal specimens also reflects the quality of care provided by surgeons, pathologists and hospitals (25, 34-38). In a study from the US conducted from 1989 to 2005 with more than 1000 patients and 40 surgeons, Dillman et al. (25) demonstrated that colorectal fellowship training and case volume strongly correlated with the number of LN investigated. In this study the proportion of cases in which > 12 nodes were found was 77% vs 63% for the fellowship trained vs non fellowship trained surgeons respectively, the influence that case volume added to the odds of getting 12 nodes was reflected by the significant gap between fellowship trained surgeons, 77% vs low case volume-non fellowship trained surgeons, 51% (p<0.0001). Furthermore pathology practice was also found to influence LN yield, the initial search for lymph nodes was performed only by slicing, visualization and palpation of the mesenteric/mesorectal fat with a median yield of 9 nodes during the period from 1991 to 1997, however as a new standard protocol that used ethanol clearing of the fat in cases in which less than 12 LN were found was applied, the median number of nodes raised to 15 during 1998 to 2005. The role of the pathologist in LNH is critical; Rieger et al. (39) compared the LN yield of a single high volume surgeon who operated at two different institutions with separate pathology departments. In this study, the median count of dissected nodes was 10 and 19 for each pathology department respectively. Furthermore, a change in the lymph node dissection protocol within the former pathology department was able to increase the median number of nodes from 10 to 12.

HOW CAN PATHOLOGISTS ENHANCE LYMPH NODE RECOVERY FROM SURGICAL SPECIMENS?

The use of techniques aimed at facilitating the identification of LN in the mesenteric/mesorectal fat has been established for a long time (40, 41). It was actually a surgeon form Chicago, Gilchrist (43) who in 1938 introduced a fat clearing method that required 6 days for completion, the chemicals employed in this procedure included, besides increasing concentrations of alcohol, substances that have been proven toxic for the operator (xylene and methyl salicylate). The latter fact combined with the
extra time required to process the specimens and the cost added have been the main reason for the lack of adherence to these techniques by the pathology community around the world. An indisputable fact is that the application of these methods result in an increased number of lymph nodes dissected from surgical specimens (40, 41, 42). Furthermore, an important point to emphasize is that not all enhancing methods require fat clearing, the use of other substances free of xylene have produced excellent results at minimal cost. We have introduced a simple approach to handle the pericolonic/perirectal fat that has yielded excellent results (44), in our experience, immersion of the mesentery/mesorectum in pure alcohol for 24 to 48 hours has led to a significant increase in the number of retrieved nodes, and this technique is simple, safe and cheap.

The application of enhancing techniques is particularly pertinent in rectal cancer specimens following neoadjuvant chemoradiation as this treatment modality has been shown to significantly reduce lymph node yield secondary to the lymphoid tissue involution and the consequent reduction in node size (44-48). The significance of this finding remains undefined; Habr-Gamma and Rullier did not find any adverse impact on outcome related to the reduced number of nodes dissected in rectal cancer specimens following preoperative treatment (45, 49). In our experience the use of this method in these cases increased the mean number of dissected LN from 5.2 to 17.3. Surprisingly we were able to demonstrate that the application of our technique led to a significant increase in the number of metastatic LN (44). This finding is of outmost relevance as patients with stage III disease are routinely offered further postoperative chemotherapy. Our results are supported by a more recent study conducted in Spain by Hernanz et al. (50) with a group of 50 consecutive TME specimens, in which LN dissection was attempted initially by traditional palpation and visualization technique followed by a second search applying fat clearance. The authors were able to dissect a mean of 10 more LN per specimen, more importantly, subsequent to the use of this clearing method, three patients were upstaged.

Other enhancing chemical components that do not contain xylene have been applied, one of the more popular substances used for this purpose is the Schawrtz solution similarly to our technique this solution contains ethanol as well as other components that add some toxicity (diethyl ether and glacial acetic acid) and expense, furthermore the mixture of these chemicals has to be performed under a fume hood.

HOW MANY LN WE NEED FOR AN OPTIMAL LNH TO ACHIEVE ACCURATE STAGING?

As a result of the large body of evidence demonstrating the influence of LNH on prognosis, numerous organizations around the world (American College of Surgeons, College of American Pathology, National Quality Forum and others) have proposed that a minimum of 12 lymph nodes needs to be assessed from the surgical specimen. Furthermore, the 12 lymph-node target is currently a requirement for certification by the ACS and CAP. Not surprisingly, insurance companies in the USA have been quite expeditious in implementing this requisite as a way to deny payment. As expected, both surgeons and pathologists have loudly and clearly voiced their discontent regarding the 12 LN rule.

The history of the “12” number commenced in 1990 based on the Working Party Report to the World Congress of Gastroenterology in Sydney (51), this report was based on data from a non-randomized observational UK one-centre study (n=103) published in 1989 by Scott and Grace (41). The authors examined colorectal specimens using conventional LN counting before and after fat clearance (xylene and alcohol). The mean number of LNs identified before and after fat clearance was 6.1 and 18.2 respectively. In LN positive cases (Dukes C/stage III; n=50) they showed that 26/50 cases revealed a positive node after examining 6 LNs vs. 47/50 (94%) when 13 nodes were examined. The total number of Dukes C/stage III cases pre and post-fat clearance was 43.7% and 48.5% respectively. Using these data, Nelson et al in 2001 (52) extrapolated that if one examines 12 LNs, node positivity is correctly identified 90% of the time. No other study to date has been able to unequivocally support the 12 cut-off value. Subsequent publications have recommended using minimum LN values that ranged from 7 to 40 nodes (9, 11, 23, 24, 53). This wide variation maybe related to dissimilar statistical endpoints such
as detection of metastatic nodes vs patients’ outcomes and more relevant, to the median or mean number of LN examined.

The lack of scientific evidence to support a set, arbitrary number of dissected LN that could predict outcomes have prompted researches to find alternative statistical and mathematical methods to assess the adequacy of LN dissection and several studies have addressed this issue. Le Voyer et al. (9) showed that for colon cancer with 1-3 LNM there was an absolute 23% improvement in 5 years OS if more than 40 LN were retrieved and in patients with 4 or more LNM the 5YS following examining >35 V <35 was 71% and 51% respectively. Similarly others (20) showed that the median survival in colon cancer when 1-7, 8-14 and ≥15 LNs were examined was 46, 52 and 67 months respectively. Vather et al. (11) reported that the mean number of LNs examined in stage III who die within 5 years was 13.1 vs. 14.8 in those who remained alive.

Some of these studies have sought a cut off value by applying specific statistical tests, including recursive partitioning or stratification by quartiles. The inaccuracy with these methods lies on the fact that it appears that the survival benefit attached to more nodes examined is in general a continuum without a clear threshold. As already mentioned the threshold that defines quality may be a different one as the one to predict prognosis or the one necessary to LN metastasis. In a study by Hashiguchi (54) with 859 patients evaluated by ROC analysis, the authors demonstrated that the calculated cut-off point of 20 and 18 for all node positive and node negative patients respectively offered the most accurate prediction of relapse free survival at 5 or more years.

It is apparent that the adequate number of lymph nodes that need to be assessed both for prognosis and adequate staging continues to evolve and as we deepen our knowledge into the different factors that influence LN harvest, it is possible that different values apply according to the patient and tumor settings and the target of the analysis.

DOES THE LYMPH NODE RATIO RELATE TO PROGNOSIS?

It has been suggested of late that due to the variability of LNs across individuals it may be more appropriate to investigate the percentage of positive nodes rather than the absolute number. The lymph node ratio (LNR) is defined as the ratio between positive LNs and total number of LNH. The concept was first proposed for colorectal cancer by Berger et al. (55) and subsequently adopted by many others. The question remains concerning the exact figures for the LNR in order to predict outcome but evidence suggests that a higher LNR equates to worsening survival.

Vather et al. (2009) (11) split the LNR of 2,364 stage III colon cancers into deciles, showing a 5-year mortality of 40% to 45% in the lowest deciles (LNR 0–0.10) increasing to 80 to 90% in the highest (LNR 0.91–1.0). Wang et al. (2009) (56) presented the 5-year OS of 24,477 stage III colon carcinomas sub-grouped into 4 LNR groups <0.07, 0.07-0.25, 0.25-0.50, and >0.50 with 5-year OS of 64.8%, 56.2%, 45.1% and 29.6% respectively. The important point remains that whilst many authors present evidence supporting the use of the LNR as a superior prognostic indicator to total LN harvest, calculation of an accurate LNR still relies on an adequate total LNH.

SHOULD LYMPH NODE HARVEST BE USED AS A MEASURE OF QUALITY CONTROL?

Quality improvement has become a major area of development in health care. Finding metrics that reflect high value medical treatment, however, remains a challenge. The use of LNH as a quality indicator in colorectal cancer care was quickly adopted by physician’s organizations as it is simple to measure and clearly associated with outcomes. Indeed, since this indicator was established as a measure of quality of care a significant increase in the number of retrieved nodes was achieved. There is heated discussion among surgeons, pathologists and hospitals in regards to the implementation of LNH as a marker of quality control in colorectal cancer surgery, mainly because the number of LNs dissected from a surgical specimen depends on a variety of parameters that are independent of the quality of care. As already mentioned, several other factors related to the tumour location, stage, differentiation and to each individual patient (age, immune status) need to be considered.
SUMMARY

1. LN harvest is important to accurately stage CRC.
2. Improved laboratory methods enhance full or almost full retrieval of LN in the specimen.
3. Using various figures as optimal numbers needs re-evaluation.
4. There are various factors influencing the LN harvest. Factors influencing LNH like age, size of the mesentery, preoperative treatment, tumour biology, pathologist’s diligence, laboratory method of LNH and quality of surgery is only one of them. Therefore LN harvest should not be used as the sole indicator of good surgery.

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