Lymph node dissection in bladder cancer

Impact on staging and prognosis

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This thesis is based on the following publications which will be referred to in the text by their Roman numerals:

I. Jensen JB, Ulhøi BP, and Jensen KM. Estimation of the true number of lymph nodes in lymphadenectomy specimens from radical cystectomy. Scand J Urol Nephrol. 2009;43(4):288-92
II. Jensen JB, Høyer S, and Jensen KM. Incidence of occult lymph node metastasis missed by standard pathological examination in patients with bladder cancer undergoing radical cystectomy. Scand J UrolNephrol. 2011;45(6):419-24
III. Jensen JB, Ulhøi BP, and Jensen KM. Lymph node mapping in patients with bladder cancer undergoing radical cystectomy and lymph node dissection to the level of the inferior mesenteric artery. BJU Int. 2010;106(2):199-205
IV. Jensen JB, Ulhøi BP, and Jensen KM. Size and volume of metastatic and non-metastatic lymph nodes in pelvis and lower abdomen in patients with carcinoma of the bladder undergoing radical cystectomy. Scand J Urol Nephrol. 2010;44(5):291-7
V. Jensen JB, Ulhøi BP, and Jensen KM. Evaluation of different lymph node variables as prognostic markers in patients undergoing radical cystectomy and extended lymph node dissection to the level of the inferior mesenteric artery.BJU Int. 2012;109(3):388-93
VI. Jensen JB, Ulhøi BP, and Jensen KM. Extended versus limited lymph node dissection in radical cystectomy: impact on recurrence pattern and survival.IJU. 2012;19(1):39-47
VII. Jensen JB, Ulhøi BP, and Jensen KM. Prognostic value of lymph-node dissection in patients undergoing radical cystectomy following previous oncological treatment for bladder cancer. Scand J Urol Nephrol. 2011;45(6):436-43
VIII. Jensen JB, Munksgaard PP, Sørensen CM, Fristrup N, Birkenkamp-Demtroder K, Ulhøi BP, Jensen KM, Ørntoft TF, and Dyrskjøt L. High expression of karyopherin alpha 2 (KPNA2) defines poor prognosis in non-muscle invasive bladder cancer and in patients with invasive bladder cancer undergoing radical cystectomy. Eur Urol. 2011;59(5):841-8

INTRODUCTION

Epidemiology

The term bladder tumour covers non-invasive and invasive bladder cancer (BC). Measured by incidence, BC is the 4th and 10th most common neoplasm in Danish males and females, respectively. Measured by prevalence, BC is the 2nd most common neoplasm in Danish males, only outnumbered by carcinoma of the prostate [1].

In 2009, 1,674 new cases of BC were registered in Denmark; 1,231 males and 443 females (male-female ratio: 2.78). Age corrected incidences have remained practically unchanged throughout the last three decades. In both genders, BC is most common in the 6thto the 9th decades of life with the highest incidence around the 70thlife year [1,2].

At the time of diagnosis, approximately 50% of the patients have non-invasive Ta-tumours, 25% T1-tumours invading the subepithelial connective tissue, and 20% muscle-invasive tumours. The remaining less than 5% of the patients have carcinoma in situ (CIS) without concomitant tumour (i.e. Tis) or flat dysplasia only [2].

Incidence of nodal and visceral metastasis at the time of diagnosis is less clarified in BC patients in general. Based on numbers from the Swedish bladder cancer registry, a minimum of 4% of all patients with BC and a minimum 13% of patients with muscle-invasive disease have LN metastasis. A minimum of 4% of all patients,12% of patients with muscle invasive disease, have distant metastasis at the time of diagnosis [3]. In autopsy studies and clinical studies of patients with BC, it has been shown that the incidence of metastatic disease is correlated to T-stage of the primary tumour [4-6].

Long term survival of BC patients in Denmark has not increased significantly during the last decades [2,7,8]. In a recent population based cohort study of patients with invasive BC from Central and Northern Denmark regions, the predicted long term survival of patients diagnosed from 2007 to 2009 reveals, however, a slight tendency towards improved survival compared to patients diagnosed with invasive BC from 1998 to 2006 [9].

Stratification

Epithelial-derived neoplasms of the urinary bladder cover a heterogeneous group of disease entities with urothelial carcinomas (i.e. transitional cell carcinomas, TCC) accounting for approximately 95% of all tumours. The remaining tumours are squamous cell carcinomas (SCC), adenocarcinomas, small cell carcinomas, sarcomas and other rare histological subtypes.

TCC is a heterogenic disease extending from small indolent papillomas with no or minimal malignant potential and no impact on

survival to high grade invasive BC with metastasis resulting in BC-related death.
The term BC covers the whole spectrum of the disease irrespective of the benign, or at least non-invasive, character of almost half the tumours. Bladder tumour is a more appropriate term, but in the English literature, BC is the most used term.
BC can be stratified according to presence of invasion in two groups: benign versus malignant; or non-invasive versus invasive. The term 'benign' is somewhat misleading and should preferably be replaced with 'pre-malignant' to indicate the true potential of the non-invasive neoplasms. However, not all benign, 'pre-malignant' tumours will progress to malignant, invasive disease if left untreated.

	6th edition	7th edition
Tumour-stage		
Tx	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
Ta	Non-invasive primary tumour	
Tis	Carcinoma in situ: 'flat tumour'	
T1	Tumour invades subepithelial connective tissue	
T2	Tumour invades detrusor muscle	
T2a	Invasion of superficial muscle (inner half)	
T2b	Invasion of deep muscle (outer half)	
T3	Tumour invades perivesical tissue	
T3a	- microscopically	
T3b	- macroscopically (extravesical mass)	
T4	Tumour invades adjacent organs	
T4a	Invasion of prostatic stroma, seminal vesicles, uterus, or vagina	
T4b	Invasion of pelvis wall or abdominal wall	
Nodal-stage		
Nx	Regional LNs cannot be assessed	
N0	No regional LN metastasis	
N1	Metastasis in a single regional LN*, 2 cm or less in greatest dimension	Metastasis in a single LN in the true pelvis#
N2	Metastasis in a single regional LN* more than 2 cm but not more than 5 cm in greatest dimension, or multiple LNs, none more than 5 cm in greatest dimension	Metastasis in multiple LNs in the true pelvis#
N3	Metastasis in a regional LN*more than 5 cm in greatest dimension	Metastasis in a common iliac LN
Metastasis-stage		
Mx	Distant metastasis cannot be assessed	*<Mx eliminated from the classification>*
M0	No distant metastasis	
M1	Distant metastasis	

Table I: *Classification of bladder cancer according to the 6th and the 7th editions of the TNM classification.*
** Regional LNs according to the 6th edition of the TNM are LNs below the bifurcation of the common iliac arteries.*
 # LNs of the true pelvis according to the 7th edition of the TNM are the hypogastric, obturator, external iliac, and presacral LNs.

A more commonly used stratification is based on the presence of invasion in the detrusor muscle: non-muscle-invasive BC (NMIBC) versus muscle-invasive BC (MIBC). Earlier, NMIBC was classified as 'superficial' BC. This stratification reflects the traditional treatment strategy where conservative methods (transurethral resections (TURB) and intravesical chemo- or immuno-therapy) are the predominant treatment methods of NMIBC and local radical treatment (radical cystectomy (RC) or radiotherapy) are the predominant treatment methods of MIBC if no metastasis are found. In patients with distant metastasis, no curative local treatment can be undertaken. Furthermore, conservative treatment does not suffice for all NMIBCs. Research of the molecular biology of BC indicates closer resemblance between T1- and T2-tumours than between Ta- and T1-tumours [10]. It is possible that the most important difference in tumour biology is found when comparing different grades rather than different stages of BC [11]. However, it is evident that some T1-tumours can be treated safely by conservative methods whereas others cannot. Given the morbidity of radical treatment of BC, co-morbidity of the individual patient has to be taken into account when deciding for treatment modality.
This thesis predominantly deals with MIBC and NMIBC treated by RC as the local radical treatment.

Staging
Staging of BC is done according to the Tumour, Node, Metastasis (TNM) classification as described by the Union International Contre le Cancer (UICC) – in the most recent editions in conjunction with the American Joint Committee on Cancer (AJCC). The reasons for classification are different treatment and control regimens undertaken depending on stage and the corresponding different prognosis. Several alterations of the TNM classification have been made since the first edition was published in 1968 [12]. Changes predominantly reflect developments in diagnostic tools, different treatment regimens, and new insight into the disease. Because of continuous changes in the classification it is imperative to specify the edition of TNM classification used in different patient series. The current 7th edition became effective January 1st 2010 (Table I) [13].
Based on the TNM classification, patients can be further classified as having stage 0–IV disease. This classification suggested by AJCC has never been generally applied in Denmark.

3rd and 4th editions	5th to 7th editions	Re-classification
Tx	Tx	Tx
T0	T0	T0
Ta	Ta	Ta
Tis	Tis	Tis
T1	T1	T1
T2	T2	T2
	T2a	
T3a	T2b	
T3b	T3	T3
	T3a	
	T3b	
T4a	T4a	T4a
T4b	T4b	T4b

Table II: *Comparison of T-stages according to the 3rd and 4th editions versus 5th to 7th editions of the TNM classification and re-classification in order to make comparisons between historical series.*

Tumour stage (T-stage)
The only major revision of T-stages in BC since the 2nd edition [14] was made with the 5th edition in 1997 [15]. The previous editions classified superficial muscle invasion as T2, deep muscle invasion as T3a and perivesical invasion as T3b [16]. The following editions have classified unspecified muscle invasion as T2, superficial muscle invasion as T2a, deep muscle invasion as T2b and perivesical invasion as T3, T3a or T3b depending on unspecified, microscopically or macroscopically invasion. The clinical relevance

of substratification of T2 (T2a versus T2b) as well as T3 (T3a versus T3b) has been questioned [17-19].
However, substratification makes it possible to compare historical BC series using the 4th,or earlier, editions of the TNM classification with series using more recent classifications if analyses do not substratify T2 and T3. Therefore, re-classification of previous T2 and T3a to T2 and reclassification of all present T3a and T3b to T3 makes the patient groups comparable without pathology revision (Table II). T-staging according to the current 7th edition of the TNM classification is illustrated in Figure 1. Substratification of T1 tumours based on depth of invasion (T1a versus T1b) is recommended in the current Danish guidelines but is not part of the official TNM classification.

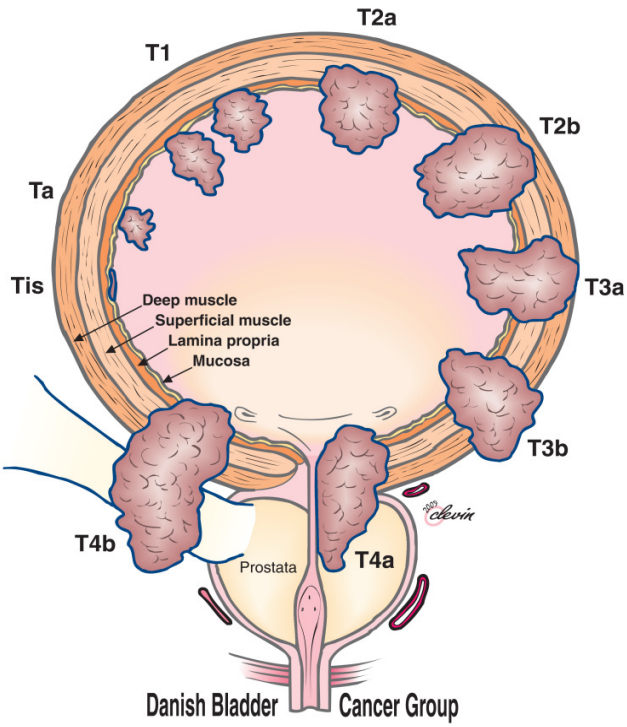


Figure 1:*Tumour stages in bladder cancer according to the 7th edition of the TNM classification.*
(Reprinted with permission from the Danish Bladder Cancer Group – DaBlaCa)

Nodal stage (N-stage)
N-staging is based on presence of lymph node (LN) metastasis in the regional LNs. Before N-staging can be made, the regional versus non-regional LNs have to be defined. Non-regional LN metastasis is classified as M1 disease and not as nodal involvement in N-stage. However, most patients classified as M1 because of non-regional LN metastasis have synchronous regional LN metastasis, thus being N positive.
N-staging of BC has undergone more substantial changes than T-staging. In the 1st TNM classification, N-staging was based on whether or not LNs were deformed on lymphography (N1 vs. N0) [12]. More recent editions have based N-staging on pathological examination of fine needle aspiration cytology (FNAC) or histology of removed LNs. Until the most recent revision of the TNM classification, local LNs in BC were defined as LNs below the bifurcation of the common iliac arteries. In the 7th edition, the common iliac LNs are included in the regional LNs as N3-disease if positive [13]. These LNs were considered non-regional in the 4th to the 6th edition (M1-disease if positive). In the previous 3rd edition, the concept of juxta-regional LNs included common iliac, para-aortic and inguinal LNs [20].
In the 3rd edition of the TNM classification, N-stage was influenced by number and laterality of the positive LNs. In this way, N1 was metastasis in a single LN ipsilateral to the tumour in the bladder, whereas a single positive contralateral LN was classified as N2 together with multiple regional LN metastases. N3 was involvement of fixed regional LNs without juxta-regional LN metastasis (N4 disease if positive).
In the 4th to the 6th edition of the TNM classification, N-staging was based on size of the metastasis in the involved LNs in addition to the number of involved LNs [21]. The size-criterion was abandoned with the 7th edition in favour of a location based classification. According to the general definitions in the TNM system it is, however, still possible to distinguish LN positive patients with 'macrometastasis' (metastasis measure more than 0.2 cm in greatest extent) in a single LN from patients with 'micrometastasis' (metastasis measures not more than 0.2 cm) by addition of '(mi)' to the latter, i.e. pN1(mi). Moreover, if the LN contains only isolated tumour cells or small clusters of cells not more than 0.2 mm in greatest extent, the classification should be pN0(i+), whereas negative morphological findings for isolated tumour cells should be classified as pN0(i-). If isolated tumour cells are suggested by non-morphological techniques, e.g. flow cytometry or DNA analysis, classification should be pN0(mol+). The reason for classification of the patient as N0 despite (i+) or (mol+) is that isolated tumour cells typically do not show evidence of metastatic activity (e.g. proliferation or stromal reaction) or penetration of vascular of lymphatic sinus walls [13]. This possibility of substratification of N-staging by adding (mi), (i+), and (mol+) is not generally used in BC.

Grading
In addition to stage, dedifferentiation of the tumour cells, i.e. grade, should be determined in all tumours. In Denmark, grading has until very recently been done according to the classification described by Bergkvist [22]. From January 1st 2009, the new World Health Organisation grading (WHO 2004 [23]) has been used in Denmark. This grading system classifies a non-invasive tumour as true papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), low grade (LG), or high grade (HG) urothelial tumour. Invasive tumours are classified according to histological subtype (different types of urothelial carcinomas). During construction of a tissue microarray (TMA) consisting of tumour samples from 425 patients with invasive urothelial carcinoma undergoing RC at the Department of Urology, Aarhus University Hospital, all tumours were re-graded according to the WHO 2004 classification by a single uropathologist. This resulted in 409 classical urothelial carcinomas (96%) of which 401 were classified as HG tumours (94% of all tumours) and only 8 as LG tumours (2% of all tumours). Sixteen tumours (4%) were classified as other histological subtypes of urothelial carcinoma, predominantly micropapillary and nested type carcinomas [24]. This very unequal distribution between WHO categories of invasive BC, with the vast majority being classical HG tumours, illustrates why grading in RC materials is less important than in NMIBC series.

Metastasizing BC
BC can be spread from the bladder to adjacent or distant locations. Different molecular pathways are thought to be involved in different metastatic mechanisms.

The simplest way for BC to involve other organs is by direct invasion. At the time of RC, only a minority of tumours are classified pT4a or pT4b because of direct invasion. Theoretically, local recurrences following RC can, however, be considered as extravesical disease originating from direct micro-invasion of the perivesical vessels in the connective tissue left behind in the RC cavity. This accounts only for non-LN recurrences. Therefore, local LN recurrences should be distinguished from other non-LN local recurrences if possible.

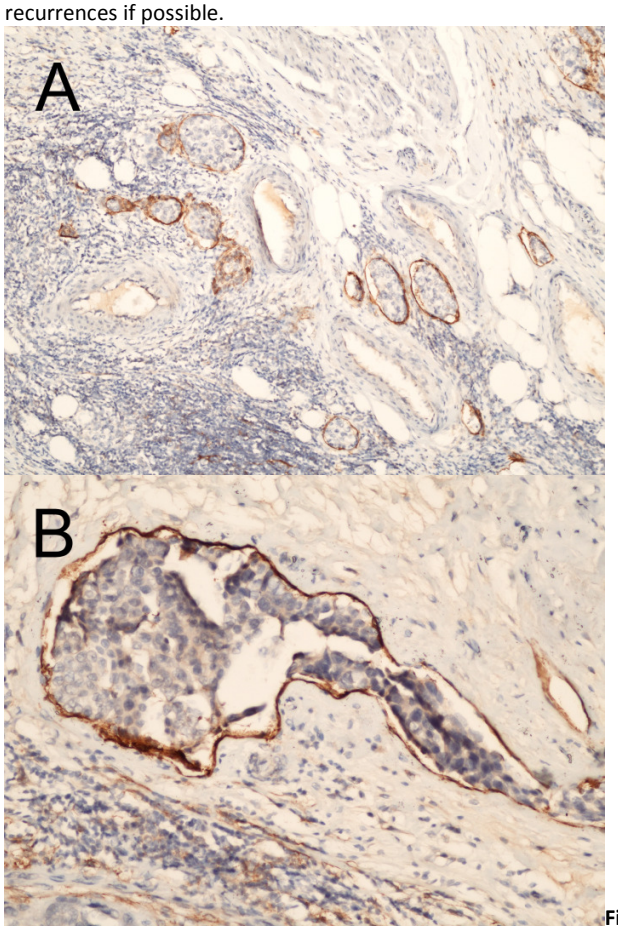


Figure 2: *Lymphatic vessels with tumour thromboses caused by urothelial carcinoma in an RC specimen. Lymphatic vessels have been stained specifically with IHC following incubation with D2-40. (A) 10x and (B) 20x magnifications.*
(By courtesy of Søren Høyer, Institute of Pathology)

Implantation metastasis from BC is thought to occur more often during a TURB with circulating tumour cells in the bladder than during a RC where spillage of tumour cells is avoided. Implantation metastasis may, however, account for some local recurrences and, more evidently, recurrences in the surgical wound or port holes following laparoscopic RC. As for implantation metastasis, transcoelomic spread of BC resulting in carcinosis requires the ability of the carcinoma cells to invade and survive in the new location. The capability of invasion may enhance if implantation is made in non-epithelial covered sites or if susceptibility of the epithelium has been altered by infection of inflammation [25].
Lymphatic spread is probably the most common way for BC to metastasize. Lymphatic vessels are present throughout the bladder wall, mostly pronounced in the submucosa. Apparently, there are more lymphatic vessels in the invasive tumour than in the normal bladder wall. This is thought to be a result of angiogenesis stimulation by the carcinoma [26-29]. LN metastases are thought to origin from LVI, i.e. tumour thrombosis in the vesical or perivesical lymphatic vessels [30,31] (Figure 2). Carcinoma cells from LN metastasis can migrate further, enter circulation and from there invade distant organs.
Haematogenous spread of BC is preceded by invasion of the peritumoural blood vessels or by seeding of carcinoma cells from LN metastasis into the venous system by lymphatic drainage through the thoracic duct, as mentioned above. Because not all patients with visceral metastasis harbour LN metastasis, direct haematogenous metastasis is evident in some BC patients. In autopsy studies, as much as one third of the patients with metastatic disease have had no sign of LN involvement but potential haematogenic visceral metastasis [4,32-35] (Table III).

Author	Year	No. pts	No. pts with metastasis (%)	Distribution of metastases		
				LN metastasis only	LN and visceral metastasis	Visceral metastasis only
Colston et al. [32]	1936	98	55 (56%)	25%	51%	24%
Jewett et al. [4]	1946	107	53 (50%)	13%	51%	36%
Friedell et al. [33]	1968	31	20 (65%)	40%	40%	20%
Babaian et al. [34]	1980	107	107 (100%)	32%	43%	25%
Wallmeroth et al [35]	1999	367	251 (68%)	14%	69%	17%

Table III. *Sites of metastases found in autopsy studies of patients with BC.*

There are several possible explanations to the preference of lymphatic spread rather than by the bloodstream. Lymph vessels are larger in dimension than blood capillaries and the wall is more permeable because of the lack of a proper basement membrane and cellular junctions [36,37]. Tumour cells entering lymph vessels are subjected to weaker shear stress forces and lower serum toxicity compared to tumour cells entering the bloodstream. Thus, micro-nutrition of the carcinoma cells is more favourable in LNs compared to viscera [38].
Most common sites of visceral metastases from BC are bone, liver and lungs but any organ may be involved, especially in terminal disease [32-35]. Studies of metastatic patterns are based on autopsy studies, imaging studies, or clinical series using several diagnostic tools. Risk of visceral metastasis and organ distribution varies considerably depending on the way to investigate this.

Clinical staging and TURB
Clinical staging prior to RC entails a high risk of understaging. Thus, series have found that 8–46% of patients undergoing RC because of clinical Ta–T1 had MIBC (T2+) in the RC specimen [39-41].
Bimanual palpation of anaesthetized patients can be decisive of whether patients with BC are suitable for radical local treatment. A fixed tumour (clinically T4b) excludes the patient from primary RC and radiotherapy. Instead, systemic chemotherapy should be initiated if not contraindicated. Apart from fixed tumours, the palpation criterion in T-staging was abandoned with the 4th edition of the TNM classification, leaving pathological examination of the TURB specimen as decisive for T-stage. Therefore, pre-RC staging can only clearly differentiate between Ta, Tis, T1, T2+ (indicating invasion of the detrusor muscle which can be T2 as well as T3 disease), T4a (if invasion in the prostate is found), and T4b as mentioned above.

Differentiation of patients into T2 versus T3 prior to RC is not important if neoadjuvant chemotherapy is not given based on this diversity. However, the lack of differentiation of depth of invasion in patients undergoing radiotherapy and neoadjuvant chemotherapy makes comparison with 'surgery only series' difficult. Pre-RC N-staging was previously, done by operative staging with removal of the local LNs [42]. This was considered rational in a time where imaging modalities were less sensitive and surgery in node positive patients was undertaken only for palliative reasons [42]. As a result of more sensitive preoperative imaging modalities, patients with massive positive LN burden can nowadays be diagnosed more safely without the need for surgical staging. Moreover, reports of acceptable prognosis after RC despite positive LNs have rendered the preoperative surgical staging obsolete [43,44]. Operative LN staging is therefore more appropriate performed at the time of RC.

Imaging

Conventional X-ray of the lungs has traditionally been performed to exclude pulmonary metastasis. Computed tomography (CT) scan of the thorax is more sensitive in that perspective but also less specific [45]. Excretory urography based on conventional X-ray is not relevant in nodal staging and is considered inferior to CT-based urography regarding investigation of the upper urinary tract [46]. In addition, CT scan of the abdomen as part of a CT urography can diagnose intra-abdominal metastases including bulky LN metastases. Ultrasound of the abdomen is inferior to CT scan regarding exclusion of intraabdominal metastasis. However, ultrasound may be useful as a supplement when CT scan is inconclusive, or when an ultrasound-guided FNAC of presumed metastasis, including bulky LNs, is required.

Magnetic resonance imaging (MRI) has not been generally applied as a staging modality in BC. Less availability and lack of clinically significant superiority to CT is the possible explanation to this. Ferumoxtran-10-enhanced MRI has shown promising results in that perspective but has not yet been implemented as a standard protocol [47,48]. The sensitivity of CT scan regarding diagnosis of metastasis can be improved by combining the CT scan with positron emission tomography (PET) in a PET-CT scan. The most commonly used tracer for PET is Fluorine-18 2-fluoro-2-deoxy-D-glucose (FDG). Excretion through the urine of FDG makes conclusions regarding the urinary tract, hence the primary tumour, difficult but is useful in diagnosis of distant metastasis. In a recent study of FDG-PET-CT in 57 patients with BC, more advanced disease was diagnosed compared with conventional CT or MRI in 40% of the patients. Furthermore, clinicians changed their planned management in 68% of patients based on the FDG-PET-CT results [49]. Combination of CT with single photon emission computed tomography (SPECT) in a SPECT/CT has been investigated in BC as a way of improving sentinel node (SN) detection. For the time being SPECT/CT is still experimental and the relevance in BC undetermined [50].

The gold standard regarding diagnosis of local LN metastasis is LN dissection (LND). Sensitivity and specificity of different imaging modalities compared with LND is shown in Table IV. The low sensitivity and specificity may be explained by the size criterion of LNs to determine the presence of metastasis used in most studies. The size of an LN is not necessarily significantly enlarged if the metastatic burden is minimal. Moreover, other causes of LN enlargement exists (e.g. anatomical variation, reactive because of infection, or inflammation following TURB or BCG). Shape and architecture can be more informative in that perspective [51]. Despite a low sensitivity of most imaging modalities, it is important to emphasize that preoperative imaging is the only reasonable way to rule out distant metastasis at present time. The presence of distant metastasis makes primary local radical treatment superfluous and potentially harmful to the patient. Moreover, it is important to emphasize that low sensitivity of imaging modalities compared to histopathological results from LND predominantly accounts for a minimal metastatic burden in local LNs that are removed at the time of RC.

Modality	Author	Year	No. patients	% N+	Sensitivity	Specificity	NPV	PPV	Accuracy
CT	Herr et al. [52]	1996	105	27%	32%	84%	77%	43%	70%
CT	Paik et al. [53]	2000	82	26%	19%	97%	78%	67%	77%
CT	Picchio et al [54]	2006	27	30%	50%	68%	76%	40%	63%
CT	Baltaci et al. [55]	2008	100	13%	31%	94%	90%	44%	86%
CT	Swinnen et al. [56]	2009	51	24%	42%	93%	84%	63%	82%
CT	Lodde et al. [57]	2010	33	45%	33%	100%	64%	100%	70%
MRI	Jager et al. [58]	1996	71	41%	83%	98%	89%	96%	92%
MRI	Thoeny et al. [48]	2009	20*	25%	80%	73%	92%	50%	75%
MRI	Jensen et al. [59]	2011	18	17%	0%	80%	80%	0%	67%
MRI-DW	Thoeny et al. [48]	2009	20*	25%	80%	87%	93%	67%	85%
MRI-fer.enh	Thoeny et al. [48]	2009	20*	30%	67%	93%	87%	80%	85%
11C-Cholin-PET	Picchio et al [54]	2006	27	30%	63%	100%	86%	100%	89%
11C-Acetat-PET-CT	Schoder et al [60]	2011	17	18%	100%	64%	100%	38%	71%
FDG-PET	Heicappell et al. [61]	1999	8	38%	67%	100%	83%	100%	88%
FDG-PET-CT	Swinnen et al. [56]	2009	51	25%	46%	97%	84%	86%	82%
FDG-PET-CT	Kibel et al. [62]	2009	43	23%	70%	94%	91%	78%	86%
FDG-PET-CT	Lodde et al [57]	2010	43	53%	57%	100%	67%	100%	77%
FDG-PET-CT	Jensen et al. [59]	2011	18	17%	33%	93%	88%	50%	83%

Table IV: *Statistical findings per patient regarding identification of LN metastasis in series evaluating different imaging modalities. Gold standard is histopathological examination of LND specimens. Only series published within the last 15 years specifically stating results from LN status and results given per patient are included. NPV: Negative predictive value, PPV: Positive predictive value, DW: Diffusion weighted, fer.enh.: ferumoxtran-10-enhanced*

**Five of the included patients had carcinoma of the prostate and not of the urinary bladder*

Pathological examination

Standard pathological examination (SPE) in a patient undergoing RC consists of examination of the RC specimen and examination of the LND specimen. Histological type, pathological T-stage (pT), surgical margins (negative vs. positive), malignancy in the urethral resection margin, malignancy in the ureteral resection margins, presence of LNs within the perivesical fatty tissue, and metastasis

in these should be described for the RC specimen. Presence of lymphovascular invasion (LVI) is a pathological feature that has been found to have impact on prognosis and risk of LN metastasis in several RC series and should therefore also preferably be registered [31,63-70]. Pathology reports regarding the LND specimen should include registration of LN metastasis and if so, number, location, and size of positive LNs. Correct TNM staging and prognostic estimation can only be determined if registration includes this minimum of details.

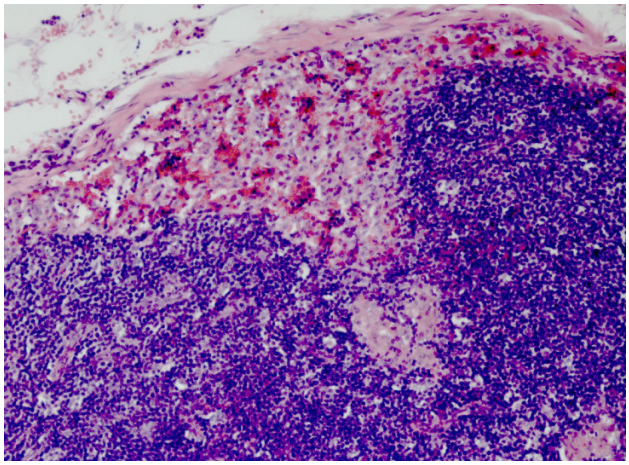


Figure 3:*True lymph node with fibrous capsule andlymphatic nodules separated by trabeculae.(By courtesy of Benedicte Parm Ulhøi, Institute of Pathology)*

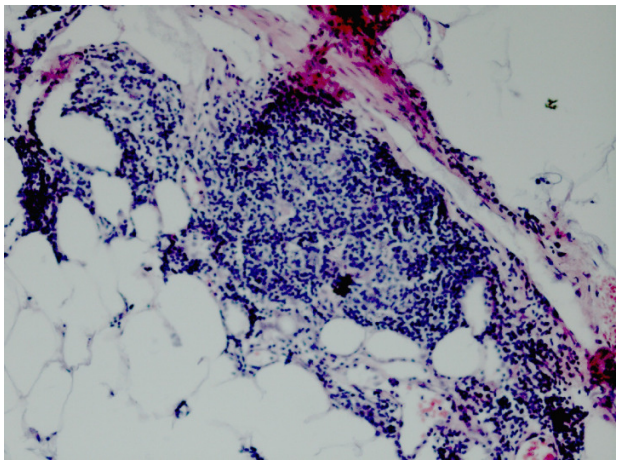


Figure 4.*Lymphocyte accumulation in adipose tissue. Note the absence of capsule and trabeculae.(By courtesy of Benedicte Parm Ulhøi, Institute of Pathology)*

The total number of retrieved LNs should also be noted. This number may serve as a surrogate marker of surgical quality and extent of LND. Several reservations should, however, be noted here. First, pathological examination of the specimen can be more or less thorough in the search for LNs. Fat-clearing solutions or LN revealing solutions have been suggested to make a better visualization of the LNs within the fatty tissue of the specimen [71-73]. The submission of the LND specimen as separate packages from different locations as opposed to en bloc submission have been found to increase the number of LNs in the pathology report significantly despite the same surgical quality and extent of LND [74,75]. Second, an inter-person variation in the total number of LNs within the pelvis exists. This has been suggested in several mapping studies and in an in vivo mapping study using SPECT/CT, where Roth et al. suggested a true variation rather than variation because of different surgical quality [5,76,77]. Third, LN count can be influenced by the pathologist's definition of an LN [78]. Lymphocyte accumulations in adipose tissue can be present; this is by definition not an LN but may be interpreted as such by some pathologists. At the Institute of Pathology, Aarhus University Hospital, an LN is defined as an organized lymphoid structure with sinus system surrounded by a fibrous capsule and with visible lymph vessels (Figure 3), whereas lymphocyte accumulations in adipose tissue without these features is not registered as an LN (Figure 4). As a result, a lower number of LNs is listed in the pathology report than if a more liberal definition is used. Altogether, these reservations make it difficult to evaluate the quality of LND by node counts. Furthermore, a high number of LNs does not always reflect removal of the most important LNs [79].

Molecular markers

Ideally, molecular markers can be used as a supplement to conventional histopathological features or replace these in identification of clinically relevant subgroups of BC patients. This stratification can be made with different entities regarding staging, risk of recurrence, risk of progression, prognosis, or treatment response. In BC patients undergoing radical treatment, supplementary diagnostic and prognostic tools are urgently needed to select patients for the optimal treatment modality and to avoid unnecessary potentially harmful treatment. Thus, individualized neoadjuvant or adjuvant treatment could be initiated to complement local radical treatment in patients with a genetic signature predicting high risk of metastasis and recurrence. Potential harmful chemotherapy could, on the other hand, be avoided if a poor treatment response is predicted.

Different multigene expression models have been suggested to predict risk of LN metastasis at the time of RC, degree of pulmonary metastasis potential, and poor prognosis [80-82]. Smith et al. has developed a gene expression model consisting of 20 genes that significantly improves prediction of LN metastasis when incorporated into a model with age, gender, pT-stage, and presence of LVI [80]. The genes were selected by microarray technique from paired frozen and formalin-fixed tissue. Relevant genes and cut-offs to stratify patients were developed by use of two separate training cohorts. A third cohort was used to validate the findings. Conclusively they found that the 20 gene model could be safely applied to formalin-fixed paraffin embedded tissue, making implementation in routine diagnostic tissue feasible [83]. This particular work is probably the most relevant and significant study published so far. In another work, Sanchez-Carbayo et al. suggested a molecular profile consisting of 100 genes to identify patients with LN metastasis and poor prognosis following RC [82]. They concluded that identification of this poor outcome profile could assist in selecting patients who could benefit from more aggressive treatment. Experience from other cancer forms has shown promising results with gene expression signatures as prognostic predictor. Thus, Van't Veer et al. established a 70 gene prognosis profile from genetic signature of 98 primary breast cancers [84]. This gene expression profile was later validated in a series of 295 consecutive patients with primary breast carcinomas [85]. An ongoing study is now trying to validate the signature in a prospective study comprising 6,000 patients [86]. Single genes associated with more accurate staging or poor prognosis in BC have been investigated in several studies. If relevant single genes can be identified and furthermore validated on the protein level, IHC can be used to identify high risk patients. More-

over, single genes involved in the metastatic process can be potential therapeutic targets in future molecular based treatment of BC. The use of IHC based technique is a potential advantage compared to laborious genetic analyses that are more bothersome and difficult to implement in the daily clinical practise. However, IHC may introduce other test difficulties because of the subjective nature of this technique.

Studies of single genes have found a significantly higher risk of LN metastasis in patients with tumours showing a high expression of nucleosomal binding protein 1 (NSBP1) [87] or vascular endothelial growth factor C (VEGF-C) [88-90]. Furthermore, blockage by soluble VEGF receptor-3 (VEGFR-3) has shown promising results as a way of suppressing lymphatic metastasis in a mouse model [91]. Low expression of laminin V gamma 2 (LAMC2) is also suggested to be associated with a higher risk of LN metastasis, whereas a high expression of LAMC2 is associated with a high risk of visceral metastasis supposedly through haematogenous dissemination rather than LN metastasis [92].

Pre-treatment diagnosis of metastasis is of course of relevance regarding better selection of patients for neo-adjuvant chemotherapy or avoiding unnecessary surgical treatment in patients with disseminated disease. However, research in molecular genetics will hopefully provide biomarkers that not only subsidize conventional clinical and pathological investigations but also provide new insights in cancer biology by identifying prognostic biomarkers that are independent of the conventional clinicopathological prognostic markers. At the present time, several molecular markers have been suggested as prognostic factors in patients with invasive BC undergoing radical treatment. In most studies, the investigated marker is based on genetic expression in the primary tumour. Thus, high expression of pRb, p21, p27, p53, VEGF-C, RhoGDI2, HER-2, phosS6, c-myc, and E-cadhesin, or a combination of a number of these markers, have all been reported as being correlated with a poor long-term prognostic outcome independent of conventional prognostic risk factors [88,93-98]. Serum based markers have also been investigated. Thus, high serum urokinase-type plasminogen activator (uPA), matrix metalloproteinase-7 (MMP-7), and endostatin levels have been reported to be correlated to poor prognosis following RC in patients with invasive BC [99-102]. Interestingly, high serum level of E-cadhesin have been suggested to be correlated to poor prognosis following RC in one study [103], whereas another study found sustained E-cadhesin expression in the primary tumour to bee a good prognostic factor when compared with patients with tumours with decreased E-cadhesin expression. These two findings do not necessarily exclude E-cadhesin as a true prognostic marker but illustrates the difficulties associated with clear-cut conclusions based on the different studies. Other potential markers have been reported with similar diverging results. Thus, high expression of the cell proliferation marker Ki-67 has been associated with poor prognosis following RC [104-107]. This prognostic value of Ki-67 expression was recently confirmed in a multi-institutional validation [108]. However, other studies found no significant adverse impact on prognosis by adding Ki-67 expression to conventional risk factors in series with BC patients [104,109]. Likewise, Aziz et al. reported overexpression of cyclo-oxygenase-2 (COX-2) to be associated with a better RFS and DSS [110], whereas Shariat et al. found that COX-2 was not a prognostic factor when adjusted for conventional risk factors [111].

Prediction of chemosensitivity and response to chemotherapy based on genetic signature has been investigated in small series that show promising results [112-115]. Prospective validation studies and intervention studies are needed to confirm these findings before implemented into clinical practise at a large scale.

Most recent molecular research in BC biology includes investigation of micro-RNA, i.e. small non-coding RNAs with modulator activity of gene expression [116]. One of these micro-RNA, miR-129, was associated with poor outcome in BC patients in a recent study by Dyrskjot et al. [117].

Molecular markers have also been investigated as a tool to detect occult metastases in LNs found to be negative by SPE. Reverse transcription-polymerase chain reaction (rt-PCR) assays of different markers have identified genetic evidence of LN metastasis in 14–35% of LN negative patients and in 9–29% of negative LNs [118-122].

Despite several promising results, it is notable that the only biomarker clinically used in larger scale is conventional urinary cytology in NIMBC. Presently, no molecular marker has been widely accepted as standard supplement to conventional histopathology in BC. Explanations for the lack of implementation of new molecular markers in daily practise may be several. Potential markers found in one study may not always result in the same promising results when a validation study is performed. Future studies should therefore focus on validation of known markers or reports of new markers including validation in an independent dataset. A major problem in the current literature is that most markers have been identified in a relatively small number of tumours. Subsequent validation in independent dataset may therefore fail. A more reliable method is to use substantially larger dataset and patient series for identification and validation of prospective markers. This is inevitable more comprehensive and costly research but may result in more reliable and clinically applicable markers.

Another explanation to the lack of clinical use of molecular markers could be that initial selection of molecular candidates predominantly is made on RNA or DNA level. Transferral to a protein level available for IHC is not imperative but an advantage if the marker should be easily accessible for the pathologist. The validation of a marker on IHC staining is not always successful; even transferral of results based on IHC of TMAs to whole-section IHC may fail [123]. An explanation to the latter could be heterogeneity of the tumour.

Publication bias is another potential problem, thus, positive findings of a prospective new marker are more likely to be submitted and accepted for publication than negative validation studies.

Hopefully, implementation of new technologies, like next generation sequencing, will result in identification of several new markers, e.g. from non-coding RNA that subsequently can be validated in large prospective patient cohorts.

Radical treatment

Local radical treatment of BC can be either RC or external beam radiotherapy, whereas chemotherapy alone is not recommended as primary therapy but may be part of multimodality treatment regimens [124]. There are no recent randomized clinical trials (RCT) of RC versus external beam radiotherapy. Moreover, in a review by the Cochrane Collaboration, only 439 patients from three historical RCTs were included in intention-to-treat analyses of the meta-analysis [125]. Whether long term results following modern radiotherapy regimens, with salvage RC if local failure occurs, are more favourable compared to RC is not clarified at present. However, local control is evidently better following RC [125,126]. RC is therefore considered the treatment of choice in localized BC if radical treatment is indicated [127,128].

RC can be performed as open surgery through a traditional midline incision or through a minilaparotomy [129]. Endoscopic procedures can be applied as a way of reducing the surgical trauma. As a result, conventional laparoscopic RC and especially robotic assisted laparoscopic RC have been implemented widely recently [130,131] The oncological results should not be compromised despite less traumatizing surgical approaches. Therefore, the same basic oncological surgery regarding RC and LND must be undertaken irrespective of surgical modality.

In male patients, RC includes removal of the prostate and the seminal vesicles. Urethra is removed if malignancy is present at the resection margin of the prostatic urethra. A prostate and seminal vesicle sparring approach have been suggested but is still considered experimental and controversial [132-134]. In female patients, RC includes removal of the internal female genitalia and urethrectomy. If a neobladder is to be constructed, the female urethra is preserved up to the bladder neck. LND is performed at the time of RC. Urinary diversion is mandatory and can be performed as incontinent or continent diversion. Ileal conduit as first described by Bricker is still the most common incontinent diversion [135], whereas the typical continent diversion is an orthotopic ileal neobladder, and more rarely a continent cutaneous reservoir [136].

Systemic chemotherapy as primary radical treatment cannot be recommended in presumed localized disease because of a low rate of complete responders [128,137,138]. Instead, chemotherapy of recurrent disease following RC should be given if no contraindications are present. Cisplatin based combination chemotherapy has been found to be superior to cisplatin alone [139]. At Aarhus University Hospital, a combination of gemcitabin and cisplatin (GC) has been used for more than a decade instead of the previous regimen of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC). GC has been found to provide the same long term results as MVAC but with less morbidity [140,141].

Systemic chemotherapy can be applied in combination with RC in a neoadjuvant or adjuvant setting. The rationale for applying chemotherapy to surgical patients without evident metastatic disease is prospective eradication of occult micrometastasis not removed by surgery. Theoretically, adjuvant chemotherapy is more favourable than neoadjuvant chemotherapy because an indication relies on more accurate surgical staging than provided by preoperative clinical staging and staging by imaging. Thus, patients with locally advanced disease (non-organ-confined tumours or LN metastasis) who have the highest risk of recurrence can undergo adjuvant chemotherapy while low risk patients can avoid the potential harmful neoadjuvant treatment. Furthermore, delay of the surgical procedure in non-responding patients is avoided. Delay of chemotherapy because of post-operative morbidity and lack of tools to assess response to chemotherapy are some of the drawbacks of adjuvant chemotherapy [128]. RCTs have shown a possible survival benefit of adjuvant chemotherapy in high risk patients [142-144]. However, major methodological problems are present in these studies and a meta-analysis of individual patient data from all available studies concluded that the current evidence is too limited to support a survival benefit of adjuvant chemotherapy compared to chemotherapy given at the time of recurrence [145]. Adjuvant chemotherapy is therefore not recommended for routine use at the present time [128].

Neoadjuvant chemotherapy before RC relies on less accurate staging and delays final radical treatment. However, chemotherapy can be administered at an earlier point of metastasis and in patients more tolerant to the treatment than if given postoperatively in an adjuvant setting [128]. RCTs comparing neoadjuvant cisplatin-based chemotherapy and RC to RC alone have failed to show a significant survival benefit of neoadjuvant chemotherapy [146-149], whereas a meta-analysis of all available studies found a significant overall survival (OS) benefit of neoadjuvant chemotherapy and RC compared to RC alone (hazard ratio (HR): 0.86, 95% confidence interval (CI): 0.75–0.98) [150]. Conclusions from this meta-analysis suggested a 5% absolute survival benefit at 5 years in favour of neoadjuvant chemotherapy for all radical local treatments (RC, radiotherapy, or both).

Lymphatic drainage of the bladder

The lymphatic system is a complex endothelial lined drainage system interspaced by LNs. Lymphatic drainage of the urinary bladder begins in a series of capillary structures in the submucosa that drains into lymphatic vessels extending through the bladder wall to the paravesical lymph vessels and LNs. Drainage continues to the regional pelvic LNs and further to non-regional LNs above the bifurcation of the aortae. Lymphatic vessels increasing in calibre continue to the thoracic duct which empties into the venous bloodstream at the conjunction of the left subclavian and the left internal jugular veins. Pelvic LNs are clustered in groups along the large blood vessels and named predominantly according to these (Figure 5). The external iliac LNs are in continuity with the LNs draining the lower limb. They are arranged along the external iliac vessels and can be divided into a medial, lateral, and obturatoric group. The obturatoric group is considered as a separate group by most urologists and is located between the external iliac vessels, the obturator nerve and the pelvic wall. The internal iliac LNs are located lateral of the internal iliac artery and anterior below the obturator nerve in continuity with the lateral paravesical LNs. LNs immediately medial to the internal iliac artery are usually named the lateral presacral LNs, whereas the true sacral LNs are located directly anterior of the sacrum. The common iliac LNs are located along the common iliac vessels. LNs medially to the common iliac vessels are the medial, promontoric, and subaortic common iliac LNs that are usually considered part of the presacral LNs. The common iliac and presacral LNs drain into the parietal lumbal LNs named by location according to the aorta and inferior caval vein [51,151-153].

Based on several mapping studies, the regional LNs have been shown to consist of all pelvic LNs below the bifurcation of the aortae [5,76,153,154]. Thus, solitary LN metastasis can be encountered in all these locations. Mapping studies have also found that skip lesions to locations above the bifurcation of the aortae without more distally located LN metastases are extremely rare and only reported in very few patients in the available literature [154,155]. Peritumoural injection of radioactive Technecium-99-labelled nanocolloid, preoperative lymphoscintigraphy, and intraoperative detection by the aid of a gamma ray detection probe to make an in vivo dynamic SN identification have been applied to identify LNs involved in drainage of the tumour-bearing part of the bladder only [156,157]. An unacceptably high false negative rate of 19% found in one of the studies has, however, rendered this technique unsuitable for clinical practise [156]. If the SN detection concept in RC involves identification of possible SNs and does not exclude dissection of other LNs, the technique can, however, be used to select LNs for a more thorough pathological examination and to ensure a more thorough removal of all relevant LNs. In a study by Liedberg et al., LNs were identified with the gamma probe when assessing the nodal basins after presumed completion of LND in 7 of 75 patients (9%) [156]. This

study found the common iliac nodes to harbour the SN in 2 of 65 patients (3%) with identified SN, whereas in the remaining 63 patients (97%), the sentinel LNs were located in the obturator, external iliac, or internal iliac LNs.

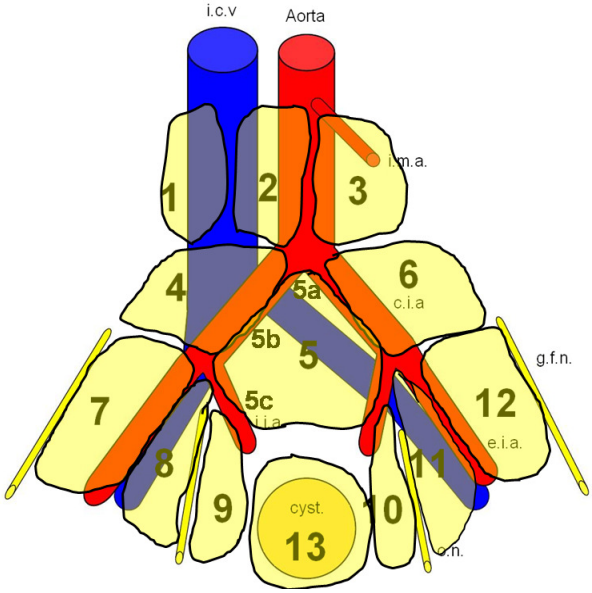


Figure 5:*Lymph node localization in the pelvis and lower abdomen. 1: Para-caval, 2: inter-aortocaval, 3: para-aortic, 4: right common iliac, 5: presacral (5a: subaortic common iliac, 5b: promontoric common iliac, and 5c: lateral presacral), 6: left common iliac, 7: right external iliac, 8: right obturator fossa, 9: right internal iliac, 10: left internal iliac, 11: left obturator fossa, 12: left external iliac, and 13 perivesical lymph nodes.*
Abbreviations: i.c.v.: inferior caval vein, i.m.a.: inferior mesenteric artery, c.i.a.: common iliac artery, i.i.a.: internal iliac artery, e.i.a.: external iliac artery, g.f.n.: genitofemoral nerve, o.n.: obturator nerve, cyst.: cystectomy specimen.

In a recent study by Roth et al., preoperative SPECT/CT was used to assess LN distribution and drainage patterns in 60 consecutive patients with BC undergoing RC. Intravesical injection of radioactive Technecium-99-labelled nanocolloid was made not only in the tumour-bearing part but also in the non-tumour-bearing part of the bladder [77]. In addition to finding a variable number of pelvic LNs among the patients, this study confirmed the ability of midline crossing of lymphatic drainage of the urinary bladder. This has also been shown in mapping studies [76,158]. Bilateral LND is therefore warranted in all patients despite the presence of a strictly unilateral tumour.

Historical aspects of lymph node dissection

One of the pioneers of radical cancer surgery, William S. Halsted, noted in an article on surgery of carcinoma of the breast in 1891 that he, eight years earlier, had begun to clean out the axilla in all cases of cancer [159]. In the same article, he referred to other surgeons in favour of methodically cleaning out the axilla as 'surgeons of the first rank'. This was one of the first suggestions of the curative potential of LND in cancer surgery. In bladder cancer, LND, at the time of RC, was first described by Godard and Koliopoulos in 1932 [160] but it was not until the late 1940s that the feasibility of RC with LND was reported in larger patient series. Marshall and Whitmore described in 1949 a technique of RC with LND beginning above the bifurcation of the aortae [161]. A more restricted LND template was later described by the same group by performing LND only to the uretero-iliacal crossing [162,163]; a template still considered gold standard for pelvic LND in some centres [79] (Figure 6).

In 1950, Leadbetter and Cooper reported their first 15 cases of RC with LND (10 in patients with BC, 5 in patients with other malignancy) in an article meticulously describing presumed lymphatic drainage of the bladder and instructions to a LND from the femoral canal distally to the bifurcation of the aortae proximally [164]. Moreover, they reported that one of the two patients they had operated with BC and positive LNs was doing well after RC and LND [164]. They concluded, however, that follow-up was too short for evaluation of a true benefit in this patient. The same year, Kerr and Colby suggested LND to be curative in 2 of the 4 patients they had operated with nodal metastasis because they had not died immediately postoperatively [165]. During the following decades, BC with LN involvement was considered an almost incurable disease [166]. Thus, LND was regarded as a diagnostic procedure and rarely as part of the curative intended treatment. This interpretation was consolidated with the series, published in 1981 by Smith and Whitmore, that reported a 5-year survival rate of only 7% in 134 patients with positive LNs [167]. Based on the diagnostic staging approach and little chance of cure in LN positive patients, Wishnow et al. advocated in 1987 for an LND restricted to the obturator fossa and the area inferior of the obturator nerve. In case of unilateral tumour they advocated for a strictly ipsilateral dissection [168]. However, 5 years earlier, Skinner had found a 5-year survival rate of 36% in 36 patients with nodal metastasis undergoing RC and LND to the level of the inferior mesenteric artery [43]. Therefore, he suggested a meticulous LND to be potentially curable in LN-positive patients. Several series have later confirmed these findings by demonstrating acceptable long term results in patients with limited nodal disease treated by surgery only [6,158,169-173]. Even patients with grossly metastatic LNs have been described to have a benefit from RC with a thorough LND [44].

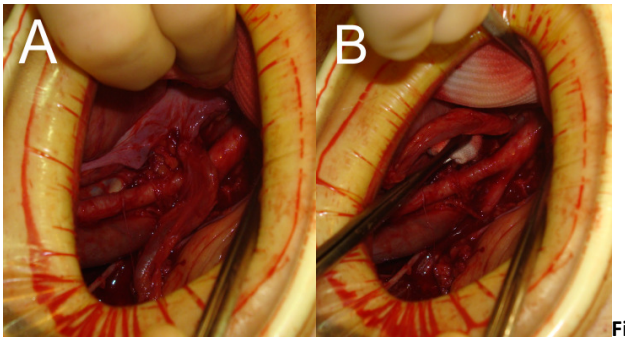


Figure 6:*The right iliac vessels following extended LND. In (A), the ureter is in situ at the ureteroiliac crossing, whereas in (B) the ureter is pushed aside to visualize the iliac bifurcation. In this patient the ureteroiliac crossing was situated exactly at the level of the bifurcation.*

In 1998, Poulsen et al. compared two historical cohorts of patients undergoing RC and LND according to two different templates: one limited proximally by the bifurcation of the common iliac artery (standard LND) and one extended up to the bifurcation of the aortae [174]. They found improved survival for patients with organ-confined tumours and without LN metastasis undergoing extended LND, whereas statistically, there was no significant difference in survival of other patient categories. Contrary to these findings, more recent historical comparisons and comparisons across continents have suggested the most pronounced survival benefit by extending the limits of LND in patients with locally advanced disease [175-178]. This is in agreement with a

higher risk of LN metastasis in the patients with non-organ-confined tumours.
Despite the general agreement of a possible therapeutic effect of LND, there is variation in the use of LND [179]. Moreover, there is no agreement to the proximal limits of the optimal LND template and different templates are therefore used at different institutions.

Survival benefit of lymph node dissection

Prognosis is evidently improved in patients undergoing RC if an LND is performed as part of the surgical procedure. This is most clearly illustrated by the patients with positive LNs that turn out to be long term survivors following surgery only. The improved survival also accounts for some supposedly LN-negative patients.

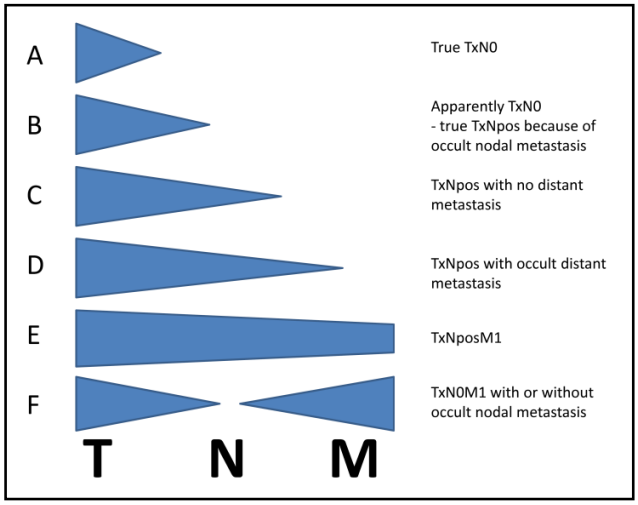


Figure 7:*Theoretical classification of patients undergoing RC based on the presence of nodal and/or distant metastases. Group A, B and C should undergo primary RC. Group D will develop extra-pelvic recurrence shortly following RC. Metastatic disease is found by preoperative imaging in group E and F. Group B and C benefits from a thorough LND, whereas group A, D, E and F do not. See text for further details.*

However, even very extensive radical surgery cannot always cure patients, even if no sign of distant metastasis is diagnosed at the time of surgery. This paradox can be explained as illustrated in Figure 7. Group A patients have a true localized disease, whereas group B patients are diagnosed as N0 because of the inability of SPE to prove the LN metastasis but harbour occult LN metastasis. Group B patients will, therefore, benefit from a thorough LND at the time of RC, provided that the LND template includes removal of all occult LN metastasis, whereas group A patients will be cured by RC irrespectively of the LND template applied. Group C and D patients are LN positive but only group C patients benefit from LND, given that the positive LNs are contained within the LND template. In group D patients, positive LNs are part of a more widespread metastatic disease. Theoretically, patients can be replaced from group D to the more favourable group C by extending the boundaries of the LND. Group E and F patients with evident distant metastasis should undergo RC only for palliative reasons or if preceded by systemic chemotherapy as part of a multimodality treatment.

Lymph node dissection templates

The literature often refers to different LND templates using the same terms. Thus, extended LND can be described by a proximal limit as high as the level of the inferior mesenteric artery or as low as at the uretero-iliac crossing [76,176]. In the current literature, limited LND covers a spectrum of templates ranging from dissection of the obturator nodes only, to dissection of all LNs caudal to the bifurcation of the common iliac artery [175,176]. The present author has contributed to this confusion by referring to an LND to the level of the inferior mesenteric artery as an 'extended LND' [III]. 'Super-extended LND' would have been a more appropriate term.
To obtain consistence, the following definitions will be used throughout the remaining contents of the present thesis:
Limited LND: Bilateral removal of LNs and fatty tissue from the obturator fossa (Figure 8a).
Standard LND: Bilateral removal of LNs and fatty tissue from an area limited proximally by the bifurcation of the common iliac artery, laterally by the genitofemoral nerve and inferiorly by the inguinal ligament and the pelvic floor including the external iliac LNs and LNs anterior of the internal iliac artery along with the obturator LNs (Figure 8b).
Subtotal LND: Like standard LND but proximally extended to include the common iliac LNs inferior to the uretero-iliac crossing. Some LNs located medially to the internal iliac artery are also included but a complete removal of the presacral LNs is not made (Figure 8c).
Extended LND: Removal of LNs and fatty tissue from an area limited proximally by the bifurcation of the aortae, laterally by the genitofemoral nerves and inferiorly by the inguinal ligaments and the pelvic floor. Extended LND includes therefore the LNs removed by a standard LND and removal of the common iliac LNs and all the presacral LNs (Figure 8d).
Super-extended LND: Removal of LNs and fatty tissue from an area as defined by the extended LND and removal of the para-caval, inter-aortocaval, and para-aortic LNs located proximally to the bifurcation of the aortae up to the level of the inferior mesenteric artery (Figure 8e).

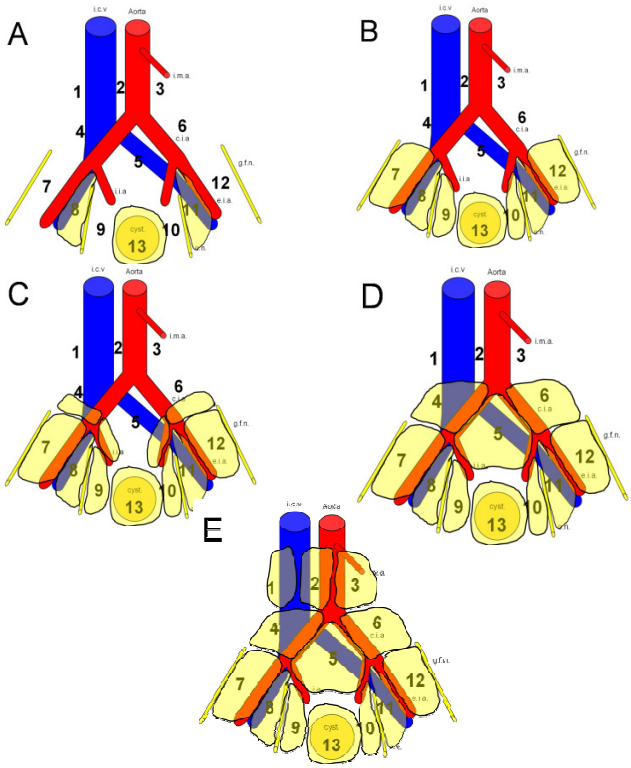


Figure 8:*LND templates: (A) limited LND, (B) standard LND, (C) subtotal LND, (D) extended LND, and (E) super-extended LND*

AIMS OF THE STUDIES

Two different aspects of LND have driven the present thesis: Evaluation of the impact on nodal staging and evaluation of the possible influence on prognosis.

Because most results rely on the ability of SPE to identify LNs and LN metastasis, we included evaluation of SPE in the present work. Hence, the aims of this thesis were as follows:

1. To evaluate the sensitivity of SPE of LND specimens regarding identification of LNs and LN metastasis (papers I and II).
2. To make a mapping of regional LN metastases in patients undergoing RC and LND and thereby evaluating the impact of different LND templates on nodal staging (papers III and IV).
3. To evaluate the prognostic value of different LN variables regarding prognosis (paper V) and to estimate the prognostic value of different LND templates in 'standard' patients and 'non-standard' patients (papers VI and VII, respectively). Furthermore, we wanted to evaluate the influence that different LND templates had in a material evaluating a potential prognostic molecular marker (paper VIII).

PATIENTS AND METHODS

In the following, an overview of the patients and applied methods is presented. A more detailed description is given in the individual articles.

Patients

The study population included all consecutive patients undergoing RC because of BC at the Department of Urology, Aarhus University Hospital, Skejby from 1999 until January 2009.

Patients operated from January 2004 to January 2009 were prospectively enrolled in 'the lymph node project'. From the start of this research project, we aimed at performing a super-extended LND at the time of RC in all patients. Clinical and histopathological data were prospectively registered in a database designed for the project. This cohort formed the base of papers I–V and part of the patient material in papers VI–VIII.

Retrospective registration of clinical and histopathological data of a historical cohort of patients undergoing RC and limited LND between 1999 and 2003 accounted for the remaining patient material included in paper VI. This thesis does not include patients in paper VIII with NMIBC undergoing TURB only.

Of 282 patients included in 'The Lymph Node Project', 32 were excluded from the material in paper I–VI and paper VIII. Exclusion criteria were previous oncological treatment (systemic chemotherapy or radiotherapy because of BC), previous radical prostatectomy with LND, and patients with gross metastatic LNs left behind in the pelvis at the time of surgery because of widespread metastatic disease diagnosed peroperatively. Patients undergoing previous oncological treatment were addressed in paper VII.

Eighty patients that did not undergo super-extended LND were excluded from the mapping study (paper III). The reasons for these incomplete LNs were various: surgical technical problems because of earlier surgical procedures, extensive atherosclerotic disease, massive bleeding, fibrous tissue difficult to resect, anaesthetic problems requiring short operation time, and nervesparing technique where omission of dissection of the presacral and most proximal LNs was decided based on the operating surgeons preference. We found it methodologically more correct to exclude these patients with an insufficient LND.

Thus, the mapping study consisted of 170 patients, whereas additionally three patients who were given adjuvant chemotherapy because of a non-organ-confined tumour were excluded from the follow-up study of this patient cohort. Hence, none of the patients included in paper V underwent neoadjuvant or adjuvant chemotherapy and all included patients underwent super-extended LND according to the same uniform template.

If not specified otherwise, T-stage used for analyses was the highest of the pre-RC T-stage (pathologically verified by transurethral resection (TURB)) and the pathological pT-stage of the RC specimen. T- and N-staging was classified according to the 6th edition of the TNM classification [21].

Preoperative staging

Preoperative clinical staging of patients included in 'The Lymph Node Project' consisted of TURB, bimanual palpation under general anaesthesia, CT-scan of the abdomen, and CT or X-ray of the chest. Neither MRI nor bone scintigraphy was performed as a routine examination but by special indication. Patients with signs of metastatic disease, including large retroperitoneal LNs above the bifurcation of the aortae, underwent FNAC or histological biopsy to confirm the diagnosis and were referred to chemotherapy instead of RC, if positive.

Follow-up

Patients were followed up by a routine schedule including clinical examinations and regular CT-scans of the chest, abdomen, and pelvis for at least 5 years or until censoring. All clinical data were updated as of January 2011. Patient alive at the end of study follow-up were followed for a minimum of 24 months. Patient follow-up regarding recurrence-free survival (RFS) was from RC to radiologically, histologically, and/or clinically proven recurrent disease or until the most recent follow-up with no suspicion of recurrence. Primary recurrence site was in each patient registered to be either local recurrence (adjacent organs, pelvic wall or undefined soft tissue within the RC cavity), LN metastasis (within the pelvis or distant), or visceral metastasis.

Time of death was given by a search in the Danish Central Personal Registry (a unique identification number is given to all Danish citizens; daily updates contain information on address, vital status, etc.). None of the patients were lost to follow-up regarding overall survival (OS). Cause of death in patients with known recurrence was regarded as BC-related when calculating disease-specific survival (DSS), whereas cause of death in patients with no evidence of recurrent disease, and with known other cause of death was regarded as BC-unrelated.

Standard pathological examination

SPE of the RC specimens included as a minimum histological type, pT-stage of the tumour, and presence of malignancy in the surgical, urethral, or ureteral resection margins. Moreover, presence of LNs within the perivesical fatty tissue and potential metastasis in these were recorded. Meticulous sectioning of the perivesical fat or other techniques to identify LNs in the RC specimen was not applied.

LND specimens were sent to pathological examination from 12 pre-designed anatomical locations as separate packages in all patients undergoing super-extended LND. SPE of LND specimens included meticulous palpation in bright light and sectioning of the tissue into thin slices if required. All identified LNs were excised. LNs larger than 4 mm were cut in 3–4 mm thick sections and processed routinely into paraffin embedded blocks. Sections were

stained with haematoxylin and eosin (H-E). Immunohistochemistry (IHC) was used when in doubt but not as a routine. Only organized lymphoid structure with sinus system surrounded by a fibrous capsule and with visible lymph vessels was registered as LN, whereas lymphocyte accumulations in adipose tissue without these features were not.
All LNs were prospectively registered as metastatic or non-metastatic, and extranodal extension of metastatic LNs was noted. The number of LNs, longitudinal length, and transverse diameter of each individual LN were registered for each anatomical location. All specimens were examined by experienced uropathologists.

Additional pathological examination
In paper I, 15 consecutive patients were selected for evaluation of the sensitivity of SPE regarding identification of LNs in LND specimens. Basically, the remaining fatty tissue from the LND specimens with no palpable or visible LNs was cut into 3 mm slices and paraffin-embedded. One sectioning of each tissue block was made and stained with H-E. Additional LNs missed by SPE were identified microscopically from these slides.
Based on random numbers, 10 LN negative patients were selected from the study database of all patients. All LNs of more than 2 mm within the pelvis in these patients were step sectioned and stained with IHC to evaluate the sensitivity of SPE regarding identification of metastasis within LNs.
In paper VIII, a TMA was constructed. The TMA consisted of tumour samples from 425 patients undergoing RC between 1992 and 2008. Tissue samples from all patients with TCC where sufficient formalin fixed paraffin embedded tissue was available at the Institute of Pathology were included in the TMA. The TMA was stained by IHC for expression of two different molecular markers: Karyopherin alpha 2 (KPNA2) and Ki-67.

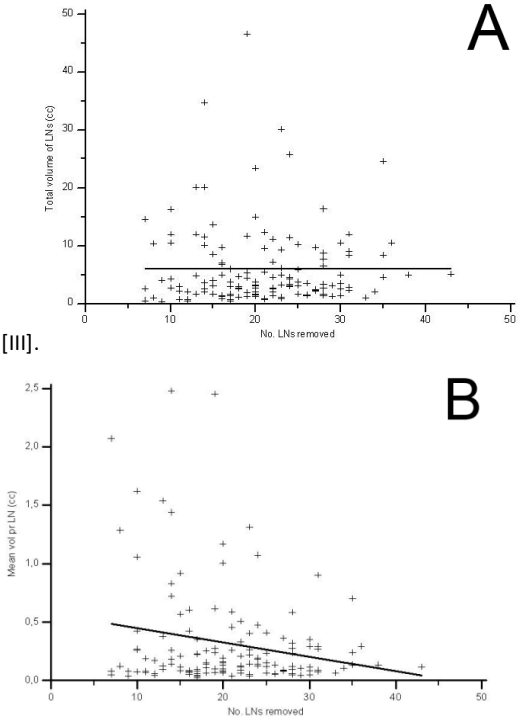
Ethics
Inclusion of patients in the project was approved by the Regional Committee on Biomedical Research Ethics of Aarhus County (later included in the Central Denmark Region Committees on Biomedical Research Ethics) (approval no.: 1994/2920 and 20040110). The database was approved by the Danish Data Protection Agency (approval no.:2007-41-0629).

Statistical analyses
Comparison of incidences was assessed using Fisher's exact test or chi-square where appropriate. Receiver operating characteristics (ROC) curves were constructed for detection of LN metastasis according to the size of an LN (paper IV). Survival estimates were calculated in life-table analyses using the Kaplan-Meier method with log-rank test for significance and univariate and multivariate Cox regression analyses. P values were based on two-sided testing at a 5% significance level. Statistical analyses were performed using the MedCalc® computer software (MedCalc Software, Mariakerke, Belgium).

RESULTS
An overview of the results that forms the thesis is summarized in the following. A detailed description is given in the individual articles.

Pathological examination
Sensitivity of SPE regarding identification of LNs in LND specimens was found to be 95% per LN (95% CI: 92–97%). Additional positive and negative LNs up to 15 mm in length missed by SPE were found in 6 of 15 patients following meticulous step sectioning of the fatty tissue [I].
Step sectioning of 173 presumably negative LNs revealed one LN with metastasis missed by SPE. This LN was found in a patient with non-organ-confined disease (pT3a). Negative predictive value (NPV) of SPE regarding identification of metastasis within an LN was 99% per LN (95% CI: 97–100%) and 90% per patient (95% CI: 56–100%). Assuming the frequency of the findings was representative of the entire RC cohort, sensitivity of SPE was estimated to be 76% per patient (95% CI: 42–99%). Estimated sensitivity increased to 91% per patient (95% CI: 34–100%) when adjusting for different T-stages [II]

Mapping and staging
Twenty-five percent of the patients undergoing super-extended LND had positive LNs (N+). Incidence of N+ increased significantly with higher T-stage, whereas location of positive LNs, including distribution of regional versus non-regional LN metastases, was independent of T-stage. A 'sentinel node' (i.e. the first draining LN from the tumour bearing part of the bladder) was estimated to be located below the bifurcation of the common iliac artery, and therefore within the template of a standard LND in most patients. In one patient, the SN was located in the presacral area, and in one patient in the common iliac area. The presacral LNs were involved in 3% of all patients, 12% of N+ patients. The common iliac LNs were involved in 6% of all patients, 26% of N+ patients. No patient had positive LNs above the bifurcation of the aortae without more distally located positive LNs. Limited, standard, and extended LND templates would have identified 70%, 99%, and 100% of N+ patients. The same templates would have left positive LNs behind in at least 16%, 11%, and 4% of all patients (65%, 42%, and 16% of all N+ patients)



[III].

Figure 9: *Correlation between the total number of LN removed per patient and total volume of LNs (A) and mean volume of per LN (B). Only patients without pos LNs included in the figure.*

Increase in size and volume of LNs in the pelvis and lower abdomen was correlated to a significantly higher risk of LN metastasis. The predictive value of length or diameter increased with a more cranial location of the relevant LN. Though significant, the correlation was poor and had only minimal clinical impact [IV].
The number of retrieved LNs varied considerably within the same LND template. Moreover, the volume of dissected lymphatic tissue was found to be independent of the number of LNs retrieved (figure 9). This suggested that a fixed volume of lymphatic tissue rather than a fixed number of LNs is present in the pelvic region [IV].

Prognostic value

Survival estimates in patients undergoing super-extended LND showed that female gender, advanced T-stage, presence of LN metastasis, number of positive LNs (1 vs. more than 1), and the presence of non-regional LN metastasis above the bifurcation of the aortae were independent adverse prognostic predictors. The number of LNs retrieved had no influence on prognosis of neither LN negative nor LN positive patients. Several other evaluated LN variables had no independent prognostic value [V].
Recurrences within the pelvic LNs were significantly more frequent in patients undergoing limited LND compared with patients undergoing a more extensive LND. When patients undergoing RC according to different LND templates were compared, recurrence patterns were remarkably identical. Patients with locally advanced disease (non-organ-confined tumour or LN metastasis) apparently had a better prognosis following a more extensive LND, whereas this was less evident in patients with organ-confined disease. Some patients were identified as possible 'stage migrators' because of a more accurate staging following the more extensive LND. These 'stage migrators' were a minority of the patients (approximately 6% of a consecutive RC series) that evidently had a better prognosis following LND according to a more extensive template than according to the limited LND template [VI].
In patients with recurrence of BC, 80% of all recurrences were diagnosed within 24 months after RC. Time from recurrence to death was significantly longer in patients with primary LN recurrence compared to patients with local recurrence or visceral metastasis (figure 10) [VI].

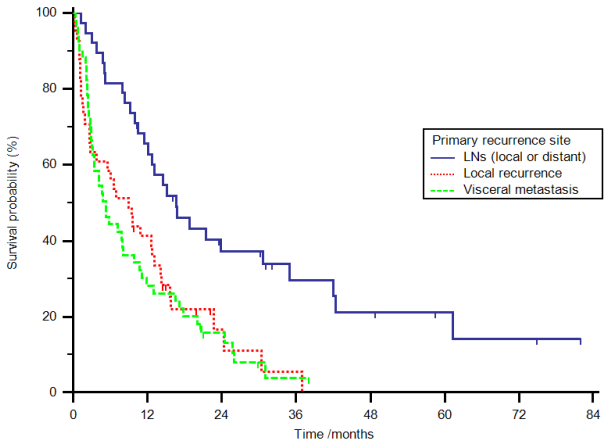


Figure 10: *Time from postoperative recurrence to death stratified according to primary recurrence site. Patients with more than one recurrence site at diagnosis were excluded from analysis.*

In patients undergoing salvage RC because of local failure of intended curative radiotherapy, 2-year and 5-year DSS were 47% and 31%. Difficulties with LND because of fibrous tissue were accounted in most patients. No metastatic LNs were found in the field of irradiation in patients undergoing LND and pelvic LN recurrences were not registered during follow-up. This suggests that LND can be omitted in this patient category [VII].
In patients subjected to preoperative chemotherapy because of preoperatively diagnosed non-regional LN metastasis, 2-year and 5-year DSS were 67% and 50%. Despite chemotherapy, some patients had vital tumour cells in the removed LNs [VII].

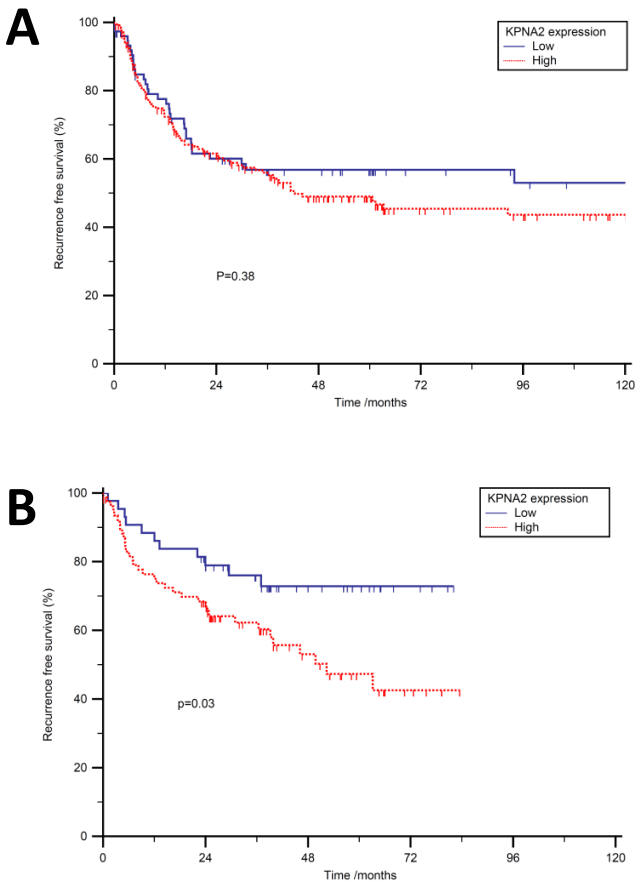


Figure 11: *RFS of patients with invasive BC undergoing RC. Stratification is made according to KPNA2 expression in patients undergoing limited (A) and extended (B) LND. Log-rank p-values are shown.*

High nuclear expression of KPNA2 (positive KPNA2) was found to be an adverse prognostic marker, independent of conventional clinic-pathological features. This correlation was not evident in patients undergoing limited LND. However, in patients undergoing extended or super-extended LND, a significantly poorer prognosis was found in patients with KPNA2 positive tumours (Figure 11). An extended or super-extended LND was associated with a better survival in patients with KPNA2 negative tumours, whereas extent of LND had no prognostic influence in patients with KPNA2 positive tumours (Figure 12). In agreement with this, patients with KPNA2 positive tumours had a significantly higher risk of

developing visceral metastasis, and a significantly lower risk of LN recurrence, compared to patients with KPNA2 negative tumours [VIII].

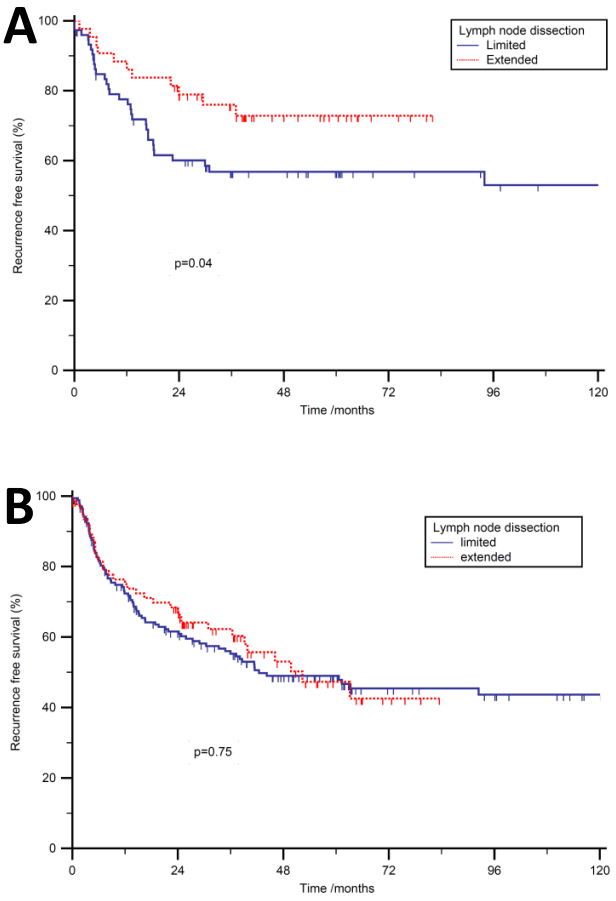


Figure 12:*RFS of patients with invasive BC undergoing RC. Stratification is made according to LND template in patients with negative (A) and positive (B) KPNA2 expression.*

DISCUSSION

Detailed discussions of the different aspects of the thesis are included in the individual articles. The main and central issues are further discussed in the following.

Analyses in the present and similar studies are predominantly based on the identification of LN metastasis by SPE. It is therefore essential that SPE have a high sensitivity regarding identification of LNs and LN metastasis. The present studies found SPE to have a high sensitivity regarding identification of LNs (95%) and identification of LN metastasis (91% per patient if adjusted for T-stage) [I,II]. However, we also found that even positive LNs could be missed in specimens. Apparently, there is a higher risk of occult LN metastasis in patients with locally advanced disease as a consequence of the higher incidence of LN metastasis in these patients. The probability of identification of LN metastasis depending on pathological sectioning technique was first addressed in a theoretical model published by Wilkinson and Hause in 1974 [180]. More recently, 2 different mathematical models estimating the probability of identifying metastasis in sentinel LNs depending on sectioning technique have been published by Meyer et al. and Farshid et al. [181,182]. To no surprise, all models show that the probability of identifying metastasis within an LN is dependent on size of the LN, size of the metastatic lesion, and number of sections per LN. Findings of additional LN metastases by step sectioning technique can therefore explain the improved survival following more extensive LND in apparently LN negative patients in RC series [174-176].

Step sectioning technique has been widely applied in SN diagnostics in oncological surgery where investigation is restricted to a few LNs. In urological malignancies, SN diagnostics has been used for more than a decade in penile carcinoma [183,184]. In BC, the use of SN technique has been limited to a few studies with somewhat disappointing results because of low detection rate [156,157].

As a supplement to standard staining, IHC can be used as a guide if normal H-E staining is inconclusive but the standard use of IHC is costly and of uncertain relevance. Yang et al. stained 159 negative LNs with IHC in 19 patients with MIBC [185]. Only in one LN, where the original H-E staining was without metastasis, they found metastasis in the IHC stained slide. However, new H-E staining of a section made adjacent to the IHC section also revealed metastasis. Likewise, we found one occult LN metastasis positive by IHC to be positive in H-E staining when an adjacent section was stained [II]. This is in agreement with the findings of a study on penile carcinoma where step sectioning technique at three levels rather than IHC helped identifying additional minimal metastatic disease in non-sentinel LNs [186].

Molecular markers have been suggested to increase detection of micrometastatic disease in LNs. Rt-PCR analysis for uroplakin II (UPII) have been found positive in approximately 10% of all LNs retrieved in LN negative patients with BC. Molecular upstaging was made in 14–35% of the LN negative patients [118,120,121]. In a study using rt-PCR analysis for mucin 7 (MUC7), 29% of histologically classified negative LNs were positive for MUC7 indicating occult metastasis [122]. Marín-Aguilera et al. compared survival of histologically LN negative patients stratified according to molecular LN staging by rt-PCR analysis for two different genes (FXYD3 and KRT20) [119]. Despite molecular upstaging of 20.5% of the patients, this patient group had the exact same prognosis as patients without genetic sign of LN metastasis. They concluded that the non-inferior survival was a consequence of removal of residual disease by LND. Another explanation could be the inadequacy of molecular staging by the suggested methods.

Laborious efforts to identify occult LN metastasis missed by SPE should be considered in context with the consequences of diagnosing micrometastasis. Patients diagnosed with minimal metastatic burden in the local LNs have a favourable prognosis following surgery only and at the present time, adjuvant chemotherapy is not recommended irrespective of LN metastasis [128]. Therefore, it remains highly speculative whether additional pathological or molecular examinations of the retrieved LNs will have a positive influence on survival of BC patients undergoing RC.

Interestingly, Fang et al. reported a significantly better prognosis in an RC cohort undergoing a more thorough pathological examination to retrieve more LNs in the specimens when comparing to a historical cohort in which an identical LND template was used but where a less thorough pathological examination was applied [187]. More patients with organ-confined tumours in the latter cohort may have given the improved prognosis rather than a more thorough pathological examination. It could be argued that patients are not cured at the Institute of Pathology but may be cured by means of a thorough LND.

Specificity of SPE is thought to be 100% regarding identification of LN metastasis but as shown in a recent study by Parkash et al.,

the definition of an LN is variable among pathologist, thereby giving a variable number of retrieved LNs depending on the pathologist [78]. In a study where the same 15 slides containing a variable number of LNs were presented to 10 pathologists, a variability of 11% in LN count was found. The greatest inter-observer variation was in two slides where LN count varied from 1 to 11 and 5 to 16. Moreover, intra-rater variability was found when the same slide was presented to the same pathologist on 2 different occasions. This illustrates some of the difficulties of comparing LN counts in different RC series or in multi-centre studies without central pathology revision.

When comparing advantages and disadvantages of different LND templates, several issues have to be taken into account. Extending the boundaries of LND can be justified from a staging perspective, prognostic perspective, or both. Theoretically, this can result in recommendation of one template for staging, whereas another template is more ideal from a prognostic perspective. However, because the chance of cure by surgery alone is higher in patients with minimal metastatic burden compared to patients with widespread LN metastases, inclusion of the 'SN region' seems appropriate from a staging as well as from a prognostic perspective. Dissection of LNs outside the staging template can provide a better prognosis in some cases but more often, non-regional LN metastases are part of a disseminated rather than localized disease. Instead, acceptable long term survival can be provided in these patients if pre-operative chemotherapy is administered [188-191,VII]. In addition, morbidity, complications, and increased operative duration associated with a more extensive LND should be considered. Brössner et al. compared two non-randomized cohorts undergoing RC and limited LND or RC and super-extended LND [192]. They found the duration of surgery to be 63 minutes longer in median in the super-extended group. However, the two cohorts were operated by two different surgeons so other causes of increase in duration of surgery may have existed. On the other hand, they could not prove differences in peri-operative complications or mortality. El-Shazli et al. found that removal of the lower para-aortic LNs increased the duration of surgery by 10–20 minutes (mean 15 minutes) when performing a super-extended LND compared with an extended LND [193]. Dissection of the para-aortic, inter-aortocaval, and para-caval LNs is laborious though and can be associated with a higher risk of peri-operative bleeding. In our experience, performing a super-extended LND takes a median of 25 minutes longer than a standard LND [III]. This was the result of chronographic timing of selected parts of the procedures, whereas comparison of average operative duration in two historical cohorts with different LND templates could not identify a prolonged operative duration [VI]. This illustrates that operative duration is affected by other variables than LND template.

The total number of retrieved LNs has been suggested as a surrogate marker of surgical quality. Leissner et al. found improved prognosis in patients with 16 or more LNs removed compared to patients with a lower number [194]. Most prognostic impact of number of LNs have been found in large registry studies like the studies published from the Surveillance, Epidemiology, and End Results program cancer registry (SEER) [195,196], or other multi-institutional studies [197]. A major limitation of this type of studies is lacking information of the LND template used in each patient. It is therefore likely that by adjusting LND template, the total number of LNs would have had less influence on prognosis, if any. Recently, Park et al. published a study on 450 patients undergoing RC and standard LND [198]. They found no impact on prognosis by a higher number of LNs in neither LN positive nor LN negative patients. This is in agreement with our findings in patients undergoing super-extended LND [V]. Moreover, we found that the total number of LNs retrieved from the same super-extended template varies considerably among patients [III]. This is in agreement with the findings of other studies [5,76,77,194]. Studies suggestive of improved survival with more LNs removed have either included variable LND templates, failed to report of results of multivariate analysis, or have found the number to fail as an independent predictor in multivariate analysis [194-196,199-201]. Moreover, a high number of LNs does not always mean that the correct LNs have been removed [79].

The total number of retrieved LNs is, however, a good surrogate marker of surgical quality when comparing LN counts from identical templates, submitted in the same way (separate package or en bloc), examined by the same methods, by the same pathologist. This is utilized if different surgical approaches to RC and LND are compared and has been widely used in laparoscopic, robotic-assisted, and minilaparotomy RC series comparing immediate oncological outcome with contemporary series of standard open RC [129,202-208]. LN number is, on the other hand, not a good marker of surgical quality when comparing different surgical series where a central pathology revision is not applied.

LN density has been suggested to be an independent prognostic marker by taking LND template, surgical quality and metastatic burden into account in one single parameter [209]. LN density is defined as the number of positive LNs divided by the total number of retrieved LNs. The number of retrieved LNs is, as indicated above, less important if a uniform LND template is used in the RC series evaluated. LN density is possibly a true significant prognostic marker in registry based RC series where correct adjustment for different LND templates cannot be made. However, proper adjustment for LND template and metastatic burden will result in LN density as a less influential prognostic marker, possibly with no prognostic impact at all. As mentioned above, the number of LNs was of no prognostic value in patients undergoing LND according to the same super-extended template in our study. Thus, LN density was only significant regarding prognosis in univariate analysis and not in multivariate analysis adjusting for metastatic burden given in the N-stage [V].

In the present thesis, we found that the quantity of lymphatic tissue was independent of the number of LNs retrieved in each patient. In other words, patients can have many small LNs or a few larger LNs. This variation in size of non-metastatic LNs makes pre-operative imaging less sensitive when a size criterion is used to identify potential LN metastasis. A metastatic LN in a patient with many small LNs will not necessarily increase beyond the size of non-metastatic LNs in a patient with few larger LNs. This could explain the increase in LN size as a poor predictor of LN metastasis found in the present thesis [IV]. Despite significant correlation between size of an LN and presence of metastasis, this had neglectable clinical relevance. Moreover, it could be the result of many LNs with minimal metastatic burden giving minimal increase in size. Other criteria than pure size (e.g. shape, internal architecture, and functional or metabolic features like FDG metabolism in FDG-PET-CT) should therefore be used in imaging to increase sensitivity [51].

The primary LNs involved in drainage of the urinary bladder have been investigated in several mapping studies [5,76,155,167,168,210-212]. Patients with metastasis to a single LN or a single group of LNs without other positive LNs are of special value in these studies. These LNs represent the SN region

necessary to dissect to get a correct N-staging. Moreover, patients with metastasis to this SN can potentially be cured by surgery alone if the LND template includes this LN region. The most common location of LN metastasis is the obturator fossa [76,167,168,III]. This location has been reported to be involved in up to 74% of all LN positive patients [167]. The second most common location is the external iliac LNs. One publication by Wishnow et al., has, however, disputed this [168]. Furthermore, they recommended limited and preferably unilateral LND. The unilateral approach has later been rejected by several later studies based on contralateral LN metastasis and LN drainage [76,77,III].

Approximately 75–90% of all LN metastases in patients undergoing RC are located below the bifurcation of the common iliac artery and therefore within the standard LND template [5,76,167,213,III]. We found the sensitivity regarding identification of LN disease of limited, standard, and extended LND templates when compared to a super-extended LND template to be 70%, 99%, and 100%,. In agreement with this, Dangle et al. reported of sensitivities of 75%, 88.9%, and 100% using these templates in an identical setup [214]. These findings are in agreement with the in vivo SPECT/CT mapping study by Roth et al. [77]. They found 92% of the LNs involved in primary drainage of the bladder located distally to the uretero-iliac crossing, whereas extrapolations from the mapping figure of the original article suggest that by including LNs up to the bifurcation of the aortae, 98% of all primary LNs are encountered. Isolated LN metastasis to the common iliac LNs or presacral LNs have been found in a minority of LN positive patients in several studies [5,76,156,211,213,III]. Isolated skip-lesions to the lower aortic LNs have been scarcely reported only in single patients in two studies [155,213], whereas the remaining available mapping studies found metastasis in these LNs only in patients with other more distally located synchronous metastasis.

Limitations of surgical mapping studies based on histopathology of removed LNs include possible sampling errors and the individual interpretation of location in LNs located at the border between two LN regions. Thus, LNs located at the bifurcation of the common iliac artery can be included in the external iliac, obturator, internal iliac, presacral, or common iliac LNs depending on the operating surgeon's interpretation. In order to include these specific LNs at, or above, the bifurcation of the common iliac artery, a subtotal LND has been suggested instead of the standard LND where these LNs potentially will be left behind. Ideally the subtotal LND therefore includes removal of the inferior part of the common iliac LNs up to the uretero-iliac crossing. However, the ureter crossing can be located at, or even below, the bifurcation of the common iliac artery in some patients (Figure 6). Potentially, this provides a less thorough LND than standard LND in these patients if subtotal LND to the uretero-iliac crossing is undertaken.

Frozen section examination of obturator and internal iliac LNs has been suggested to be reliable and to be decisive of performing additional LND [215]. Results from a Turkish multicentre study found a high correlation between frozen section examination and the final histopathological result. Subsequently, they recommended extended LND if the frozen section was positive but no further LND if negative [216]. The presence of primary draining LNs outside this diagnostic template and the inability of even final histopathology to identify all LN metastasis are major arguments against this strategy.

The proximal limit of LND should not only be designated in order to gain a perfect staging but also from a prognostic perspective. In our mapping study, we found that the extended template to the aortic bifurcation is sufficient regarding identification of all LN positive patients. However, 16% of the LN positive patients (4% of all patients) had metastatic LNs removed from the para-caval, inter-aortocaval, or para-aortic LNs because a super-extended LND was performed [III]. Despite removal of these additional positive LNs, 6 of 7 patients developed recurrent disease within 7 months and 5 had died within 13 months from the surgery. Yet, one patient with positive para-aortic LN metastasis is still alive and without sign of recurrence more than 5 years after RC and super-extended LND without neoadjuvant or adjuvant chemotherapy. This shows that super-extended LND can be curative and provide better prognosis than extended LND in selected individuals. It is, however, likely that dissection of these very cranially located LNs does not provide a better prognosis in the vast majority of patients. This is because metastasis in these locations usually is associated with widespread disease that cannot be cured by surgery alone [5,76,158,193,209,III,V]. Therefore, adjuvant therapy rather than more extensive surgery seems plausible in these high risk patients.

Patients with minimal metastatic burden in few LNs rather than multiple LN metastases are the patients that potentially benefit from RC with a thorough LND, whereas location of minimal LN burden within the regional LNs is not important if all regional LNs are removed in the LND [158,209,217,V,VI]. In other words, dissection of LNs outside the extended 'staging-template' with removal of non-regional LNs is not relevant from a prognostic perspective in other than selected casuistic individuals. However, the presence of occult LN metastasis missed by SPE in these proximally located LNs is not clarified. Thus, patients with evident LNs metastasis located below the aortic bifurcation may have micrometastasis in the para-aortic LNs that are removed by a super-extended LND and not by an extended LND.

When evaluating survival benefit of performing a more extended LND, it is important to estimate whether a survival benefit in LN negative and LN positive patients is a true survival benefit or a result of better staging. The 'Will Rogers-phenomenon' describes how stage migration of patients with intermediate prognosis 'migrates' from the low risk group (N0) to the high risk group (N+) because of a better staging and thereby increasing the survival in both groups without a true survival benefit of the overall patient group [218]. By performing a more extended LND, some patients with LN metastasis outside the limited LND template, were without sign of recurrent disease in long term follow-up. Hence, it can be concluded that the improved survival of LN positive patients is a true survival benefit, at least for some of the stage migrators, whereas an apparent survival benefit of LN negative patients most likely is a result of 'stage-migration' of LN positive patients [VI]. Adjustment for this potential stage migration can be made by analyzing survival according to T-stage irrespective of N-stage but if adjuvant chemotherapy is administered based on nodal status, a potential bias that can influence survival despite T-stage only analysis, will be introduced. Therefore, long term survival, especially RFS, is a more reliable parameter in patient series where no adjuvant chemotherapy is given.

Results from the ongoing RCT in Germany (NCT01215071), investigating survival of patients undergoing RC and super-extended LND versus standard LND, are awaited and will hopefully clarify some of the true prognostic benefits of LND. However, some node positive patients in the standard LND cohort will inevitably

be incorrectly staged as node negative if they have isolated LN metastasis outside the standard template. Results from the study will therefore only provide information on the percentage of patients with evident benefit from a more extended LND and only if this percentage reaches statistical significance, the study will be able to prove a survival benefit by performing super-extended LND instead of a standard LND. Despite the ability of proving this survival benefit, the study will unfortunately not be able to clarify the optimal proximal boundaries of an LND from a prognostic perspective, i.e. super-extended, extended, or subtotal. An upcoming randomized study (SWOG S1011) aims at evaluating the survival benefit of a standard versus an extended LND performed at time of RC MIBC.

Despite the lack of RCTs at the present time, LND per se is of true prognostic importance, at least in selected patients. This is most evident in node positive patients that are long term survivors without recurrence in surgery-only-series. Agreement to the boundaries of a perfect LND template is, however, still controversial. Consequently, super-extended, extended and subtotal LNDs are all referred to as 'extended LND' in the current literature [176,193,219].

In the absence of RCTs, comparison of survival estimates from different RC series using different LND templates has been used to estimate differences in prognosis achieved by the different LND templates. The first report using historical follow-up cohorts going from one template to another, was made by Poulsen et al. [174]. They compared patients undergoing RC and standard LND with more recently operated patients undergoing RC and extended LND. They found a survival benefit in a sub-group of LN negative patients with disease confined to the bladder wall. Later, Holmer et al. made a similar study where they compared two historical patient series undergoing limited or extended LND [175]. They found the survival benefit to be more evident in patients with locally advanced disease. This was also the patient category benefitting most in the most recent study published by Abol-Enein et al. where they compared two cohorts of patients undergoing standard LND with dissection also of the distal inch of the common iliac LNs (i.e. comparable to a subtotal LND) or super-extended LND [177]. A limitation of the study by Holmer et al. is the use of adjuvant chemotherapy based on histological findings, thus, making stage migration a possible explanation to the improved survival in the patient group undergoing more accurate staging.

Another way to compare different LND regimens was first applied in a the two-centre study by Dhar et al. where they compared two patient cohorts undergoing RC at the same time but at two different institutions, in two different countries [176]. One cohort underwent standard LND and one subtotal LND. They found a significantly better survival for patients with locally advanced disease in the cohort undergoing subtotal LND. Except for the submission of LN specimens en bloc at one institution and as separate LN packages at the other and different regimens of liberal adjuvant chemotherapy, there may have been other factors not taken into account when analyzing survival benefits. Recently, the Bern patients included in the Dhar study were included in another transatlantic two-centre study comparison between subtotal LND and super-extended LND [178]. Interestingly, this study found exactly the same survival in the two patient cohorts suggesting that LND above the ureter crossing is unnecessary. This is, however, not in agreement with the study of Abol-Enein et al. as mentioned above [177]. Moreover, it is striking that extending the limits from standard LND to subtotal LND presumably can improve survival significantly as shown in the study by Dhar et al., whereas inclusion of all common iliac, presacral and lower para-aortic LNs in the study by Zehnder et al., has no influence on prognosis despite the possibility of single positive LNs in these locations. The risk of type II error because of an underpowered material in the latter mentioned study seems imminent. Furthermore, it is noticeable, that a significantly higher incidence of LN metastasis was found in the cohort undergoing super-extended LND (35% vs. 28%, p=0.02), whereas the 5-year RFS was similar in the two cohorts when comparing pT2pN0-2 and pT3pN0-2 separately [178].

					LN pos pts			LN neg pts			All pts		
Authors	Period	LND	No. pts	No. N+ (%)	RFS	DSS	OS	RFS	DSS	OS	RFS	DSS	OS
Bassi et al. [221]	1982-1994	Limited	369	78 (21%)			15%			65%			54%
Holmer et al. [175]	1997-2000	Limited	69	12 (17%)							65%#	67%#	
Jensen et al. [VII]	1999-2003	Limited	204	43 (21%)	8%	14%	12%	75%	80%	66%	62%	67%	55%
Poulsen et al. [174]	1990-1993	Standard	68	15 (22%)	7%						56%		
Dhar et al. [176]*	1987-2000	Standard	336	(13%)	7%		7%						
Honma et al. [201]	1990-2002	Standard	146	25 (17%)		22%#			62%#			55%#	
Hautmann et al. [222]	1986-2003	Standard	788	142 (18%)	21%			75%			66%	68%	58%
Osawa et al. [223]	1990-2005	Standard	435	83 (19%)			27%#						
Park et al. [198]	1991-2001	Standard	450	129 (29%)	25%	33%		73%	79%		60%	68%	
Smith et al. [167]	1966-1977	Subtotal	662	134 (20%)			7%						
Vieweg et al. [224]	1980-1990	Subtotal	686	193 (28%)		31%			67%			57%	
Madersbacher et al. [225]	1985-2000	Subtotal	507	124 (24%)	33%		26%				62%		59%
Ghoneim et al. [226]	1970-2000	Subtotal	2.720	555 (20%)	27%			62%			55.5%		
Zehnder et al. [178]	1985-2005	Subtotal	405	114 (28%)	42%		38%	69%		60%			
Jeong et al. [227]	1990-2007	Subtotal	543	112 (21%)	22%	34%							
Abol-Enein et al. [177]	1999-2003	Subtotal	200	48 (24%)	28%			66%			55%		
Steven et al. [217]	1993-2005	Extended	336	64 (19%)	39%		41%	76%			69%		68%
Holmer et al. [175]	2001-2005	Extended	101	38 (38%)							75%#	74%#	
Roehrborn et al [170]	1971-1986	Super-ext	280	42 (15%)			19%						
Stein et al. [6]	1971-1997	Super-ext	1.054	244 (23%)	35%		31%	78%		69%	68%		60%
Zehnder et al. [178]	1985-2005	Super-ext	554	195(35%)	40%		34%	71%		59%			
Abol-Enein et al. [177]	1999-2003	Super-ext	200	48 (24%)	48%			72%			67%		
Jensen et al. [VII]	2004-2008	Super-ext	265	61 (23%)	29%	39%	38%	74%	82%	75%	64%	72%	67%

Table V: *Five-year survival estimates in RC series stratified according to LND. Only the most recent or most representative publication is shown if several publications exist from the same institution based on the same patients. Only series where extent of LND and relevant survival estimates are sufficiently stated in the original publica-*

tion are included. If more than one template is used in a series, the patients are either stratified according to template or registered by the predominant template.
** Cleveland Clinic patients only. The Bern patients from the same publication are included in the publication of Madersbacher et al.*
 # Survival estimate extrapolated from Kaplan-Meier curves in the original manuscript.

Both two-centre studies excludes up to half of the patients undergoing RC at the involved institutions in the period for various reasons and includes only clinically N0 patients with T2- and T3-tumours [178,220]. The potential prognostic benefit of a thorough LND in patients with T1-, T4-tumours, or gross LN metastasis is therefore not explored.

Conclusions from these and similar non-RCTs should be made with caution. Table V shows different survival estimates found in RC series with different LND templates. Survival estimates from RC series are variable, not only depending on the LND template but also depending on other non-corrected biases. Biases include different time periods, variable follow-up time, different survival estimates used, and different chemotherapy regimens in the different series. Furthermore, survival can be influenced by exclusion criteria in the reported series. Thus, 5-year OS in a cohort of LN-positive patients from Bern, Switzerland varies from 26–34% in different publications [176,225,228]. In 2003, Madersbacher et al. reported a 5-year OS of 26% for the entire cohort of 124 LN-positive patients undergoing RC and subtotal LND between 1985 and 2000 [225]. By excluding patients with non-TCC and N3-disease and adding longer follow-up time, Fleischmann et al. found a 5-year OS of 30% of 101 of the patients [228], whereas Dhar et al. excluded patients operated before 1987 and patients with pTa-, pT1-, or pT4-disease. This resulted in an increase in 5-year OS to 34% in 83 of the same patients [176]. By analysing the available series it is, however, evident that survival following limited LND is inferior to other more extensive templates.

As noted, the different RC series in table V have different inclusion- and exclusion criteria and are from different time periods. Furthermore, different adjuvant chemotherapy regimens, if any, have been used. The survival estimates are therefore not comparable. However, if a comparison of the different 5-year RFS estimates is made despite these seriously conflicting reservations, this could be illustrated as in figure 13. There is a slight tendency towards improved survival by extending the limits of LND when analyzing all patients irrespectively of LN status (Figure 13a). When comparing pN0-patients there is no tendency towards improved survival (Figure 13b), whereas a survival benefit is indicated in LN-positive patients (Figure 13c).

Increase in survival by extending the limits of a LND beyond the limits of an extended LND to a super-extended LND is less evident. This could be the result of a survival benefit in a minority of patients only as indicated in the present thesis [VI]. This minority of patients cannot safely be selected preoperatively at the present time. This emphasizes the need for performing LND according to a standardized template in all patients irrespective of perioperative findings.

The truth is, most likely, that there is a true survival benefit by extending the limits of LND at the time of RC, especially in patients with true locally advanced disease. The survival benefit obtained by moving the proximal limit of LND more and more cranially probably decreases when the limit is extended beyond the true pelvis. Therefore, despite the potential benefit of LND including all LNs above the aortic bifurcation potentially up to the diaphragm, the potential oncological benefit must be compared to the potential morbidity of this extensive surgical procedure in the majority of patients not benefitting from the procedure. A reasonably proximal limit should be chosen based on these considerations. Based on the current literature and the present thesis, this limit is at the aortic bifurcation except in patients with known high risk of para-aortic metastasis (i.e. known LN metastasis at lower locations or suspicious LNs on preoperative imaging) and no sign of disseminated disease.

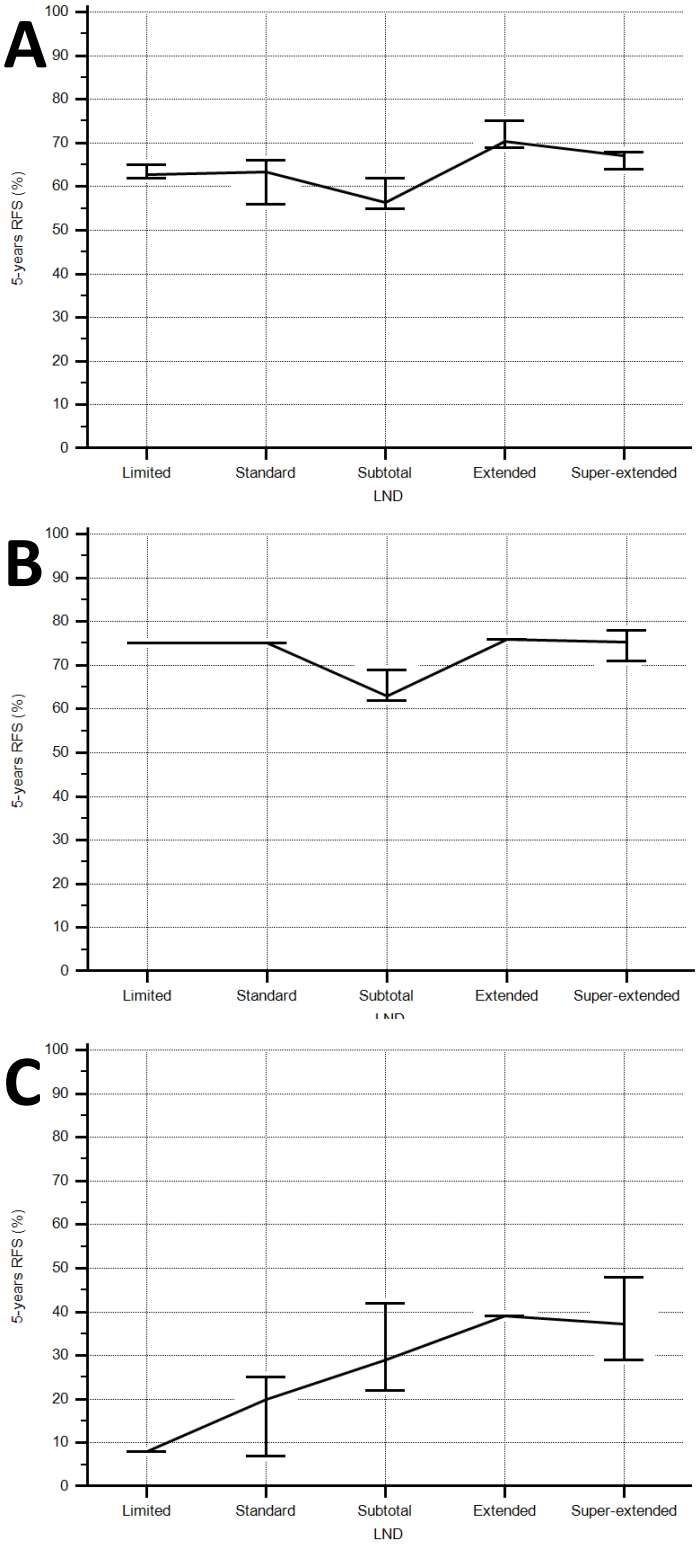


Figure 13:*Actuarial 5-year RFS achieved in RC series with different LND templates. Range is shown with bars. Weighted means are connected with line. A: All patients, B: Only LN negative patients, C: Only LN positive patients.*

Mapping studies and survival studies tend to include 'standard' patients and exclude all 'non-standard' patients. Therefore, relevance of LND and boundaries of an ideal template in patients undergoing preoperative radiotherapy or chemotherapy is less clarified. Some authors suggest 'non-standard' patients to represent the majority of patients undergoing RC [79]. The present thesis has evaluated the relevance of LND in patients undergoing prior oncological treatment. Despite the inability of eradicating disease within the bladder in a high number of patients, curatively intended radiotherapy apparently eradicates LN metastasis efficiently [229,VII]. This is indeed fortunate because in most patients undergoing salvage RC following failed radiotherapy, LND is difficult without doing more harm than good to the patient because of irradiation induced fibrosis [230].

In patients undergoing preoperative systemic chemotherapy, vital tumour cells within some of the retrieved LNs have been found in 30%–82% of the patients despite apparently complete response estimated by imaging [188-191,VII]. This emphasizes the inability of chemotherapy to efficiently eradicate all tumour cells and emphasizes the importance of doing a thorough LND in this patient group too. Excellent long term survival can be achieved in this manner. By applying these findings to patients undergoing neoadjuvant chemotherapy without suspected LN metastasis on pre-treatment imaging, all patients should preferably undergo the same thorough LND as patients not submitted to neoadjuvant chemotherapy. Neoadjuvant chemotherapy can, on the other hand, efficiently eradicate some occult LN metastasis in RC patients. This is shown by the good chemotherapy response in some of the patients undergoing preoperative treatment because of widespread LN metastasis and also illustrated by the long term survivors following nodal recurrence without visceral metastasis [VI+VII].

A meta-analysis of all available RCTs investigating the survival benefit of neoadjuvant chemotherapy found a 5-year absolute survival benefit of 5% and most evident in patients with advanced T-stages [150]. These findings mimic the results found in the present thesis where approximately 4% of the patients had an evidently better prognosis by extending the limits of LND. Theoretically, these patients could have been cured by neoadjuvant chemotherapy and a limited LND if chemotherapy had eradicated all occult LN metastasis outside the limited LND template. Considering the higher morbidity of neoadjuvant chemotherapy compared to merely extending the limits of the LND, more extensive surgery rather than multimodality treatment is preferred. Interestingly, most patients included in the RCTs in the meta-analysis did not undergo an extended or super-extended LND. A third of the included patients were part of the MRC/EORTC study where the external iliac LNs were removed only if involvement was suspected at the time of surgery [146]. Twenty percent of the included patients were from the two Nordic cystectomy trials. In the first of these trials patients were given 20 gray (Gy) preoperatively and only enlarged LNs were removed at the time of surgery, whereas in the second trial, removal of the obturator and iliac LNs was recommended but great variations in LND existed and LND was not performed in all patients [147,231,232]. The overall survival benefit for the treatment arms of the RCTs in the meta-analysis was predominantly a result of improved survival on this half of the patients undergoing insufficient LND. In the remaining half of the patients in the meta-analysis, information regarding extent of LND is not sufficiently specified in the available literature [148-150].

Therefore, at the present time, it is not clarified whether neoadjuvant chemotherapy and 'sufficient' extended LND have an additive impact on prognosis or simply are two different methods of achieving the same favourable results in a minority of the patients with minimal metastatic burden in the local LNs. Proper nodal staging—and treatment—by the means of an extended or super-extended LND in a large prospectively randomized patient material is needed to evaluate the true prognostic benefits of neoadjuvant chemotherapy in patients undergoing optimal surgical treatment.

The relevance of proper staging, including N-stage, when evaluating prognostic markers is illustrated in a study included in the present thesis that evaluated the prognostic impact of a molecular marker in patients undergoing RC [VIII]. Patients with high KPNA2 expression had a higher risk of developing visceral metastasis, whereas patients with low KPNA2 expression more often developed LN recurrences. In patients undergoing RC and limited LND, survival estimates were not significantly different when stratifying according to KPNA2 expression. However, in patients undergoing RC and extended or super-extended LND, there was a significant better survival in patients with low KPNA2 expression compared with patients with high KPNA2 expression. A possible explanation is the lower risk of local LN recurrence in patients undergoing RC and extended or super-extended LND. Because of a higher risk of LN recurrence in KPNA2-negative patients, the most significantly improved prognosis is noted in this group of patients, whereas the assumed association with visceral metastasis eliminates the survival benefit of a more extended LND in patients with high KPNA2 expression.

CONCLUSIONS

- LNs and LN metastasis can be missed by SPE in a minority of patients. Conclusions based on materials with SPE of LND specimens should be interpreted with this reservation. Because of small patient materials in the present studies, conclusions regarding the true incidence of occult LN metastasis missed by SPE cannot be made.
- Increasing size of an LN is associated with a higher risk of metastasis but not to a degree that makes a size criterion clinically useful in patients undergoing RC. Preoperative imaging is, however, important to avoid unnecessary, potentially harmful procedures in patients where preoperative imaging in fact reveals gross LN metastasis or visceral metastasis. These patients should be offered systemic chemotherapy prior to radical local treatment.
- A fixed volume of lymphatic tissue rather than a fixed number of pelvic LNs is present in patients undergoing RC. Moreover, variation in number of LNs retrieved was not correlated to prognosis in patients undergoing RC and LND according to a standardized super-extended template in the present series.
- Extended LND to the aortic bifurcation provides a more accurate staging of nodal disease compared to more limited templates. Extending the limits of LND to include LNs above the aortic bifurcation is not indicated from a staging perspective.
- Information given by the N-stage of the conventional TNM classification (N0 vs. N1 vs. N2–3) was found to be the only LN variable that was an independent prognostic factor in a series of patients undergoing super-extended LND. Therefore, despite suggestions of several different prognostic variables based on

conventional histopathological examination, the most important prognostic factor was the presence of LN metastasis and the metastatic burden.

- Thorough LND evidently improved prognosis in patients with nodal metastasis, not submitted to adjuvant chemotherapy, and no recurrence in long term follow up, whereas improved survival by the means of an extended or super-extended LND compared to a limited LND is evident only in a minority of patients. This minority of approximately 5% can, however, not safely be identified pre- or per-operatively. Therefore, the same LND should be undertaken in all patients undergoing RC, if possible. Given that some patients have LN metastasis missed by SPE, the true number of patients who benefits from a more extended LND is most likely higher.
- From both a staging and a prognostic perspective, the common iliac LNs should be included in the regional LNs draining the bladder. This is in agreement with the most recent edition of the TNM classification.
- Selected individuals can benefit from LND to the level of the inferior mesenteric artery compared to a proximal limit at the aortic bifurcation. Most patients with non-regional LNs have, however, more widespread disease indicating the need for systemic treatment. Thus, inclusion of LNs above the aortic bifurcation in LND remains controversial and without clarification regarding true prognostic value.
- LND in the previously irradiated pelvis can be difficult and remains controversial. Acceptable long term survival can be achieved by salvage cystectomy in this otherwise critical patient category.
- Patients presenting with non-regional LN metastasis have a high risk of harbouring vital tumour cells within the LNs despite preoperative chemotherapy. This emphasizes the need for thorough LND following chemotherapy in this patient category. Good long term survival can be achieved in this manner.
- The impact of neoadjuvant chemotherapy remains to be evaluated in patient series undergoing relevant extended or super-extended LND for staging and prognostic reasons.
- When evaluating molecular markers or other prognostic factors in RC series, it is imperative to do the evaluation in patients who have undergone sufficient LND to provide the correct nodal staging and most favourable prognosis. Otherwise, bias from un-corrected conventional risk factors can influence the results and conclusions significantly. In the present thesis we found that KPNA2 was a predictor of visceral metastasis rather than LN metastasis. As a prognostic marker, KPNA2 was only independent of conventional risk factors in patients undergoing at least extended LND and not in patients undergoing only a limited LND.

FUTURE ASPECTS

The results obtained in the present thesis have clarified some of the controversies in LND in BC, whereas others still remain. Thus, there are several unresolved problems.

Better imaging modalities to provide more accurate preoperative nodal staging are needed. We found that patients in whom positive LNs were diagnosed before RC had a better prognosis compared to patients with LN metastasis diagnosed preoperatively. There is a potentially, pronounced selection bias leading to these results because patients with no response to primary chemotherapy were not offered RC. If patients with non-regional LN metastasis could be identified more accurately by more sensitive imaging modalities, unnecessary radical treatment could be avoided in patients with widespread microscopic disease without response to chemotherapy. PET-CT has shown promising results in that perspective but should be further prospectively investigated in large series with histopathological verification of the findings. Furthermore, a more ideal tracer than the currently used, FDG, should be developed e.g. as molecular imaging in collaboration with research in molecular markers. Presently, we have initiated a study where meticulous mapping of LNs on preoperative FDG-PET/CT is correlated to peroperative findings in the LND specimens to evaluate the accuracy of this modality regarding diagnosis of LN metastasis. A method based on immediate preoperative systemic FDG administration and peroperative findings guided by a "PET"-probe to identify small LN metastases potentially missed by PET/CT imaging is under preparation. Hopefully this new technique can identify with LN metastasis with a higher sensitivity than conventional FDG-PET/CT. In both these upcoming studies, FDG-positive LNs will be investigated thoroughly with step-sectioning technique to identify metastasis.

The prognostic impact of performing super-extended versus extended LND should be investigated further to make final conclusions regarding the optimal proximal limit of LND. At the Department of Urology, Aarhus University Hospital, Skejby, we have changed the standard procedure following results from our mapping study. Therefore, at present time, we are performing an extended LND instead of a super-extended LND. Follow-up of this new cohort compared to the super-extended cohort of the present thesis can indicate poorer prognosis following extended LND if this is a true consequence. There are, however, major limitations of this approach with non-randomized historical cohort studies as indicated in the present thesis. Ideally, an RCT could clarify the true impact on prognosis. The risk of type II error is most likely high in an RCT because of the presumed minimal influence on prognosis achieved by resection of these proximal LNs. Therefore, a very high number of patients are needed. Another approach could be removal of the para-caval, inter-aorto-caval, and para-aortic LNs in a smaller consecutive series of RC patients. These LNs should be submitted to meticulous step sectioning technique or molecular staging to evaluate the true number of LNs involved at this proximal level. In combination with long term survival, this could provide a theoretical prognostic impact of super-extended LND compared to extended LND. Neoadjuvant chemotherapy has been found to provide a better prognosis in RC patients undergoing less than an extended LND. The prognostic benefits of neoadjuvant chemotherapy should be further validated in patients undergoing at least extended LND to evaluate the relevance of neoadjuvant chemotherapy in modern RC series. Future studies involving neoadjuvant chemotherapy should preferably include validation of diagnostic and prognostic biomarkers, and markers of response to chemotherapy in an intervention study.

As part of the current study, samples from primary tumours and LNs were sampled and fixed in Tissue-Tek to preserve mRNA. Laser micro dissection was performed to retrieve carcinoma cells only. Subsequent genetic analysis was performed with Affymetrix U133 plus 2 arrays to identify differences between metastasizing tumours and non-metastasizing tumours, and to investigate correlations between the primary tumour and corresponding LN metastasis. The preliminary results from this part of the project were presented at the European Association of Urology (EAU) annual conference in Stockholm 2009 [233]. We found that the genetic signature of an LN metastasis resembled the primary tumour more than LN metastases in other patients. Thus, it was

suggested that the genetic signature of an LN metastasis could be predicted from a sample of the primary tumour. Further validation of these findings and validation of suggested genetic markers of metastasis and poor prognosis is part of an ongoing PhD-project from the Departments of Molecular Medicine and Urology, Aarhus University Hospital.

KPNA2 expression was found to be an independent prognostic marker. However, the clinical relevance of KPNA2 expression in RC patients is not clear. KPNA2 should be prospectively validated in other RC series and, if successful, in future intervention studies, preferably in combination with other prospective prognostic biomarkers. Future studies will include functional studies to evaluate whether biomarkers can be used as a therapeutic target in molecular medicine as part of a multimodality treatment.

Looking beyond urothelial carcinoma of the bladder, urothelial carcinoma of the upper urinary tract is an entity with biological similarity to BC where the prognostic impact and extent of LND is unclarified [234]. Presently, we have initiated a retrospective registration of metastatic patterns in all patients undergoing radical nephro-ureterectomy because of upper urinary tract urothelial carcinoma at our institution in the last 20 years. Hopefully, this can be the basis of a prospective study including a thorough LN mapping in future patients with upper urinary tract urothelial carcinoma based on PET/CT, peroperative PET-probe, or SN technique to identify the potential metastatic LNs. A RCT with randomization between more or less extensive LND at the time of nefro-ureterectomy should be conducted as a multicentre study because of the rarity of this disease.

SUMMARY

The present thesis consists of 8 original articles focusing on lymph node dissection (LND) in patients undergoing radical cystectomy (RC) because of bladder cancer.

LND is considered an essential part of the surgical procedure when performing an RC to achieve the correct staging and for prognostic reasons. However, the boundaries of LND have been the subject of debate. Proximal limit above, at, or below the aortic bifurcation has been suggested to define the perfect LND. Two questions have driven the present thesis. First, which extent of LND is needed to make the most accurate identification of patients with nodal involvement? And second, which extent of LND is needed to provide the most favourable prognosis in patients undergoing RC?

During a 5-year period, all patients undergoing RC and LND to the level of the inferior mesenteric artery at the Department of Urology, Aarhus University Hospital, Skejby were prospectively enrolled in meticulous registration of several LN variables including burden and location of metastasis based on standard pathological examination. From these patients, mapping of the metastatic LNs were made. Moreover, we included patients from a historical cohort undergoing limited LND to evaluate the possible prognostic impact of a more extended LND.

Standard pathological examination was found to be reliable regarding identification of LN metastasis. A proximal limit of LND at the aortic bifurcation was found to be sufficient from a staging perspective, whereas less extensive LND was associated with a risk of under-staging.

From a prognostic perspective, LND at least to the aortic bifurcation should be performed. It is still controversial and unclarified whether LND above the aortic bifurcation has any prognostic value. By extending the limits of LND from a limited dissection involving only the LNs in the obturator fossae to a dissection including all pelvic and lower lumbar LNs, a survival benefit in at least 5% of the patients was found.

We also found that an extensive LND should be performed in all patients irrespective of T-stage of the primary tumour and in patients undergoing chemotherapy before RC.

Previous radiotherapy, on the other hand, apparently eradicated LN metastasis in the irradiation field within the pelvic region and made subsequent LND difficult and possibly superfluous.

In evaluation of a molecular marker, KPNA2, we found that the more accurate staging and more favourable prognosis achieved by extended LND compared to a limited LND was essential in evaluation of the prognostic impact of KPNA2.

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