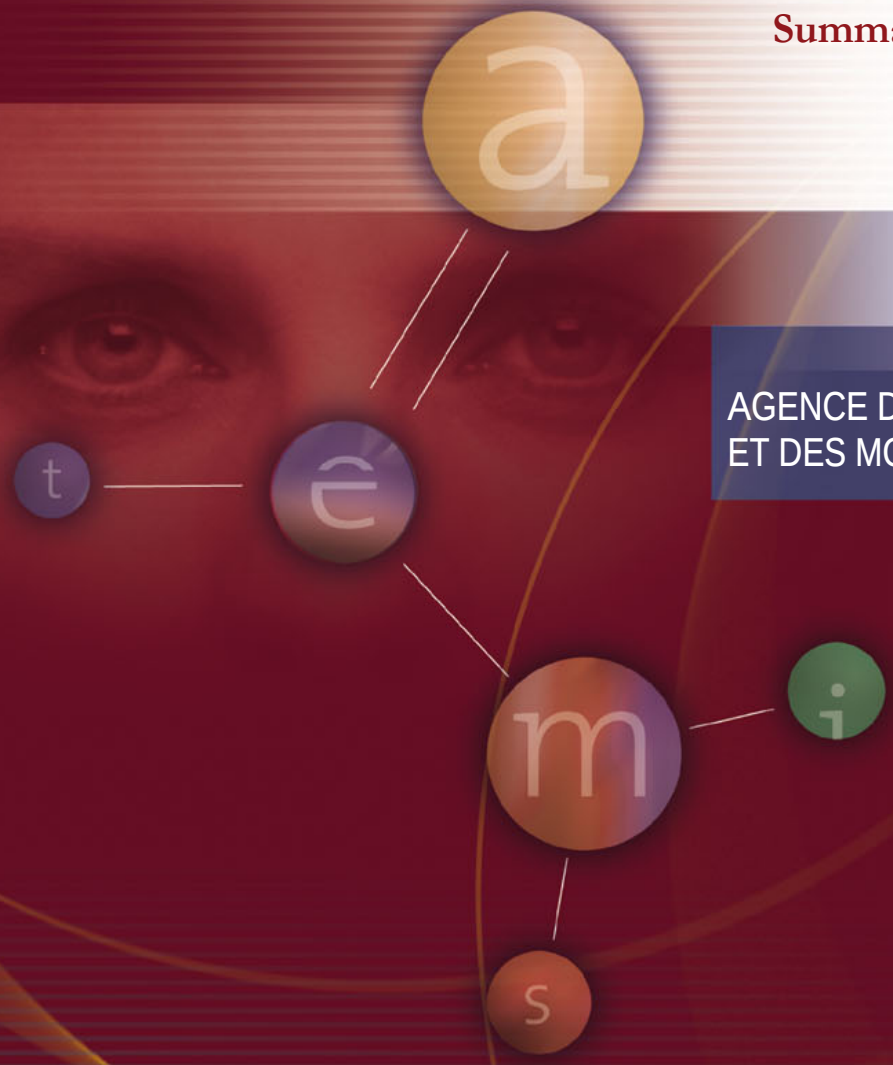
Diagnostic Performance of Techniques Used for HER-2 Testing in Breast Cancer

Summary



AGENCE D'ÉVALUATION DES TECHNOLOGIES
ET DES MODES D'INTERVENTION EN SANTÉ

Québec

Diagnostic Performance of Techniques Used for HER-2 Testing in Breast Cancer

Summary

Report prepared for AETMIS by

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20 ans AVEC VOUS POUR LA SANTÉ

Agence d'évaluation des technologies et des modes d'intervention en santé
Québec

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Mission

The mission of the Agence d'évaluation des technologies et des modes d'intervention en santé (AETMIS) is to help improve the Québec health-care system. To this end, it advises and supports the Minister of Health and Social Services and decision-makers in the health-care system with regard to the assessment of health services and technologies. The Agency makes recommendations based on scientific reports assessing the introduction, diffusion and use of health technologies, including technical aids for the disabled, as well as the methods of providing and organizing services. The assessments examine many different factors, such as efficacy, safety and efficiency, as well as ethical, social, organizational and economic issues.

Preface

In 2007, an estimated 5900 women were diagnosed with breast cancer in Québec. Despite a decrease in the breast cancer mortality rate in all age groups, breast cancer remains the second leading cause of death from cancer in women and the leading cause of death among those under the age of 50. Approximately 1400 women died of breast cancer in 2007.

About 18 to 20% of breast cancer patients have a poor prognosis associated with the biomarker HER-2. The HER-2 protein, human epidermal growth factor receptor 2, can be found in excessive numbers on the surface of tumour cells, thus promoting their proliferation. These patients, referred to as "HER-2-positive", are observed, among other things, to have an increased risk of postsurgical recurrence and reduced survival.

Trastuzumab (Herceptin®), a drug specifically developed to inhibit the activity of the HER-2 receptor on the surface of tumour cells, is able to significantly improve the prognosis in HER-2-positive women. However, it is very expensive (approximately C$45,000 per patient) and carries a risk of cardiotoxicity.

Initially approved for the treatment of metastatic stages, trastuzumab is now indicated for the adjuvant treatment of early invasive breast cancer. To ascertain if patients are likely to benefit from the drug, it is now the standard of practice to determine the HER-2 status of all invasive breast cancers at the time of diagnosis.

Given the clinical and economic impact of administering trastuzumab, a patient's HER-2 status should be determined as accurately as possible, minimizing false-positive and false-negative results.

To this end, there are several different diagnostic tests, each with its advantages and drawbacks. As well, the tests and utilization algorithms recommended by the various international organizations differ from country to country. The Comité de l'évolution des pratiques en oncologie (CEPO) therefore asked AETMIS to carry out a comparative assessment of the methods for determining the HER-2 status of a tumour.

This report is a systematic review of the diagnostic performance of techniques used for HER-2 testing in breast cancer. The conclusions and recommendations may serve as a basis for the CEPO to develop clinical practice guidelines for testing and for the Direction de la lutte contre le cancer to organize services provided by pathology laboratories.

Juan Roberto Iglesias, M.D., M.Sc.
President and Chief Executive Officer

Executive summary

The standard of practice now consists in determining the HER-2 status of all women with invasive breast cancer so as to evaluate their eligibility for trastuzumab (Herceptin®) therapy. This assessment shows that the initial diagnostic test generally used in the province and country, immunohistochemistry (IHC), requires a high level of expertise on the part of the laboratories that perform it and the pathologists who interpret its results. Furthermore, the different types of *in situ* hybridization (ISH) tests, some of which are presently used in Québec to confirm equivocal IHC results, are not always equivalent, in terms of accuracy, to the reference test, fluorescence *in situ* hybridization (FISH). Moreover, internal and external laboratory quality assurance is absolutely essential, and in this context, preference should be given to central or reference laboratories. In light of these considerations, AETMIS recommends:

1) that the authorities concerned implement an HER-2 testing quality assurance program in Québec and, as part of this program:
   - that at least one reference laboratory be designated;
   - that this or these reference laboratories develop and apply internal quality assurance measures;
   - that this or these laboratories' results be periodically checked against those of other reference laboratories, provincial and Canadian or foreign;
   - that all laboratories that perform the tests in question be affiliated with a reference laboratory and be required to participate in the external quality assurance program to be established;
   - that the methods used by all the laboratories that perform the tests in question be validated initially and periodically and that these laboratories be required to meet the quality requirements set out by the reference laboratory or laboratories;
   - that the laboratories be required to meet the minimum number of cases to be tested annually recommended by Canadian standards, namely, 250 for IHC and 100 (ideally 200) for ISH tests, with a limit on the number of pathologists responsible for interpreting them, so as to maintain their expertise;
   - that the pathologists and the laboratory personnel involved receive ongoing training;
   - that the samples which hospitals are to submit to the laboratories that perform the tests in question be prepared in accordance with the best practices;
   - that the feasibility of setting up a central database containing the results of these tests and other relevant data for all patients with invasive breast cancer be assessed;
2) that the Canadian recommendations concerning the use of IHC and FISH tests be followed by the laboratories, in particular, the use of IHC initially, followed by FISH (or other validated ISH tests) in cases of equivocal results;
3) that FISH be the initial test performed when the quality of the sample received by the laboratory is questionable;
4) that chromogenic *in situ* hybridization (CISH) be performed with two probes to confirm an equivocal IHC result, one for the HER-2 gene, the other for the chromosome 17 centromere;
5) that the diagnostic performance of silver-enhanced *in situ* hybridization be the subject of a literature watch until the evidence confirms the validity of this technique.

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CONFLICT OF INTEREST

None declared.

Summary

Introduction

An estimated 5900 women in Québec were diagnosed with breast cancer in 2007. Approximately 18 to 20% of them have a poor prognosis associated with the biomarker HER-2. In this subgroup of patients, tumour cells express, on their surface, excessive numbers of a membrane growth factor receptor, the protein HER-2, which promotes tumour proliferation. This results in, among other things, an increased risk of postsurgical recurrence and a decreased tumour response to conventional chemotherapy.

Trastuzumab (Herceptin®), a drug that targets the HER-2 protein, may significantly improve these patients' prognosis. However, this therapy is very expensive and carries a risk of serious adverse effects. Its administration must therefore be strictly reserved for patients with invasive cancer that overexpresses the HER-2 protein, the only ones likely to derive benefit from it.

The standard of practice now consists in determining the HER-2 status at diagnosis for all invasive breast cancers. Because of the extent of the clinical and economic impact of administering trastuzumab, HER-2 status must be determined as accurately as possible. Several diagnostic tests are currently available, each with its advantages and drawbacks. The Comité de l'évolution des pratiques en oncologie (CEPO) therefore asked AETMIS to carry out a comparative assessment of the techniques for determining HER-2 status in breast cancer. This assessment could be used as a basis for the CEPO to develop clinical practice guidelines and for the Direction de la lutte contre le cancer to organize services provided by pathology laboratories.

Techniques for determining HER-2 status

Immunohistochemistry (IHC) is an inexpensive and rapid technique that is readily available in most pathology laboratories. It uses antibodies and an enzyme reaction to determine the level of HER-2 protein expression on the surface of tumour cells. The stain produced by the reaction is interpreted semiquantitatively on a scale of 0 to 3+, a score of 3+ indicating HER-2 protein overexpression.

Other indirect methods, such as *in situ* hybridization (ISH), are used instead to detect amplification of the gene that encodes the HER-2 protein. Amplification is an increase in the gene copy number per chromosome and appears to be the mechanism most often responsible for the overexpression of this protein. Consequently, a tumour with gene amplification is also considered HER-2 positive.

Fluorescence *in situ* hybridization (FISH) is the most widely known type of ISH. It uses fluorochrome-labelled DNA probes that bind specifically to the gene copies and make it possible to count them. FISH is more time-consuming and requires expensive equipment that is available in only a few pathology laboratories. FISH seems to be more reproducible than IHC.

Usually, FISH is performed with two different probes, one specific to the HER-2 gene, the other to the chromosome 17 centromere (CEP17). Since an increase in the HER-2 gene copy number per cell attributable to polysomy 17 does not constitute true amplification, the simultaneous use of both probes permits this correction.

Chromogenic *in situ* hybridization (CISH) is a type of ISH that uses a chromogenic reaction to reveal the DNA probes hybridized to the gene locus. It has a considerable advantage over FISH in that it does not require expensive, specialized equipment. However, the two probes, HER-2 and CEP17, cannot be used simultaneously and require two separate hybridization reactions. In practice, it is not unusual for the HER-2 probe to be used alone in CISH.

Silver-enhanced *in situ* hybridization (SISH) is a new ISH technique that uses the precipitation of silver ions to locate hybridized DNA probes. This technique has been adapted for automated slide preparation and to permit a correction for polysomy 17.

Lastly, real-time PCR and RT-PCR are molecular biology techniques that can be used to quantify tumour cell DNA or messenger RNA (mRNA) content specific to a given gene sequence (HER-2 versus a reference gene). Thus far, they have been used mainly in research.

The recommended techniques and algorithms for determining HER-2 status differ from country to country. In the United States, either FISH or IHC is recommended as the initial test. In Canada, IHC is recommended as the initial test, followed by one of the three ISH tests (FISH, CISH or SISH) to confirm an equivocal result. In Europe, IHC, CISH or FISH is recommended as the initial test.

Research method

This report is a systematic review of the literature on the diagnostic performance of IHC, CISH, SISH and real-time PCR and RT-PCR in breast cancer. The gold standard used for the analysis is the FISH which utilizes both the HER-2 and CEP17 probes. The distribution of IHC scores in breast cancer patients is estimated, and the concordance between FISH and CISH results for each IHC score (0, 1+, 2+ and 3+) is presented. The interobserver and interlaboratory variability for each technique is evaluated as well. This report includes an analysis of studies published from January 2000 up to and including November 2007.

Results

Nearly three fourths (approximately 73%) of breast cancer patients test negative on IHC (a score of 0 or 1+), and the vast majority (97.4%) of them can be confirmed by FISH. As for patients who test positive on IHC (a score of 3+), only 89.9% test positive on FISH. Thus, it can be said that 10.1% of these cases are discordant. However, the concordance between IHC and FISH results is better when the tests are performed at central or reference laboratories as opposed to local laboratories.

The diagnostic performance of CISH is very good, but only when the HER-2 and CEP17 probes are used in a complementary manner. Otherwise, both the sensitivity and specificity of this test fall below 95%. For cases with an equivocal IHC result (a score of 2+), the CISH result varies and is thus less concordant with the FISH result.

Only two studies comparing SISH and FISH were identified, one of which took into account the correction for polysomy 17. The use of two probes (HER-2 and CEP17) seems to improve the performance of SISH, but its sensitivity nonetheless remains low.

Real-time PCR and RT-PCR have good specificity but have difficulty detecting FISH-positive cases. Selecting the tumour cells to be analyzed by laser microdissection seems to increase the accuracy of PCR, but the evidence in this assessment is based on only one study.

The level of agreement among pathologists in interpreting IHC test results varies considerably from study to study and is not always satisfactory, given the issues involved. Although less studied, the interobserver variability in FISH, CISH and SISH seems to be very good.

As for the tests' interlaboratory reproducibility, it is low to average in the case of IHC and seems to be better between laboratories that use the same antibodies. Several studies have reported, for local laboratories, high proportions (18.4 to 36.4%) of positive IHC results (a score of 3+) that subsequently turned out to be negative when the cases were retested with IHC at a central or reference laboratory.

The concordance of FISH results is good between local and central laboratories and excellent between central laboratories. Results for the interlaboratory reproducibility of CISH are scarcer but seem to be good, whereas those for SISH and real-time PCR and RT-PCR are not yet available.

Conclusions

The use of IHC as the initial test seems to be a good strategy for rapidly and less expensively identifying patients who are not likely to benefit from trastuzumab therapy. On the one hand, they account for the vast majority of breast cancer cases, and on the other, negative IHC results tally well with FISH results.

However, many IHC-positive cases (3+) turn out to be negative on FISH. Given the variability of IHC results, some of these cases could be false positives due to technical difficulties, problems calibrating the method, or interpretation problems. However, their proportion out of the total number of discordant cases is not known because certain biological mechanisms can also lead to the overexpression of a given protein without there being any gene amplification. At this time, the response to trastuzumab in discordant cases still appears to be uncertain.

However, since the treatment targets the protein, the Canadian consensus guidelines automatically consider eligible for trastuzumab therapy cases that test positive on IHC performed on a good-quality sample at laboratories with recognized expertise that have internal and external quality assurance measures.

To improve diagnostic accuracy, several laboratories in the province perform, for each patient, not one, but two IHC tests with antibodies of different sensitivities. They then perform ISH on all discordant or equivocal cases. Even if it seems reasonable to think that this algorithm can improve test accuracy, there is still little evidence to support this.

Whatever the case, this strategy of performing two rather than one IHC test should not make up for the absence, in Québec, of a formal external quality assurance program specific to these tests or for the insufficient number of IHC tests performed by some Québec laboratories. Indeed, it appears that several laboratories do not perform tests on the minimum annual case load stipulated in the Canadian standards to ensure the desired level of competence.

The Canadian consensus guidelines recognize CISH as an alternative to FISH, but this technique, when performed with an HER-2 probe alone, does not appear to be reliable enough to replace FISH. In Québec, CISH is performed without a chromosome 17 centromere probe, with the result that a certain number of these tests have to be repeated with FISH to confirm the diagnostic accuracy.

Furthermore, the Canadian recommendation of using SISH as a possible alternative to FISH seems to be supported more by expert opinions than evidence. The diagnostic

performance reported in the literature is clearly inadequate. Just the same, this technique is in the process of being introduced in Québec.

None of the evidence presented in this report supports real-time PCR or RT-PCR as an alternative to FISH in a clinical setting.

Recommendations

In light of these considerations, AETMIS recommends:

1) that the authorities concerned implement an HER-2 testing quality assurance program in Québec and, as part of this program:
   - that at least one reference laboratory be designated;
   - that this or these reference laboratories develop and apply internal quality assurance measures;
   - that this or these laboratories' results be periodically checked against those of other reference laboratories, provincial and Canadian or foreign;
   - that all laboratories that perform the tests in question be affiliated with a reference laboratory and be required to participate in the external quality assurance program to be established;
   - that the methods used by all the laboratories that perform the tests in question be validated initially and periodically and that these laboratories be required to meet the quality requirements set out by the reference laboratory or laboratories;
   - that the laboratories be required to meet the minimum number of cases to be tested annually recommended by Canadian standards, namely, 250 for immunohistochemistry (IHC) and 100 (ideally 200) for *in situ* hybridization (ISH) tests, with a limit on the number of pathologists responsible for interpreting them, so as to maintain their expertise;
   - that the pathologists and the laboratory personnel involved receive ongoing training;
   - that the samples which hospitals are to submit to the laboratories that perform the tests in question be prepared in accordance with the best practices;
   - that the feasibility of setting up a central database containing the results of these tests and other relevant data for all patients with invasive breast cancer be assessed;
2) that the Canadian recommendations concerning the use of IHC and fluorescence *in situ* hybridization (FISH) tests be followed by the laboratories, in particular, the use of IHC initially, followed by FISH (or other validated ISH tests) in cases of equivocal results;
3) that FISH be the initial test performed when the quality of the sample received by the laboratory is questionable;
4) that chromogenic *in situ* hybridization (CISH) be performed with two probes to confirm an equivocal IHC result, one for the HER-2 gene, the other for the chromosome 17 centromere;
5) that the diagnostic performance of silver-enhanced *in situ* hybridization be the subject of a literature watch until the evidence confirms the validity of this technique.

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