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Review

Sentinel node biopsy in gastrointestinal-tract cancer

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Abstract

Forty three years after Gould's first description of the sentinel lymph node (SN) technique in malignant tumours of the parotid, sentinel lymph node biopsy (SLNB) has become an invaluable tool for the treatment of solid tumours. In some tumour types, it has been shown to reliably reflect the lymph node (LN) status of the tumour-draining LN basin. In melanoma and breast cancers, it has become a widely accepted element in the routine surgical management of these malignant diseases. In gastrointestinal tumours, the technique is currently under intense investigation. First reports on its application in other solid tumours like non-small cell lung cancer, thyroid carcinoma, oropharyngeal carcinoma, vulvar carcinoma, and Merkel Cell carcinoma of the skin were published more recently. In the following review, we will give a synopsis of the fundamentals of the SN concept and will then proceed to an overview of recent advances of SLNB in gastrointestinal cancers.

1. Introduction

During recent decades, the treatment of solid cancers has progressively evolved to an individualised multimodal therapeutic concept involving different specialists i.e., medical and surgical oncologists, pathologists, radiologists and radiotherapist. The therapeutic concept chosen depends on the exact histopathological staging and the presence of specific molecular or immunohistochemical markers. Surgical oncologists play a pivotal role in the treatment process. Their primary aim is to obtain the complete resection of the tumour (RO). The surgical procedure provides the material for the histopathological staging of the tumour. Nevertheless, the surgical trauma should be as limited as possible in order to decrease intra- and postoperative morbidity and mortality. The sentinel lymph node biopsy (SLNB) provides an invaluable tool to achieve these goals. First, it allows for a minimally invasive assessment of the nodal status. Thus, patients with positive sentinel nodes (SN) can be selected for elective lymph node (LN) dissection. In patients with negative SNs, LN dissection may be avoided in certain tumour types. Second, aberrant lymphatic drainage can be assessed and considered when deciding on the extent of the surgical resection. Finally, histopathologists may concentrate their methodological repertoire, including step-sectioning and immunohistochemistry, on the LN that has the highest risk for metastatic deposits.

While SLNB has found its place in routine treatment for melanoma and, more recently, for breast cancer patients [1], its role in gastrointestinal cancer patients is currently the subject of intensive clinical research.

2. Techniques of SN-detection: blue dye versus radiocolloid methods

The Blue dye-method is a strictly intraoperative technique. In Europe, patent blue is the most commonly used dye [2]. Evans blue, methylene blue, indigo carmine, indo-cyanine green and other dyes have also been injected, notably by American or Japanese investigators. Soluble dyes, like Evans blue and patent blue, are bound to endogenous proteins by sulphonation and are thus trapped within lymphatic capillaries [3]. Since dye

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migration towards the SN through the lymph channel is quite fast, the Blue dye-method is essentially an intraoperative technique. Preparation time of the efferent lymph channel should be kept to a minimum. In cases of prolonged preparation, the risk of secondary, third and subsequent tier nodes also showing blue staining will increase. A traumatic preparation may result in the rupture of the afferent lymphatic channel and diffuse contamination of the operative field with blue dye compromising any further detection of the SN. Alternative lymphatic drainage is difficult to visualise by this method. A further potential drawback of this technique is the possibility of inducing anaphylactic reactions. Allergic incidents of varying severities were reported in up to 1.5% of SLNB procedures [4]. Nevertheless, the Blue dye-method is an invaluable tool for SN-detection in the hands of an *experienced* surgeon. Moreover, this method is inexpensive compared with the use of radiocolloids.

Less training appears to be necessary to achieve good detection rates with the radiocolloid method [5,6]. Several hours before the surgical procedure (2–24 h), a radioisotope is injected around the tumour. In Europe, a commonly used tracer is $^{99\,m}$Tc–radiolabelled colloidal albumin (Nanocoll, particle size under 80 nm) [7,8], whereas the most employed tracer in the United States of America (USA) is $^{99\,m}$Tc–sulphur colloid [9], which is characterised by its larger particle size. In Japan, $^{99\,m}$Tc–tin colloid with particle sizes larger than that of the other colloids is currently the most commonly used radiotracer [10]. Migration kinetics and uptake in LN of different colloids can vary considerably according to the particle size. One hour after the injection, 3% of the activity is drained from the injection site with a speed that is inversely proportional to the particle size. After 17 h, 83% of the original activity still remains at the injection site. Accumulation of radioactivity in single LNs ranges from 0.01% to 1% [11]. After preoperative lymphoscintigraphic imaging with a γ-camera, intraoperative detection of the SN is done with a hand-held γ-probe.

In recent years, there is a growing tendency to consider the Blue dye and the radiocolloid-method as complementary techniques. It is controversial whether the further improvement of the detection rate appearing in some studies and in some tumour types as result of complementary use of blue dye and radioisotopes reflects the increasing experience of the surgeons or is due to a true benefit from the combined technique [12]. Morrow and colleagues [13] identified the number of cases performed by the individual surgeon as the most important predictor for SN-detection.

Recently, several series with radioactively labelled dyes or labelled liposomes containing blue dye showed the feasibility of these approaches [14,15].

3. Biological aspects

The growing popularity of the SLNB has led to renewed interest in the anatomy and physiology of the lymphatic system.

Lymph flow is generated by lymph formation, active contraction of the lymph channel and external interstitial pressure. Lymph formation and interstitial pressure, in turn, are influenced by the volume of the injected tracer. This may, at least partly, account for the variation in the detection rates found in studies injecting different tracer volumes [16]. Furthermore, lymphatic flow can be influenced by a variety of drugs, e.g., halothane [17].

Lymph capillaries are lined with a single layer of endothelial cells with openings 10–25 nm wide. These openings permit the entrance of small particles, i.e., blue dye. Larger particles such as nanocolloid enter the lymph system more slowly by active pinocytosis [16]. Radiocolloid is retained in the LN through active saturable phagocytosis by antigen presenting cells lining the sinusoid spaces [18]. Prerequisite for this uptake is a negative surface charge and preliminary opsonisation by a variety of proteinaceous compounds e.g., C3, C4B and C5 [19]. Blue dye is assumed not to be subjected to phagocytosis. Its capacity to enter and remain in the lymphatics seems to be determined by the presence of at least one sulphonic acid group, resulting in optimum protein binding conditions. With the blue dye method, second level LNs may be stained in absence of any blue colour in first line LNs when time between injection and detection was sufficiently long for the dye to completely exit the first level LN.

The capacity of radiocolloid retention also seems to be determined by the grade of malignant infiltration. Several authors reported that false-negative LNs showed extensive tumour infiltration. Following their theory, tumour-free SNs or SNs harbouring only single malignant cells will take up radiocolloid normally. If numerous tumour cells infiltrate the LN, radiocolloid uptake is reduced. After complete infiltration and destruction of the nodal reticuloendothelial system and in cases of malignant obstruction of the afferent lymphatic vessel, tracer uptake will be abolished. However, a small study set up to determine the reasons for high false-negative rates of SLNB in gastrointestinal cancers could not establish a correlation between tumour infiltration and the rate of false-negatives [20].

4. Immunological aspects

Although direct priming of the anti-tumoural immune response can occur directly in the tumour under certain conditions, it is an established consensus that cross-priming in lymphoid organs is an important step

in the induction of tumour rejection. The SN may be the place of cross-presentation of tumour-derived antigens to the immune system. This lymphoid organ may thus be essential for the induction of an effective immune response.

An important observation was published by Cochran *et al.* and Essner *et al.* in 2001 [21,22]. They found a downregulation of antigen-presenting cells in SNs. Both authors investigated differences between SN and non-SNs on a histological and molecular level. There was a significantly reduced number of antigen-presenting cells, i.e., dendritic cells in the SNs compared with non-SNs. Expression of B7.1, B7.2 and CD40 on dendritic cells, as well as expression of CTLA-4 and CD28 on T-lymphocytes was downregulated, indicating a depressed activational state of antigen-presenting cells in SNs. Although these studies may be criticised for technical reasons, they at least give some indications that SNs have an immunosuppressed phenotype. Leong and colleagues compared the expression of interferon (Interferon-γ, interleukin (IL)-2, IL-10 and granulocyte macrophage-colony stimulating factor (GM-CSF) in SNs and non-SNs [23]. There were no statistically significant differences in IL-10 production between SNs and non-SNs. In contrast, IFN-γ, IL-2 and GM-CSF were produced at significantly higher levels in SNs without micrometastasis compared with non-SNs from the same patients. Interestingly, this difference disappeared when SNs with micrometastasis and non-SNs were compared.

Some authors have raised concerns about the safety of SLNB due to a putative immunosuppressive effect from the procedure. Several investigators showed that tumour-specific lymphocytes have been present in the excised SNs [24,25]. However, in a case report, Schrama and colleagues showed the presence of those tumour-specific T-lymphocytes in other non-SNs of the same patient as well [25]. Further investigations on a molecular level are required to elucidate the role of the SN in lymphatic metastasis as well as in the induction of an anti-tumoural immune response.

5. Sentinel lymph node mapping in oesophageal cancer

Early lymphatic metastasis is a characteristic of oesophageal cancer. Since tumours of the oesophagus are mostly detected in advanced tumour stages, the number of patients suffering from early oesophageal cancer without clinically apparent nodal metastasis is limited. Although, these patients may profit from improved staging by SLNB.

The lymphatic drainage of the oesophagus is marked by a great complexity with extended lymphatic networks in the lamina mucosa and submucosa. Regional LN stations are located in different anatomical sites. The two most frequent malignancies of the oesophagus, e.g., adenocarcinoma and squamous cell carcinoma (SCC), seem to differ in their characteristic metastatic pattern. Although the location of the primary tumour has a strong influence on the site of metastasis in SCC, early tumours of this type most frequently metastasise to perigastric LN basins or the LNs at the thoracocervical junction. Metastasis even to distant anatomical LN stations appears frequently and metastatic disease may be present in up to 26% of pT1 tumours [26,27]. In contrast, LN metastasis occurs in ≈22% of patients with pT1 adenocarcinoma of the oesophagus. In early stages of oesophageal adenocarcinoma, the first involved LN was most frequently located in the lower posterior mediastinum, the right or left paracardiac region or along the lesser gastric curvature. Skip metastasis occurred in only 5% of patients [28,29].

Two larger trials investigated the practical application of SLNB in oesophageal cancer. In SCC, Kato and colleagues reported detection rates of 92% using technetium 99-m colloidal rhenium sulphide injected endoscopically in 25 patients. The sensitivity of the procedure was 86.7%. In two patients with advanced disease, nodal metastasis were not detected by SLNB, resulting in a false-negative rate of 13.3%. Fifty percent of patients with negative LNs on routine haematoxylin–eosin (HE) staining, including both patients with false-negative SNs, had micrometastasis detected by immunohistochemistry [30].

Yasuda *et al.* reported on 21 SLNB performed in patients with oesophageal cancer. The detection rate was 100%, the false-negative rate reached 25%. Two of the three patients with nodal metastasis that was not detected had advanced tumour stages [31].

Burian and colleagues investigated the applicability of SLNB to patients with Barretts and cardia cancer. Twenty patients were injected with technetium colloid preoperatively, 10 received an additional intraoperative injection of blue dye. The detection rate was 85%. The accuracy of the method varied between 100% and 75%, with reduced values for Type I and II tumours [32].

SLNB and the consecutive intensified histopathological work-up of the SN may improve the quality of the histopathological staging of oesophageal carcinoma. However, further studies including larger patient numbers are needed to support the present data.

6. Sentinel lymph node mapping in gastric cancer

Similar to malignant melanoma and breast cancer, lymph node involvement is an important prognostic factor in gastric carcinoma. In pT1 tumour, 2–8% of patients were found to have nodal metastatic disease. The number increased to ≈50% when the tumour infiltrated the muscular tunic or subserosa (T2) [33]. The

optimal extent of lymphadenectomy in gastric cancer is still under discussion and is thought to vary with the individual characteristics of the primary tumour, i.e., location, depth of invasion, maximal diameter, macroscopic and histological type [34]. Notably, in patients with early gastric cancer, two randomised trials reported no survival benefit for patients treated with D2 lymphadenectomy compared to D1 lymphadenectomy [35,36], whereas other retrospective studies found a survival benefit for patients with D2 lymphadenectomy [37,38]. In Asian countries, and especially Japan, D2 lymphadenectomy is part of standard treatment for early gastric cancer, whereas in Europe the clinical practice is less standardised and does not routinely include a D2 lymphadenectomy in all European countries.

Due to the complex embryological development, lymphatic drainage of the stomach is considerably more complex than that of ectodermal organs, e.g., breast and skin [39]. Frequency of skip metastasis is as high as 15–20% [33,40]. Thus, SLNB in gastric cancer seems more complex than in breast cancer or melanoma. During the past 2 years, a growing number of clinical trials evaluating the feasibility and accuracy of SLNB in gastric cancer have been published. Results of the trials with the largest patient numbers are summarised in Table 1 [41–50].

In summary, these clinical trials show a higher percentage of false-negative results than use of SLNB in melanoma or breast cancers. Moreover, the number of patients with the SN as the only LN with metastatic involvement is lower than in the aforementioned tumour types. The number of SNs per patient varies significantly (2–7 SN per patient) according to the techniques and SN definitions employed by the authors [44–46,51].

In recent years, has been considerable debate on the advantages and disadvantages of different detection methods, i.e., radiocolloid and vital dye, in the detection of SN in gastric cancer patients. While early studies of SLNB preferred the vital dye method, a growing number of investigators used radiocolloid or a combination of both methods in more recent studies. Uenosono and colleagues examined the influence of particle size of radiocolloids on the detection rate. Uptake of large size of radiocolloids (500 nm) was reduced compared with that of smaller particle sizes (50 and 100 nm). Thus, the authors recommend the use of radiocolloids with particle sizes of around 100 nm [52]. Hayashi and colleagues compared blue dye and radiocolloid in the same patient group. Each method had detection rates of 90%, whereas the combination of both techniques achieved a detection rate of 100%. The false-negative rate was 14% in the Blue dye- and 29% in the radiocolloid group. Again, the combination of both techniques resulted in a reduction of the false-negative rate to 0% [48]. Consequently, the authors consider both techniques as complementary and recommend their combined use. Results from our own Group confirm these findings [50].

Table 1
Results of SLNB in gastric carcinoma

Author (Reference)	No. of patients	Stage	Method	Detection rate (%)	SLN-positive (%)	Sensitivity (%)	False-negative (%)	Only SLN-positive (%)	Remarks
Palaia and colleagues [41]	25	T1–T2	CDT	100	40	100	0	n.s.	–
Hiratsuka and colleagues [42]	74	T1–T2	CDT	99	12	90	10	30	–
Ichikura and colleagues [43]	62	T1–T2	CDT	100	21	86	14	n.s.	–
Kitagawa and colleagues [44]	145	T1–T2	RCT	95	n.s.	92	8	n.s.	–
Miwa and colleagues [45]	211	T1–T3	CDT	96.2	5.2	89	11	32	–
Tonouchi and colleagues [46]	17	T1	RCT + CDT	100	27	100	0	66	Laparoscopic
Ryu and colleagues [47]	71	T1–T2	CDT	91.5	17	61	39	27	–
Hayashi and colleagues [48]	31	T1–T2	RCT + CDT	100	22.5	100	0	22.5	–
Song and colleagues [49]	27	n.s.	CDT	96.3	30.8	100	0	0	–
Gretschel and colleagues [50]	34	T1–T3	CDT	100	n.s.	100	0	n.s.	
			RCT	93		89	11		–
			RCT + CDT	100		100	0		

n.s., Not specified; RCT, radiocolloid technique; CDT, coloured dye technique; SLNB, sentinal lymph node biopsy; SLN, denital lymph node.

The first trials of laparoscopic SLNB gave results comparable to the standard laparotomy procedure. Thus, the minimally invasive assessment of the nodal status by SLNB may potentially constitute the basis for a limited minimally invasive surgery in early gastric cancer patients [42,53]. However, further prospective studies are needed to confirm the findings of the aforementioned studies in larger patient groups. Especially in non-Asian countries with a lower incidence of early gastric cancer, the clinical significance of SLNB in this disease entity remains to be shown. At the moment, the available data does not justify reduced extent of lymphadenectomy on the basis of SLNB in gastric carcinoma, but provides strong evidence for an improvement in tumour staging using this procedure.

7. Sentinel lymph node mapping in colorectal cancer

The 5 years survival rate of Stage III colorectal cancer patients falls to around 50% compared with ≈75% and 90% for Stage II and I patients, respectively. Adjuvant chemotherapy can significantly improve the 5-year survival of patients with node-positive colorectal cancer [54,55]. This emphasises the importance of the correct LN staging as prognostic factor as well as criteria for patient selection for neoadjuvant therapy [56].

The number of LNs that have to be submitted to histopathological analysis in order to obtain a reliable nodal staging remains the subject of debate. Recommendations for LN retrieval vary between six and 17 [57,58]. The pTNM classification of colorectal cancer is based on the histopathological work-up of 12 LNs [59]. However, in present practice, ≈20–30% of nodal-negative patients will develop locoregional or systemic disease [54]. In these cases, an understaging of the primary disease may have occurred, either due to an incomplete harvest of LNs or inadequate histopathological evaluation. Several authors have reported on techniques to improve LN detection in surgical specimens by LN revealing solutions [60] or fat-clearing methods [61,62].

Unfortunately, these techniques are labour-intensive and expensive. Modern diagnostic methods like immunohistochemistry and reverse transcriptase–polymerase chain reaction (RT–PCR) can increase the sensitivity of detection of even micrometastatic disease. However, these techniques are also labour- and cost-intensive and thus inappropriate for large-scale application on all LNs found in the surgical specimen. The restriction of the refined histopathological examination to intraoperatively suspicious or enlarged LNs is also not useful, since 69% of metastatic nodes are smaller than 5 mm in size [63].

In this setting, SLNB may constitute a method to limit the use of the full range of very sensitive histopathological techniques, like serial-sectioning, immunohistochemistry and RT–PCR, to the LN with the highest probability of metastatic involvement.

SN mapping is currently evaluated for its ability to refine the staging of the nodal status in colorectal cancer. In malignant melanoma or breast cancer, SN biopsy can reduce the invasiveness of the surgical therapy by improving the patient's nodal staging, with subsequent restriction of unnecessary LN dissections in nodal-negative patients. By contrast, in most cases of colorectal cacinomas, the extent of the surgical procedure will remain unchanged by the results of the SN mapping, since all regional LNs are routinely removed *en bloc* with the resected bowel segment. The improved nodal staging by means of SN mapping and subsequent selective application of immunohistochemical staining and RT–PCR techniques results in an upstaging of up to 20% for patients with lymphonodal micrometastasis.

As yet, the question persists of whether the presence of micrometastasis detected by immunohistochemistry is a prognostic indicator for disease-free and overall-survival in patients staged node-negative by routine HE-staining. Some studies report a significant survival benefit for patients without micrometastasis [64–69], whereas others could not detect any difference in disease-free survival between patients with or without micrometastatic LN involvement [70–77].

Published studies on SLNB in colorectal cancer show considerable heterogeneity in the detection techniques used, in the practical definition of the SN, in the time interval chosen between dye injection and SN-detection, in the histopathological techniques applied and in the composition of the patient groups. Consequently, the detection rate, sensitivity and rate of false-negative LNs varies considerably (Table 2). As in melanoma and breast carcinomas, the detection rate and sensitivity are thought to vary according to the experience of the surgeon. In general, the rate of false-negative SLNB increases with the size and depth of infiltration (pT) of the primary tumour. This may be due to partial obstruction of the lymphatic channels by tumour cells with consecutive blockage of tracer migration.

While SLNB provides good results in colon cancer, it has been shown to be less reliable in rectal cancer [20,82,89–91]. In our series, we performed SLNB in 48 patients with rectal cancer. A SN was detected in 46 of the 48 patients, resulting in detection rate of 96%. Metastatic disease was detected in the LNs of 16 patients. Nine of these patients had metastatic disease in non-SNs while the SN proved to be tumour-free. The false-negative rate was therefore 56%. There was no statistically significant difference between patients with early or advanced tumour stages [89]. The significantly reduced sensitivity of SLNB in rectal cancer compared with colon cancer patients may be due to the close vicinity of the primary LNs in the pararectal tissue and to the tumour. Signals from the primary tumour and the

Table 2
Results of SLNB in colorectal carcinoma

Author (Reference)	No. of patients	Mapping technique	Detection rate (%)	SLN-positive			Only SLN-positive (%)	False-negative (%)	Atypical LN
				Total (%)	HE only (%)	Upstaging by IHC (%)			
Cserni and colleagues [78]	25	Blue dye	100	32	n.s.	n.s.	25	38	n.s.
Jossten and colleagues [79]	50	Blue dye	70	28	22	6	0	50 (HE + IHC)	n.s.
Saha and colleagues [80]	86	Blue dye	99	34	n.s.	n.s.	52	9	2
Merrie and colleagues [81]	26	Blue dye, radiocolloid	88	23	n.s.	n.s.	n.s.	45	n.s.
Wood and colleagues [82]	75	Blue dye	96	40	29	11	49	14	7
Saha and colleagues [83]	203	Blue dye	98	37	31	6	49	10	11
Paramo and colleagues [84]	55	Blue dye	82	32	18	14	64	7	1
Bilchik and colleagues [85]	100	Blue dye	97[a]	40	22	18	0–100[b]	11	8
Broderick-Villa and colleagues [20]	51	Blue dye	92	21	21	0	5	50	n.s.
Kitagawa and colleagues [86]	56	Radiocolloid	91	32	32	n.s.	n.s.	18	n.s.
Trocha and colleagues [87]	50	Blue dye Radiocolloid	98 94	38	22	16	n.s.	16	n.s.
Bembenek and colleagues [88]	45	Blue dye	84	39	16	23	n.s.	21	n.s.

HE, Haematoxylin–eosin; IHC, immunohistochemistry; n.s., not specified; LN, lymph node.
[a] Open, 90%; laparoscopic, 100%; *ex-vivo*, 92%.
[b] T1, 100%; T2, 24%, T3, 53%, T4, 0%.

LN can be confounded. However, many patients in the study suffered from advanced tumour stages requiring neoadjuvant therapy including radio-chemotherapy. The high percentage of advanced tumour stages in the patient population, as well as the effects of the neoadjuvant therapy may also be responsible for the observed reduced sensitivity of SLNB in rectal cancer. However, currently available clinical data does not justify the application of SLNB in patients with rectal cancer.

Bilchik and colleagues reported that immunohistochemistry led to an upstaging of 18% of patients with negative HE-staining. Interestingly, the use of RT–PCR for β-hCG, c-Met and uMAGE resulted in upstaging of 46% of histopathologically negative patients [85]. In this patient series, the number of positive PCR-markers seems to correlate with the tumour stage. The multimarker approach (β-hCG, c-Met and uMAGE) may present some advantages over the classical single marker approach (CEA or GCC or CK20) [92–94], since it eliminates some classical problems caused by tumour heterogeneity, loss of expression of tumour markers, clonal selection and unspecific positive results due to low level antigen expression in non-malignant tissue. However, due to the extreme sensitivity of RT–PCR and the lack of morphological information, the specificity of the method is low and positive results can be the result of scattered apoptotic malignant or epithelial cells transported passively to the LN or from contamination during the surgical procedure or pathological work-up. In summary, the clinical relevance of micrometastasis detected by RT–PCR and/or immunohistochemistry has not yet been proven.

Histopathological ultrastaging leads to an upstaging of up to 23% of patients and to a considerable reduction in the SNs false-negative rate. However, as long as the non-SNs in those trials are not examined with the same sensitive techniques as for the SN-lymph nodes, no definitive conclusion on this reduction of false negative rate can be drawn because these techniques will not only lead to an increase in the reduction in the detection of metastasis in the SN, but also in non-SNs.

Intraoperative SN mapping can identify patients with aberrant or atypical lymphatic drainage outside of the field of the planned resection. Aberrant lymphatic drainage has been reported to be present in 2–8% of patients with colon cancer [84,85,89]. In these patients, the margins of surgical resection can be adjusted [83,95] our own results in [89].

The growing acceptance of laparoscopic colectomy [96,97] for colorectal cancer has raised the question of whether SLNB can be applied in laparoscopic procedures. Wood and colleagues reported on nine laparoscopic SLNB procedures, with a detection rate and sensitivity of 100%. The rate of false-negative SNs in this small patient group was 0% [82]. Kitagawa and colleagues reached a sensitivity of 83% and an accuracy of

96% in a series of 12 patients [53]. Recently, Bilchik and colleagues published a series of 30 patients undergoing laparoscopic SLNB. The detection rate was 100%, accuracy was 93%, but the false-negative rate was relatively high (33%), since there was quite a low number of patients with nodal disease (six patients; 20%). Interestingly, in eight cases, aberrant lymphatic drainage was detected [98].

As yet, most SLNB trials in colorectal cancer have used the Blue dye-method. In a recently published clinical multicentre trial on 50 patients, Trocha and colleagues could not show an increased sensitivity or detection rate following the combined use of blue dye and radiotracer in patients with colorectal cancer [87]. However, metastasis was found more frequently in the blue/hot LNs than in blue-only LNs.

In contrast to the standard procedure consisting of intraoperative subserosal injection of blue dye, several authors have reported on the feasibility of *ex vivo* SN-detection with detection rates and sensitivities comparable to the *in vivo* technique [90,99]. Wong and colleagues injected blue dye postoperatively in the submucosa of the colorectal specimen. Detection rate of this procedure was 92%. The sensitivity of SLNB in identifying metastatic involvement was ≈94%. Immunohistochemical staining of the SN resulted in upstaging of 29% of patients with a negative nodal status after HE-staining. This postoperative approach may reduce the operation time needed for classical first-detection. Moreover, the potential risk of dislodging tumour cells through manual manipulation during the SN procedure is eliminated. Aberrant lymphatic drainage can not be detected by this method, constituting a potential inconvenience of postoperative SN detection [99].

8. Sentinel lymph node biopsy in anal carcinoma

The primary therapeutic approach to anal carcinoma without clinical evidence of LN involvement is a combination of radio- and chemotherapy [100]. Systematic inguinal LN dissection which was included in the historical surgical management of anal carcinoma was abandoned due to the absence of a significant survival benefit and the considerable morbidity associated with this technique [101–103].

However, the problem of the potential metastatic involvement of the inguinal LNs and the appropriate therapeutic approach remains controversial. In cases of clinically suspected inguinal LN involvement, histopathological confirmation of the diagnosis by "core" – or open biopsy is required. If the presence of inguinal lymph node metastasis is confirmed, selective boost radiation, with or without surgical inguinal dissection of the involved side, is the therapy of choice. The management of patients with clinically uninvolved groins varies considerably from prophylactic irradiation of the groins in some centres to a 'watch-and-wait' policy in others [104].

Most anal carcinomas are situated proximal to the dentate line drain to pararectal and subsequently to LNs at the origin of the inferior rectal artery, whereas tumours distal to the dentate line drain mainly to the inguinal LNs. However, lymphatic drainage in this region is very variable, and both drainage patterns may exist along the entire anal canal. Moreover, the localisation of the primary tumour does not always reliably predict the side of the draining inguinal LN basin. SLNB is a appropriate tool to identify the tumour-draining LN basin.

Only a few patients (10–20%) present with clinically apparent synchronous inguinal LN metastasis [104,105], but the sensitivity and specificity of their clinical detection is poor. In several studies, clinically suspicious LNs contained malignancy in only up to 55% [106,107]. Wade and colleagues reported that 44% of LN metastasis were located in LNs smaller than 5 mm [108].

SLNB in anal cancer patients may prove to be a reliable tool for improved histopathological staging of inguinal LN basins leading to the identification of a patient subgroup that may benefit from inguinal therapy, and also sparing patients without inguinal metastasis the risks of irradiation, including lymph oedema, vascular stenosis or necrosis of the femoral head.

So far, three feasibility studies, as well as several case reports, have been published. The first feasibility studies reported detection rates of 80–100% [109,110]. All patients were discharged from hospital on day of their surgery, and no major complications occurred. In the largest study including 17 patients from our institution, the detection rate was 76.5%. Metastases were found in five patients; in two patients, metastases were only detected by immunohistochemistry. No major complications occurred. One patient developed a lymphocutaneous fistula that resolved spontaneously after 10 days. During a 10 month follow-up, only one patient developed an inguinal recurrence after radiation (45 Gy) for SN-involvement to the groin [111]. Since SLNB was not followed by systematic LN dissection, the sensitivity and the SN false-negative rate could not be assessed. However, these promising results show that SLNB is highly efficient in detecting metastatic deposits in inguinal LNs compared with other currently available techniques. Further studies are required to assess the clinical relevance of this technique in anal cancer patients.

9. Sentinel lymph node biopsy in pancreatic carcinoma and other gastrointestinal malignancies

To date, there is one publication reporting on the application of SLNB in locally advanced tumours of the

pancreatic head. Ohta and colleagues performed SLNB in nine patients with advanced pancreatic cancer. One SN was detected in the posterior pancreaticoduodenal LN group in eight patients. Of these, 4 SN showed metastatic involvement, prompting LN dissection of the abdominal paraaortic LN group. In patients without metastatic involvement of the SN, the routine lymphadenectomy of the paraaortic LN basin was omitted. Three of four patients who are alive after 3 years of follow-up belonged to the groups without lymphadenectomy of the paraaortic LNs [112]. However, it remains to be shown whether SLNB is a suitable technique for reliably predicting the nodal status of the paraaortic LNs, and whether this approach leads to a reduction of therapy-associated morbidity and mortality.

One group published their experience with SLNB in patients with liver metastasis from colorectal carcinomas. This procedure was performed in 11 patients. No metastasis were detected, either in the SN or in the additionally removed unstained LNs [113]. No conclusion concerning the clinical value of this procedure in patients with liver metastasis of colorectal carcinoma can be drawn from this described experience due to the small patient numbers.

10. Conclusions

Forty three years after Gould's first description of the SN technique in malignant tumours of the parotid [114], SLNB has become an important component of the diagnotic and therapeutic concept for solid tumours. Today, SLNB finds broad application in the routine management of malignant melanoma and breast cancer. Promising results for SLNB in patients with tumours of the gastrointestinal-tract has stimulated the interest of a growing number of investigators in this area that is documented by the growing number of publications in the past 3 years. This technique may have the potential to improve the staging of gastrointestinal cancer and to reduce treatment-associated morbidity. The application of SLNB in gastrointestinal cancers is an important step towards a individualised therapy for patients. Thus, in the years to come, it will be essential to learn more about the influence of SLNB on the long-term outcome of patients. A further critical issue will be evaluation of the clinical significance of micrometastasis found by ultrasensitive detection methods, as well as their influence on the choice of adjuvant treatment options.

In the future, technical progress will lead to a growing competition between SLNB and improved imaging techniques, such as fluorine-18 flurodeoxyglucose (^{18}FDG) positron emission tomography (PET) imaging or other molecular imaging techniques. As yet, the limited spatial resolution of these tools does not permit as precise a nodal staging as from SLNB techniques. Thus, SLNB remains the technique of choice for lymphonodal staging.

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