Use of a Newly Developed Ultrarapid Immunohistochemical Method to Improve Security in Sentinel Node Investigation and Timing in Interdisciplinary Cooperation

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In the strategies to detect SLNs in different primaries, such as breast cancer, melanoma, lung and gastrointestinal carcinomas and also neuroendocrine tumors and urogenital cancers, exact timing of the various diagnostic and therapeutic procedures has an important role. As a general rule, it is extremely helpful to have an exact histopathological and/or cytopathological diagnosis before sentinel lymphadenectomy is performed. In certain tumor entities, such as melanoma, primary biopsies before radioactive SLN labeling cannot be recommended because the procedure entails a high risk of tumor spread in these. However, in breast cancer, oropharyngeal cancer, lung cancer, and gastrointestinal tumors, for example confirmation of the cancer diagnosis is the basis for all further labeling and treatment procedures.

Whereas biopsies can be taken directly from organ tissues that are covered by mucosa and from the gastrointestinal tract, in the case of a deeper location of a suspected cancer (e.g. breast cancer), it is necessary to use puncture methods to take the specimens. The most common and best evaluated methods are: fine needle aspiration biopsy (FNAB), core biopsy and, for limited indications, vacuum biopsy (see Chapters 15, 16). In this context it must be stated that FNAC is also appropriate during follow-up for the evaluation of small, suspicious lesions, some of which may not be palpable. However, in highly suspicious cases, when a primary is palpable or has been disclosed by mammography or MRI, core biopsy methods are now increasingly preferred. With these methods, it is possible to check the cancer diagnosis, on the one hand, and on the other to perform additional immunohistochemical evaluations for prognostic factors in addition.

However, routine immunohistochemical techniques are time consuming; they take at least 1 day. A newly developed method, the “ultrarapid immunohistochemical staining procedure,” makes it possible to obtain results within approximately 20 min. With this faster availability of the results, the method offers the opportunity of developing an exact time schedule for confirmation of the diagnosis, nuclear medical labeling and surgical excision (interdisciplinary timing). A second problem, in addition to the initial confirmation of the diagnosis, is the question of whether the lymph nodes can already be investigated intraoperatively to look for cancer cell infiltration, especially by means of techniques that help to detect isolated cancer cells or small cancer cell clusters. These methods are used in many pathology laboratories and their use is acceptable for differentiated cancers with high cancer cell adhesion (differentiated adenocarcinomas or squamous cell carcinomas). However, HE staining alone is insufficient in intraoperative evaluations, especially in cases with a high degree of tumor cell dissociation. Therefore, the use of frozen sections for intraoperative lymph node examination can hardly be recommended in cases without macroscopically obvious clear-cut cancer infiltration and with high-grade tumor cell dissociation. The degree of high-grade dissociation can be detected even in the initial investigations of the primaries and can also be suspected from the behavior of the cancer within the nodes. Because intraoperative sectioning of small lymph nodes with diameters of 3–5 mm leads to the loss of parts of the lymph node tissue and further loss must be expected after paraffin embedding, the intraoperative search for single tumor cells or small clusters of tumor cells must be regarded very critically within the SLN concept. In these circumstances it seems advisable never to investigate the sentinel nodes except after paraffin embedding in serial and step sections by the newly elaborated immunohistochemical method, because a false-negative diagnosis can lead to a fatal outcome for the patient.

Because the histopathological investigations of the tumor tissue from the primaries and of the sentinel node(s) must fit in with the time schedule
for clinical labeling, imaging and operative procedures and accelerated staining procedures, especially those for immunohistochemical staining of cancer cell markers, are highly valued. An “ultra-rapid staining method” newly developed in the research laboratories of Höfler and Nährig supports this ambition fundamentally.

To give readers an idea of the practical management of this accelerated, very fast-working, immunohistochemical staining procedure (e.g., demonstration of cytokeratins in epithelial cancer cells and vimentin in soft tissue tumors and differentiation products in neuroendocrine cancers) that gives satisfactory results within 2 h, the essentials of this method are described below.

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**Methodical Options**

The time required depends on whether frozen sections are used after ultrarapid immunohistochemical staining or paraffin sections requiring embedding.

- When the ultrarapid immunohistochemical staining of frozen sections of sentinel node tissue (selection of cases see above) is used, a clear-cut result can be obtained according to the following time schedule:
  - Transport from the operating room to the pathology laboratory: approx. 5–15 min
  - Preparation of the frozen sections: approx. 10 min
  - Staining procedure for different markers: approx. 1.5 h
  - Evaluation of the stained sections: approx. 10–15 min

According to this time schedule a significant result can be obtained within approximately 2 h. This means that further surgical treatment is possible on the same day.

- When paraffin sections are used approximately 2–4 h must be added for quick embedding in paraffin, and 30 min for sectioning and drying of the sections.

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**Technical Procedure for Ultrarapid Immunohistochemistry**

Fresh SLN specimens are usually obtained within 10 min after surgical removal for frozen section examination. The number of lymph nodes resected generally varies from 1 to 3, and it is rare for more nodes to be identified. Each lymph node is cut through the longitudinal axis to give slices of 2–3 mm. Specimens are snap-frozen and 5-µm cryostat sections are prepared. Fresh sections are fixed in 4% neutral buffered formalin for 2 min and then rinsed in Tris-buffered saline containing sodium chloride (TBS, 0.01 M, pH 7.4) for 15 s. Endogenous peroxidase is blocked using 1% H₂O₂ dissolved in methanol for 5 min. The fixed slides are then washed again with Tris buffer plus 1% Tween-20 detergent to reduce nonspecific background staining. A direct immunoperoxidase method is applied for ultrarapid immunostaining using the EPOS R system (DAKO, Copenhagen, Denmark). Prediluted EPOS antibodies are incubated in a humid chamber at 54°C for 5 min. Then slides are rinsed in tap water and nuclear counterstaining of the specimens is done with hemalaun for 10 s. Finally, slides are rinsed in tap water and dehydrated through increasing concentrations of alcohol, isopropanol, and xylene and then coverslipped (Eukitt), or alternatively rinsed in tap water and coverslipped with Kaiser’s glycerin gelatin (Merck, Darmstadt, Germany).

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**Preliminary Experience with Ultrarapid Immunohistochemistry Staining**

We conducted a clinical study to evaluate the ultrarapid immunohistochemistry method in the examination of sentinel nodes in primary breast cancer using pancytokeratin antibody clone MNF 166 (EPOS, DAKO; Fig. 1). The preliminary results suggest, first, that this technique can well be applied to intraoperative examination of lymph nodes, owing to its short turnaround time of about 20 min; and secondly, that its specificity and sensitivity were high. The major advantage of the method seems to be that it requires less time than microscopic screening. Especially in cases where metastatic disease presents with isolated tumor cells and in the case of lobular carcinoma infiltration, it may help to detect tumor cells more easily.