Experimental Diagnostics and Therapeutics of Invasive Bladder Cancer

BY

AMIR SHERIF
The two purposes of this thesis were to evaluate new diagnostic techniques of lymphnode staging in invasive bladder cancer and to evaluate the results of neoadjuvant chemotherapy in invasive bladder cancer.

Sentinel node detection was performed in 13 patients. The method showed to be feasible, and the results displayed the occurrence of metastatic nodes outside the traditional area of diagnostic dissection in a majority of patients. Four patients were metastasized, each one with one metastatic node detected with the help of the sentinel node procedure.

Four randomly selected sentinel nodes from four different unmetastasized patients were compared to the four metastatic sentinel nodes from the first series. After microdissection, p53 genomic structure, immunohistochemical expression and MVD (microvessel density) were assessed in the primary tumors and corresponding sentinel nodes. The results suggested that invasive bladder cancer mainly involved monoclonal proliferation with predominantly homogenous bimarker profile, but there were also signs of clonal evolution.

The Nordic Cystectomy Trial 2 (NCT2), is a randomized prospective trial investigating the possible benefit of neoadjuvant chemotherapy versus cystectomy only, in 311 eligible patients with urinary bladder cancer T2-T4aNXM0. Evaluation of overall survival did not show any statistically significant benefit in the experimental arm. This probably due to lack of statistical power.

To increase the statistical power we performed a combined analysis of randomized patients from both the Nordic Cystectomy Trial 1 (NCT1) and NCT2, n = 620. Eligible patients from NCT1 had T1G3, T2-T4aNXM0 urinary bladder cancer. Standard meta-analysis methods were used. The only end-point analysed was overall survival. Neoadjuvant platinum based combination therapy was associated with a 20% reduction in the relative hazard in probability of death.

Key words: bladder cancer, lymph node excision, radionuclide imaging, gene expression, cystectomy, neoadjuvant chemotherapy

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To Nona, Deborah and David
It’s a mystery! It’s a mystery, wrapped in a riddle inside an enigma!

Joe Pesci portraying David Ferrie in the film “JFK” by Oliver Stone, 1991
This dissertation is based on the following papers, which are referred to by their roman numerals

I. LYMPHATIC MAPPING AND DETECTION OF SENTINEL NODES IN PATIENTS WITH BLADDER CANCER

A. SHERIF, M. DE LA TORRE, P.-U. MALMSTRÖM AND M. THÖRN
From the Departments of Urology, Pathology and Surgery, University Hospital, Uppsala, Sweden

II. EARLY METASTATIC PROGRESSION OF BLADDER CARCINOMA: MOLECULAR PROFILE OF PRIMARY TUMOR AND SENTINEL LYMPH NODE
PER-UNO MALMSTRÖM, ZHI-PING REN, AMIR SHERIF, MANUEL DE LA TORRE, KENNETH WESTER AND MAGNUS THÖRN
From the Departments of Urology, Pathology and Surgery, University Hospital, Uppsala, Sweden

III. Neoadjuvant Cisplatin-Methotrexate Chemotherapy for Invasive Bladder Cancer. Nordic Cystectomy Trial 2
Amir Sherif, Erkki Rintala, Oddvar Mestad, Jonas Nilsson, Lars Holmberg, Sten Nilsson, Per-Uno Malmström and other co-workers in the Nordic Urothelial Cancer Group
From the Department of Urology, Akademiska University Hospital, Uppsala, Sweden, Department of Urology, Helsinki University Hospital, Helsinki, Finland, Urological Service, Central Hospital of Rogaland, Stavanger, Norway, Regional Oncological Centre, Akademiska University Hospital, Uppsala, Department of Surgical Sciences, Akademiska University Hospital, Uppsala, Department of Oncology, Radiumhemmet, Karolinska Hospital, Stockholm, Sweden

IV. NEOADJUVANT CISPLATINUM BASED COMBINATION CHEMOTHERAPY IMPROVES OVERALL SURVIVAL IN PATIENTS WITH INVASIVE BLADDER CANCER. A COMBINED ANALYSIS OF TWO NORDIC COLLABORATIVE STUDIES
Amir Sherif1, Erkki Rintala2, Oddvar Mestad3, Jonas Nilsson4, Lars Holmberg4,5, Sten Nilsson6, Per-Uno Malmström1 and other co-workers in the Nordic Urothelial Cancer Group

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To be submitted

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CT</td>
<td>Computerised tomography</td>
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<tr>
<td>IVP</td>
<td>Intravenous pyelogram</td>
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<tr>
<td>MRT</td>
<td>Magnetic resonance tomography</td>
</tr>
<tr>
<td>MVD</td>
<td>Microvessel density</td>
</tr>
<tr>
<td>NCT 1</td>
<td>Nordic cystectomy trial 1</td>
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<tr>
<td>NCT 2</td>
<td>Nordic cystectomy trial 2</td>
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<tr>
<td>Te99</td>
<td>Technetium 99</td>
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<tr>
<td>TURB</td>
<td>Transurethral resection of bladder tumor</td>
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INTRODUCTION

These four studies hereby presented are attempts to understand more about the nature of invasive urothelial bladder cancer, and to investigate therapeutic modalities that might have efficacy in the treatment of this tumor. Metastatic spread or absence of it, is one of the most important factors to influence occurrence of relapse and overall survival in this tumor. The earliest and most common metastatic spread, is found in regional lymphnodes. It is well known from clinical trials, that in those patients who have not been subject to metastatic spread to the regional lymphnodes, the survival prospects are significantly better than in those having metastatic disease. Previous attempts to stage lymphnodes with the help of CT-scan or MRT, have not been sufficient. Routines for intraoperative surgical staging of presumed target-areas for affected nodes, are more or less questionable. Many a patient have undergone excellent surgery with artistically fantastic urinary diversions of different kinds, but due to lack of proper staging procedures, their actual nodal status remains unknown or only partially known. This lack of knowledge has implications for decisions to be taken postoperatively in relation to adjuvant oncological treatment and general follow up. The innate nature, and the molecular secrets of a bladder cancer that results in metastases, and also the intricate changes of the metastatic deposit itself, warrants detailed studies. Increased knowledge might be gathered of early metastatic progression by studying some well-known aspects of this kind of tumor. Thus the second paper is based on the findings and primary results of the first paper and consequently based on detection of early metastatic spread, a result of new suggestive methods for lymphnode staging.

Preoperative treatment for eradication of micrometastatic deposits, is an option that is being investigated increasingly in different urological centers. A previous prospectively randomized Nordic trial (nordic cystectomy trial 1, NCT 1), had some seemingly interesting results on overall survival in a subgroup of patients. This warranted further nordic efforts (nordic cystectomy trial 2, NCT 2) resulting in the third paper hereby presented. In order to increase the statistical power, and further investigate the impact on overall survival by neoadjuvant chemotherapy regime, we decided to perform a combined analysis of both the nordic cystectomy trials. The third and fourth papers were prepared in
close cooperation with members of the Nordic Urothelial Cancer Group, part of the Nordic Urological Association.

BACKGROUND

In general

The incidence of urinary bladder cancer has increased markedly in Sweden in the years 1961 – 1998 (Fig1). Approximately 2000 new cases are diagnosed every year in Sweden. In the years 1997 - 2000, the absolute amount of performed cystectomies for muscle-invasive bladder cancer has slightly increased from 149 operations 1997 up to 167 in the year 2000. The relative occurrence of muscle-invasive urothelial bladder cancer (>T1) has been almost the same, ranging from 25 -27.6 % of all newly diagnosed bladder cancer tumors (Nationellt kvalitetsregister). Although surgical techniques and anaesthesiological measurements have rapidly developed during the last few decades, and also oncological options have become more refined, we are still faced with high mortality rates in the majority of affected patients (Ghoneim, Hall).
Staging

Some of the most important tools we have for taking clinical decisions in treating this tumor, are the procedures of staging, like first-time assessment with a visual impression of the tumor by the cystoscope, outcome of bimanual palpation and microscopic staging of the TURB-material. Roentgenological tools like IVP and CT-scan of abdomen and chest (alternatively plain chest X-ray) can help the clinician to find out the nature of function and anatomy of the upper urinary tract, in gathering information for a treatment-strategy. Although, we must be well aware of the limitations when it comes to assessing nodal status and T-stage. CT-scan (Paik) and even MRT have not been considered being the most reliable of instruments in these aspects (See). CT-scan is therefore mainly used for the general routine work-up (Fig 2).

Post-cystectomy staging

Once the bladder and adjacent organs are removed, the bladder specimen is re-examined by the pathologist, and a pT-stage can now be determined. Retrieved nodes are being searched for metastatic deposits, and a pN-stage can be assessed.

Limited surgery

A combination of tethered tumor on bimanual palpation, advanced primary pT-stage in TURB specimens and even grossly enlarged multiple nodes on CT-scan, may either discredit the patient completely from any further attempts to perform major surgery, or in the case of a clinically fit patient, lead to chemotherapy in cooperation with an oncologist and further on to a so called salvage cystectomy. In that case the ambition would be to perform simplest and easiest form of urinary diversion. In the unfit patient, even chemotherapy would be hazardous, and external irradiation would be an option but without any following attempt to cystectomy. In some patients, the clinician needs to revert to decisions colored by palliative thinking.
Basic Flow Chart - main diagnostic work-up for locally advanced urothelial bladder tumors

First clinical suspicion of bladder cancer

Diagnosis I
- Visual detection by cystoscope
- Cold-cup biopsy and/or cytology

First clinical indication of bladder cancer

Diagnosis II
Primary surgery TUR-B
- Resection of bladder tumor
- Cold-cup biopsy - optional
- Diagnostic resection of prostatic urethra - optional for determination of local engagement
- Bimanual palpation

Determination of T-stage

CT-scan
IVP
Plain chest X-ray

Determination of M-stage, and in some few instances N-stage

Determination of urinary upper tract status and function

Diagnosis of T2-T4bNX-N+M0-1 urothelial bladder cancer

Clinical decision for further treatment-options

Fig 2: Basic Flow chart for main diagnostic work up
Radical surgery

Most patients with invasive tumors are considered being candidates for major surgery, i.e. cystectomy, lymphnode dissection and construction of some kind of urinary diversion for the outlet of urine. These are patients with well-defined tumors, even if they may be rather advanced on the TURB-specimen, but free on bimanual palpation, free from massive nodal disease on the CT-scan, free from any detected distant metastases and with an acceptable performance status.

Peroperative lymphnode staging

In many centers frozen sections of lymphnodes harvested at cystectomy, are considered being crucial for deciding what kind of urinary outlet the patient deserves or is considered to be most suitable for in that specific situation. A positive outcome on frozen sections, with metastatic deposits indicating a systemic disease, may be the final culprit for reverting to simplest possible surgery with for instance an ileal conduit, i.e. non-continent urinary stoma. This strategy is the most common, but has been challenged in some recent reports where the investigators have seen benefit in performing advanced reservoir surgery even in patients with proven metastatic disease in the lymphnodes (Lebret).

Adjuvant therapy

A finding of metastatic lymphnode/s and/or an advanced pT-stage may also be indicative for further oncological attempts to control the disease in a postoperative setting. Adjuvant chemotherapy is thus a part of the therapeutic arsenal being utilized (Logothetis, Bono, Studer).
Fig 3: Different treatment options in invasive bladder cancer, where staging has an impact for choice of treatment.

"*" Indicates new considerations in surgical treatment (Lebret).
Present problems

Our problems arise from the fact that present clinical staging-tools still are too blunt and limited. TURB-specimens do not always confer the true picture of the actual primary T-stage of the tumor. CT-scan is an inaccurate instrument when it comes to detection of micrometastases or even small and medium sized macrometastatic deposits. Nodal dissection during major surgery has its own drawbacks relating to extent of surgery, inadequate harvesting and prolonged surgical exposure. Even in meticulous and ambitious attempts to perform wide and extensive lymphnode surgery, a single viable node with a micrometastic deposit might be remaining in the patient after surgical closure. This may lead to extended metastatic disease and the ultimate propagation of the already established systemic disease. Node dissection in many centers is limited to the fossa obturatoria. Such an approach is based on established concepts of metastatic spread primarily to this specific regional site. Thus it is supposed that this area should be indicative for the individual patient, relating to his/her nodal status. Refined instruments for detection of nodal metastases need to be developed.

Background of Sentinel Node detection

The initial concepts of detailed sentinel node mapping and detection, were originally highlighted and further formulated in the 1960s and 70s (Gould, Cabanas). The sentinel node concept proposes that lymphatic drainage from a primary tumor goes to a particular regional lymph node called the sentinel node and then continues to other nodes. Thus it is also postulated that tumor cells cannot metastasize to lymph nodes in the rest of the regional lymphatic area before metastasis also exist in the sentinel node. A detected sentinel node devoid of metastatic deposit, can be considered being primed for future metastatic acceptance. This especially in case no surgical or other therapeutical measurements take place. The sentinel node is specific for each individual. The possible tumor status of the sentinel node
supposedly reflects that of the regional lymphatic field. Colleagues working with surgical
treatment of malignant melanoma and breast cancer have developed research and practical
implementations of the principles of sentinel node detection (Morton, Krag, Keshtgar). The
methodology has also been applied in patients with colorectal and gastric cancer (Thörn
2000). In the field of urological surgery, some inspiring investigations have been made in
penile cancer (Horenblas) and recently a number of pioneering articles on prostate cancer and
sentinel node detection have started to appear (Wawroschek 2001 & 2002, Kocjancic). These
and other newly published articles with detailed surgical and pathoanatomical dissection in
prostate cancer and also sentinel node detection in cancer of the cervix uteri have seriously
challenged the established belief in the obturator fossa as the one and only receiver of early
nodal metastases in malignancies emanating from the pelvic region (Burkhard, Levenback).

*Improved staging requested*

The need for more exact and detailed nodal staging in bladder cancer seems warranted. Our
knowledge about original stage (TNM), postcystectomy stage (pTNM) and our knowledge
about prognosis of the patient and actual response to treatment, whether relating to adjuvant
or neoadjuvant treatment, might become confused and inadequate even in the most refined
and correctly controlled studies. This due to only having a limited and unspecified knowledge
about the actual nodal extent of the tumors, a consequence of limited and suboptimal
assessment. Nodal status as a predictor of survival is well known (Ghoneim, Bassi), but the
reality about macro- and micrometastatic spread is almost unknown. Many crucial decisions
being taken in major surgery for locally advanced bladder cancer depends on a proper
assessment of nodal status in every individual patient case.
**Sentinel node detection and early metastatic progression**

The inherent nature of early metastatic progression is still almost undiscovered territory. Utilizing the sentinel node concept, biological material can be gathered not only for molecular studies on macrometastatic deposits, but also on early metastatic progression and on micrometastases. The function of the p53-gene as a guardian of the genome, and the research previously done in the area of both superficial and invasive urinary bladder cancer, heralds further investigations with this gene in focus (Esrig, Gao, Cordon-Cardo, Cote). By examining the molecular changes from different parts of the primary tumor and following the dynamic changes in different aspects all the way out to the tumors first metastatic deposits we might start to have an insight in the nature of the tumors innate changes and adaptations for survival in remote sites. In order to elucidate some qualities in this kind of early progression, it might be of value to study some of the basic aspects in molecular and clonal development.

**Neoadjuvant therapy**

In an attempt to eradicate micrometastatic disease prior to major surgery, the concepts of neoadjuvant radiation (Anderström, Crawford) and furthermore neoadjuvant chemotherapy have gradually been developed since the early 80s (Soloway, Fagg, Rhagavan). The concept of cisplatinum-based combination therapy has gradually emerged and these regimes have previously in phase II clinical studies shown efficacy on both survival and time to progression, in patients with invasive urinary bladder cancer. Actually only a few controlled prospective trials have been performed, and the results of end-points like overall survival and time to progression have been inconclusive (Abol-Enein, Hall, Natale, Martinez-Pineiro). Although, it has been noticed in some of the trials that patients with neoadjuvant regime versus control have had a statistically significant downstaging relating to T versus pT-stage, these findings have not translated into a substantial survival benefit (Malmström, Natale). One
reason for the more or less inconclusive results on survival, have been the number of randomized patients. So far most presented studies have had too few patients randomized for statistical detection of discrete and subtle differences. The first nordic cystectomy trial (NCT1) indicated a possible survival benefit in the T3-T4 subgroup of the experimental arm, but still the statistical power was too low for detecting this, and also for displaying any statistically significant survival benefit in the whole experimental arm. In NCT 1, all patients had received preoperative radiation, both the patients in the experimental arm and control.

The difference between the two study arms was the intended neoadjuvant chemotherapy (Malmström). Therefore it was of interest to study in NCT 2 the effect of an experimental arm with a neoadjuvant cisplatinum-based regime versus a control arm devoid of any neoadjuvant treatment at all. In order to increase the statistical power by increasing the number of patients investigated, we decided to proceed with a combined analysis of the two nordic cystectomy trials mentioned (NCT1 & NCT2). The only end-point to be studied would be overall survival. Any survival advantage to be found in the result, could have clinical implications on selection of patients for future neoadjuvant treatment. Therefore we also recognized a secondary aim when we explored if there were signs of heterogeneity in effect by T-stage, by gender and in age groups. To detect any subtle signs of survival-differences in defined subgroups, might be of help for future clinical decisions. Still our preconceived hypothesis was that the biological effect would be similar over these different strata.
AIMS OF THE INVESTIGATIONS

Study 1:
To study the possibility of sentinel node detection in invasive urothelial bladder cancer, and to compare any positive findings with corresponding histopathology.

Study 2:
To characterize early metastatic progression of advanced urothelial bladder cancer from the primary tumor, separated in the central part and invasive front to the first lymphatic metastasis.

Study 3:
To investigate if neoadjuvant cisplatinum-based combination therapy could affect overall survival in patients with advanced urothelial bladder cancer undergoing cystectomy.

Study 4:
To perform a combined analysis of two nordic cystectomy trials, in order to increase the statistical power, and investigating if neoadjuvant cisplatinum-based combination therapy could affect overall survival in patients with advanced urothelial bladder cancer undergoing cystectomy.
PATIENTS AND METHODS

Study 1:

Thirteen patients with locally advanced urinary bladder cancer and meeting the qualifications for radical cystectomy were elected. 11 male patients and 2 female. The preoperative staging ranged from T1G3NXM0 to T4aG3NXM0, whereof 10 were muscle-invasive and three had advanced T1G3-tumors. Three modalities of sentinel node detection were used. Preoperative lymphoscintigraphy by injecting radioactive tracer [Albures, Technetium 99] peritumorally transurethrally. Followed by lymphoscintigraphy, recording any uptake in lymphnodes. Peroperative $\gamma$-detection after a renewed injection prior to planned major surgery, and following peroperative measurements. At that occasion besides Albures, also a defined amount of Patent blue was injected in the same locations. Thus the third modality being visual detection of dye-uptake to regional nodes.

Cystectomy including lymphnode dissection of obturator fossa bilaterally and of any positive findings, guided by the preoperative lymphoscintigraphy detection and the two ongoing peroperative detections abovementioned. Postoperative histopathology being performed, for final correlation to status of extracted nodes.

Study 2:

Based on the previous sentinel node-detection study, nodes from 8 of the original 13 patients were examined. The actual 4 having detected sentinel lymphnodes with metastatic deposits and 4 with detected sentinel lymphnodes without metastatic spread. The last 4 were randomly chosen. Microdissection was performed, and p53 genomic structure, and immunohistochemical expression of p53, pRB, Ki 67 and E-cadherin were analyzed. Microvessel density (MVD) and apoptosis were also examined.
Study 3:
The study recruited 317 patients with T2-T4aNXM0 urothelial bladder cancer. The patients were randomly allocated to three courses of cisplatin-methotrexate versus no pretreatment prior to cystectomy. Eight patients were excluded due to protocol violation. Estimates of 5-year survival figures were performed, comparing the two study arms. All 309 eligible patients were followed in respective study arm, according to the intention to treat principle. Calculations of downstaging, relating to relative outcome of pT0 were performed. Risks of locoregional relapse and distant metastases were assessed.

Study 4:
A combined analysis of two nordic collaborative cystectomy trials, NCT 1 and NCT 2, was performed. The trials included 1985 -1997, 620 eligible patients with clinically T1G3, T2-T4a NX M0 urothelial bladder cancer and WHO performance status ≤ 2. Individual patient data were used. The median follow-up was 4.7 years. Follow-up was nearly complete. Platinum was combined with adriamycin in the first and with methotrexate in the second trial. In the first of the studies, preoperative radiotherapy was used in both arms. Standard meta-analysis methods were used to combine results of the two trials. Subgroup analyses were performed for T-stages, gender and age groups. All analyses were done according to the intention to treat principle.
RESULTS AND CONCLUSIONS

Study 1:

Results: Sentinel nodes were detected in 11 of 13 patients (85 %). Detection rate varied with all three described methods, and where all of $\gamma$-probe detections were successful (Fig 5). Four of the patients had metastatic deposits in detected sentinel nodes. There were no false-negative nodes. Three of the metastatic sentinel nodes, in three different patients, were located in places other than the obturator fossa. The mean number of nodes excised were 8.5, ranging from 5 to 22.

Conclusions: Sentinel node detection in patients with advanced urothelial bladder cancer can be performed. The histopathological status of the identified sentinel nodes was diagnostic for all other excised lymph nodes in our 13 presented cases. Sentinel nodes and metastatic sentinel nodes often seem to be located outside the obturator lymphatic field. An area traditionally examined during peroperative staging of bladder cancer.

Study 2:

Results: In 5 patients there were p53 gene mutations in the primary tumor, while 3 had the wild type gene. The genotypes were identical in the central part and invasive front. All sentinel node metastases harbored p53 mutations, in contrast to all nonmetastatic sentinel nodes. Two patients had the same mutation as the primary tumor and one had an additional mutation. In the patient with a wild-type gene in each compartment of the primary tumor a mutation appeared in the corresponding sentinel node metastasis. There was poor concordance of p53 mutation with protein status. The expression of p53, pRB, Ki67, E-cadherin, and the evaluation of apoptosis and angiogenesis showed in most cases only slight
variations in tumor compartments and the sentinel node. Thus there were results partly elucidating the homogenous biomarker profile, but also showing signs of clonal evolution.

**Conclusions:** Invasive bladder carcinomas are monoclonal proliferations with a mainly homogenous biomarker profile. Corresponding early metastases have a similar molecular profile but sometimes signs of clonal evolution appear.

**Study 3:**

**Results:** Chemotherapy was administered to 74 % of the patients in the experimental. No chemotherapy related mortality was observed. All 309 eligible patients were followed. In the experimental arm 131 patients were cystectomyed and in the control arm 139 patients. Estimated 5-year overall survival was 53% in the experimental arm and 46% in the control arm (n.s. log-rank test). The proportion of patients with pathological stage pT0 was 26.4% in the experimental arm and 11.5%. This difference was statistically significant ( p = 0.001 ). Risk of locoregional relapse and distant metastases was similar in the study arms.

**Conclusions:** The chemotherapy regimen was well tolerated. Despite substantial downstaging no statistically significant survival benefit with the neoadjuvant therapy could be seen after 5,3 years (mean) of follow up. A statistically significant downstaging was observed in the experimental arm. The study arms did not differ in risk of relapse and occurrence of distant metastases.
Study 4:

**Results:** The combined study results showed a hazard ratio of 0.80 (95% confidence interval 0.64-0.99) for overall survival. Survival was 56% at five years in the experimental group versus 48% in the control group, thus corresponding to an eight percent absolute risk reduction after neoadjuvant chemotherapy. We could not substantiate any differences in effect by subgroup.

**Conclusions:** Neoadjuvant platinum based combination chemotherapy was associated with a 20% reduction in the relative hazard in probability of death corresponding to an absolute risk reduction of eight percent at five years of follow up. We could not substantiate different effects in subgroups defined by T-stage, gender or age group. The subgroup analyses were explorative, had low power and should be interpreted cautiously.
GENERAL DISCUSSION AND SUMMARY

Sentinel node detection is possible to perform in invasive bladder cancer, actually this is the first ever published study on sentinel nodes detection in urinary bladder cancer. In the presented pilot study, the histopathological status of the identified sentinel nodes was diagnostic for all other excised lymph nodes in every patient. The finding of a substantial percentage of detected metastatic sentinel lymph nodes in locations unexpected, warrants further investigations and also tempts us to re-evaluate the dogma of node-staging based on outcome solely from dissection in the obturator fossa. In order to better evaluate the method, and to establish further data relating to percentage of false-negative cases we are in need of larger series, and also more extended lymphnode dissection (fig 5). This is a process already started, with a prospective multi-center trial initiated by our group in close cooperation with another Swedish center, and we are awaiting the results (Liedberg). The p53-gene, the guardian of the genome, is investigated in terms of mutational status in different tumor-locations and the active metastatic extension of the cancer. The results of our extended pilot study encourages us to look further into the molecular basis for early metastatic progression. One of our most interesting findings were that only metastatic sentinel nodes displayed p53-mutations, while the other four unmetastatic sentinel lymphnodes had an unmutated p53. In two of the metastatic cases we found an identical p53-pattern all the way out from the different tumor compartments of the primary tumor to the metastatic sentinel node. In two we found signs of clonal evolution taking place solely in the sentinel node. It is still not known, how the presence or absence of clonal evolution in the primary tumor and sentinel nodes translates into aspects like survival and response to chemotherapy. There might not be any correlations at all, but there might also be patterns to be assessed. First when we have collected and analysed larger series over a long period of time, can we return with further results relating to mentioned aspects of outcome. The method of microdissection has been
crucial for the results of this study, other indirect methods for examining genomic alterations and clonal changes, might be too inconclusive. Although microdissection can be a time- and resource-consuming method, it seems to be an accurate way for further investigations on larger series of sentinel node detected patients. Other aspects of genomic alterations are being elucidated in the scientific literature, thus it is important to search further for more bits and pieces belonging to this giant jigsaw-puzzle.

Eradication of micrometastases and tumor-downstaging are the two main aims with neoadjuvant chemotherapy. The theoretical result of the neoadjuvant regime would be a prolonged survival. Our NCT 2 could not detect a statistically significant survival benefit, but there was a clear tendency in favor of neoadjuvant chemotherapy. The study was underpowered for detecting any possible survival benefit and one of the main drawbacks with both the two nordic trials separately presented, was the lack of statistical power to detect a moderate or small difference in overall survival. In order to increase the power, our main intention with the combined analysis was to increase the numbers of patients being analyzed in terms of overall survival as the only end-point. The advantage of neoadjuvant chemotherapy that is hereby displayed is consistent with some other trials previously presented.

**Final words**

Our investigations have led to attempts to more precisely define and stage nodal status in advanced urothelial bladder cancer. The sentinel node concept has been introduced into bladder surgery, and the possibilities of further molecular research based on this concept, has been elucidated. Ultimately we are trying to treat this cancer form with new therapeutic modalities. Neoadjuvant chemotherapy is one way to search for efficient impact on micrometastases and hopefully improved survival. Although the nordic cystectomy trial 2
could not prove the efficacy of this regime, the trend was discernable. Our combined analysis of the two nordic cystectomy trials, almost doubled the amount of patients being investigated. The statistical power increased consequently, and the results of the analysis showed a statistically significant survival benefit in the experimental arm.
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ERRATA - LIST 1

Summary-discussion (page 1-36)

Page 10:

The illustration on that page should have following legend

Fig 1: Incidence of urinary bladder cancer in Sweden

Page 22:

On the third line, chapter “Study 1: Results”, the reference to “(Fig 5)”, should be removed

Page 23:

In chapter “Study 3: Results” on the first line, following sentence

Chemotherapy was administered to 74 % in the experimental.

should be replaced with:

Chemotherapy was administered to 74 % in the experimental arm.

Page 25:

In chapter “General discussion and summary”, 10th line from the bottom, following sentence

One of our most interesting findings were that only metastatic sentinel nodes displayed p53-mutations, while the other four unmetastatic sentinel lymphnodes had an unmutated p53.

should be replaced with:

Only metastatic sentinel lymph nodes displayed p53-mutations.