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PROGNOSTIC INDICATORS OF LYMPH NODE METASTASIS IN UROTHELIAL BLADDER CANCER

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ABSTRACT

Pelvic lymphadenectomy remains the most accurate means to diagnose nodal metastasis from bladder cancer. Retrospective studies have suggested that there may be a therapeutic benefit to an appropriately performed lymphadenectomy at the time of radical cystectomy. The optimal extent of the dissection continues to be debated. Nodal involvement is often considered a poor prognostic finding, although some may experience long-term disease-free survival. We reviewed the literature regarding several possible prognostic variables for patients with lymph node positive bladder cancer, including extent of node dissection, nodal yield, lymph node density, extranodal extension, and lymph node tumor burden. Molecular markers may ultimately provide more accurate prediction of tumor biology.

Introduction

Pelvic lymph node dissection (LND) is a critical component of radical cystectomy for patients with muscle-invasive bladder cancer. Lymph node metastases are identified in approximately 25% of patients undergoing cystectomy for curative intent[1,2,3]. Although lymph node involvement is often associated with a poor prognosis, some patients may experience long-term survival when treated with an appropriate lymphadenectomy with and without systemic chemotherapy. Of those who have lymph node metastasis at cystectomy, 70% will develop a recurrence within 5 years of surgery with a 5-year survival of 35%[1]. Several lymph node variables have been identified that may provide insight to survivorship of patients with both node-positive and node-negative disease. These variables include lymph node yield, extent of LND, number of positive nodes, location of positive nodes, pathologic stage of the primary tumor, presence of extracapsular nodal extension (ECE), size of lymph node metastasis, and lymph node density (LN-d). These factors may be utilized when counseling patients and directing adjuvant therapy. Herein, we review the literature regarding several lymph node prognostic variables for patients undergoing radical cystectomy for urothelial bladder cancer.

Lymph Node Template

Extended versus Standard Template

The benefits of an extended lymph node dissection (eLND) compared to a standard lymph node dissection (sLND) for muscle-invasive bladder cancer have been well documented[1,4]. Many contemporary series reaffirm the benefits of an eLND. A meta-analysis by Mandel and colleagues reviewed eleven studies comparing patients undergoing standard versus extended LND at the time of radical cystectomy. While the template was not uniform across the studies, a majority of the papers defined the superior extent of the sLND as the bifurcation of the iliac vessels and defined the superior extent of an eLND as either the bifurcation of the aorta or level of the inferior mesenteric artery. The meta-analysis revealed an improved 5-year recurrence free survival (RFS) in those patients who underwent eLND compared to those who had a sLND (62% vs. 55%, $p<0.001$). After controlling for confounding variables, the survival benefit remained, even though preoperative prognostic factors were worse in the eLND cohort. Notably, no single study included in the meta-analysis reported a significant difference in rates of complications between sLND and eLND[5]. More recently, Abdi et al compared outcomes of a single cohort of cystectomy patients who underwent eLND versus sLND. The superior extent of the sLND was the level of the common iliac artery while the superior extent of the eLND was the aortic bifurcation. Again, eLND was associated with a better RFS (HR=0.63, $p=0.005$), but was not an independent predictor of overall survival (OS) with a HR of 1.63 ($p=0.84$). There was no difference in terms of hospital stay and complication rates, however, rates of blood transfusion were higher in the eLND group[6].

While many studies have suggested a therapeutic benefit to a more extended lymphadenectomy, these all suffer from relatively small numbers, short follow-up, and retrospective study design. Despite the lack of prospective data, the extent of lymphadenectomy appears to be primarily dependent on surgeon preference and training as opposed to patient age or comorbidity or even the perceived risk of disease. In other words, surgeons tend to perform the same LND for all patients undergoing cystectomy[5,6]. The optimal extent of LND has yet to be proven in a prospective trial. One such trial has been completed in Germany by the Association of Urogenital Oncology, but has not yet been reported. Another prospective randomized multi-institutional trial from the Southwest Oncology Group (S1011) is currently accruing.

Positive Nodes Outside Standard Template

The survival benefit reported for eLND has been ascribed to the more accurate identification of node-positive patients and improved clearance of disease outside of the standard lymph node template. Dorin et al reported the distribution of lymph node metastases in patients undergoing radical cystectomy with a pre-defined lymph node template. Of the node-positive patients, 41% had metastases outside the template of a sLND (above the level of the bifurcation of the common iliac artery)[7]**.** Similarly, Jensen et al analyzed 170 patients uniformly treated with a "super-extended" LND to the level of the inferior mesenteric artery. The authors determined how lymph node staging and positivity are affected if limits of the dissection are changed. If a limited LND (restricted to the obturator fossa and perivesical tissue) was performed, 30% of the node-positive cohort would have been under-staged as node-negative. If a standard template was used, 11% of patients would have lymph nodes left behind. This number drops to 4% if an extended template was utilized. There was no difference in nodal staging if a standard, extended, or super-extended template was used. This study concluded that eLND should be completed to at least the level of the aortic bifurcation and highlighted the importance of extending the limits of LND above the level of the aortic bifurcation if a curative intent is the goal[8].

The benefits of an eLND may also apply to those who undergo cystectomy for non-muscle invasive bladder cancer. Bruins et al described a prospective lymph node mapping study evaluating the incidence and location of nodal metastases in patients with non-muscle invasive bladder cancer. Of the 114 patients included in the study, 9 patients (7.6%) were node-positive; one third of which had involvement of nodes above the level of the aortic bifurcation. All patients with positive lymph nodes above the aortic bifurcation also had positive lymph nodes that would have been identified using a sLND. Thus, extension of the lymph node dissection above the level of the aortic bifurcation may not alter staging in this subgroup, but again may be justified to maximize therapeutic benefit[9].

Lymph Node Yield

The prognostic significance of lymph node yield (total number of lymph nodes reported) remains controversial. Several factors impact the number of nodes reported in the pathologic specimen, including extent of the dissection, meticulousness of the surgeon and pathologist in removing and counting nodes, pathologic processing, patient body habitus, prior therapies, and individual anatomy. Many retrospective studies have concluded that lymph node yield itself is a good prognostic indicator of survival for bladder cancer. This benefit has been demonstrated in node-negative cohorts, but inconsistently proven in node-positive patients. A multi-institutional study of eight tertiary care centers across Germany reviewed over 1200 patients who underwent radical cystectomy with node-negative disease. Those with removal of at least 16 lymph nodes (LNs) showed an increased 10-year disease specific survival (DSS) compared to those with removal of <16 LNs (75% vs. 64%, p=0.001). This study, however, did not review the cohort of lymph node positive patients[10]. Morgan et al studied the effects of lymph node yield on node-negative and node-positive populations. In the node-negative cohort they found that individuals with a LN yield of 1-5 had significantly worse DSS and OS when compared to cohort with >14 LNs removed (OS: HR 1.33, p=0.001; DSS: HR 1.36, p=0.003). The survival benefit of lymph node yield did not hold true for the node-positive population in which no significant difference was seen in OS and DSS[11]. Similarly, a population-based retrospective study showed that lymph node yields of >13 had improved OS and DSS compared to those with yields <5 (OS: HR=1.33 95% CI: 1.12-1.57; DSS: HR=1.36 95% CI: 1.12-1.64). However, on multivariate analysis this significance was only seen in node-negative patients[12].

The seemingly isolated benefit of lymph node yield on node-negative patients can be due to a number of reasons. First, removal of lymph nodes with micrometastatic disease not detected by standard pathologic analysis can increase the likelihood of being oncologically free of disease. Additionally, the number of lymph nodes may represent a confounding variable for improved outcome. Patients with fewer comorbidities may undergo a more extended LND and may have improved survival due to their lack of comorbidities rather than the extent of dissection. Lastly, the Will Rogers phenomenon may be in effect. This phenomenon can be explained by the fact that when a greater number of nodes are examined, more patients are upstaged from node-negative disease to node-positive disease. The removal of true node-positive patients from the node-negative cohort explains the survival benefit seen with increased lymph node yield for node-negative patients.

Limitations of Lymph Node Yield

The association between higher lymph node yields and improved survival remains controversial. Contrary to the aforementioned studies that demonstrated a benefit of lymph node yield on node-negative patients, Park et al did not show a correlation between lymph node yield and long term survival in either node-positive or node-negative populations. Using 18 as cutoff to separate the cohort into two groups, they found that the overall 5-year DSS was 67% vs 69.4% (p=0.679), while the 5 year RFS was 59.4% vs 60.6% (p=0.725)[13].

How is one supposed to reconcile this seemingly conflicting data? One explanation may be rooted in the adequacy of the lymphadenectomy performed and the potential for under-diagnosing nodal involvement with a limited LND. Park et al had median lymph node yield of 18, which was higher than other studies. Most of the studies that showed a correlation between lymph node yield and survival had a cohort of patients with lymph node yields <10 [13]. It may be that there is a threshold above which the number of lymph nodes does not have prognostic value. If, however, one falls below this threshold, long term survival may be impacted, specifically in node-negative populations. This may be due to a failure to diagnose and treat node-positive disease when an inadequate LND is performed.

The definition of an adequate lymph node yield is clouded by the variabilities in nodal counts between patients and institutions. Studies have suggested that the more lymph nodes removed, the better the survival outcome[3,14,15]. May et al and Leissner et al showed a significantly better DSS beyond a cutoff of 16 lymph nodes[2,10]. Alternatively, Herr at al found the most significant cutoff to be 8, while Konety et al using the SEER database found it to be 4[16,17]. It appears that adequacy of nodal yield remains to be determined.

Methods to Increase Lymph Node Yield

If greater lymph node counts are presumed to be representative of a more extensive and adequate LND, how does one change practice to increase lymph node yield during pelvic LND? Bochner et al reported the benefits of submitting pelvic lymph nodes as separate specimens compared to en bloc submissions for pathologic review. Submitting pelvic lymph nodes as separate specimens improved overall yield in patients who underwent sLND (8.5 vs 2.4 nodes, p=0.003) and in those who underwent eLND (36.5 vs 22.6 nodes, p=0.02). It was unclear from this study, however, whether submitting separate specimens increased lymph node yield due to a more extensive dissection by the surgeon, a more meticulous review by the pathologist, or both[18]. Fang et al reviewed the effect on lymph node yield, lymph node positivity, and survivorship after implementation of an institutional policy change which required at least 16 lymph nodes to be examined during pelvic LND. Specimens with less than 16 nodes were resubmitted to detect any additional lymph nodes. The median number of lymph nodes increased from 15 to 20 over an 8 year period.

Lymph node positivity did not change significantly, however, OS increased from 41.5% to 72.3% (p<0.01). Interestingly, there was a corresponding increased use of adjuvant therapy from 7% to 15%[19].

Lymph Node Density

LN-d has been proposed to be a good prognosticator for node-positive patients following radical cystectomy. It is defined as the number of positive lymph nodes divided by the total number of nodes removed. We previously examined ten retrospective studies published from 1993 to 2008 regarding the prognostic significance of LN-d[20]. These studies included 2,027 unique patients with a median follow-up which ranged from 1.8 years to 15.5 years. The median number of nodes removed ranged from 9 to 31 and the median number of positive nodes ranged from 2 to 3. The most common LN-d cut-off point was 20% among these studies. All the studies revealed statistically significant differences between survival outcomes for patients with lymph node densities below the cutoff point on univariate analysis and all but one showed a significant difference after multivariate analysis. Multiple studies dating back to 2003 have demonstrated superiority of LN-d to other prognostic factors such as number of positive lymph nodes, lymph node yield and TNM stage[1,2,16].

Ku et al performed a meta-analysis looking at node-positive patients to determine whether LN-d is a good prognosticator for long term outcomes. 14 studies including 3,311 patients between 2003 - 2014 were analyzed looking specifically at DFS, DSS and OS and their relationships to LN-d at established cut-off points. Pooled HR for DFS, DSS, and OS was 1.45 (95% CI 1.10–1.91), 1.53 (95% CI, 1.23–1.89) and 1.45 (95 % CI, 1.11–1.90), respectively. There was no uniform cut-off point used for LN-d, therefore the threshold for clinically relevant LN-d has yet to be agreed upon. This analysis again confirms the utility of LN-d as a prognostic tool and that higher LN-d is associated with worse survivorship[21].

Minimum Lymph Node Yield for LN-d

Lymph node density simultaneously accounts for both nodal disease burden (number of positive nodes) as well as nodal yield (which as previously described may have prognostic significance as well). Despite the theoretical advantages of LN-d as a prognostic marker, ideal cut-off points have not been established. Variable cut-off points have been proposed with the most common still being 20%[1,2,3,14,15,16]. Others have proposed cutoffs of 18%[22], 25%[23], 12.5%[24] and 11%[25]. Simone et al attempted to establish a range of cutoff points for LN-d and used a tertiary mode of distribution. They found the most significant cutoff range to be between 12 and 30% and thus categorized patients into three groups: <12%, 12-30% and >30%. When comparing groups <12% and 12-30%, they demonstrated a difference in DSS with a HR of 1.51 (p=0.047). This HR increased to 2.89 (p<0.001) when comparing <12% and >30%. These findings were re-demonstrated utilizing an externally validated cohort[26]. Kassouf et al demonstrated similar results using tertiary distribution with cut-offs of <6%, 6%-41% and >41% with corresponding DSS of 47%, 36% and 21% (P<0.001) respectively[27]. It appears that the most useful means of stratifying patients utilizes a 3-tier model, however, further work has to be done to establish the most reliable cut-off.

The minimum lymph node yield required to calculate a clinically relevant LN-d has not been established. It is, however, evident that the utility of LN-d is negatively impacted in the setting of low lymph node yields[22,28]. Kassouf et al demonstrated the improved prognostic accuracy of LN-d in the setting of an eLND compared to that of a limited LND in terms of DSS (eLND HR 4.63, 95% CI 2.50–8.57, P < 0.0001; limited LND HR 1.62, 95% CI 1.05–2.49, P = 0.03). In this study, limited lymph node yield was defined as <25 nodes with LN-d cutoff of 20%[27]. Similarly, Jeong et al found that lower lymph node yield compromised the utility of LN-d. In those with lymph node yields <15, LN-d was a poor prognostic indicator for survivorship. However, in patients with yields >15, LN-d was the only independent predictor of DSS (HR = 4.08, 95% CI 1.10-15.1, P=0.036)[22]. From these studies we can conclude that LN-d is less reliable in the setting of a limited lymph node yield as there is likely an underdiagnosis of nodal involvement. Although the concept of LN-d was originally designed to account for the extent of the lymphadenectomy, it is clear that the utility of this factor is directly associated with nodal yield.

Extracapsular extension

Extracapsular extension has been found to be prevalent in 35-60% of node-positive specimens from radical cystectomy and has long been theorized to have a prognostic role[29]. Mills et al analyzed 83 node-positive patients following radical cystectomy and LND and found that median survival was 16 months in those patients with ECE compared to 93 months in those whose capsule was not violated by tumor (p=0.0004)[30]. Since then, multiple studies have confirmed and replicated their findings, suggesting the important role of ECE in predicting clinical outcomes. Ahn et al performed a meta-analysis looking at the prognostic role of ECE. 1,892 patients from 10 studies met eligibility criteria and RFS, DSS and OS were analyzed. They found significant hazard ratios for RFS and DSS of 1.56 (95% CI 1.13-2.14) and 1.6 (95% CI 0.71-3.05) respectively, but no significance in OS. This study suggests that ECE is likely a reliable predictor of the degree of lymph node involvement and may be used to better stratify node-positive patients[29]. Some limitations of many ECE studies include heterogeneity in the number of lymph node yields, variable templates and differing practices of adjuvant therapy. Still, ECE status may help with decisions regarding adjuvant therapy and counseling of patients.

Size of lymph node metastasis

Size of lymph node metastases has been proposed as a prognostic indicator of survivorship. Two methods of calculating nodal tumor burden have been proposed. The first is aggregate lymph node metastasis diameter (ALNMD) and the second is the size of the largest metastatic focus. Stephenson et al analyzed a cohort of 134 node-positive patients and found ALNMD to be an independent predictor of RFS and OS (adjusted HR 1.1, p=0.02) using a cutoff of 20 mm. In addition, there was improved predictive accuracy when survivorship models incorporated ALNMD to pathologic T-stage, lymphovascular invasion, LN-d, surgical margins, ECE, and Charlson score[31].

Rather than using an aggregate measurement of nodal metastases, Mills et al looked at 83 node-positive patients and analyzed whether the size of the largest metastasis has prognostic value. Using a cut-off of 5 mm they found a significant difference in survival between the two groups (64 vs 16 months, p=0.024)[30]. Similarly, using a cut-off of 1 mm Jensen et al demonstrated an association between lymph node size and survival on univariate analysis (HR: 2.47; 95% CI: 1.01–7.60)[32]. Though these differing concepts of lymph node size have potential prognostic value, they have gained little traction in the literature and need further evidence before being incorporated into clinical practice.

Genetic markers for lymph node metastases

Genetic markers sampled from primary tumors have been applied to predict lymph node metastases in several types of cancers including breast, prostate, and head and neck[33,34,35]. Similarly, gene expression signatures predicting lymph node metastases have been found from primary bladder tumors. These genetic markers can help identify patients who are at higher risk to have lymph node metastases and could benefit from a more extended lymph node dissection. Laurberg et al evaluated 18 primary tumors and 12 matched lymph node metastases from 18 different patients who underwent cystectomy with eLND and identified two separate markers - EDNRA and GEM. Using immunohistochemistry both of these markers' protein expression was localized in the primary tumor cells as well as the lymph node metastases, but not in normal urothelium. They also investigated the similarity of GEM and EDNRA protein expression between matched primary tumors and lymph node metastases and found there was a 94% match rate for EDNRA and 71% match rate for GEM[36]. Smith et al utilized two independent cohorts of patients who underwent cystectomy and eLND for bladder cancer and developed a gene expression model for nodal prediction. They discovered a signature of 20 protein coding genes named LN20 which significantly predicted nodal metastases utilizing an independent validation cohort with an area under the curve (AUC) of 0.67[37]. More recently, Seiler et al generated whole transcriptome expression profiles of 199 patients who underwent RC and eLND. They identified a novel protein coding gene named KNN51 and compared it to two previously described signatures including RF15 and LN20. KNN51 achieved an AUC of 0.82 to predict lymph node metastases and significantly outperformed RF15, which had an AUC of 0.62 and LN20, which had an AUC of 0.46. Expression of KNN51 increased the overall risk of lymph node metastases by 2.65 for every 10% increase in score ($p<0.001$)[38]. While research to identify genomic markers to help stratify patients with various types of cancer is booming, the clinical usefulness of the markers has yet to be proven. Still, these markers have great potential to stratify patients with higher or lower risk disease. Specific to nodal metastases, these markers can help identify patients who would potentially benefit from a more extensive LND and/or perioperative chemotherapy.

Conclusion:

Lymph node dissection remains a critical component in the treatment of muscle-invasive bladder cancer. The presence of nodal disease is often associated with poor clinical outcome, however, some may experience long-term disease-free survival. Identification of these patients would be helpful in selecting more aggressive multimodality therapies for those at highest risk for recurrence and cancer-specific mortality. Several variables, including extent of LND, nodal yield, LN-d, extranodal extension, and lymph node tumor burden, have been proposed as prognostic factors. Molecular markers may ultimately provide more accurate prediction of tumor biology.

REFERENCE

1. Stein, J.P. et al., 2001. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 19(3), pp.666–675.
2. Leissner, J. et al., 2000. Lymphadenectomy in patients with transitional cell carcinoma of the urinary bladder; significance for staging and prognosis. BJU international, 85(7), pp.817–823.
3. Poulsen, A.L., Horn, T. & Steven, K., 1998. Radical cystectomy: extending the limits of pelvic lymph node dissection improves survival for patients with bladder cancer confined to the bladder wall. The Journal of urology, 160(6 Pt 1), pp.2015–9; discussion 2020.
4. Steven, K. & Poulsen, A.L., 2007. Radical cystectomy and extended pelvic lymphadenectomy: survival of patients with lymph node metastasis above the bifurcation of the common iliac vessels treated with surgery only. The Journal of urology, 178(4 Pt 1), pp.1218–23; discussion 1223–4.
5. Mandel, P., Tilki, D. & Eslick, G.D., 2014. Extent of lymph node dissection and recurrence-free survival after radical cystectomy: a meta-analysis. Urologic oncology, 32(8), pp.1184–1190.
6. Abdi, H. et al., 2016. Balancing risk and benefit of extended pelvic lymph node dissection in patients undergoing radical cystectomy. World journal of urology, 34(1), pp.41–48.
7. Dorin, R.P. et al., 2011. Lymph node dissection technique is more important than lymph node count in identifying nodal metastases in radical cystectomy patients: a comparative mapping study. European urology, 60(5), pp.946–952.
8. Jensen, J.B., Ulhøi, B.P. & Jensen, K.M.-E., 2010. Lymph node mapping in patients with bladder cancer undergoing radical cystectomy and lymph node dissection to the level of the inferior mesenteric artery. BJU international, 106(2), pp.199–205.
9. Bruins, H.M. et al., 2014. Incidence and location of lymph node metastases in patients undergoing radical cystectomy for clinical non–muscle invasive bladder cancer: Results from a prospective lymph node mapping study. Urologic Oncology: Seminars and Original Investigations, 32(1), pp.24.e13–24.e19.
10. May, M. et al., 2011. Association between the number of dissected lymph nodes during pelvic lymphadenectomy and cancer-specific survival in patients with lymph node-negative urothelial carcinoma of the bladder undergoing radical cystectomy. Annals of surgical oncology, 18(7), pp.2018–2025.
11. Morgan, T.M. et al., 2012. Lymph node yield at radical cystectomy predicts mortality in node-negative and not node-positive patients. Urology, 80(3), pp.632–640.
12. Siemens, D.R. et al., 2015. Lymph node counts are valid indicators of the quality of surgical care in bladder cancer: a population-based study. Urologic oncology, 33(10), pp.425.e15–23.
13. Park, J. et al., 2011. Does the greater number of lymph nodes removed during standard lymph node dissection predict better patient survival following radical cystectomy? World journal of urology, 29(4), pp.443–449.
14. Fleischmann, A. et al., 2005. Prognostic Implications of Extracapsular Extension of Pelvic Lymph Node Metastases in Urothelial Carcinoma of the Bladder. The American journal of surgical pathology, 29(1), pp.89–95.
15. Koppie, T.M. et al., 2006. Standardization of pelvic lymphadenectomy performed at radical cystectomy. Cancer, 107(10), pp.2368–2374.

16. Herr, H.W. et al., 2002. Impact of the number of lymph nodes retrieved on outcome in patients with muscle invasive bladder cancer. The Journal of urology, 167(3), pp.1295–1298.
17. Konety, B.R., Joslyn, S.A. & O’Donnell, M.A., 2003. Extent of Pelvic Lymphadenectomy and Its Impact On Outcome in Patients Diagnosed With Bladder Cancer: Analysis of Data From the Surveillance, Epidemiology and End Results Program Data Base. The Journal of urology, 169(3), pp.946–950.
18. Bochner, B.H., Herr, H.W. & Reuter, V.E., 2001. Impact of separate versus en bloc pelvic lymph node dissection on the number of lymph nodes retrieved in cystectomy specimens. The Journal of urology, 166(6), pp.2295–2296.
19. Fang, A.C. et al., 2010. Effect of a minimum lymph node policy in radical cystectomy and pelvic lymphadenectomy on lymph node yields, lymph node positivity rates, lymph node density, and survivorship in patients with bladder cancer. Cancer, 116(8), pp.1901–1908.
20. Quek et al., 2009. The role of lymph node density in bladder cancer prognostication. World journal of urology, 27(1), pp.27-32.
21. Ku, J.H. et al., 2015. Lymph node density as a prognostic variable in node-positive bladder cancer: a meta-analysis. BMC cancer, 15, p.447.
22. Jeong, I.G. et al., 2011. Comparison of 2002 TNM nodal status with lymph node density in node-positive patients after radical cystectomy for bladder cancer: analysis by the number of lymph nodes removed. Urologic oncology, 29(2), pp.199–204.
23. Osawa, T. et al., 2009. Role of lymph node density in predicting survival of patients with lymph node metastases after radical cystectomy: a multi-institutional study. International journal of urology: official journal of the Japanese Urological Association, 16(3), pp.274–8; discussion 278.
24. Wright, J.L., Lin, D.W. & Porter, M.P., 2008. The association between extent of lymphadenectomy and survival among patients with lymph node metastases undergoing radical cystectomy. Cancer, 112(11), pp.2401–2408.
25. Wiesner, C. et al., 2009. Cancer-specific survival after radical cystectomy and standardized extended lymphadenectomy for node-positive bladder cancer: prediction by lymph node positivity and density. BJU international, 104(3), pp.331–335.
26. Simone, G. et al., 2012. Development and external validation of lymph node density cut-off points in prospective series of radical cystectomy and pelvic lymph node dissection. International journal of urology: official journal of the Japanese Urological Association, 19(12), pp.1068–1074.
27. Kassouf, W. et al., 2013. Critical analysis and validation of lymph node density as prognostic variable in urothelial carcinoma of bladder. Urologic oncology, 31(4), pp.480–486.
28. Karl, A. et al., 2009. The impact of lymphadenectomy and lymph node metastasis on the outcomes of radical cystectomy for bladder cancer. European urology, 55(4), pp.826–835.
29. Ahn, T.S. et al., 2015. Extracapsular Extension of Pelvic Lymph Node Metastasis is an Independent Prognostic Factor in Bladder Cancer: A Systematic Review and Meta-analysis. Annals of surgical oncology, 22(11), pp.3745–3750.
30. Mills, R.D. et al., 2001. Pelvic lymph node metastases from bladder cancer: outcome in 83 patients after radical cystectomy and pelvic lymphadenectomy. The Journal of urology, 166(1), pp.19–23.
31. Stephenson, A.J. et al., 2010. Aggregate lymph node metastasis diameter and

survival after radical cystectomy for invasive bladder cancer. Urology, 75(2), pp.382–386.

32. Jensen, J.B., Ulhøi, B.P. & Jensen, K.M.-E., 2012. Evaluation of different lymph node (LN) variables as prognostic markers in patients undergoing radical cystectomy and extended LN dissection to the level of the inferior mesenteric artery. BJU international, 109(3), pp.388–393.
33. Sørensen, K.P. et al., 2015. Long non-coding RNA expression profiles predict metastasis in lymph node-negative breast cancer independently of traditional prognostic markers. Breast cancer research: BCR, 17, p.55.
34. Prensner, J.R. et al., 2014. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. The lancet oncology, 15(13), pp.1469–1480.
35. Roepman, P. et al., 2005. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. Nature genetics, 37(2), pp.182–186.
36. Laurberg, J.R. et al., 2014. High expression of GEM and EDNRA is associated with metastasis and poor outcome in patients with advanced bladder cancer. BMC cancer, 14, p.638.
37. Smith, S.C. et al., 2011. A 20-gene model for molecular nodal staging of bladder cancer: development and prospective assessment. The lancet oncology, 12(2), pp.137–143.
38. Seiler, R. et al., 2016. Prediction of Lymph Node Metastasis in Patients with Bladder Cancer Using Whole Transcriptome Gene Expression Signatures. The Journal of urology. Available at: http://dx.doi.org/10.1016/j.juro.2016.04.061.

