SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Comparative Analyses in HER-2 Status Determination in Breast Cancer

by Csaba Kósa MD

UNIVERSITY OF DEBRECEN

Doctoral School of Clinical Medicine

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1. Introduction

Breast cancer is the third most frequent cause of death among women. In Hungary, with nearly 7000 diagnosed cases a year, breast cancer is responsible for 2200 to 2400 deaths each year. Establishment of the diagnosis and design of the treatment plan require a multidisciplinary approach involving complicated algorithms. In most cases, diagnosis is based on a pathology report.

A wide variety of pathological procedures are available for correct diagnosis of breast cancer, including cytology, classic histology, immunohistochemistry or molecular tests. These procedures are used to assess the tumour size, presence of multifocality, the exact histological type, grade of differentiation, spread to blood and lymphatic vessels, as well as the status of surgical resection edges and regional lymph nodes. Determination of the tumour proliferation rate, as well as of hormone receptor and HER2 status is also required; furthermore, there is an increasing demand for identifying the genetic properties of the tumour, which carry invaluable prognostic information. Current international and Hungarian guidelines contain optional methods leaving the decision with the expert: one can choose from the recommended procedures or, after careful review, opt for one which is not included in the guideline.

HER2-positivity in breast cancer means overproduction of the HER-2 protein, an important negative prognostic indicator, as these tumours grow more quickly, produce distant metastases sooner, have a higher recurrence rate, and are more resistant to conventional therapies. HER2 status determination has been a requirement since 1998, when targeted treatment of HER2-positive tumours with trastuzumab (HERCEPTIN) was introduced. The HER2 test is routinely performed in all cases where breast cancer is confirmed in the first histological diagnosis. The practical importance is increasing in the anticipation of HER-2 positive breast cancers with the change in clinical management

practices. In the modern treatment of breast cancer and definition of personalized mechanism aspiration cytology significantly overshadowed by the more detailed information bearing core biopsy sampling. As a result, information may have been significant with regard to the biological behavior of tumors before treatment, and this may change for example the treatment plan for the HER-2 positive breast cancer. More and more often we choose the initiation of primary systemic treatment prior to the surgical care in case of HER-2 positive breast cancer.

According to Hungarian and international protocols, the first method of choice is immunochemistry (IHC), yielding scores expressed in crosses, with 3+ corresponding to positivity, 0 or 1+ corresponding to negativity; 2+ is considered borderline, requiring another test for clarification, which is usually an in situ hybridisation assay suitable for identifying a potential HER2 gene section multiplication.

There are several in situ hybridisation techniques, such as chromogenic in situ hybridisation (CISH), metallographic in situ hybridisation, gold-facilitated in situ hybridisation (GOLDFISH), silver in situ hybridisation (SISH) and fluorescence in situ hybridisation (FISH), currently the most widespread of all. The wide variety of options also includes dual-colour in situ hybridisation techniques (DISH), like dual-colour chromogenic in situ hybridisation or dual-colour dual-hapten brightfield in situ hybridisation (DDISH). These methods produce more reliable results as they facilitate simultaneous assessment of HER2 status, polysomy 17 and tissue structure; furthermore, they have higher spatial resolution, do not require special equipment other than a conventional light microscope, are highly reliable and easily reproducible, and also more cost effective than FISH commonly used in everyday practice.

Multiplication of the HER2 gene section and the resulting overproduction of the HER2 protein, a factor greatly impacting on therapy and the outcome of the disease, may be due not only to gene amplification but also polysomy. The HER2 gene is located on the long arm of chromosome 17, near the centromeric region. In the case of gene amplification, the section containing HER2 is multiplied; with polysomy, the number of chromosomes is increased, along with the number of centromeric regions and that of HER2 sections in the vicinity. Gene amplification occurs in 20% of breast cancers, while polysomy has been identified in one-third, 10 to 50% of the cases, depending on the criteria used. Differentiation of the two has an impact on the patient's fate: they have prognostic value and also influence the effectiveness of medication. Polysomy 17 is associated with poorer prognosis; furthermore, it is resistant to targeted treatment with trastuzumab, a factor which must be taken into account, considering the significant cytotoxicity and costs of the treatment. Polysomy can be tested by several techniques, including in situ hybridisation (ISH), spectral karyotyping (SKY), microarray analysis, restriction fragment length polymorphism analysis (RFLP), flow cytometry etc. Gene amplification and polysomy can be distinguished either by the comparison of two in situ hybridisation assays, with the HER2 gene section staining in one and the centromeric region of chromosome 17 staining in the other, or, in a much simpler way, by using dual colour in situ hybridisation techniques, where the centromeric section of the chromosome and the HER2 gene stain two different colours and their ratio serves as basis for the identification of amplification and/or polysomy. In the present study, the HER2 status of breast cancer cases was reclassified, with the presence of polysomy 17 taken into account.

2. Objectives

2.1. Comparison of dual-colour dual-hapten brightfield in situ hybridisation (DDISH) and fluorescence in situ hybridisation (FISH) in detection of HER2 amplification in breast cancer.

2.1.1. Comparison of the results of dual-colour dual-hapten brightfield in situ hybridisation (DDISH) and fluorescence in situ hybridisation (FISH).

2.1.2. Summary of experiences with the dual-colour dual-hapten brightfield in situ hybridisation (DDISH) technique.

2.2. Reclassification of HER2 status in breast cancer patients by evaluation of the centromeric region of chromosome 17.

2.2.1. Importance of polysomy 17 in HER2 status determination.

2.2.2. Comparison of HER2 status in breast cancer patients by gene amplification and chromosome 17 centromere testing using alternative methods, which may specify the uncertain situations and assessment of therapeutic effects.

3. Materials and methods

3.1. Materials and methods in the dual-colour dual-hapten brightfield in situ hybridisation (DDISH) validation study

3.1.1. Selection of tumour samples

One hundred and five patients diagnosed with invasive breast cancer between 2010 and 2011 were selected. A pre-condition of inclusion was the availability of paraffin blocks from the surgical preparations. The tumour-containing parts undergoing further testing were selected from sections prepared from the paraffin blocks. Earlier haematoxylin-eosin (HE) sections and immune reactions were reviewed by a pathologist, who also checked the validity of the earlier diagnosis. The samples were coded to avoid patients being identified, in accordance with the regulations of medical ethics.

3.1.2. Fluorescence in situ hybridisation

The sections were deparaffinated in xylol and rehydrated in ethanol. The slides were treated with Paraffin Treatment II kit (Abbot-Vysis, Downers Grove, IL, USA) according to the user's guide. In short, the slides were treated for 6 min with proteinase-K solution in a water bath of 37 ºC. After rinsing and drying in air, 10 µl of dual-colour assay (PathVysion, Vysis) was applied to each slide. After covering and closing by gum sealing, the sections were denatured at 73 ºC for 5 min, then hybridised overnight at 37 ºC. Hybridisation was performed using a StatSpin Thermobrite hybridisation equipment (Abbott-Vysis, Downers Grove, IL, USA). After being washed, the sections were stained with 20 µl of 4,6-diamino-2-phenylindole (DAPI).

3.1.3. Dual-colour dual-hapten in situ hybridisation (DDISH)

DDISH is a fully automated process; in the present study it was performed using Roche Diagnostics' BenchMarkXT™ equipment, according to the instructions of the user's guide. In brief, the sections were deparaffinated, then treated in citrate buffer (pH 6) at 90 ºC in two cycles for 8 and 12 min, then treated with proteinase-3 for 16 min. Hapten-labeled assay, dinitrophenol (DNP)-labeled HER2 assay and digoxigenin (DIG)-labeled chromosome 17 centromere specific assay were applied. The probes and the samples were denatured at 80 ºC for 20 min, then hybridised at 44 ºC for 6 hours. The signals were detected one after the other for the chromosome 17 centromere as well as the HER2 loci. After washing, the HER2 signal was incubated with rabbit anti-DNP monoclonal antibody for 20 min, then treated with HRP-conjugated goat anti-rabbit antibody for 16 min, then subjected to silver-detection assay, which produced a black signal. The chromosome 17 centromere was treated with mouse anti-DIG monoclonal antibody for 20 min, then with alkaline phosphatase-conjugated goat anti-mouse antibody for 24 min, and finally subjected to FastRed/Naphthol detection assay, which produced a red signal. The slides were treated with a few drops of detergent mixed with tap water, then rinsed with ultra-clean water three times, dried at 37 ºC for 15 min, and finally covered. In each sample, 30 separate, non-overlapping nuclei were examined for determination of the HER2/CEP17 ratio and the number of HER2 and chromosome 17 signals. SISH signals were estimated using a reference signal. Cases with a HER2/CEP17 ratio lower than 1.8 were considered as negative, i.e. HER2 non-amplified; those with a ratio higher than 2.2 were classified as positive. In borderline cases, i.e. those with a ratio between 1.8 and 2.2, further 20 to 40 cells were examined and the ratio was re-calculated. If the average number of chromosomes 17 per cell was higher than 2.5, polysomy was established.

3.1.4. Statistical analysis

Concordance correlation coefficients (RC) and Bland-Altman 95% limits of agreement were calculated to evaluate agreement between techniques for results expressed as continuous variables. A straight line passing through the intersections of means with a steepness corresponding to scatter ratios, i.e. a reduced major axis, was plotted to visualise the centre of the data pool. Agreement of categorical outcomes was evaluated by Kappa-statistics, defining confidence intervals (CI) using a bootstrap-based analytical technique in accordance with the number of classes.

3.2. Materials and methods in HER2 status reclassification in breast cancer

3.2.1. Selection of patients

Four hundred and five patients with known HER2 status were selected. Earlier FISH reports were available for all of them in the pathology data base. In the majority of the cases, the tumour samples were evaluated in tissue multi-blocks (TMAs), previously tested by the FISH technique. In the remaining cases, conventional blocks were used for determination. From the TMAs and other blocks, conventional sections were obtained and applied on surface-treated slides.

3.2.2. Evaluation of fluorescence in situ hybridisation

The filter pair was optimised for the PathVysion kit. The ratios of the HER2 locus specific identification signal and the centromere identification signal (HER2:CEP17) were counted in 60 tumour cells in each tumour, using 100-time immersion enlargement.

Positive HER2 amplification was defined as a HER2 / CEP 17 ratio of 2.2 or higher; ratios lower than 1.8 were considered negative. Ratios below 4 were classified as low-grade (LG) amplification; ratios of 4 or higher were considered as high-grade (HG) amplification. In borderline cases, where the ratio fell between 1.8 and 2.2, another set of 20 to 40 cells were tested and the scores were re-evaluated.

4. Results

4.1. Results of the dual-colour dual-hapten brightfield in situ hybridisation (DDISH) validation study

4.1.1. Fluorescence in situ hybridisation

The HER2 FISH analyses by PathVysion were invariably successful. None of the cases had to be excluded due to technical error. Staining and the quality of the signals were excellent. No amplification was detected using the PathVysion reagent in 71 cases; the reaction was positive in 28 cases.

4.1.2. Dual-colour dual-hapten in situ hybridisation (DDISH)

Modification of the DDISH protocol during protocol optimisation had only a minimum impact on the results. The protocol requires protease digestion for 16 min and washing at 72 ºC After this procedure, 8 of the samples failed to produce a red signal. Pre-treatment was modified and hybridisation was repeated, which reduced the number of samples without a red signal to 6. These samples were excluded from the study. By the DDISH technique, no amplification was detected in 75 cases; 24 cases were found positive.

4.1.3. Comparison of the two techniques

Assessability of the reactions was similar by subjective judgement. The HER2/CEP17 ratio was evaluated as a continuous variable. Deviation from FISH findings occurred in 4 out of 99 samples. These 4 samples proved to be false negative by DDISH. The concordance correlation coefficient (RC) showed a nearly perfect agreement in HER-2/CEP17 ratios between the PathVysion and the DDISH techniques ($RC=0.959$, $P<0.0001$). Plotting the reduced major axis produced a similar result, with the major axis running in close proximity of the straight line signifying complete concordance.

Agreement of the two techniques by Kappa-statistics was as high as 95.9% (Kappa=0.8712, P<0.0001).

4.2. Results of HER2 status reclassification in breast cancer patients

In the HER2 status reclassification study of the 405 patients with breast cancer, cases were regarded as HER2-positive if the number of HER2 loci in a cell was higher than 6; cases with 6 or less loci were considered negative. Amplification was detected in 143 of the 405 cases, while 243 were found negative by the FISH methodology used. A total of 19 (4.69%) controversial cases were identified; in 5 of these (1.23%), no amplification had been detected originally, while the number of HER2 loci was over 6. They were accordingly reclassified as HER2-positive. In 14 other cases (3.46%), amplification had originally been identified, with the number of HER2 loci being 6 or lower.

5. Discussion

5.1. Dual-colour dual-hapten brightfield in situ hybridisation (DDISH) validation study

Recently, an increasing number of pathological diagnostic techniques have become available in breast cancer, making the process more and more complicated. Factors determining the molecular type of breast cancers, such as oestrogen receptor, progesterone receptor, HER2 status and proliferation markers, have come to play an increasingly important role. They help to get a more comprehensive picture of tumour properties and, as a consequence, make it easier to choose the right therapy. Among these markers, HER2 status is of special importance as HER2 protein overproduction in HER-2 positive tumours can be targeted with trastuzumab (HERCEPTIN).

Amplification of epidermal growth factor receptor type 2 (EGFR-2), also known as c-ERBB-2 or HER-2/neu gene, leads to overproduction of the HER-2 protein, which can be detected by various methods. Immunohistochemistry (IHC) is the most widespread of them, probably because it can be performed on paraffin blocks and fits into routine pathological procedures. According to the FDA-approved protocol, immunohistochemical results are classified into 4 groups, scored by crosses by the degree of intensity (0, 1+, 2+, 3+). Samples producing 0 or 1+ are considered as negative. Cases of 3+ are classified as positive. The interpretation of 2+ is controversial as there have been cases which failed to respond to trastuzumab adequately although the immunohistochemical reaction was positive, a phenomenon explained by the fact that HER2 gene multiplication is not always due to amplification but may be the result of chromosome 17 polysomy: it means there are more chromosomes 17 in the cell and, as a consequence, there are more HER-2 coding gene sections and larger quantities of proteins detectable by immunohistochemistry, falsifying the results of immunohistochemical tests. In such cases, further tests must be

performed to establish the correct HER2 status and detect potential gene amplifications and this is usually done using in situ hybridisation techniques. Of these, fluorescence in situ hybridisation (FISH) is the most common, which too can be performed on paraffin tissue blocks and fitted into routine pathological diagnostics, but requires a fluorescence microscope for the evaluation of the results. Immunohistochemistry can be used as a pre-screening test, the agreement with the FISH assay being 89-100% in HER2-positive 3+ cases and 92-98% in negative 0 or 1+ cases in comparative studies; gene amplification was detected in only 23-25% of borderline 2+ cases. These days, several in situ hybridisation techniques are available and their efficiency and reliability are intensely discussed in the literature. The so-called dual-colour in situ hybridisation tests represent an interesting new methodology providing a more detailed picture of the HER2 status of an individual tumour. In dual-colour assays the centromeric region of chromosome 17 and the HER2 coding gene section are stained simultaneously with two different colours, making it possible to distinguish between genuine gene copy number multiplication, gene amplification, and chromosome 17 multiplication, i.e. polysomy. This is essential for choosing the right therapeutic approach as chromosome 17 polysomy will fail to respond properly to targeted treatment with trastuzumab while the patient is exposed to severe side-effects, including cardiotoxity. Of the several dual-colour techniques in use, dual-colour dual-hapten brightfield in situ hybridisation (DDISH) was validated in the present study, a technique which also enabled assessment of the HER2 gene copy number and chromosome 17 centromere ratio by using DNA assays directly labelled with fluorescence. As discussed in the international literature, FISH is a well-established method, its use, however, is limited due to its high cost, the time-consuming nature of its implementation and other technical requirements, which make the operation and maintenance unfeasible in most pathology labs. With this consideration in mind, one

might reasonably ask whether a cheaper, quicker and simpler method can produce just as reliable results. An important advantage of dual-colour techniques is that their scoring system is practically identical with those of other methods, they are fully automated, do not require any special technical equipment, and produce more information in less time, based on HER2 locus and centromere region stainings and their ratio; last but not least, they are more cost effective compared to FISH. Another benefit is that signal intensity is not affected significantly by prolonged storage at room temperature, which means that it can provide reliable information when a difficult case is reviewed. Concordance of dual-colour techniques is over 90%, an outstanding figure. If found reliable, dual-colour assays could make HER2 status determination in breast cancer more economical, quicker and more effective.

Dual-colour dual-hapten brightfield in situ hybridisation has been optimised for HER2 gene copy number determination, with the data corrected on the basis of chromosome 17 centromere (CEP17) number. In the assay, excitation and emission wavelengths can be detected using the same filter pairs which have been developed for the PathVysion. Under these conditions, the dual-color dual-hapten brightfield in situ hybridisation assay has been found to produce easily assessable, nice and unequivocal signals. The concordance correlation coefficient calculated from HER2 and CEP17 numbers and the HER2/CEP17 ratio showed almost complete agreement between the two tests. Categorised variables in Kappa-statistics also provided similar results. Eight samples failed to produce red signals in assessment. Repeated hybridisation after pre-treatment modifications reduced the number to 6. These samples were excluded from the study. No amplification was found in 75 cases and 24 proved to be positive by DDISH. Of the 99 cases tested, there were 4 whose amplification status was different by the two techniques. DDISH produced false-negativity in 4 cases. Since the ideal of 100% security scanning is not available new

techniques' value is determined in accordance with the "gold standard" PathVysion FISH results. This results indicate that the method DDISH demonstrates high specificity (100%), sensitivity (85.8%), a high positive (100%) and negative predictive value (94.7%). Immunohistochemistry and FISH have been found to produce similar results, therefore it can be claimed that HercepTest is capable of a precision of a similar degree.

The cost-effectiveness of DDISH is that - although the cost of the test is only slightly less compared to FISH - it does not require costly fluorescence microscope, the shorter the incubation time, the process is fully automated. Another advantage is that tissue structure can be examined on the same slide, learning the technique is faster and easier so all these lead to significant working time and human resource savings with its financial related areas (unfortunately that is not yet a decisive aspect in Hungary). Given the archivability, this also means cost savings by not having to repeat the test, in contrast to FISH.

5.1.1. Novel claims in the validation study

Dual-colour dual-hapten brightfield in situ hybridisation (DDISH) is a reliable test method in clinical practice.

- It shows high concordance compared with the conventional FISH technique, in terms of the identification of amplification as well as the determination of the degree of amplification.
- It detects amplification at a rate comparable to those demonstrated by standard assays in immunohistochemically positive and negative breast cancers.
- Compared to FISH, it requires a less complicated technical background.
- Its use can save considerable cost while providing more information on the genetic profile of the tumour.

5.2. Significance of chromosome 17 centromere assessment in HER2 status determination in breast cancer.

HER2 amplification leads to HER2 protein overproduction appearing on the cell surface in 20 to 30% of mammary tumours. HER2 amplification cannot always be detected by FISH in histochemically positive (3+) cases, a phenomenon explained by chromosome 17 polysomy. Genuine gene amplification occurs when the HER2 locus (17q12) on the long arm of chromosome 17 is multiplied. If, however, it is the number of chromosomes 17 that is increasing, giving rise to an increase in HER2 loci, polysomy is present. Such cases are positive immunohistochemically and negative by FISH, and fail to respond to trastuzumab. Distinguishing between polysomy and gene amplification is essential because of the costs and cardiotoxicity involved, as well as for choosing the most effective therapy. Several studies have examined the 17 polysomy phenomenon and its importance with significant difference of the results. In the background is the definition of polysomy in most cases. The question is, from how many CEP17 signals / nucleus shall we talk about polysomy. There are studies in terms of the relatively frequent polysomy 17, detected in 3-46% of breast cancers. During our investigation quantitative definition of polysomy was far from uniform, different studies used different values between 2.1 and 4, and as a result – together with the diversity of criteria- could develop the situation that the incidence of polysomy could vary from such a wide range. In our study, we used a value of 2.5, but it's probably ≥3 CEP17 copy number/nucleus that formed the largest consensus. In fact, the classification criteria used in my work was not affected by the fact that the case proved to be polysomy or not so the results are not altered by a different limit.

According to the literature, polysomy is not associated with gene amplification in the majority of the cases, which suggests that the two genetic phenomena evolve through different mechanisms. Nevertheless, polysomy 17 is associated with poorer prognosis as aneuplodity in chromosome 17 represents a state of genetic instability, which contributes to breast cancer through the accumulation of genetic errors. Indeed, breast cancers with chromosome 17 polysomy have been shown in several studies to be associated with higher nuclear atypia, higher rate of lymphogenic metastasis, increased proliferation index and higher Nottingham Prognostic Index (NPI), leading to lower survival time. Polysomy can be detected by several methods. Today, in situ hybridisation techniques are the most common, which stain the centromeric region of chromosome 17, providing information on the number of chromosomes. There are also so-called dual-colour in situ assays, which stain the HER2 coding section on the long arm of chromosome 17 and the centromeric region of chromosome 17 simultaneously with different colours (red and green, respectively). This makes it possible to test HER2 and CEP17 together and assess their ratio within an individual nucleus. Several recommendations have been published on how to relate the number of signals per nucleus to gene amplification or polysomy. More than 6 signals per nucleus is usually interpreted as gene amplification. Although there is no universally accepted score for polysomy, 4 signals per nucleus or more is generally accepted and used. By this definition, the incidence of polysomy has been found relatively high, accounting for 3 to 46% of breast cancer cases. A pitfall of the method is that co-amplification of the HER2 gene section and chromosome 17 centromere may occur in rare cases, producing high CEP17 and HER2 counts in a dual-colour in situ assay suggesting polysomy, while in reality genuine amplification occurs in both gene sections. In this way, the test may yield false results. To solve the problem, alternative chromosome

17 assays have been designed, such as SMS (Smith-Magenis syndrome, 17p11.2) or RARA (retinoic acid receptor, 17q21.2), which allow safer definition of genetic status.

Perception of polysomy and HER2 status of polysomic cases is a very difficult task. A consensus proposal published in 2014 gives a hand to that. In the event of polysomy (CEP17 signal amplification) - similar to the method we used - takes only HER2 signal number and 6 or above comments positive. Thus, if the CEP17 numbers high, it is not amplified in 4 / nucleus based on the copy number of the HER-2, 6 / nucleus or above considered an amplified HER-2 status. In case of HER-2 numbers between the two values - if it is not already happened - IHC study suggested and classification according to the results. If it is still uncertain - with IHC 2+ - in clinical cases a different tissue block immunohistochemistry testing is suggested. If results are the same then case should be considered negative. For research studies alternative chromosome 17 probes ISH testing (SMS, RARA) or molecular karyotyping is recommended.

In the present study, the HER2 status of mammary tumours was reclassified, with chromosome 17 polysomy taken into account. In the 405 cases studied, routine FISH tests were checked and centromere regions were examined. Amplification was detected by both methods in a total of 143 cases (35.31%) and negativity was registered in 243 cases (60.00%). The tests yielded different results in 19 cases; no amplification had originally been found with HER2 signals over 6 in 5 of them (1.23%) and they were reclassified as HER2-amplified cases; 14 cases (3.46%) originally demonstrating amplification with HER2 signals below 6 were reclassified as HER2-negative. This means that 1.23% of the patients did not receive biological therapy although it would have been justified, while the approved and administered anti-HER2 treatment was probably ineffective in 3.46% of the patients. Our study created the basis for a more precise HER2 status determination, greatly contributing to the success of targeted therapies.

Controversial cases are divided into two groups studied. In 5 cases the initially negative (HER-2 / CEP17 ratio <1.8) status was modified to positive due to HER-2 signal numbers over 6. I managed to collect 4 patients' data. Interestingly, in spite of the intention to treat two patients received Herceptin, in one case 3+ immunohistochemistry was the reason for treatment, this patient is still tumor-free, is the sole alive from this group. In another case, a very high (over 12) HER-2 copy number was the reason for treatment, this patient has died because of a very rapid disease course 14 months later. The 5-year survival was 75% (3/4).

In the other group (14 cases) the initially positive (HER-2 / CEP17 ratio> 2.2) assessment was modified to negative due to HER2 copy number <6. I could collect data of 11 cases. Among them - despite positive HER-2 status - only four patients were treated with Herceptin. In the background we have identified several reasons: early stage tumours (without trastuzumab treatment only additional 3 patients received chemotherapy), patients' age, poor general condition, and the valid financing environment at the time of sampling or surgery (on an individual basis) all contributed to only a proportion of patients receiving anti-HER-2 therapy. We have lost six patients, 3 were free of cancer other diseases led to death. The 5-year survival rate was 82% (9/11), the median survival of nearly 7 years (6 years 10 months).

The above detailed patient information has not yet been published. Of course, the already small number of cases also makes it unsuitable for the data to draw any conclusions, but the heterogeneity of cases and treatments do not justify it. In a large number of cases, prospective, randomized study may give an answer to the question of whether the re-classification - considering the disease-free and overall survival – gives clinically proven advantage to the patients in part the use of effective therapy, partly by avoiding unnecessary therapy side effects.

5.2.1. Novel claims in HER2 status reclassification

Comprehensive chromosome 17 centromere tests have an important role in a more precise determination of the HER2 genetic status.

- For the success of targeted therapy, exclusion of false-positive and false-negative results is essential.
- The study allows identification of false-positive results due to 17 polysomy.
- With the recognition of false-negative results, targeted therapies are approved, giving patients the chance for recovery.

6. Summary

Exploration of the genetic profile of an individual tumour, including determination of its HER2 status, is essential for the successful design and implementation of therapy in breast cancer. The present dissertation deals with techniques used for the determination of HER2 expression, a factor playing a key role in the choice of therapy in breast cancer.

In the first part, the FISH assay is compared with DDISH, a so-called dual colour technique. In the validation study, DDISH has been shown to have high concordance in the statistical analysis, suggesting similar reliability for the two methods. Therefore, it has been concluded that owing to its lower price and less demanding technical background dual-colour dual-hapten in situ hybridisation can be a suitable alternative for laboratories where FISH is not available.

The second part of the paper deals with chromosome 17 polysomy, a genetic phenomenon complicating HER2 status determination in breast cancer. Multiplication of chromosomes 17, with a resulting increase in the number of HER2 coding sections, may yield false-positive results by the FISH test. In the present study, the HER2 status of mammary tumours was reclassified, with the HER2/CEP17 ratio taken into consideration. On the basis of the statistical analysis it can be concluded that the use of CEP17 testing in HER2 determination is justified as the sensitivity of the two techniques is different.

Relevant experiences with HER2 status determination in breast cancer are summarised.

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List of publications related to the dissertation

1. **Kósa, C.**, Kardos, L., Kovács, J., Szöllősi, Z.: Comparison of dual-color dual-hapten brightfield in situ hybridization (DDISH) and fluorescence in situ hybridization in breast cancer HER2 assessment.
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Pathol. Res. Pract. 207 (8), 468-471, 2011.
DOI: http://dx.doi.org/10.1016/j.prp.2011.05.008
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3. Lázár, G., Bursics, A., Farsang, Z., Harsányi, L., **Kósa, C.**, Maráz, R., Mátrai, Z., Paszt, A., Pavlovics, G., Tamás, R.: III. Emlőrák Konszenzus Konferencia: az emlőrák korszerű sebészi kezelése.
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Total IF of journals (all publications): 2,775
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