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MammaPrint

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DISCLAIMER

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EXECUTIVE SUMMARY

MammaPrint is an in vitro prognostic test which is able to measure the activity of 70 genes. The MammaPrint is the first commercially available product in a new category of multivariate in vitro prognostic tests. MammaPrint is reported to add information (rather than replace) the current clinical, histological, laboratory and prognostic assessment. It claims that it can be used to predict subsequent behaviour of breast cancer and specifically individualizing ongoing treatment. MammaPrint claims that it could give better prediction of cancer behaviour compared to other techniques.[1] MammaPrint is CE marked and it also has the FDA Approval by the FDA (February 2007). At present MammaPrint is only available at UK to private patients.

The review on this technology showed that MammaPrint may be used as a diagnostic tool. This review also revealed that there was lack of evidence to support the clinical utility of MammaPrint assay for any subset of breast cancer patients. The systematic reviews retrieved currently indicated that there was no evidence to support the benefit of this test to predict chemotherapy benefit and improvement in clinical outcomes by the gene expression profiling test. The underway RCT trials might be useful to determine the benefit of clinical utility of MammaPrint and Oncotype DX for the breast cancer patients. However, some limitation was listed on microarray assay such as instability of gene lists, overoptimistic performance indicators and inadequate validation.

Cost effective study by Agency For Health Care Research and Quality (AHRQ) , which showed inconclusive economic outcomes for all the gene profiling tests. There was one study which showed the direct cost of MammaPrint which was estimated about $ 3,500 per test. Hence, the cost per test for Mammaprint assay is costly.

There are currently two ongoing RCTs such as TAILOR (Trial Assigning Individualized Options for Treatment) and MINDACT (Microarray for Node- negative Disease may Avoid Chemotherapy) trial that could provide significant answers about the clinical value of the multigene predictors of this microarray technology. Hence, it is suggested that this technology should not be adopted currently until the findings of the above two clinical trials have been published. More quality evidence is warranted to support the effectiveness and cost effectiveness of MammaPrint as a diagnostic and prognostic tool for patients with breast cancer.

MAMMAPRINT

1. INTRODUCTION

Breast cancer is the commonest cancer among Malaysian women in all ethnic groups of Malays, Chinese , Indians and others. The National Cancer Registry reported that breast cancer is the leading of cancer death among women in Malaysia**.** Data from the National Cancer Registry of Malaysia for 2004 provide an age-standardised incidence rate (ASR) of 46.2 per 100,000 women. This means that approximately 1 in 20 women in the country develop breast cancer in their lifetime. However, the rate differs between the three main races, the Malays, Chinese and Indians. The age standardised incidence in Chinese is the highest, with 59.7 per 100,000, followed by the Indians at 55.8 per 100,000. The Malays have the lowest incidence of 33.9 per 100,000. This translates into 1 in 16 Chinese, 1 in 16 Indian and 1 in 28 Malay women developing breast cancer at some stage in their lives. The commonest age at presentation is between 40-49 years, with just over 50% of the cases under the age of 50 years, 16.8% below 40, and 2% under 30. Some 55.7% of all cases were found to be estrogen-receptor-positive (ER positive) . Hence, prognostic tests are very important to predict the outcome of breast cancer patients. [1]

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MammaPrint is currently being used as a prognostic assay for breast cancer. There are three types of prognostic breast cancer tests are available at the market. Those are the Oncotype DX (Genomic Health, Redwood City, California), MammaPrint (Agendia BV, Amsterdam, the Netherlands), and H/I (AvariaDX, Carlsbad, California). These tests are based on gene expression that is currently introduced to the clinical application. Oncotype DX is based on a 21 – gene profile developed by Paik and colleagues [2] (MammaPrint is based on a 2- gene signature (*HOXB13-IL17BR)* developed by Ma and colleagues[3] The gene sets on which these tests are based have minimal overlap. The 21 – gene and 71 – gene expression signatures. Two technologies are used to determine gene expression: real time RT- PCR (Oncotype DX and H/I) and DNA microarray (MammaPrint).

All the tests used pathologic review of specimens to check for tumor content and evaluate the RNA preparation. Fresh unfixed tumor tissue is required for MammaPrint whereas the other tests need to use formalin-fixed in paraffin embedded tumor tissues.[4] This technology review mainly focused on MammaPrint test, using the microarray technology for the clinical utility of breast cancer patients.

This technology review was requested by the development group of the Clinical Practice Guidelines on Management of Breast Cancer , in view of recommending MammaPrint as a diagnostic and prognostic tool in the management of breast cancer patients.

2. OBJECTIVE

The objective of this review is to determine the effectiveness and cost effectiveness of MammaPrint as a diagnostic or prognostic assay for breast cancer.

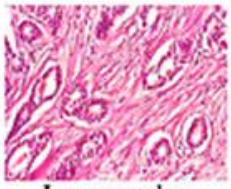
3. TECHNICAL FEATURES

MammaPrint

The MammaPrint assay was the first fully commercialized microarray-based multigene assay for breast cancer. This test is currently designed as a pure prognostic assay and has received 510(k) clearance from the FDA, and is offered as a prognostic test for women under the age of 61 with either ER-positive or ER-negative, lymph node–negative breast cancer. The test was also the first assay to be approved by the FDA's, as a in vitro diagnostic multivariate index assay classification. The test is not yet marketed in the U.S. The test was originally developed at the Netherlands Cancer Institute in Amsterdam as a single site using stored frozen samples from breast cancer patients under the age of 53 years and using the Rosetta Inpharmatics DNA microarray system (Merck and Co., Whitehouse Station, NJ) and then commercialized on the Agilent microarray platform (Agilent Technologies, Wilmington, DE).[5]

3.1 MammaPrint (Microarray technology)

The MammaPrint test looks at the expression of 70 genes linked to breast cancer. It is believed that the 70 genes show a different expression pattern in correlation to the aggressiveness of a tumour. The technique of gene expression profiling or 'genomic profiling' allows the comparison of RNA expression levels of all or a subset of genes in different types of cells or tissues. Seventy essential genes can show different expression pattern in correlation to the aggressiveness of the tumour.



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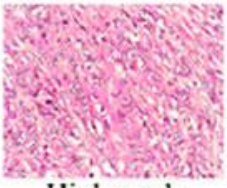
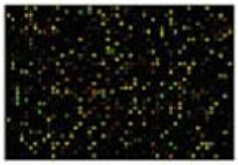


High grade

MammaPrint®



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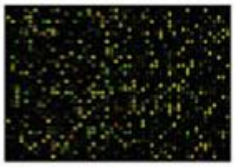

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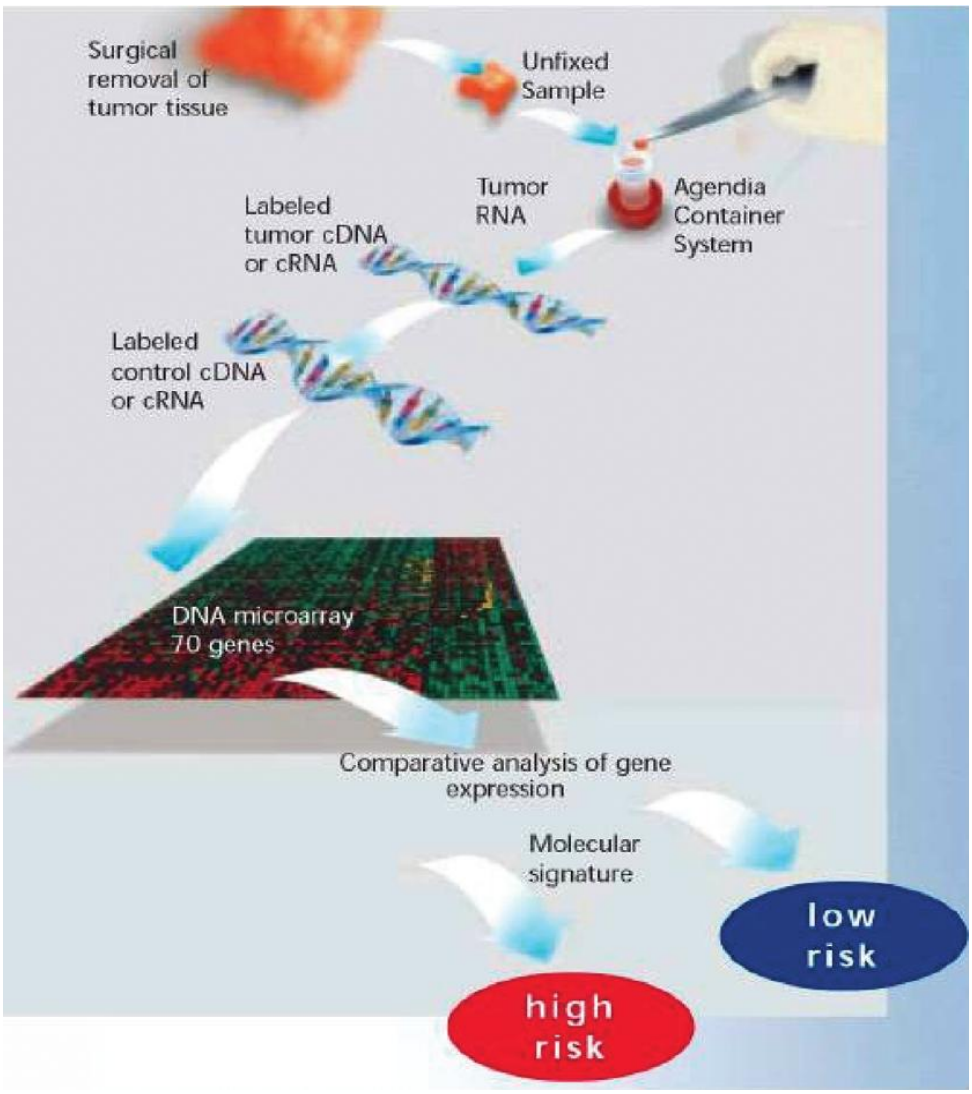

3.2 Microarrays exploit the preferential binding of complementary nucleic acid sequences. A microarray is typically a glass slide onto which DNA molecules are attached at fixed locations (spots or features). There may be tens of thousands of spots on an array, each containing a huge number of identical DNA molecules (or fragments of identical molecules) of lengths from 20 to hundreds of nucleotides.

3.2.1 Sample collection

Fresh sample such as breast tissue is taken and send for further analysis. The RNA is extracted at the laboratory and quality controlled. Isolated RNA is treated with DNAse and translated into cDNA and then into cRNA. Tumour cRNA is fluorescently labeled using Cy5, whereas reference cRNA is fluorescently labeled using Cy3. After purification, hybridization of cRNAs of tumour and of reference samples to the MammaPrint microarray takes place.

Microarray results are scanned and data analysis is performed according to a specific MammaPrint algorithm. The correlation of an expression profile of a tumor sample to a reference is calculated. The molecular signature of the tumour sample is determined as low risk or high risk.

3.2.2 Interpreting the results

MammaPrint test results indicate either a "high risk" or "low risk" of cancerous spread. "High risk" indicates a chance of 50% that patient will experience metastasis (regrowth of tumor) within 10 years. "Low risk" means chances of developing metastasis within 10 years is 10 % - 15%.

3.3 Other Competing Technology

3.3.1 Onco*type* DX assay

The Onco*type* DX assay is a validated genomic test that predicts the likelihood of breast cancer recurrence, the likelihood of patient survival within 10 years of diagnosis and the likelihood of chemotherapy benefit in early-stage, node-negative, ER-positive breast cancer. The Onco*type* DX assay uses a reverse-transcriptase (RT) polymerase chain reaction (PCR) process to quantify the expression of specific mRNA for 16 cancer genes and 5 reference genes in paraffin samples obtained from a breast cancer biopsy, combining the expression results into a single score called the Recurrence Score® result.[6]

3.3.2 Immunohistochemeistry (IHC)

IHC has been used as the platform for multigene predictors in breast cancer, combining a series of antibodies with some form of digital image analysis slide scoring. Having the advantage of using a morphology-driven signal and thus not requiring tissue microdissection, IHC is nonetheless exposed to preanalytic tissue processing and antigen retrieval variables that can significantly impact the test results.[6] IHC is limited in the number of markers that can be used whether fluorescent or bright field signal development procedures are used, but this "limitation" also allows for a less complex statistical algorithm required for data analysis, reducing the likelihood of false biomarker discovery.[6]

3.3.3 Flourescence in situ hybridization (FISH)

The FISH method has been primarily used for determining the copy number of the human epidermal growth factor receptor 2 (*HER-2)* gene for purposes of selecting HER-2–targeted therapies such as trastuzumab and lapatinib. Testing for *HER-2* gene amplification using the FISH technique has now been widely used in prospective clinical trials evaluating HER-2–targeted therapies in both the adjuvant and neoadjuvant settings in both the U.S. and Europe. Although FISH technology has been used to measure a number of other prognostic factors, including chromosomal aneusomies and amplifications of cell proliferation–associated genes, until recently these assays were not formally commercialized and have not been widely used in clinical studies. In 2007, a

three-color FISH assay was commercialized to assess stand-alone prognosis in ER-positive and ER-negative stage I breast cancers. [7] Level II -3

3.3.4 Real- time Polymerase Chain Reaction (RT-PCR)

RT-PCR procedures designed to predict outcome in breast cancer can be performed on either fresh or formalin-fixed paraffin-embedded tissues from breast cancer (FFPE) samples. The heterogeneous expression of important mRNAs, such as ER, HER-2, and Ki-67, often reflected in the varying histologic grades seen in larger tumors can influence the predictive accuracy of transcriptional profiling measurements. Although the number of genes that can be simultaneously assessed by multiplex qRT-PCR is significantly greater than that for IHC, this requires a more complex statistical evaluation of the gene-expression profiles. However, RT-PCR does allow multiple biologic processes to be assessed simultaneously, including proliferation, hormone receptor, and HER-2 pathways. The RT-PCR technique has been used to predict overall prognosis and response to both hormonal and cytotoxic therapies [7] Level II -3

4. Methodology

4.1. Searching

Electronic databases were searched, which included Pubmed, Medline, CINAHL, and Cochrane database of systematic reviews, HTA Databases, Horizon scanning databases (CADTH, ASERNIP-S, Defra, Euroscan), FDA website and Google for relevant articles. Additional articles were identified from reviewing the bibliographies of retrieved articles. This review mainly focused on studies published after 2007 until up to 2008.

The search strategy used the terms, which are either used singly or in various combinations: Mamaprint, "MammaPrint AND Breast cancer", microarray AND breast cancer AND prognostic "effectiveness OR efficacy, "cost effectiveness" and "cost analysis".

4.2. Selection

All articles published and unpublished related to effectiveness and cost effectiveness of MammaPrint were selected. Critical appraisal of relevant literature was performed and evidence graded according to US/Canadian Preventive Services Task Force (Appendix 1)

5. RESULTS AND DISCUSSION

The search strategies yielded one HTA report and one systematic review regarding multigene predictors of clinical outcome for breast cancer. Another systematic review was on the gene expression profiling assays in early – stage of breast cancer. Besides that other relevant laboratory experiments were also included.

5.1. Effectiveness

5.1.1. MammaPrint (MicroArray assay) as a Diagnostic tool

Currently in the market there are several techniques available for breast cancer gene expression assays. In a Health Technology Assessment (HTA) report by Agency for Healthcare Research and Quality, 2008 (AHRQ) showed the impact of Gene Expression Profiling Tests on breast cancer outcomes. In this HTA report three gene expression assays namely Oncotype DX ^{TM}, MammaPrint and Breast Cancer Profiling (BCP or H/I ratio test) were compared.

Evidence on analytical validity and variability for MammaPrint were obtained from two diagnostic studies which showed success rate of 80.9 %. The HTA report revealed that MammaPrint could be used as a reliable diagnostic tool.[7 Level 1I-3]

The specificity and sensitivity of the MammaPrint assay and the Adjuvant Online (a software model that predicts the benefit of adjuvant therapy for women with early-stage breast cancer) were compared for distant metastases within 5 years and for death within 10 years. The study found that similar sensitivities but higher specificity was demonstrated for MammaPrint. The areas under the receiver operating characteristic (ROC) curves were comparable between MammaPrint and Adjuvant online (0.68 vs. 0.66) for distant metastases at 5 years. However, this study predicted in the context of no adjuvant hormonal or chemotherapy treatment. Thus its applicability to women over 60 years old and treated with tamoxifen is unknown. [7 Level 11-3]

In addition, MammaPrint may allow young patients (<61 years) with early-stage breast cancer to be categorised as having a high or low risk of distant metastasis. High-risk patients may then be managed with more aggressive therapy. [8 Level III]

5.1.2 MamaPrint (Microarray assay) as a Prognostic Tool

The findings of a Health Technology Assessment report revealed that MammaPrint had no published studies evaluated on the ability of the 70 genes signature to predict chemotherapy benefit. [7 Level 11-3] Similarly another narrative review reported that microarray based prognostic test for breast cancer in terms of clinical utility might never be formally established. [12 level III] Amongst the limitations mentioned on microarray prognostic signature were instability of gene lists, overoptimistic performance indicators and inadequate validation.

In another systematic review conducted by Marchioni l et al [11 Level II- 3,] revealed that MammaPrint test was validated in a multicenter Eropean study of 302 patients not treated with chemotherapy or tomoxifen. It provided prognostic information beyond the Ajuvant Online (a software model that predicts the benefit of adjuvant therapy for women with early-stage breast cancer). MammaPrint provided better reclassification of patients in risk groups. Hazard ratio (HR) estimated between high and low -risk categories for distant recurrence **(**Metastatic Breast Cancer) in Van de Vijver et al [9, Level 11-3] study was substantially higher compared to another validation study by Buyse et al[10, Level II-3](unadjusted Hazard ratio (HR) > 15 vs. 2.3, respectively ; adjusted HR, 4.6 vs. 2.1) . Buyse et al, study had conducted validation cohort that observed for a longer period (median, 13.6 vs 6.7 years) which included older women and excluded patients who received adjuvant therapy. The HRs for all end points decreases steadily with an artificial increase in censoring time from 5 to 10 years. [11 Level II- 3,]

5.3. COST EFFECTIVENESS

The cost-effectiveness of the Netherlands Cancer Institute gene expression profiling (GEP) assay (MammaPrint) was compared to the U.S. National Institutes of Health (NIH) guidelines for identification of early breast cancer patients who would benefit from adjuvant chemotherapy. The GEP assay was projected to yield a poorer quality-adjusted survival than the NIH guidelines (9.68 vs. 10.08 QALYs) and lower total costs ($29,754 vs. $32,636). In order to improve quality-adjusted survival, the GEP assay would need to have a sensitivity of at least 95 percent for detecting high risk patients while also having a specificity of at least 51 percent. [8 Level II-3]

C***ost***

The cost anticipated for Microarray assay such as Mammaprint and RT-PCT was about $ 3,500 per test. [7 Level II-3]

6. CONCLUSION

6.1. EFFECTIVENESS

MammaPrint may be used as a diagnostic tool. There was lack of evidence to support the clinical utility and clinical validity of MammaPrint assay as a prognostic tool for any subset of breast cancer patients. The systematic reviews retrieved currently indicated that there was no evidence to support the benefit of this test to predict chemotherapy benefit and improvement in clinical outcomes by using the gene expression profiling test. However the underway RCT trials might be useful to determine the benefit of clinical utility of MammaPrint and Oncotype DX for the breast cancer patients. One must be cautioned that there were some limitations listed on microarray assay such as instability of gene lists, overoptimistic performance indicators and inadequate validation. [8] Level 11-3

6.2. COST EFFECTIVENESS

Cost effective study by Agency for Healthcare Research and Quality (AHRQ) report showed inconclusive economic outcomes for all the gene profiling tests. The direct cost per test for MammaPrint was more costly compared to other technology such as ONCOTYPE DX , IHC and FISH assays.

7. RECOMMENDATION

There are currently two ongoing RCTs such as TAILORx (Trial assigning individualized Options for Treatment Rx) and MINDACT trial (Microarray for node- negative Disease may Avoid Chemotherapy) that could provide significant answers about the clinical value of the multigene predictors of the microarray technology. Hence, it is suggested that this technology should not be adopted currently until more quality evidence is present to support its effectiveness and cost-effectiveness.

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9. APPENDIX

9.1 Appendix 1

DESIGNATION OF LEVELS OF EVIDENCE

I Evidence obtained from at least one properly designed randomized controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)