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ORIGINAL ARTICLE

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Prostate lymphoscintigraphy for sentinel lymph node identification in canines: reproducibility, uptake, and biokinetics depending on different injection strategies

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Abstract At present there are neither clinical nor experimental data available on the influence of technical details on the quality and reproducibility of prostate lymphoscintigraphy. Six adult fox hounds received repeated transrectal ultrasound guided intraprostatic injections of a technetium 99m labeled nanocolloid to prove the influence of different techniques of injection (one central injection in both prostate lobes vs two peripheral injections in both lobes) on tracer accumulation in sentinel lymph nodes (SLN) and other organs. The reproducibility of the favored technique was examined and in a last step it was subject to scrutiny following a reduction of the injected volume to 1% of the prostate volume. The number of scintigraphically visualized SLN varied between four and seven. They were located in the region of the internal and external iliac vessels, presacrally, paravesically, and directly paraprostatically. In five of six cases, the localization was reproducible both with the central application of an identical volume as well as with the volume reduced central injection. Tracer accumulation of SLNs and other organs varied enormously. We expect that with the combination of both injection techniques, even with the reduced injection volume, an optimized prostate lymphoscintigraphy will be the outcome.

Keywords Prostate lymphoscintigraphy · Sentinel lymph node · Canine · Prostate cancer · Lymph node metastases

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Introduction

The first experience with prostate lymphoscintigraphy and intraoperative sentinel lymph node (SLN) identification proves to be a feasible procedure, not only in breast cancer and malignant melanoma, but also in prostate cancer [15].

Despite the encouraging first results in the identification of lymph node positive patients (sensitivity 96%, 95% confidence interval 79.7–99.9), questions regarding the method remained unsolved. These can only be answered by repeated investigations on the same individual.

First, the reproducibility of the results in identical test conditions as a requirement for the validity of the method used has to be reviewed. In addition it is important to examine whether the quality and the reproducibility of prostate lymphoscintigraphy are influenced by the intraprostatic injection volume and the area of administration.

In order to transfer results from animal studies to humans, the anatomy of prostate lymph drainage and injection requirements need to be comparable. There is evidence from both the anatomy of prostatic lymph drainage in humans as well as in canines, that different areas of the prostate have a variable drainage region [12]. The different lymphatic drainage from the periurethral and peripheral zone is discussed as the only difference between the human and canine prostate [1]. More extensive investigations, comparable to those in humans [4], which concentrate on the lymphatic density in the canine prostate do not, to our knowledge, exist.

Due to the fact that several studies have already shown that transrectal ultrasound guided manipulation of the canine prostate [3] and prostate lymphoscintigraphy are possible, we chose canines as our animal model.

To our knowledge, this is the first study examining different strategies of injection and the reproducibility of prostate lymphoscintigraphy. It is compelling, because the inter-individual variability of the prostatic lymph drainage both in canine as well as in humans is quite marked.

Materials and methods

Study design

This study was carried out on six canines. In case the same strategy of injection was successful in all canines, the lower limit of the 95% confidence interval to estimate the effectiveness of this strategy was set at 54%. In this group, the 50% threshold value for this lower limit was exceeded for the first time, which was a requirement for the given number of cases.

Experimental protocol

All investigations on canines (adult male fox hounds, weight 36–40 kg) were carried out under general anaesthesia. Similarly to humans, an intraprostatic injection of a technetium 99m labeled nanocolloid (100 MBq) was administered under ultrasound guidance. Following volumetric measurements of the prostate, a bilateral, centrally placed application of the tracer substance was carried out (Fig. 1). The injected volume (1.6–3.8 ml) was equivalent to approximately 10% of the prostate volume. To determine the uptake and biokinetics of the tracer static, lymphoscintigraphies after approximately 10, 30, 60, 90, 120 and 140 minutes and in a second general anaesthesia 24 h later using anterior, posterior and single lateral views were carried out. Biodistribution (max. uptake as a percent of injected activity) and biokinetics in the respective organ could be determined using a region of interest (ROI) evaluation. In addition, the activity of the urine was measured at the end of the first part of the investigation (approximately 140 min p.i.). The concentration of activity in the blood was determined by taking repeated venous blood samples. The whole activity in blood was calculated with an estimated 2,000 ml blood volume. To demonstrate the biokinetics without being obscured by the radioactive decay, all activities and count rates were adjusted to decay at the time of injection. For the precise localization of SLN ex vivo technetium 99m labeled erythrocytes (sodium diphosphate decahydrate 20.12 mg, tin (II) chloride-dihydrate 4.05 mg) were injected at the end of the third investigation (Table 1). The resulting vascular imaging made the anatomical relation of SLN easier.

Variation of injection technique and injection volume

The first investigation (Table 1) was repeated with an identical volume but with bilateral dorsal and ventral intraprostatic injections close to the prostate capsule (Fig. 1). The third investigation repeated the one in which more SLN where shown, to prove reproducibility. In the presence of an identical number of SLN, the less invasive and easier technique (central injection) was selected. To test the reproducibility of the preferred technique, the experiment

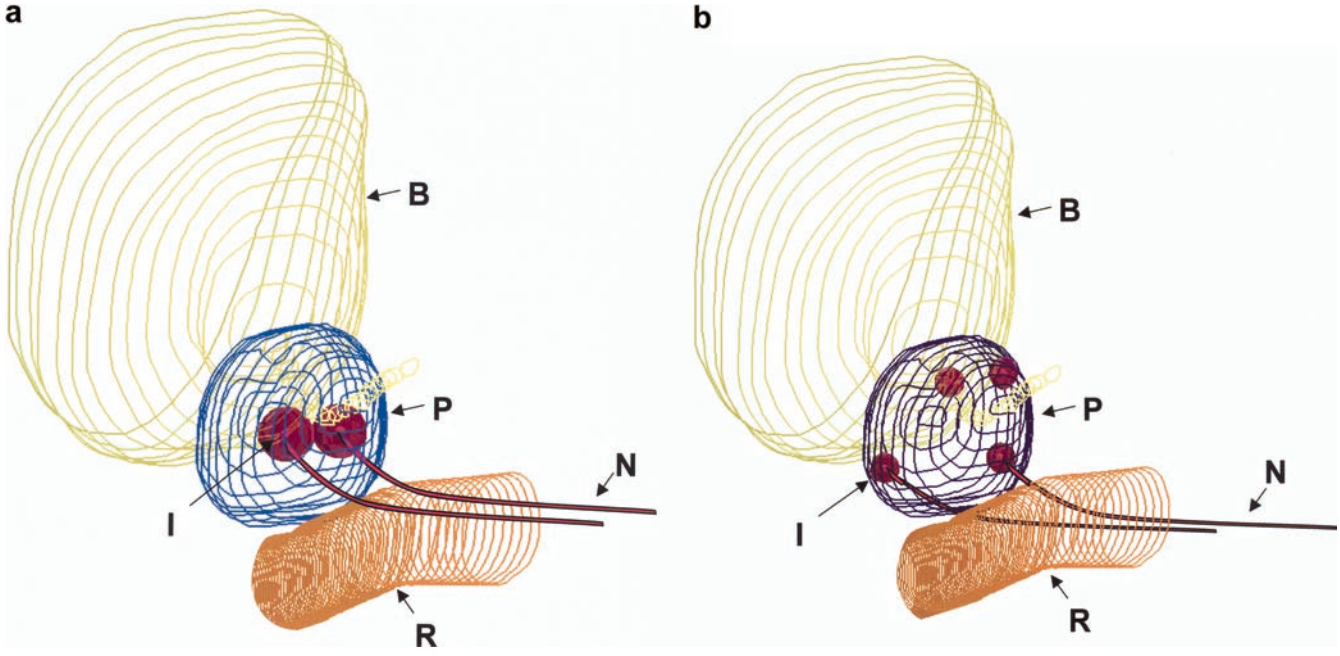

Fig. 1 Scheme of the: **a** intraprostatic central and **b** peripheral injection technique (*B* = bladder, *P* = prostate, *N* = needle, *R* = rectum, *I* and *red points* = injection sites)

Table 1 The different techniques used in the study

	Number of injections	Location of injections	Injection volume
First investigation	2	Central, both prostate lobes	10% of prostate volume
Second investigation	4	Peripheral, both prostate lobes	10% of prostate volume
Third investigation	2	Central, both prostate lobes	10% of prostate volume
Fourth investigation	2	Central, both prostate lobes	1% of prostate volume

was repeated with an injection volume reduced to 1% of the prostate volume (forth investigation). In the case of reproducibility not being achieved, it was planned to increase the injection volume by half of the initial volume.

Sentinel lymph node criteria

Anatomical localization and the time of appearance of radioactivity in lymph nodes were the main SLN criteria. The visualization of lymphatic pathways in prostate lymphoscintigraphy is a rare, and therefore unreliable, criteria. In some cases, identification was made more difficult by the close proximity of the lymph nodes to each other and their low activity uptake.

Results

Number and localization of sentinel lymph nodes

Between four and seven SLN (median five) were shown in each canine (Fig. 2). These were located in the internal and external iliac pathway as well as presacrally, paravesically and directly paraprostatically (Fig. 3).

Preferred technique and reproducibility

The central injection technique was superior, or at least equal, to the fractionated, multiple, peripheral injection for the detection of SLN (Table 1). The localization of all SLN was reproducible in five out of six cases (83.3%, 95% confidence interval: 35.9–99.6%) in both the central application with identical volume as well as in the central injection reduced to 1% of the prostate volume in all experiments (Fig. 4).

SLN-uptake and biokinetics

The median accumulated activity of SLN varied between 0.46 and 0.69% of the injected activity (Fig. 5). The lowest median uptake was recorded with the peripheral injection technique. The small number of investigations and the strongly varying individual uptake by SLN and other organs make it impossible to determine statistical significance. The largest scatter occurred after central, volume reduced injection (0.04–1.47%). The median uptake by SLN with the lowest accumulation varied between 0.11 and 0.2% (Fig. 6). The peripheral injection was the one with the lowest value. The intra-individual variation of SLN-uptake was quite considerable. This is shown in Fig. 7, where the respective activities varied seven to eightfold with identical average SLN-uptake between the first and third investigation (SLN 1). Apart from a few exceptions, the SLN-uptake showed a steady state after approximately 150 min p.i. (Fig. 8).

Uptake and biokinetics of the prostate

The activity measured 10 min following application was equal to the maximum activity, and varied between 12

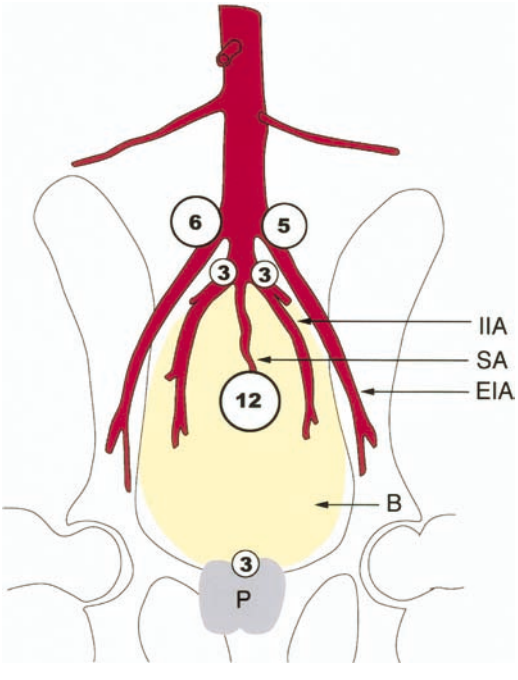

Fig. 2 Number of sentinel lymph nodes (SLN) and their location identified in all canines [*EIA* = external iliac artery (11 SLN), *IIA* = internal iliac artery (6 SLN), *SA* = sacral artery (12 SLN), *P* = prostate (3 paraprostatic SLN), *B* = bladder]

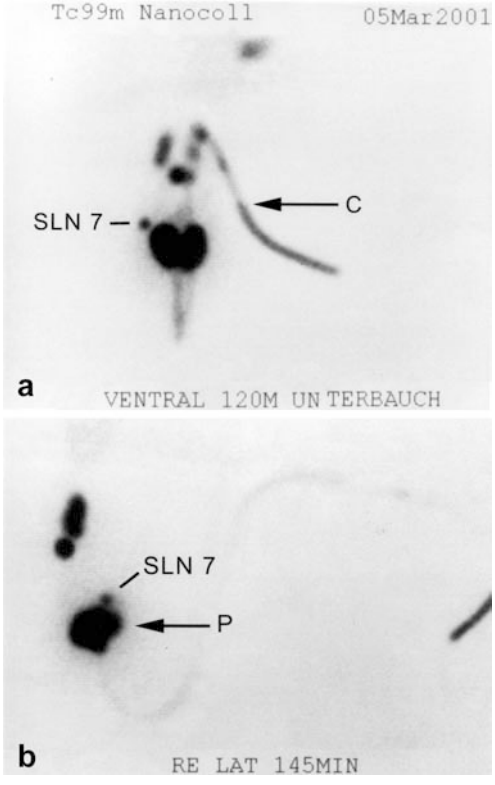

Fig. 3 a Anteroposterior and **b** lateral views of prostate lymphoscintigraphy with a paraprostatic sentinel lymph node (*SLN 7* = sentinel lymph node number 7, *C* = catheter, *P* = prostate)

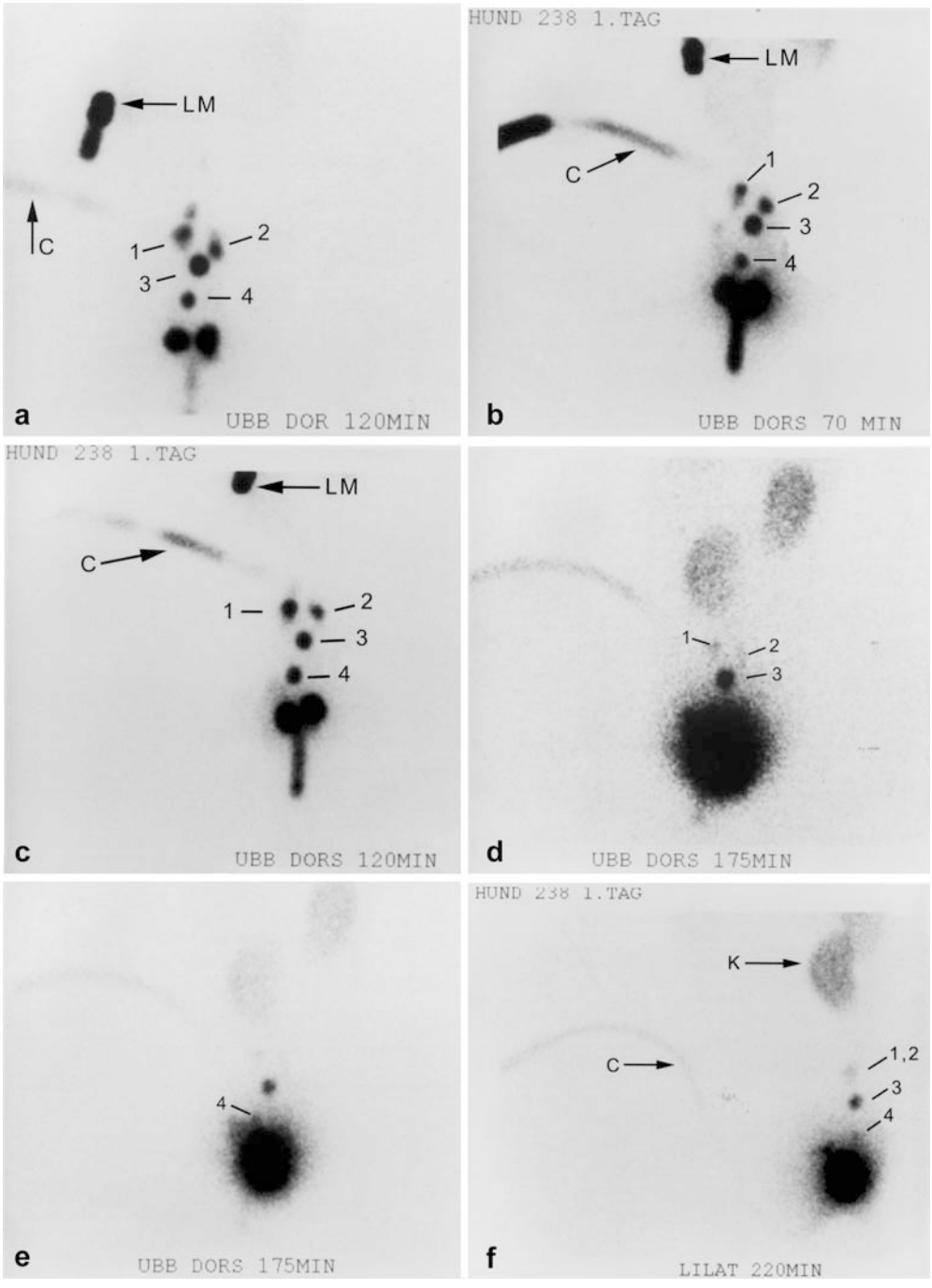

Fig. 4 An example of the reproducibility of prostate lymphoscintigraphy depending on different injection strategies in canines. **a**–**d** (investigations 1–4) demonstrating the sentinel lymph nodes in all investigations. The SLN close to the prostate (SLN 4) is only visible (weak) with **e** different imaging and **f** in a lateral view in prostate lymphoscintigraphy of the fourth investigation (**d**). (*LM* = radioactivity for landmarking, *C* = catheter, *K* = kidney, *1–4* = SLN)

and 80% of the overall activity. Different techniques of injection could not demonstrate any differences. In all investigations, a steady decline with a median biological half-life of 3.5 h (range 1.6–9.4 h) was observed within 90 min p.i. (Fig. 8).

Urine uptake

The major part of the activity (median between 35–57%) was present in the urine in the bladder (Fig. 9) immediately following injection. This most likely happens via the excretory ducts of the prostate which drains via the seminal colliculus into the bladder. A minor part is eliminated over the kidney through the haematogenic tracer transport mechanism. The desired and expected significant decrease of urine uptake with a reduced injection volume was not achieved.

Blood activity

The maximum uptake in blood (range 0.18%–7.98%, estimated 2,000 ml blood volume) was recorded in each

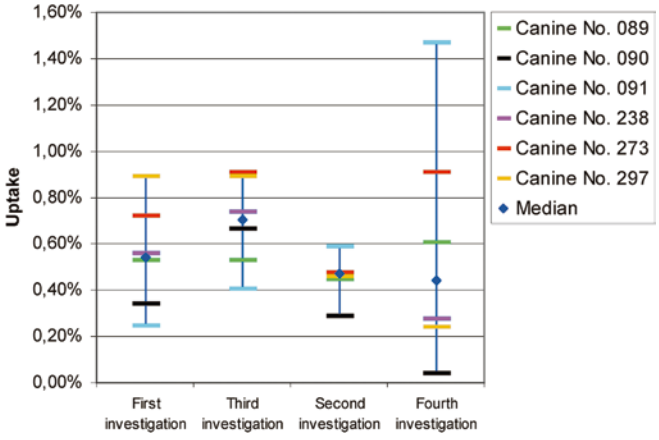

Fig. 5 Average tracer accumulation in the sentinel lymph node of each investigation

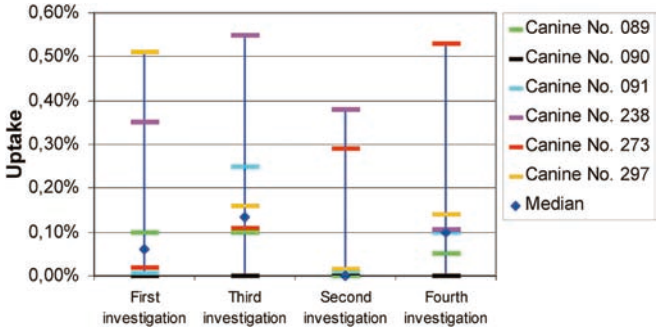

Fig. 6 Tracer uptake in the sentinel lymph nodes with the lowest activity accumulation of each investigation

first imaging following injection. During the first 90 min, a comparably steep, continuous fall in activity (median biological half-life 1.2 h) in blood was frequently observed. Later a plateau was commonly seen (Fig. 8).

Uptake and biokinetics in liver and spleen

The maximum uptake by the liver and spleen (range 0.1–34%) was observed between the first (10 min) and the last imaging of the second day (24 h p.i.) (Fig. 8). Approximately 2 h after injection, a major part of the

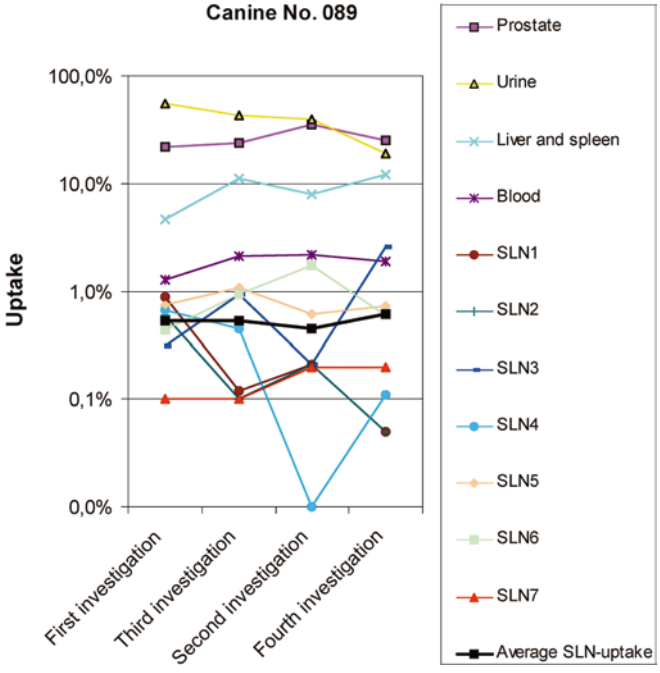

Fig. 7 An example of tracer accumulation in sentinel lymph nodes, urine, and organs of each investigation (canine no. 089)

accumulation was already completed. High activities in blood led to a higher and earlier tracer-uptake in the liver and spleen.

Discussion

Tracer uptake, biokinetics and number of visualized SLN in canines are comparable to those in humans [15, 16]. As a consequence it can be expected that the results on reproducibility and the influence of different experimental parameters can be applied to humans.

The identification of SLN in prostate cancer is different on principle from the technique in other tumor entities. Both in breast cancer [10], penis cancer [6, 14] as well as in malignant melanoma [11], a well directed peritumoral injection is placed only to observe the lymphatic drainage of the tumor. In prostate cancer, however, it is not known from which part of the organ

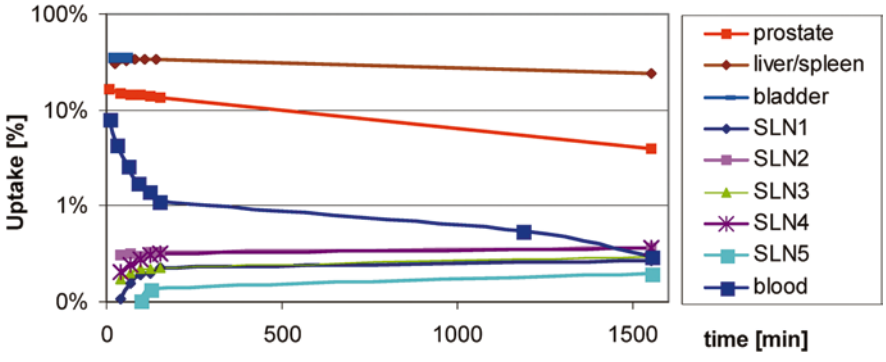

Fig. 8 An example of the biokinetics of technetium 99m labeled nanocolloid (canine 297, fourth investigation)

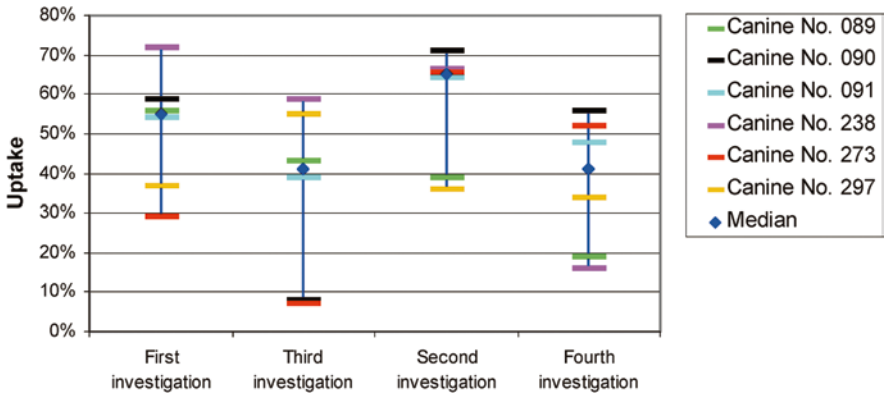

Fig. 9 Activity in the urine of each investigation

the metastatic spread originates. Therefore, the aim of prostate lymphoscintigraphy needs to be the imaging of all primarily draining lymph nodes of the prostate. Inevitably, a single or more SLN of the tumor will be present among them.

The quality of prostate lymphoscintigraphy not only has to be evaluated by the SLN detection rate, but also by the proportion of low accumulating SLNs, because the latter can be missed in intraoperative identification. Consequently the SLNs of lesser radioactivity are the problem in prostate lymphoscintigraphy. Single lymph nodes with a very high uptake (3.9% in one case) actually increase the value of average SLN uptake, but are not necessary for intraoperative identification. Therefore the aim has to be an even SLN uptake and a reduction of the number of SLN with low activity. The latter can be missed in the intraoperative gamma-probe identification, especially with the preferred 2-day-protocol. As several SLNs showed a higher uptake after peripheral injection, and in contrast other SLNs after the central one, it seemed reasonable to combine both techniques. Expected that this combination would lead to a homogenous intraprostatic tracer distribution, resulting in a more homogenous tracer uptake by all SLNs.

Lymphatic flow is initialized by an increase in the interstitial pressure triggered by a certain volume of fluid that causes distension of the endothelial cells mediated by anchoring filaments. The volume for initializing lymphatic flow therefore depends on tissue density. For subdermal application in malignant melanoma or penile cancer, small volumes (0.1–0.2 ml.) seem to be appropriate, whereas larger volumes of from 3 to 8 ml are needed for peritumoral application in breast cancer [5]. Apparently this does not reflect the physiological condition, and individual reports have demonstrated good results with much smaller volumes [13]. As the reduction of volume in prostate lymphoscintigraphy to 1% of the prostate volume demonstrated reproducible results in terms of SLN uptake, this should be preferred as the more physiological way of administration.

Furthermore the varying activities in SLN in repeated tests clearly demonstrate that the amount of activity uptake [8] is not an appropriate criteria for SLN identification.

Compared with previous prostate lymphoscintigraphies in canines [7, 9] the number of identified lymph nodes was increased and locations detected which until now could not be demonstrated with dyes or lymphoscintigraphy. However, the cause for this is not known; as compared to previous investigations most of the parameters (amount of radioactivity, radiopharmaceutical agent, injection volume, injection technique) have changed.

Conclusion

As a consequence of our investigations, we expect that with the combination of both injection techniques (central and peripheral), even with the reduced volume, an optimized prostate lymphoscintigraphy will be the outcome.

It is now necessary to test this hypothesis in humans.

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