Sentinel nodes in complex areas: innovating radioguided surgery
Vermeeren, L.

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Feasibility of sentinel node detection in renal cell carcinoma: a pilot study

A. Bex
L. Vermeeren
G. de Windt
W. Prevooy
S. Horenblas
R. A. Valdés Olmos

ABSTRACT

Purpose: Lymphatic drainage from renal cell carcinoma is unpredictable and the therapeutic benefit and extent of lymph node dissection are controversial. We evaluated the feasibility of intratumoural injection of a radiolabeled tracer to image and sample draining lymph nodes in clinically non-metastatic renal cell carcinoma.

Methods: Eight patients with cT1-2 cN0 cM0 (<10cm) renal cell carcinoma prospectively received percutaneous intratumoural injections of 99mTechnetium-nanocolloid under ultrasound guidance (0.4ml, 225 MBq at one to four intratumoural locations depending on tumour size). Lymphoscintigraphy was performed twenty minutes, two hours and four hours after injection. After the delayed images a hybrid SPECT-CT was performed. SPECT was fused with CT to determine the anatomical localization of the sentinel node. Surgery with sampling was performed the following day using a gamma probe and a portable mini-gamma camera.

Results: Eight patients, seven with right sided renal cell carcinoma, were included with a mean age of 55 years (range 45–77). Mean tumour size was 4cm (range 3.5-6cm). Six patients had sentinel nodes on scintigraphy (two retrocaval, four interaortocaval, including one hilar) with one extraperitoneal location along the internal mammary chain. All nodes could be mapped and sampled. In two patients no drainage was visualised. Renal cell carcinomas were of clear cell subtype with no lymph node metastases.

Conclusion: Sentinel node identification using preoperative and intra-operative imaging to locate and sample the sentinel node at surgery in renal cell carcinoma is feasible. Sentinel node biopsy may clarify pattern of lymphatic drainage and extent of lymphatic spread which may have diagnostic and therapeutic implications.
INTRODUCTION

In oncology, the purpose of lymph node dissection is to improve staging and survival. The rationale depends largely on the pattern of lymphatic drainage and mode of metastatic spread. Despite decades of research, the role of lymph node dissection in renal cell carcinoma remains controversial. The results of the single prospective randomized study concerning lymphadenectomy do not demonstrate a survival benefit. This may be due to the unpredictability of lymphatic drainage of renal cell carcinoma and the assumption that the main route of spread is haematogenous. In former series, between 58%–95% of patients with lymph node involvement have associated haematogenous metastases, which is why lymph node metastases is regarded as a significant indicator of systemic disease and adverse prognosis. Patients with pN0 have a five-year survival of 75%, whereas patients with pN+ survive five years in 20%. Therefore, extensive lymph node dissection may only prolong time to progression, but not survival. The high percentage of concurrent systemic disease with lymph node involvement has resulted in the view that the likelihood of identifying a patient with lymph node only involvement is low. As a consequence routine lymph node dissection for all patients in order to identify those few with lymph node only metastases who would potentially benefit from the lymph node dissection does not seem to be justified. The current consensus is that suspicious lymph nodes either at imaging or palpation should be removed during nephrectomy.

However, there is evidence from the literature, that patients with very early lymph node metastases and no metastatic disease can potentially be cured by lymph node dissection. The true incidence of early lymph node metastasis without distant metastatic disease is unknown, but seems to be significantly correlated to tumour size. In nephrectomy and autopsy studies microscopic lymph node metastases were frequently observed in smaller tumours.

Due to the widespread use of ultrasound, a stage shift is observed in renal cell carcinoma. An increasing number of patients is diagnosed at an earlier stage with consequently smaller renal tumours. At the same time, retrospective studies reveal that even small tumours have a potential for early lymphatic or distant metastatic spread. In an autopsy study 254 patients had renal tumours of less than 3cm and 3.5% revealed early lymph node metastases, which increased to 21% in tumours of 4–5cm. In nephrectomy series of Matsuyama and Hashimoto this was 2.5% and 4% respectively for tumours of 4cm and less. Thus a considerable number of patients with pT1 tumours may have early lymphatic metastases. In contrary
to the historical data, stage shift may result in an increasing number of patients with early lymph node metastases only who may benefit from removal of these lesions. In addition, the introduction of more effective therapy with tyrosine-kinase inhibitors has revived interest in adjuvant treatment concepts. More accurate lymph node staging is warranted to determine the risk of recurrence or progression. Sequential lymphoscintigraphy and sentinel node biopsy in renal cell carcinoma may enhance early detection of lymph node metastases without the associated morbidity of extensive lymph node dissection.

Sentinel node biopsy is based on the concept of step-wise dissemination of neoplastic disease through the lymph nodes. In the late 1980’s, Morton and Cochran proposed the concept of lymphatic mapping and used a tracer to visualise the lymphatic drainage from a primary tumour. They suggested that the node that receives direct drainage from a melanoma can be any node in a particular lymph node basin, depending on the location of the primary lesion and with a certain individual variability.

Sentinel node biopsy is now widely used for nodal staging of melanoma and breast cancer, as well other malignancies including urological malignancies, head and neck cancer and gynaecological malignancies. The only report available analyzing sentinel node biopsy in kidneys used application of 99mTechnetium intra-operatively in a porcine model. In this study, the tracer and blue dye were injected into the renal parenchyma after exposure with a flank incision. In four pigs, sentinel lymph nodes were successfully removed within ten minutes after injection following detection with a gamma probe. In the current study we investigated the feasibility of the procedure and the location of the sentinel node including sampling at nephrectomy in clinically non-metastatic renal cell carcinoma. Currently no data exist for application of the radiotracer in the kidney in humans. The purpose of the current study was to define the feasibility of sentinel node mapping in patients with renal cell carcinoma. Furthermore, lymphatic drainage patterns from the human kidney are described.

PATIENTS AND METHODS

Patients

Eight patients were included in the study, which was approved by the medical ethical committee. All patients signed written informed consent and were informed about the objective of the study. Inclusion criteria were localized parenchymal tumour of the kidney not exceeding 10cm (cT1—cT2), no metastatic disease on imaging and clinical examination (cN0, cM0), age 18 years, life expectancy >3 months, WHO performance status 0 or 1 and fit
for surgery, no prior systemic treatment with biological response modifiers, tyrosine-kinase inhibitors, monoclonal antibodies or chemotherapy.

**Lymphoscintigraphy**

Patients underwent preoperative lymphoscintigraphy on the day before nephrectomy. 99mTc-Technetium-nanocolloid was injected percutaneously in a volume of 0.4ml and a dose of 225 MBq into the primary lesion under ultrasound guidance. CT-guidance was only indicated in case of inappropriate imaging with ultrasound. Primary tumours of up to 4cm were injected centrally with a volume of 0.4ml. In cases of tumours between 4–7cm two to three depots of 0.4ml totally were injected around the centre to achieve sufficient application of the tracer. There were no patients with tumours larger than 7cm. Following injection, anterior and lateral lymphoscintigraphy of the affected site was obtained after twenty minutes, two hours and four hours. After the delayed planar images, SPECT and low-dose CT were acquired, using a hybrid camera (SymbiaT, Siemens, Erlangen, Germany). After correction for attenuation and scatter, corresponding SPECT and CT axial 5mm slices were generated using an Esoft 2000 application package (Siemens). Images were fused using an Osirix Dicom viewer in a Unix-based operating system (MAC OS X, MacPro; Apple Inc.) and analysed using two-dimensional orthogonal re-slicing in axial, sagittal and coronal directions. Also a three-dimensional presentation, using volume rendering, was generated to localize sentinel nodes in relation to anatomic structures. All images were available on a separate SPECT-CT screen in the operation theatre.

The nodes in each station appearing on early planar lymphoscintigraphy were considered to be the sentinel node. Nodes appearing later in the same stations were considered to be second echelon nodes. If SPECT-CT showed hotspots in other areas or on a side with no previous drainage, those were also considered to be sentinel nodes. To provide an orientation point for incision, levels of the sentinel nodes were marked on the skin. Those marks also functioned as a centring point for intra-operative radioguidance with the portable gamma camera.

**Surgery and intra-operative sampling**

Patients underwent an open or laparoscopic transperitoneal nephrectomy. In cases of tumours up to 4cm a nephron-sparing tumour resection was performed. This approach does not interfere with the feasibility investigation.

A gamma probe (Neoprobe, Johnson&Johnson Medical, Hamburg, Germany) in combination with radioguidance with a portable gamma camera (Sentinella, Oncovision,
Valencia, Spain) was used for intra-operative sentinel node detection and localization. The use of this portable gamma camera has been described previously.\textsuperscript{12}

Sentinel nodes were separately excised. A routine lymph node dissection was performed involving on the right side a hilar, paracaval and interaortocaval lymphadenectomy and on the left side a hilar, paraaortal and interaortocaval lymphadenectomy.

As this was a feasibility study involving a) the feasibility of lymphoscintigraphy and b) the intra-operative sentinel node biopsy, only sentinel node in abdominal locations were removed.

**Pathology**

Sentinel nodes were examined with step-sectioning at three levels. Paraffin sections were stained with haematoxylin-eosin staining and immunohistochemical staining using CAM5.2. The remaining lymph nodes will be analysed by routine pathological examination.

**Statistical considerations**

To gain sufficient experience with the injection technique and intra-operative sampling during a feasibility study, a total number of eight patients was included in this study. No statistical analysis was required.

**RESULTS**

Eight patients, seven with right sided renal cell carcinoma, were included. Patient characteristics are summarized in table 1. Mean tumour size was 4cm (range 3.5–6cm).

<table>
<thead>
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<th>Age</th>
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<th>Side</th>
<th>Nephrectomy</th>
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</table>

f female; m male; y years; cm centimetres
In four patients lymphatic drainage was visualised on planar lymphoscintigraphy, in two patients with non-visualisation on planar images SPECT/CT did show lymphatic drainage (two sentinel nodes in one patients and one sentinel node another patient). All sentinel nodes could be exactly localized with SPECT/CT. Figure 1 gives a synopsis of the anatomical location all visualised sentinel nodes in relation to size and location of the primary tumour. In total, two retrocaval sentinel nodes and four interaortocaval sentinel nodes were found (including one hilar sentinel node). One patient showed drainage to an extraretroperitoneal sentinel node, located along the internal mammary chain. The preoperative images of this patient are shown in figure 2. In three patients, lymphatic drainage was observed to two sentinel nodes simultaneously with preoperative imaging (patient 1, 2 and 7). In one patient, an additional hot node (sentinel node) was found intra-operatively while this node was not visualised preoperatively. Figure 3 shows lymphatic drainage in two patients, as visualised with preoperative imaging.

All other visualised sentinel nodes could be localized intra-operatively. The node in the internal mammary chain was not excised after localization for safety reasons, all other sentinel nodes were harvested without difficulty.

In two patients no lymphatic drainage could be visualised with preoperative imaging, in those patients exploration with the gamma probe did not reveal any radioactive hotspots either. Intra-operative localization and excision of the sentinel nodes did not increase operation time significantly (all patients underwent regional lymphadenectomy). No complications related to the sentinel node excision occurred. One patient who received open surgery was treated for an infection of the incision wound.
Figure 2 | A patient with a tumour of 5cm in the upper pole of the right kidney. Lymphoscintigraphy at twenty minutes (A) shows lymphatic drainage to the abdomen and thorax. Furthermore, prominent physiologic liver and spleen activity is visible, probably due to partial incorporation of the tracer into the bloodstream after injection. Fused SPECT/CT images show a para-aortic sentinel node (B) as well as a parasternal sentinel node (C). On the sagittal reconstruction (D) both sentinel nodes are visible. The three-dimensional reconstruction (E) clearly shows the parasternal node to be located in the second intercostal space, just above the third rib.

Figure 3 | Coronal fused SPECT-CT showing drainage to a sentinel node (arrow) located between the aorta en vena cava inferiorly (A). This lymph node is also displayed on axial fused SPECT-CT (B) and diagnostic CT (C). In another patient (D) paravertebral drainage is observed on coronal fused SPECT-CT (arrow). On axial fused SPECT-CT (E) and diagnostic CT (F) the sentinel node is seen dorsal from the vena cava inferior.
Renal cell carcinomas were of clear cell subtype with no lymph node metastases in the sentinel nodes and the lymph nodes in the defined retroperitoneal template.

**DISCUSSION**

Lymphatic mapping in other malignancies has shown to be a safe and feasible procedure for lymph node staging. After injection of a radioactive tracer, sequential planar images are performed to localize sentinel nodes. With SPECT/CT, those nodes can be anatomically localized. We are the first to describe lymphatic mapping for patients with a kidney tumour.

Concerning tracer injection, we choose to use a dose of around 225 Mbq. This dose was chosen because it has been validated in our centre for prostate cancer patients.\(^{13}\) The tracer was injected in one or two depots into the tumour in order to avoid variability in the lymphatic drainage due to multiple injections (as is required for peritumoural tracer injection). Intratumoural injection was validated by us for other tumours with a high reproducibility.\(^{14,15}\) Planar images after fifteen minutes, two hours as well as four hours were performed in order to guarantee visualisation of late drainage as well. In the future the four hours images will possibly be proven of no additional value. Due to logistic reasons all patients were operated the following morning. In this cases radioactivity within the nodes was sufficient to facilitate intra-operative localization, although we do recommend to operate within 24 hours after injection in order to prevent localization difficulty. In one patient an additional hot node was found intra-operatively, while not being visualised preoperatively, therefore we suggest that intra-operative exploration with the gamma probe is relevant in order to identify all sentinel nodes.

Lymphatic mapping, using preoperative planar images and SPECT/CT and intra-operative detection with the gamma probe, was shown successful in 75% of our patients, with visualisation of one or more nodes. SPECT/CT showed sentinel nodes in two patients with non-visualisation on the planar images. In other malignancies SPECT/CT has also shown to visualise additional sentinel nodes.\(^{16}\)

If sentinel node mapping in renal cell carcinoma will be used in the future, optimization and validation of dosage of radiotracer used, injection techniques, imaging protocols and timing to operation might lead to improved visualisation of sentinel nodes.

For safety reasons one intra-mammary sentinel node was not harvested, but all other nodes could be successfully localized and excised during surgery. Although the possible value of sentinel node mapping in renal cell carcinoma has to be established with further research,
our method of preoperative lymphoscintigraphy in combination with intra-operative sentinel node excision has shown to be safe and feasible.

Since lymphoscintigraphy has not been performed before in patients with kidney tumours, exact direct lymphatic drainage patterns are unknown. Lymphatic drainage patterns were previously assessed by analyzing lymphatic metastasis within lymphadenectomy specimen or lymphatic dissemination patterns on autopsy.

In nephrectomy series, metastases are generally detected in “regional” lymphadenectomies and it is not clear how extended the lymph node dissection has been performed. As in other tumours the accuracy of detecting lymph node metastases increases with the amount of sampled nodes. Terrone and co-workers found that the proportion of pN+ patients increases with the numbers of lymph nodes examined, independently of the extent of lymph node dissection. Analyzing 1828 autopsy records, Saitoh and co-workers described a broad variation of the anatomical localization of lymph node metastases. Because most patients had multiple lymphatic metastasis, it cannot be concluded which node was the first involved node. Interestingly though, ipsilateral renal hilar lymph node metastases were only found in 7%, while pulmonary hilar lymph node metastases were found in 66.2%, retroperitoneal in 36%, para-aortal in 26.8% and supraclavicular in 20.7%. Hulten and co-workers described single metastases in a peripheral supraclavicular lymph node in one and in an iliac lymph node in two patients without any further metastasis. Johnsen and Hellsten found single mediastinal lymph node metastases in eight, supraclavicular in one and axillary in one patient. Since all those patients had massive systemic metastasis, the authors regarded those rare single lymph node metastases as an expression of haematogenous tumour spread subsequently involving the lymph nodes. On the other hand, those single positive nodes may chronologically represent the first metastatic site with subsequent haematogenous spread.

By injecting blue dye at high pressure into normal cadaveric kidneys, Parker found extreme variations of lymphatic drainage between individual cases in 1935. Assouad and co-workers injected normal kidneys of sixteen cadavers with a blue modified Gerota mass and dissected lymph vessels until their termination. Renal lymphatics have been found to reach very distant nodes (e.g. aortic bifurcation, celiac or mesenteric lymph nodes and contralateral lymph nodes). Furthermore, they found that the lymphatic vessels were always connecting to the origin of the thoracic duct, some directly without traversing any retroperitoneal lymph nodes. As the authors argue, this feature may play an important role in the frequently observed pulmonary and mediastinal metastatic spread in renal cell carcinoma. Isolated mediastinal lymph node metastasis are more frequently observed in renal cell carcinoma.
compared to tumours in other organs.\textsuperscript{23-25} Direct drainage to the thoracic duct may also explain the failed scintigraphic detection of lymph nodes in two cases in our study, despite a satisfactory intratumoural location of the tracer. As we perform the first scintigraphy after twenty minutes, flow in lymphatic vessels is not detected. Therefore, we plan to observe immediate drainage during and after injection with the portable mini gamma camera.

As has often been found for other tumour entities, the lymphatic drainage may not follow the known pattern. In renal cell carcinoma it is often believed that the draining lymph nodes are in the hilar region branching off into the paracaval, interaortocaval or paraaortal retroperitoneal lymph nodes depending on the side of the renal tumour. A potential reason for the lack of evidence supporting locoregional retroperitoneal lymph node dissection and the low detection of lymph node metastases in CT-negative locoregional nodes may simply be the fact that the sentinel node of renal cell carcinoma is located outside the region of dissection in a percentage of cases. In our study, we found extraretroperitoneal drainage in one out of eight patients, though it was a simultaneous drainage together with an interaortocaval node. In view of the small numbers, mostly right sided tumours and the objective of investigating feasibility, frequency of anatomical location should be interpreted with caution. The majority of draining lymph nodes were located in the interaortocaval space with only one lymph node duly in the hilar region.

\textbf{CONCLUSION}

Sentinel node identification using preoperative and intra-operative imaging to locate and sample the sentinel node at surgery in renal cell carcinoma is feasible. Further interpretation of this study is limited by small numbers and a relatively small tumour size. Sentinel node biopsy may clarify patterns of lymphatic drainage and extent of lymphatic spread which may have diagnostic and therapeutic implications. In a diagnostic trial with a larger sample size we plan to investigate the frequency of extraretroperitoneal drainage and occult lymph node metastasis in clinically non-metastatic and node-negative renal cell carcinoma.
REFERENCES


