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Minimal residual disease assessment in sentinel nodes of breast and gastrointestinal cancer: a plea for standardization

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SUMMARY

Lymph node dissection plays an important role in staging and treatment of cancer patients with solid tumors. Sentinel node biopsy (SNB) has been introduced to minimize the extent of surgery and to enable minimal residual disease (MRD) assessment without compromising accurate staging and survival. This review addresses the variation in technical aspects and outcome of SNB and MRD assessment in patients with breast and gastrointestinal cancer. There is a need for quality control leading to standardization of SNB and consecutive pathological examination to enable reliable comparison of studies, leading to consensus of diagnostic and therapeutic strategies.

INTRODUCTION

The histological status of lymph nodes is one of the most important prognostic indicators in patients with cancer originating from solid tumors. Staging patients to determine the need for adjuvant therapy presently occurs through lymphadenectomy. Apart from lymphadenectomy as a staging tool, it may also serve a therapeutic aspect, even in patients without nodal involvement[1,2]. Overall survival of colorectal cancer patients without nodal involvement, improves with increasing number of lymph nodes recovered[3]. Also in invasive bladder cancer, both node-negative and node-positive patients had prolonged overall survival with an increasing number of lymph nodes examined[4]. This benefit is possibly due to the presence of MRD in H&E-negative lymph nodes.

Lymphadenectomy may be associated with considerable morbidity, especially in breast cancer and melanoma patients. To minimize the extent of lymphadenectomy without compromising accurate staging and survival, SNB has been introduced. Sentinel nodes are known as the first possible sites of metastasis along the route of lymphatic drainage from a primary tumor. The histopathological state of the sentinel node is presumed to reflect that of all regional lymph nodes. SNB can be performed by injecting either a vital dye, a radioactive colloid or both around the primary tumor. Techniques vary, however, substantially between institutions and researchers, which complicates reliable assessment of the role of SNB.

An amenity of the SNB is the lower number of lymph nodes that have to be examined compared to regional lymph node dissection. Laborious and expensive focused examination techniques like immunohistochemistry (IHC) and reverse transcriptase polymerase chain reaction (RT-PCR) can therefore be applied in a limited number of sentinel nodes to detect the presence of so-called minimal residual disease, also known as micrometastases. Micrometastases are defined as a cohesive cluster of malignant cells, greater than 0.2 mm and up to 2.0 mm in diameter, that are usually not detected with conventional pathological examination techniques. The prognostic significance of micrometastases and the therapeutical consequences of upstaging by MRD assessment, however, are far from clear yet. Nevertheless, in some countries treatment decisions are already based on MRD assessment, implying possible over treatment. This review addresses the role of SNB and MRD in (sentinel) lymph nodes in breast, gastric and colorectal carcinoma and pleads for standardized and randomized trials in this field.

BREAST CANCER

Axillary lymph node dissection (ALND) contributes to both treatment and staging. Overgaard reported large differences in local recurrence rates in a trial investigating the efficacy of radiotherapy following total mastectomy[5]. There were clear variations in the extent and

Table 1. An overview of the SNB studies in breast cancer

Reference	Type of tracer	Average no of SNs	Succesrate mapping (%)	Upstaging method	False-negative rate (%)
Nwariaku et al[36]	Tc + blue dye	1.84	81	s.s.	4
Borgstein et al[37]	Tc	1.2	100	IHC	2
Krag et al[38]	Tc	2.6	91	-	11
Hill et al[39]	Tc + blue dye	2.1	100	IHC	11
Veronesi et al[40]	Tc + blue dye	1.4	99	s.s.	7
Winchester et al[41]	Tc	3.1	90	s.s.	8
Bass et al[42]	Tc + blue dye	2.0	93	IHC	2
Morrow et al[43]	Tc + blue dye	1.8	79	-	13
Fraile et al[44]	Tc	2.0	96	IHC	4
Kollias et al[45]	Tc + blue dye	1.4	81	IHC	6
Tafra et al[46]	Tc + blue dye	2.2	87	IHC	13
Nano et al[47]	Tc + blue dye	-	87	IHC	7

Tc = 99m Technetium; s.s. = serial sectioning; IHC = immunohistochemistry

quality of surgery since more than half of the local recurrences appeared on the chest wall. It was concluded that radiotherapy improved local control with the current surgery. However, if surgical procedures would improve, the benefits of standard application of radiotherapy might be questionable. It is clear that the quality of surgery dictates the value of adjuvant treatment. This stresses the need for standardized and quality-controlled SNB as staging and treatment decisions depend on removing and investigating only one or a few sentinel nodes. Currently, most centres agree on using the combination of a radioactive tracer and blue dye, which improves the identification of multiple sentinel lymph nodes compared to the use of one tracer alone[6]. Table 1 highlights studies published since 1998 on SNB in breast cancer patients, with more than 100 patients included. Most centres use the combination of blue dye and radioactive colloid to detect sentinel nodes. In the displayed studies considerable variation exists in the volume of tracer used and the technique of examination of the resected sentinel nodes, which might lead to different success and false negative rates. The site of injection is often inaccurately reported and it remains unclear whether massage has been performed.

In focused examination studies of H&E negative lymph nodes, there is considerable variation in the applied technique, marker or antibody used and data analysis. Dowlatshahi showed upstaging by serial sectioning and immunohistochemistry of 9 to 33%[7,8]. The clinical relevance of MRD assessment is debatable. Studies that showed survival disadvantage due

to the presence of micrometastases included larger patient populations (range 147-921) and had more prolonged follow-up (at least 6 years) than studies that did not prove any survival difference. Moreover, most studies did not take the size of the micrometastases into account, whereas data already exist that the size of nodal metastases linearly correlates with survival[8]. Also the role of isolated tumor cells in lymph nodes has not been elucidated yet[9]. It might be difficult to distinguish isolated tumor cells from mesenchymal cells, mesothelial cells, transfer (contamination) artefact, and transport of benign or malignant epithelium. Many investigators probably often encounter these technical difficulties, but reports on these issues are remarkably scarce.

MRD assessment in sentinel nodes with immunohistochemistry and serial sectioning reveals a higher detection rate of micrometastases in sentinel nodes than in the regional lymph nodes[10]. This is in line with the sentinel node hypothesis. An overview study showed that in 38-67% of patients with breast cancer the sentinel node is the only involved lymph node[11]. When the sentinel node is the only involved lymph node it can be argued that ALND is not necessary. In the AMAROS trial (After Mapping of the Axilla Radiotherapy Or Surgery), coordinated by the European Organization for Research and Treatment of Cancer, patients with positive sentinel nodes are randomized to ALND or axillary radiotherapy. The presence of any tumour deposit, detected with either HE staining or IHC, has consequences for the local treatment of the axilla (i.e. surgery or radiotherapy) but not for systemic treatment. Recently, concern has been expressed that many pathology laboratories have adopted IHC techniques and many oncologists recommend adjuvant chemotherapy upon IHC detected metastases only[12]. Giving patients a toxic and often expensive treatment with possibly limited benefits, based upon IHC findings alone, is not backed up by the literature and should therefore not be encouraged.

It can be concluded for breast cancer patients, that the SNB is presently performed with acceptable success rates and low false negative rates despite considerable variation in SNB techniques. Special techniques to detect micometastases can lead to upstaging in a considerable number of patients, but it remains unclear whether these findings should affect the choice of adjuvant treatment.

GASTRIC CANCER

The widespread use of gastroscopy has led to increasing chance of identifying gastric cancer at an early stage. Nodal involvement occurs only in 2 to 18% in T1 tumors and in about 50% in T2 tumors[13]. This means that a larger than necessary lymphadenectomy is performed in a substantial number of patients. The debate on the benefits of D1 compared to D2 lymph node dissection is still ongoing. Also, the value of adjuvant therapy in relation to the extent of surgery is intensely discussed[14]. An extended lymphadenectomy is associated with considerable postoperative morbidity and mortality, especially in western countries[15,16]. However, reliable

tools are lacking to predict nodal involvement. SNB and its investigation might however gain a role in minimizing the surgical procedure and predicting the status of non-sentinel nodes. The studies on feasibility of SNB in gastric cancer are rather limited. Table 2 shows that different types of tracers are being used and a ranging number of SNs are retrieved. Moreover, only in one SNB study upstaging techniques were applied[17]. Endoscopic submucosal injection has shown to be a feasible route of administration of a radioactive tracer or a dye. Identification of the sentinel node using a radiolabelled colloid and perioperative detection with a gamma-ray detection probe has the drawback of detecting not only radiation from lymph nodes, but also from the adjacent injection site. Therefore, most experience has been gained so far with the application of dyes. All the displayed studies, initiated in the Far East, showed acceptable feasibility in early stage disease (i.e. T1 or T2). In Western countries however, gastric cancer is often diagnosed at an advanced stage, which questions the role of SNB in these patients.

Table 3 displays that two out of five IHC studies, using anticytokeratin antibodies showed an adverse effect of the presence of micrometastases. Remarkable are the differences in

Table 2. An overview of the SNB studies in gastric cancer

Reference	No of pts	Type of tracer	Volume of tracer (ml)	Average no of SNs (range)	Succesrate mapping (%)	False-negative rate (%)
Hiratsuka et al[48]	72	Indocyanine green	5	2.6 (1-9)	99	10
Aikou et al[17]	18	Tc + blue dye	2 (Tc)	3	94	17
Yasuda et al[49]	26	Tc	2	4 (2-8)	100	18
Ichikura et al[50]	62	Indocyanine green	4 or 8	4.5 (1-12) resp. 8.6 (1-25)	100	13
Kitagawa et al[51]	145	Tc	2.0	3.6 (1-8)	95	8
Miwa et al[52]	211	Blue dye	0.8	6 (1-19)	96	11

Tc = 99m Technetium

Table 3. Immunohistochemistry studies on H&E-negative lymph nodes in gastric cancer

Reference	Antibody	No of H&E-node-negative patients	No of nodes per patient	Node sectioning	Upstaging (%)	Prognostic value
Maehara et al[53]	CAM 5.2	34	12.4	single	23.5	adverse
Cai et al[54]	CAM 5.2	69	24.6	single	25	controversial
Morgagni et al[55]	MNF 116	139	10.7	multi	17	no difference
Fukagawa et al[56]	AE1/AE3	107	41.9	single	35.5	no difference
Lee et al[57]	AE1/AE3	70	23.7	single	40	adverse

antibodies used, the number of resected lymph nodes and proportion of patients upstaged. Noguchi et al used RT-PCR with keratin 19 as a marker to detect micrometastases and found that this was a more sensitive method than histological examination for the detection of gastric micrometastases in lymph nodes[18]. The prognostic significance of micrometastases, detected with this technique, was however not addressed.

The majority of the reports on gastric carcinoma originate from specialized centers that have been able to gain experience with the technical demanding procedure in a patient population less prone to postoperative morbidity and mortality than in Europe and the USA.

In conclusion, the initial and limited experience in SNB has a potential value in staging and treating gastric cancer patients. However, only patients with early stage disease, a patient category not very often encountered in Western population, may benefit from SNB. Moreover, the existing variation in technical aspects of SNB and MRD assessment hampers the introduction of treatment decisions based on MRD assessment.

COLORECTAL CANCER

The treatment of node-negative colorectal cancer consists of surgical resection of the primary tumor without adjuvant therapy. However, up to 30% of these patients will develop metastases possibly due to micrometastases in the regional lymph nodes. We showed that patients with CEA RT-PCR negative lymph nodes had a significantly better five-year disease-free survival than patients with positive lymph nodes (91 versus 50%, p=0.02)[19]. Three other RT-PCR studies[20-22] also showed an adverse effect on the prognosis whereas only three of ten immunohistochemistry studies showed an adverse effect[22,23]. Again, the IHC studies show clear variation in the number of resected lymph nodes, the use of serial sectioning and antibodies, and the degree of upstaging, which ranges from 10 to 76%[22,24-32]. Noura et al studied the same paraffin-embedded lymph nodes with CEA RT-PCR and cytokeratin immunohistochemistry and showed that CEA RT-PCR had prognostic value whereas immunohistochemistry did not[22].

SNB in colorectal cancer patients is still in childhood. In contrast to breast cancer patients, SNB in colorectal cancer is not performed to avoid unnecessary lymphadenectomy but to enable focused examination of few lymph nodes. An important consequence of intraoperative SNB in colorectal cancer patients is the identification of aberrant lymphatic drainage patterns occurring in up to 14% of the patients leading to an adjustment of the initial surgical resection plan[33,34]. Table 4 summarizes SNB studies on colorectal cancer patients, with more than 25 patients included. Blue dye is used in most of the studies with moderate variation in volume and site of injection. However, the number of detected SNs ranges widely. Success rates, false-negative rates and upstaging techniques vary and are influenced by disease

Table 4. An overview of the SNB studies in colorectal cancer

References	No of patients	Identification time (min)	Success rate (%)	Average no of SLNs (range)	Upstaging methods	False-negative rate (%)
Joosten et al[58]	50	15	70	3	IHC	60
Wiese et al[59]	83	5-10	99	1.9	s.s. and IHC	9
Feig et al[60]	48	-	98	2.6	IHC	38
Wong et al[61]	26	2-5	92	2.8	s.s. and IHC	6
Saha et al[62]	203	1-5	98	(1-4)	s.s. and IHC	6
Merrie et al[63]	26	20*; 26 – 106**	88	3 (0-8)	RT-PCR	45
Esser et al.[64]	31	-	58	-	-	33
Broderick-Villa et al[65]	51	-	92	1.5	IHC	50
Wood et al[66]; Bilchik et al[67]	100	-	97	2	s.s. and IHC	11
Fitzgerald et al[68]	26	5-10	88	2.5	s.s. and IHC	40
Paramo et al[69]	55	5	82	1.9	s.s. and IHC	7
Kitagawa et al[35]	56	120	91	3.5	-	18

stage. In rectal cancer, the dye method has its limitations because of the restricted visibility of the transit of dye into the SNs[35].

In summary, SNB in colorectal cancer patients is a technical demanding procedure with variable success rates. Although MRD assessment can lead to profound upstaging, there is no clear evidence yet that it should affect adjuvant treatment decisions. Still, in some countries colorectal cancer patients with sentinel node micrometastases are already receiving systemic adjuvant therapy. SNB and MRD assessment techniques are currently being optimised, which may lead to more tailored adjuvant treatment, based upon MRD assessment.

CONCLUSION

Limiting the extent of surgery in the treatment of solid tumors through SNB is technically feasible. However, when comparing studies investigating the role of SNB, there is a large variation in patient selection, and type and volume and location of tracers injected around the tumor. This variety complicates trial comparison, which hampers application of SNB into daily practise. Minimal residual disease assessment by serial sectioning, immunohistochemistry and RT-PCR is possible and may lead to considerable upstaging. The results from studies

addressing the prognostic role of micrometastases are often contradictory, which might be due to the use of different examination techniques, markers, antibodies and differences in sample size and length of follow-up. This variation in techniques of SNB and MRD assessment precludes the availability of evidence-based diagnostic and therapeutical guidelines in the near future. Quality control leading to standardization of SNB and MRD assessment is necessary to enable reliable comparison of different studies. In this way only, we can determine the prognostic role of MRD and develop tailored adjuvant treatment, based upon MRD assessment of lymph nodes retrieved after limited surgery.

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