Comparative Analysis of Methods Used in Breast Cancer HER2 and Sentinel Lymph Node Diagnosis

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1 Introduction

Breast cancer is the commonest malignant disease among Hungarian women. Its therapy is determined by prognostic and predictive factors revealed by histological examination, consequently finding the most appropriate diagnostic method has a great importance of choosing the optimal treatment. The objectives of this work are examination of HER2 amplification (A) and sentinel lymph node status assessment (B) in breast cancer cases. These methods are important in the prediction of breast cancer prognosis and therapy.

(A) Overexpression of the human epidermal growth factor receptor-2 (HER2) gene, a breast cancer marker, is associated with rapid tumour growth, increased risk of recurrence after surgery, poor response to conventional chemotherapy, and shortened survival. The availability of targeted trastuzumab (Herceptin) therapy for tumours overexpressing HER2 protein (HER2 positive) has brought the need for accurate determination of HER2 status into sharp focus. The significant benefits coupled with the high cost and potential cardiotoxicity of trastuzumab demand accurate HER2 testing. HER2 testing should be routinely performed in patients who have been diagnosed with invasive breast cancer for the first time. However, the best method to assess HER2 status, with regard to both the type of assay used and the optimal method to perform each assay, remains controversial. Immunohistochemistry (IHC) is offered in most routine histology laboratories, and several assays have been approved by the Food and Drug Administration (FDA) to assess breast carcinomas for HER-2/neu amplification. Although IHC is relatively
inexpensive and easy to perform, it has drawbacks such as antibody specificity, sensitivity variability, and subjectivity of interpretation. These problems are circumvented by fluorescence in situ hybridization (FISH), in which the fluorescent signals can be directly seen and enumerated in the tumour cells. Although more expensive than IHC, FISH is quantitative, and easier to interpret.

Currently, there are three FISH based assays that are approved for HER2 testing in the USA: the PathVysion HER2 FISH test (Abbott-Vysis, Downers Grove, Illinois, USA; also approved for predicting anthracycline sensitivity); the INFORM HER2 FISH system (Ventana Medical Systems) that is only approved for assigning prognosis; and the PharmDx HER2 FISH (Dako, Glostrup, Denmark) that is approved for prediction of trastuzumab sensitivity. Recently, a new FISH kit from Kreatech, Poseidon ERBB2/SE17 has appeared in the market, which may be eligible for use in the routine assessment of HER2 status. The current study was done as a validation study prior to setting up a clinical HER2 testing service using Poseidon HER2 FISH; however, it was felt that the experience of the authors of this study may be of interest to other laboratories when considering setting up HER2 diagnostic facility.

(B) The single most important predictor of outcome in women with breast carcinoma is the status of the ipsilateral lymph nodes. Traditionally, the axillary lymph node status has been evaluated by routine axillary lymph node dissection accompanying a lumpectomy or mastectomy. Axillary lymph node dissection is used to obtain precise staging data, provide local control for patients with metastatic breast carcinoma, and for the selection of adjuvant therapies. Unfortunately, the only patients who may truly derive
therapeutic benefit from axillary lymph node dissection are those with positive nodes, who correspond to approximately 40% of those who undergo axillary lymph node dissection. This has brought the sentinel lymph node (SLN) concept to the forefront in considering changes in the practice of axillary nodal staging.

The SLN is defined as the first lymph node to receive drainage from the site of a primary tumour. If the SLN is free of tumour, the probability of tumour involvement in the non-SLNs is about 1%. With the intraoperative identification of a tumour-free SLN, surgeons can avoid complete axillary lymph node dissection, thus sparing the patient unnecessary morbidity.

The techniques used for the intraoperative evaluation of SLNs include frozen section analysis and imprint cytology. Frozen section analysis has the advantage of high sensitivity but the disadvantages that the tissue may be damaged, and the procedure is time consuming. In contrast, imprint cytology can provide clear cytological details and a rapid diagnosis, preserving the tissue for permanent sections. However, imprint cytology has the disadvantage that the number of cells examined is small and the chance of an indeterminate result is high. In the literature, there is wide variation in the reported accuracy of these techniques. The addition of immunohistochemistry (IHC) to frozen section analysis or imprint cytology has also yielded conflicting results.

In this study, we analysed the utility of imprint cytology with rapid immunocytochemistry (ICC) and frozen section analysis for the evaluation of SLNs in breast cancer patients. We also compared these results with those from postoperative paraffin sections.
Goals:

(A) Validation of the Poseidon HER2/SE17 FISH assay in comparison with PathVysion HER2/CEP17 FISH assay:
   o comparison of Poseidon and PathVysion HER-2 FISH assays,
   o comparison of the two FISH assays with the HercepTest,
   o practical experiences in using the Poseidon HER2/SE17 FISH assay.

(B) Comparison of intraoperative sentinel lymph node diagnostic methods in breast cancer patients:
   o review of the literature,
   o introduction of the rapid immunocytochemical method in intraoperative sentinel lymph node examination,
   o comparison of the three intraoperative sentinel lymph node diagnostic methods: imprint cytology, frozen section and rapid immunocytochemical assay,
   o practical experiences in routine sentinel lymph node examination,
   o recommendation for intraoperative sentinel lymph node assessment algorithm.
2 Materials and methods

2.1 Materials and methods in validation of the Poseidon HER2/SE17 FISH assay

Tumour specimens
One hundred and twenty-two patients who had been diagnosed with invasive breast cancer during the period 2006–2007 were selected. A pathologist reviewed the previously made haematoxylin–eosin (H&E) and immunostained slides for each case and confirmed the original diagnosis.

Tissue microarrays
Tissue microarray (TMA) blocks were constructed using a tissue arraying instrument (TMA Master, 3D Histech, Budapest, Hungary) and 1-mm cores. The TMA blocks contained three cores per patient and cores of normal liver for orientation. One tissue core from each tumour was obtained from the centre of the tumour and the second and third cores were taken from the periphery of each tumour. TMA coordinates and pathological data of the cohort were stored in a spread sheet for reference. The TMA slides were sectioned and mounted on charged glass slides as 4-µm sections. H&E-stained TMA sections were used as morphological references for each core studied.

Immunohistochemistry
Immunohistochemical examination of the TMA slides was carried out with the HercepTest kit (Dako, Glostrup, Denmark), according to the manufacturer’s instructions. Briefly, epitope retrieval was carried out with
pH 7.2 citrate buffer (0.1 mol/L) for 40' in a 95°C water bath. IHC reactions were performed using the DAKO autostainer (Dako). For visualization of the reactions, 3,3’ Diaminobenzidine (DAB) chromogene was used. Positive and negative controls were included in all IHC runs. The results were scored strictly following the well-known FDA approved scoring system (score 0-3, or 0-3+) designed for HercepTest, based on the membrane staining pattern and intensity.

Fluorescence in situ hybridization

The TMA slides were deparaffinised in xylene and rehydrated in ethanol. The slides were pre-treated with Paraffin Pretreatment kit II (Vysis) according the manufacturer’s instructions. Briefly, the slides were treated with protease-K solution for 6 min at 37°C in a water bath. After rinsing and air-drying, 10 µl of probe was applied to each TMA slide. The Vysis PathVysion HER2 DNA probe kit and Kreatech Poseidon ERBB2/SE17 probe kit were used separately. The slides were cover slipped and sealed with rubber cement, denatured for 5 min at 73°C, and were subsequently hybridized overnight at 37°C. Denaturisation and hybridization were performed on the StatSpin® ThermoBrite platform (Vysis). After post-hybridization washing, the TMA slides were counterstained with 20 µl of DAPI (4,6-diamidino-2-phenylindole). The FISH signals were visualized on an Olympus BX51 fluorescent microscope using filter sets that were optimized for the PathVysion kit. The relative ratios of LSI-HER2 to CEP-17 signals were counted in 60 tumour cells for each case under immersion oil at x100 magnification. HER2 to chromosome 17 ratios equal to or more than 2.0 were considered positive for HER2 gene amplification. Low-grade amplification was diagnosed when the ratio was below 4, and high-grade
amplification, when the ratio was equal to or above this limit. When the average number of chromosome 17 signal numbers exceeded 2.5 per cell, it was considered as polysomic.

Statistical analysis
For FISH results expressed as continuous variables, between-method agreement was evaluated by calculating concordance correlation coefficients (RC) and Bland-Altman 95% limits of agreement. The reduced major axis, a line through the intersection of the means with slope given by the ratio of the standard deviations, was used to visualize the centre of the data. Outcomes of classification types were evaluated for agreement using the Kappa statistic with analytical or bootstrap-based confidence intervals that were appropriate to the number of grouping levels. Pairs of binary outcome assays, where one method could be identified as reference against which performance of another classification could be evaluated, were described in terms of specificity, sensitivity, positive and negative predictive values, and per cent correctly classified (accuracy) with exact binomial 95% confidence intervals.

2.2 Materials and methods in comparison of intraoperative SLN diagnostic methods

Patient characteristics
This study was based on the prospective collection of data, carried out at the Department of Pathology of Josa Andras University Teaching Hospital (Nyiregyhaza, Hungary). Patients who had a preoperative diagnosis of T1–T3 invasive breast cancer with clinically negative lymph-node status and who may or may not have received neoadjuvant chemotherapy were
considered for the study.

**Surgery**

Preoperative lymphatic mapping with a hand-held gamma detector was used to identify the SLNs in the axilla in all patients. Lymphoscintigraphy was performed the day before surgery, a median of 4 h after a single intratumoral injection of 100 MBq of $^{99m}$Tc-labeled human albumin colloid Nanocoll (Nycomed Amersham Sorin s.r.l., Saluggia, Italy), with particle sizes of less than 80 nm in a volume of 0.2 ml.

SLNs were defined as the hottest radioactive lymph node plus all the radioactive nodes with a target count of more than 25% that of the hottest spot. Level I–II axillary clearance (AC) was performed during the primary surgery for patients with SLN metastases identified in the frozen sections and/or touch imprints and/or rapid immunocytochemistry. Patients with false-negative findings in the rapid assessment diagnosis underwent level I–II AC as a second surgical procedure.

**Pathological assessment**

The SLNs were trimmed of excess adipose tissue and sliced into 1–1.5 mm thick sections perpendicular to their long axes. Two slides of touch imprints from each surface were made first by pressing charged glass slides gently against the slices. The slides demonstrated the same imprint pattern. One air-dried slide of the imprints was stained with Wright-Giemsa method (Hemacolor, Merck, Darmstadt, Germany). Intraoperative immunocytochemistry was performed on the second imprint. After the imprint was dried at room temperature for 2 min, the slide was fixed for 30 s in cold acetone, dried at room temperature for 1 min, washed in saline, and
rinsed in phosphate-buffered saline (PBS). Anti-cytokeratin (AE1/AE3; DAKO, Glostrup, Denmark) was used as the primary antibody for rapid ICC, together with a polymer–horseradish peroxidase (HRP)-labelled secondary antibody (EnVision Detection Kit, DAKO). After the application of the primary antibody, the slide was incubated in an oven for 3 min 10 s at 37 °C. After the samples were washed with PBS, the secondary antibody labelled with polymer–HRP was applied and incubated in an oven for 3 min 10 s at 37 °C. After the samples were washed, diaminobenzidine was applied. The staining took approximately 20 min. Hematoxylin counterstaining was also performed. While the pathologist evaluated the imprints, frozen sections were cut from one level and stained with haematoxylin–eosin (H&E). The histological and cytological findings were compared with the results of immunocytochemical staining. The results were reported to the surgeon in the operating theatre.

The remaining specimens were fixed in 4% neutral-buffered formalin. The final pathological evaluation was performed on the formalin-fixed, paraffin-embedded tissue sections of the lymph node after H&E staining of the first section and pan cytokeratin staining of the third level of each tissue block. SLN micrometastasis was defined as metastatic foci smaller than 2 mm and larger than 200 µm, and isolated tumour cells were defined as metastatic foci smaller than 200 µm.

**Statistical analysis**

The sensitivities, specificities, and positive and negative predictive values of touch imprint cytology, frozen sections, rapid immunocytochemistry, touch imprint cytology plus frozen section, frozen sections plus rapid immunocytochemistry, touch imprint cytology plus rapid
immunocytochemistry, and the combination of all three methods for the
detection of metastatic tumour in the SLNs were determined with the
corresponding 95% confidence intervals (CIs). Metastases of any kind
(macrometastases, micrometastases, and isolated tumour cells) were
considered positive results. In the calculations, the data obtained by final
histopathologic examination of the lymph nodes using serial H&E sections
and pan cytokeratin immunostaining were used as the gold standard.

3 Results

3.1 Results in HER2 FISH validation

Immunohistochemistry
IHC results for 122 cases were available. The results of the three TMA
cores per case were summarized and a score was given according to the
strongest IHC staining among the cores. Finally, 62 IHC 0, 29 IHC 1+, 17
IHC 2+, and 13 IHC 3+ cases were scored.

Fluorescence in situ hybridization
HER2 FISH analysis was successfully performed on 122 breast cancer
cases. Overall, the staining and signal quality for the majority of the cores
was excellent for both assays. Complete absence of signals was not
observed.

Comparison of the two FISH assays
Average HER2 and centromere specific signals per cell and HER2/CEP17
ratios were first examined as continuous variables. Concordance
correlation coefficients (RC) showed near perfect agreement in average
HER2 and centromere specific signal counts per cell (RC=0.993 and 0.929, respectively, p<0.0001) and in HER2/CEP17 ratios (RC=0.993, p<0.0001) between the PathVysion and the Poseidon FISH assays (Fig. 1). In all three graphs, reduced major axes covered the lines of perfect concordance almost completely. According to the HER2/CEP17 ratios, cases were categorized into three groups. Of 102 cases, 101 showed no amplification, seven cases out of nine showed low grade HER2 gene amplification, and 12 out of 13 cases showed high grade HER2 gene amplification with the PathVysion and Poseidon FISH kits, respectively. Comparing the two assays based on these groups, the Kappa measure showed perfect agreement between them (Kappa=0.9441, p<0.0001). The classification performance of the Poseidon FISH kit was characterized taking the PathVysion FISH result as reference. The sensitivity and the specificity of the Poseidon FISH kit was calculated to be 95.2% and 100%, respectively, whereas the positive (PPV) and negative predictive values (NPV) were 100% and 99%. Fifteen cases showed polysomy with both the PathVysion and the Poseidon assays; however, four cases were detected differently. With regard to the ability to diagnose HER2 polysomy, the Poseidon FISH kit had a sensitivity of 93.3% and a specificity of 99.1% with a PPV and NPV of 93.3% and 99.1%, respectively, as assessed with PathVysion classification as reference.

Comparison of the two FISH assays with the HercepTest

When the IHC results were dichotomized at 2+ and then at 3+, the overall accuracies of prediction of gene amplification as determined by PathVysion were 85.2% (CI: 77.7%, 91.0%) and 94.3% (CI: 88.5%, 97.7%), respectively. There was a lower accuracy of classification by Poseidon
results when both 2+ and 3+ cases were considered IHC positive (84.4%; CI: 76.8%, 90.4%); on the other hand, when the IHC was dichotomized at 3+, the accuracy of prediction of gene amplification with the Poseidon assay was slightly higher than with PathVysion (95.1%; CI: 89.6%, 98.2%). When IHC data were dichotomized at 2+, the Kappa statistic showed moderate agreement between HercepTest and PathVysion and with the Poseidon FISH results (Kappa=0.5645 and 0.5344, respectively; p<0.0001), whereas dichotomization at 3+ substantially improved the level of agreement (Kappa=0.7681 and 0.7960, respectively; p<0.0001).

3.2 Results in intraoperative SLN methods
Between February and November 2009, we enrolled 100 women with 127 SLNs in this study. One patient had simultaneous bilateral breast cancer. The quality of the frozen sections and touch imprint cytology was satisfactory in all patients for histological or cytological interpretation; rapid immunocytochemistry was unavailable for one SLN. Rapid immunocytochemistry revealed strong cytoplasmic and membranous staining of the metastatic tumour cells. The morphological features of these cells, in conjunction with the staining patterns, were useful for the accurate interpretation of the metastatic tumours. The final histological examination revealed metastases in 36 lymph nodes. 27 of them contained macrometastasis. Imprint cytology and rapid ICC were positive in 23, 23 cases respectively. Frozen section was positive only in 21 cases. The final inspection found micrometastases or isolated tumour cells in 9 lymph nodes. None of them were seemed in frozen sections. 2 cases were revealed by immunocytochemistry and 1 case by ICC. There
were 2 false positive cases by imprint cytology, 1 by using immunocytochemistry and none by frozen examination. The sensitivity of touch imprint cytology compared with the final histopathology result, considered the gold standard, was 69.44% (CI: 51.73%–83.08%). Similarly, the sensitivities of frozen sections and rapid immunocytochemistry were 58.33% (CI: 40.89%–74.04%) and 66.67% (CI: 48.95%-80.90%), respectively. When touch imprint cytology was added to either frozen section analysis or rapid immunocytochemistry, the sensitivity of tumour cell detection increased to 72.22% (CI: 54.57%–85.20%), which is equal to the sensitivity of all three detection methods combined. Frozen section analysis plus rapid immunocytochemistry together had a sensitivity of 69.44% (CI: 51.73%–83.08%).

The individual specificities of touch imprint cytology, frozen section analysis, and rapid immunocytochemistry relative to that of the final histopathology result (the gold standard) were 97.80% (CI: 91.53%–99.62%), 100% (CI: 94.95%–100%) and 98.90% (CI: 93.17%–99.94%), respectively. Grouping the data, the highest specificity of 98.90% (CI: 93.17%–99.94%) was associated with the combination of frozen section analysis plus rapid immunocytochemistry, whereas all other combinations had slightly lower specificities of 97.80% (CI: 91.53%–99.62%). The positive and negative predictive values showed similar tendencies.

4 Discussion

4.1 Discussion of HER2 FISH results
HER-2/neu overexpression and/or gene amplification predict poor outcome for invasive breast carcinoma and have become crucial determinants in therapeutic decisions. Therefore, it is of paramount importance to devise a
reliable, reproducible, and technically feasible method to assess HER-2/neu status. Although numerous techniques can be employed, IHC and FISH are the most widely used because they are applicable to paraffin-embedded tissue and can also be performed in surgical pathology laboratories.

FISH is used extensively for the evaluation of gene amplification. It requires standardization and monitoring of the methodology and the use of validated assays. Dual colour FISH with HER2 and chromosome 17 probes ensures the ability to determine whether the increased HER2 gene copy number is independent of polysomy of the chromosome 17. Aneuploidy has been reported in 56% to 92% of invasive breast carcinomas; thus, dual colour assays have a significant advantage over single colour assays. Both assays compared in this study use direct-labelled DNA probes, including a satellite probe against the centromere region of chromosome 17 to allow calculating ratios between HER-2/neu and chromosome 17.

PathVysion is a well-described, FDA-approved kit, which – according to available studies – is considered the gold standard of available HER2 assays. On the other hand, the costs of HER2 determination with PathVysion are rather high. The less known commercial FISH assays, one of which is the dual colour kit by Kreatech, may also ensure the same high quality results, but at a lower price per case. The Poseidon ERBB2/SE17 commercially available kit is optimized to assess copy counts of the HER2 gene region at 17q12, whereas the probe for the SE17 gene region is available to facilitate correction for chromosome 17 counts. The HER2 probe is labelled with PlatinumBright 550 fluorophore with excitation at 546+/-20nm, and emission at 580+/-30nm, and the SE17 probe is conjugated with PlatinumBright 495 fluorophore with excitation at 495+/-
20nm and emission at 525+/−30nm. Therefore, the excitation and emission wavelengths can be detected with the very same filter sets developed for the Pathvysion assay (MF101, MF102; Chroma Technology Group, Rockingham, VT, USA).

The results of this study underline that the two assays are comparable in terms of determination of HER2 gene amplification. The concordance correlation coefficients calculated from HER2 and CEP17 copy counts and HER2/CEP17 ratios as continuous variables showed perfect agreement between the two FISH assays. Moreover, Kappa statistic between categorized variables also proved to have perfect agreement between the PathVysion and Poseidon FISH kits. Of the 122 diagnosed cases, there was only one tumour in which there was significant difference between the amplification statuses as determined by the two assays: according to the HER2/CEP17 ratios, patient No. 106 exhibited low-grade amplification with PathVysion, whereas only chromosome 17 polysomy was detected by the Poseidon Kit. The tumour exhibited 2+ protein expression with the HercepTest; the exact HER2/CEP17 ratios were 2.38 versus 1.35, respectively, 2.6 centromeric probes per cell were indicated by both assays, and differences were observed between the numbers of HER2 specific signals. Unfortunately, clinical information regarding the patient was not available to effectively control the results. Taking the “gold standard” PathVysion as the endpoint, the Poseidon assay showed very high sensitivity, specificity, PPV, and NPV, even when the measured factor was the ploidy of the tumours. When comparing FISH data to the IHC results, HercepTest revealed similar accuracies in predicting the amplification status by both FISH assays. The accuracies obtained after dichotomizing IHC data at 3+ were, not surprisingly, higher because of the
observed low frequency of gene amplification among IHC 2+ cases with both assays (17.7% and 13.3%). The correspondence between IHC and FISH results – as revealed by the Kappa statistic – increased from moderate to substantial agreement when ICH results were dichotomized at 3+ rather than at 2+. Kappa values derived from both FISH results were similar; moreover, the 3+ IHC–FISH concurrence was even slightly higher with the Poseidon kit than with the PathVysion. Proceeding from these results, it may be concluded that the two FISH assays are nearly equivalent.

4.2 Discussion of SLN techniques

In our prospective study, we simultaneously applied three different intraoperative methods—frozen section analysis, touch imprint cytology, and rapid immunocytochemistry—to identify the most sensitive and specific method or combination of methods with which to detect tumour cells within an SLN. Our data show that conventional frozen section analysis was associated with the lowest sensitivity, whereas the combination of either frozen sections + touch imprint cytology, touch imprint cytology + rapid immunocytochemistry, or frozen sections + touch imprint cytology + rapid immunocytochemistry yielded the highest sensitivity. Frozen section technology did not produce false positive results, and therefore showed 100% specificity, whereas the lowest specificity, which was associated with the highly sensitive combinations of methods, was still quite high (97.80%). Not surprisingly, the highest negative predictive values were also associated with the highly sensitive combinations of methods, whereas the positive predictive values of these combinations were also relatively high. We believe that until the specificity of these techniques is sufficiently high,
sensitivity is the most important characteristic of the methods investigated. Therefore, according to our results, the most accurate combinations of intraoperative methods are touch imprint cytology with either frozen section analysis or rapid immunocytochemistry, or the combination of all three methods.

Practical experiences in routine sentinel lymph node examination
The standard method of touch imprint smear preparation described above has numerous advantages:

- it makes the diagnostic process fast, easy and safe,
- two or more equal smears might be useful in difficult cases and for applying two or more different methods at the same time,
- the method might give provide information about the approximate size of the metastasis in SLN.

We summarized our experiences about the size of lymph node metastasis. Large number of tumour cells in a well-defined area of the smear predicts macrometastasis. In cases of micrometastasis there are only a number of irregularly scattered tumour cells or clusters intermixed with lymphoid cells. We have also defined the features of dendritic cells in immunocytochemical preparations and provided differential diagnostic possibilities.

Another complex area is the detection of metastatic lobular carcinoma. The intraoperative detection of lobular carcinoma in a lymph node is difficult because of its low-grade cytomorphology and its tendency to infiltrate metastatic sites in a single-cell pattern. Our experiences revealed the usefulness of the cytokeratin immunocytochemistry in such cases.

We recommended an algorithm to choose the adequate and most cost effective method or method combination.
5 New results

5.1 New results in HER2 FISH validation

In the first part of essay we compared two FISH assays and an immunohistochemical test to make validation of the newly invented Poseidon ERBB2/SE17 dual colour FISH test. The statistical analysis of our data proved, that the two probes have nearly identical value in clinical practice. Moreover, no methodological difference was necessary to provide equally optimal hybridization circumstances for the Poseidon kit. Considering costs, however, there is significant difference between the two assays. The PathVysion probe kit is almost twice as expensive as the Poseidon equivalent. Therefore, using the latter option means a remarkable reduction of costs of HER2 testing. The price of the Kreatech assay is also comparable to that of HercepTest immunohistochemistry, which again is closer to a previous concept, shared by several different authors, of performing the more reliable in situ hybridization on all breast cancer cases, especially in high-throughput HER2 laboratories. In conclusion, data derived from two FISH assays and corresponding IHC results were compared to validate a recently developed commercial assay, the Poseidon ERBB2/SE17 probe kit. Statistical analysis confirmed that the two FISH assays are comparable in terms of detection of HER2 gene amplification. Proceeding from these findings, the genetic diagnoses obtained with the Poseidon kit can be considered as valuable as the results from the FDA approved PathVysion assay, and propose its utilization in routine HER2 diagnostics.
5.2 New results in intraoperative sentinel lymph node examination

In the second part of the work we have described our experiences in combination of intraoperative immunocytochemistry technique with frozen sections and touch imprint cytology. Our goal was to increase the accuracy of intraoperative detection of metastases in sentinel lymph node. In our study, the combined accuracy of the three methods was the same as combining touch imprint plus frozen section or touch imprint plus rapid immunocytochemistry; therefore for daily routine practice we would recommend touch imprint cytology in combination with either frozen section analysis or rapid immunocytochemistry.

The novel standard imprint technique proved to be time and cost saving, and it can be useful in appreciating the extent of metastasis.

We summarized our experiences about the size of lymph node metastasis. Our experiences revealed the usefulness of the cytokeratin immunocytochemistry in lobular carcinoma metastasis.

We recommended an algorithm to choose the adequate and most cost effective method or method combination.
6 Summary

Breast cancer therapy is determined by prognostic and predictive factors revealed by various histological examinations. Therefore, the most appropriate diagnostic method has a great importance of choosing the optimal treatment. The objectives of this work are HER2 amplification and sentinel lymph node status assessment in breast cancer cases.

Comparison of two commercially available FISH assays (PathVysion assay and newly introduced Poseidon HER2 dual color FISH kit) demonstrated nearly identical results. Considering costs, the PathVysion assay is almost twice as expensive as the Poseidon kit. Therefore, using the new HER2 kit in routine pathological testing of HER2 gene status provides reliable results with a remarkable reduction of costs.

We have also described our experiences in combination of intraoperative immunocytochemistry technique with frozen sections and touch imprint cytology in breast cancer sentinel node assessment. The combined accuracy of three methods was the same as combining touch imprint plus frozen section or touch imprint plus rapid immunocytochemistry. In conclusion, touch imprint cytology in combination with either frozen section analysis or rapid immunocytochemistry are recommended for routine practice. The novel standard imprint technique has showed time and cost saving, and it can also be well utilized in appreciating the size of metastasis

Keywords: breast cancer, HER2, sentinel lymph node
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