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Limited human epidermal growth factor receptor 2 discordance in metastatic breast cancer patients treated with trastuzumab, a population based study

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Abstract ***Background:*** Accurate assessment of the human epidermal growth factor receptor 2 (HER2) in breast cancer is essential for proper treatment decisions. HER2 positivity confirmation rates in breast cancer trials by central testing pathology laboratories were reported to be approximately 85%. The aim of our study was to assess in a population based sample concordance of HER2 status in metastatic breast cancer (MBC) patients locally tested HER2 positive and treated with trastuzumab. Moreover cost-effectiveness of *in situ* hybridisation (ISH) in patients with an immunohistochemical score 3+ (IHC3+) was explored.

Methods: MBC patients treated between 2005 and 2009 with trastuzumab-based therapy in North East Netherlands were identified by a survey of hospital pharmacies. Primary tumour samples were retested centrally for HER2 status using 1 immunohistochemical (IHC) method and two methods using ISH on tissue micro-arrays. Potential discordant patients were retested on whole tumour slides. HER2 positivity was defined as: (1) ISH amplification (according to American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) clinical practice Guideline Update) and (2) when ISH failed an IHC score of 3+. Cost-effectiveness was estimated using potential ISH and treatment costs.

Results: HER2 status could be retested in 174 of 194 (90%) patients. The HER2 concordance rate was 87%. The 21 discordant patients were in the 67% due to primary HER2 testing with only IHC. Overall survival of HER2 discordant and concordant patients was not significantly different (18 versus 25 months, $p = 0.131$). Structural ISH in the case of IHC3+ has an estimated potential saving of €87,710 per 100 patients.

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Conclusion: HER2 concordance in a population based study is comparable to those described in selected populations. Discordance is mostly due to testing with only IHC. ISH in the case of IHC3+ is cost-effective.

1. Introduction

Approximately 18–20% of all breast cancers overexpress human epidermal growth factor receptor 2 (HER2) due to *HER2* gene amplification [1]. HER2 influences differentiation, mediation of growth and survival of cells, thereby promoting more aggressive behaviour of tumours. In the era before incorporation of HER2 directed therapy as part of standard of care, these tumours were associated with a more aggressive clinical course compared to HER2 negative disease in both early and advanced breast cancer [2–5]. Trastuzumab, a monoclonal antibody directed against HER2 in combination with chemotherapy versus chemotherapy alone leads to an improved disease-free and overall survival in HER2 positive metastatic breast cancer (MBC) [6–8].

Treatment outcomes in HER2 positive MBC treated with chemotherapy plus trastuzumab were even better compared to HER2 negative MBC [5]. However, trastuzumab can cause cardiotoxicity and is expensive [9]. Therefore, it is essential to have a well validated diagnostic tool to asses HER2 positivity.

HER2 positivity is routinely assessed using immunohistochemistry (IHC) with antibodies directed against HER2 on formalin-fixed, paraffin embedded (FFPE) specimens. Cell surface HER2 protein levels are measured semiquantitatively and scored from 0–3. In the registration trials of trastuzumab in MBC, HER2 positivity was defined as IHC scores of 2+ or 3+ [6].

Subsequently *in situ* hybridisation (ISH) testing methods to determine HER2 amplification have been introduced as IHC was felt to be influenced by many (pre-)analytical factors such as choice and dilution of the HER2 antibody, cold ischaemia and fixation times of the specimens and to inter-observer variation [10–13]. *HER2* gene amplification, which highly correlated with overexpression of the HER2 protein, was introduced as an obligatory additional method in case of a HER2 IHC score of 2+. HER2 was also considered positive in the case of HER2 amplification regardless of IHC. IHC score of 3+ remained a method to consider a patient HER2 positive without *in situ* hybridisation assays [14].

However HER2 concordance rates have so far only been studied in selected populations participating in clinical trials and may not reflect routine clinical practice. We have therefore conducted a retrospective population based study of patients diagnosed and treated with trastuzumab containing therapy in routine clinical practice with HER2 positive MBC. The aim of our study is to assess HER2 discordance in routine clinical practice between the historical positive HER2 status determined at the primary treatment sites compared to a state of the art HER2 assessment during central pathology review. Moreover we analysed overall survival (OS) of patients tested positive (HER2 concordant) during central testing and the patients tested negative (HER2 discordant). In addition we explored cost-effectiveness of ISH used as a confirmatory test in all patients with an IHC score of 3+ to avoid unnecessary trastuzumab treatment.

2. Materials and methods

2.1. Patient selection and breast tumour samples

We identified patients treated between 2005 and 2009 with trastuzumab for HER2 positive MBC in North East Netherlands. This region contains 3.3 million residents and 23 hospitals. All hospital pharmacies were informed and asked for their participation. A total of 19 hospitals including the one university hospital did provide data. The participating hospital pharmacies selected the patients treated with trastuzumab for HER2 positive MBC in their own registries. Patients who had metastatic disease at breast cancer diagnosis were cross-checked with the population-based data from the nationwide Netherlands Cancer Registry (NCR) which is maintained and hosted by the Comprehensive Cancer Centre the Netherlands (IKNL). Data on patient and tumour characteristics and time of death were retrieved from the NCR. The NCR contains data on patient and tumour characteristics, stage and treatment of all newly diagnosed malignancies [15]. Subsequently, more specific data about patient, disease and survival characteristics and treatment with trastuzumab were collected by the specially trained registration clerks of the NCR. Retrieved data included the particular pathology laboratory and the accession numbers of relevant tissues. The data collection was anonymised for all participating parties except for members of IKNL to ensure patient privacy. Patients who were treated for previous non-metastatic HER2+ breast cancer with trastuzumab in the adjuvant setting were excluded.

2.2. HER2 status reassessment

All 10 pathology laboratories were requested to provide original pathology reports of resections and/or core biopsies of primary breast cancers including the report