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REVIEW

The Potential Use of Tumour-Based Prognostic and Predictive Tools in Older Women with Primary Breast Cancer: A Narrative Review

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ABSTRACT

A move is under way towards personalised cancer treatment, where tumour biology of an individual patient is examined to give unique predictive and prognostic information. This is extremely important in the setting of older women, who have treatment-specific goals which may differ from their younger counterparts, and may include conservation of quality of life rather than curative intent of treatment. One method employed to assist with this is the use of tumour-based prognostic and predictive tools. This article explores six of the most common tumour-based tools currently available on the market: MammaPrint, Oncotype DX, Mammostrat, Prosigna, EndoPredict, IHC4. The article discusses the creation and validation of these tools, their use and validation in older women, and future directions in the field. With the exception of Oncotype Dx, which has also been licensed for prediction of response from adjuvant chemotherapy, these tools have been licensed for use as prognostic tools only, mainly in the setting of adjuvant therapy following surgery. The evidence base for use in older women is strongest for Mammostrat and PAM50, although overall the evidence is much weaker than that in younger women. Where older women have been included in validation studies, this is often in small numbers, or the exact proportion of older women is unknown. In practice, all six of the tools are recommended to be utilised on surgical excision specimens, as well as in core needle biopsy (CNB) specimens in all of the tools except Mammostrat. This is extremely important in the setting of older women, of whom a large proportion do not undergo surgery. The suggested nature of the sample is formalin-fixed paraffin-embedded in all the tools except MammaPrint, which can also be performed on fresh-frozen samples. Future development of prognostic tools in older women with breast cancer should focus on treatment dilemmas specific to this population. This includes the decision of primary treatment between surgery or endocrine therapy and decisions regarding adjuvant therapy, in particular, chemotherapy.

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Key Summary Points

There are many tumour-based prognostic and predictive tools on the market; however, these tools have been primarily derived from and tested in younger women.

Assessment of tumour biology is extremely important in primary breast cancer in older women, who have specific treatment goals and concerns.

The evidence base for use of six of the most common tumour-based tools (MammaPrint, Oncotype Dx, Mammostrat, Prosigna, EndoPredict and IH4) is much weaker in the older compared to younger population.

The majority of existing tools focus on prognosis after adjuvant therapy following surgery and do not consider the unique needs of the older population.

Many of these tools have been utilised on core needle biopsy specimens, which is important when examining biology in older women, as a large proportion do not undergo surgical treatment.

INTRODUCTION

Current Challenges in the Older Population

Breast cancer is more common in older women; nearly half of all diagnoses are made in patients over 65 years of age [1], and this will continue to increase with the ageing population [2]. Despite this, older women are often under-represented in clinical trials.

There is a growing body of evidence to suggest that the biology of breast cancer differs in older compared to younger women [3–5]. A better understanding of these differences has the potential to alter the management of breast cancer in older women at every stage of the pathway, including consideration of neoadjuvant treatment, primary treatment, adjuvant treatment and treatment of recurrent disease. One example is the use of primary endocrine therapy (PET) as an alternative option to surgery in patients with oestrogen receptor (ER)-positive tumours. Although PET is only recommended for patients who have a limited life expectancy of 2–3 years [6], around 40% of older women have previously been found to receive this treatment [7, 8]. Therefore, treatment decision-making in clinical practice is not as straightforward, as there are numerous other reasons that an older patient might not want surgery, related to frailty, competing causes of death and maintenance of quality of life.

In common with other regulatory bodies, the National Institute for Health and Care Excellence (NICE) provides no age-specific guidelines on the management of breast cancer in the UK.

A move is under way towards personalised cancer treatment, where tumour biology of an individual patient is examined to give unique predictive and prognostic information. This is even more important in the setting of older women, to direct the healthcare team and the patient to decide on the most appropriate treatment plan specific to them.

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

The Role of Predictive and Prognostic Tools

Prognostic tests inform us about a possible cancer outcome (recurrence of the disease, disease regression, death), independent of treatment, over a specified time-period [9]. Predictive tests provide information on response to a specific treatment [10].

Some tools to help inform the prognosis and response to therapy do exist. The Nottingham Prognostic Index (NPI) [11] was the first tool of its kind to assess a combination of factors. This includes histological grade which reflects tumour biology, together with size of tumour

and nodal status (time-dependent factors), to inform prognosis following surgery. More comprehensive assessment tools have been developed in tools such as Adjuvant! Online [12], which uses more clinicopathological features; however, recruitment of older women in their conception is lacking. The aims of these tools are not focused on the treatment dilemmas of the older population. Furthermore, these tools do not require unique tumour material to be assessed from an individual patient, so are not truly personalised to that patient.

To provide information specific to an individual patient, unique material from the patient's tumour is required, rather than using standard clinicopathological variables such as tumour grade and size. Assessment of patient tissue can be protein or genomic based.

Patients who have been identified as 'low risk' of recurrence by tools of this kind have been shown to have a very low risk of metastatic disease after extensive follow-up. A study by Stemmer et al. [13] examined 1365 women from the Clalit Health Services Registry with node-negative, ER-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer who underwent Oncotype DX testing between the years 2006 and 2009. The median age of the group was 60 years (standard deviation 52–-66 years). The study was relatively well represented in older women: 473 women (34.7%) were aged 60–69 years, 201 (14.7%) were 70–79 years and 17% (1.3%) were over 80 years. The breakdown by Oncotype DX recurrence score (RS) score was RS 0–10, 17.8%; RS 11–25, 62.5%; and RS 26–100, 19.7%. The outcomes showed that patients with a low and median RS, of whom only 10% were treated with chemotherapy, had good outcomes. The low-score group had a 10-year distant recurrence of 2.6% and breast cancer-specific mortality of 0.7%, and the median group was 6.1% and 13.1%, respectively. These findings support the evidence for Oncotype DX as a tool for identifying low-risk patients where it is possible to treat with endocrine therapy alone.

In this paper, we will discuss six of the most well-known tumour-based tools in chronological order of their conception: MammaPrint, Oncotype DX, Mammostrat, Prosigna (PAM50), EndoPredict, IHC4. We will outline the clinical use of each tool, development and validation, with specific interest to validation in older women, as well as possible future directions for the tool. Following this, we will conclude whether there is enough evidence for use of these tools routinely in the setting of primary breast cancer in older women.

The development of each of the six tools, as well as their validation studies, were all performed on surgical excision samples.

TUMOUR-BASED TOOLS

Table 1 gives a summary of the six tumour-based tools discussed in this article.

MammaPrint

Clinical Utility

The MammaPrint assay is a prognostic test based on the assessment of a panel of 70 genes [14]. It is designed for use in women with early (stage I or II) breast cancer, $\leq$ 5 cm size, with up to three positive lymph nodes, irrespective of hormone receptor (HR) status [15].

The gene expression profile of the patient is compared with a template profile of a known risk and subsequently categorises the patient into either high or low risk of recurrence. A high-risk result means that a patient has an 11.7% chance that her cancer will recur in 5 years, and a low-risk result has a 1.3% chance [16]. This result should help to inform the healthcare team of the likely benefit from adjuvant therapy.

In Europe, the MammaPrint assay is currently only available at the Agendia laboratory in the Netherlands and takes 10 days to return a result. In the USA, it is processed centrally in California. Therefore, MammaPrint is not currently recommended by NICE for guiding adjuvant therapy; the body has deemed it not cost-effective at the present time [15].

Development of the Tool

The test was developed in 2002 at the Netherlands Cancer Institute using samples from surgical specimens of 78 patients with primary, node-negative breast cancer, tumours of

Table 1 A summary of six most common tumour-based predictive and prognostic tools

Tool	Source of sample	Nature of sample	Central laboratory required	Setting	Prognostic value and validation	Predictive value and validation	Validation of tool in older women
MammaPrint	SE, CNB, VAB	Fresh/ frozen or FFPE	Yes	Adjuvant treatment following surgery	Early-stage breast cancer, irrespective of HR status, up to 3 positive nodes Netherlands Cancer Institute, 295 patients, < 53 years of age (no age range given) [38]	No	Ongoing MINDACT trial Phase 3, RCT, 6693 patients, median age 55 years (range 23–71), assessing prognosis of low genomic, high clinical risk without chemotherapy [38]
Oncotype DX	SE, CNB	FFPE	Yes	Adjuvant treatment following surgery	Early-stage breast cancer, ER-positive, HER2-negative, up to 3 positive nodes NSABP B-14: 668 patients in total, 301 (45%) were aged > 60 years (no age range given) [41]	Early-stage breast cancer, ER-positive, HER2-negative, up to 3 positive nodes NSABP B-20: 651 patients. 196 (30%) > 60 years (no age range given) [42]	Ongoing (RxPONDER) group 9400 patients with no age limit, Phase 3, RCT, randomising patients to the use of adjuvant endocrine alone or endocrine plus chemotherapy [44]

Table 1 continued

Tool	Source of sample	Nature of sample	Central laboratory required	Setting	Prognostic value and validation	Predictive value and validation	Validation of tool in older women
Mammostrat	SE	FFPE	No	Adjuvant treatment following surgery	Early-stage breast cancer, ER-positive, LN negative Cleveland Clinic Foundation, 225 patients, 75% > 50 years (no age range given) [50] British Columbia Cancer Agency: 344 patients (no age range given) [55]	No	4598 TEAM patients, ER-positive postmenopausal patients, RCT, accurately predicted DRFS, median age 64 years (range 34–96) [56]
Prosigna (PAM50)	SE, CNB	FFPE	No	Adjuvant treatment following surgery	Postmenopausal, early breast cancer, ER-positive, HER2-negative, up to 3 positive nodes 1478 postmenopausal women with ER+ early breast cancer, ABCSG-8 group, Median age: 64 years (range 41.4–80) [61]	No	1478 postmenopausal women with ER+ early breast cancer, ABCSG-8 group, median age: 64 years (range 41.4–80) [62]

Table 1 continued

Tool	Source of sample	Nature of sample	Central laboratory required	Setting	Prognostic value and validation	Predictive value and validation	Validation of tool in older women
EndoPredict	SE, CNB	FFPE	Central lab or local pathology	Adjuvant treatment following surgery	Early breast cancer, ER-positive, HER2-negative, up to 3 positive nodes 2 cohorts of 1702 samples acquired from patients enrolled into 2 randomised trials at the ABCSG ABCSG-6 median age 65.6 years (range 43.7–80.7 ABCSG-8 median age for this group was 63.4 years (range 41.5–80.5) [65]	No	GEICAM 9906, Phase 3, RCT 555 patients randomised to receive either FEC or FEC-P, 250 (45%) of the patients were < 50, 305 (55%) were ≥ 50 (no age range given), confirmed independent prognostic marker of recurrence at 10 years [65]
IHC4	SE, CNB	FFPE	No	Adjuvant treatment following surgery	Postmenopausal, early-stage breast cancer, ER-positive, up to 3 positive nodes, 5 years of adjuvant endocrine therapy 786 patients in Nottingham Median age: 55 (range 48–63) [65]	No	TEAM trial: 2919 samples [65] postmenopausal patients with early PgR/ER-positive invasive breast cancer who had completed primary endocrine therapy. confirmed IHC4 ability to predict residual risk (no age range given)

ABCSG Austrian Breast and Colorectal Cancer Study Group, *DRFS* distant recurrence-free survival, *ER* oestrogen receptor, *FFPE* formalin-fixed paraffin-embedded, *GEICAM* Group Español de Investigación en Cáncer de Mama, *HR* hormone receptor, *HER2* human epidermal growth factor, *MINDACT* Microarray In Node-negative Disease may Avoid ChemoTherapy, *NSABP* National Surgical Adjuvant Breast and Bowel Project, *PgR* progesterone receptor, *RCT* randomised controlled trial, *RxPONDER* Rx for Positive Node, Endocrine Responsive Breast Cancer) *SE* surgical excision, *TEAM* Tamoxifen Exemestane Adjuvant Multinational, *trans-ATAC* translational arm of Arimidex, Tamoxifen, Alone or in Combination trial

size < 5 cm, irrespective of HR status and in patients aged < 55 years [17]. A panel of around 25,000 genes was measured by DNA microarray analysis. Following a series of applied supervised classification, the optimal number of marker genes was reached (70). Classification of high or low risk of recurrence is based on measurement of these 70 marker genes and whether the patients in this cohort developed distant metastases.

Validation of the Tool

MammaPrint was initially validated by the same team surgical samples from the fresh-frozen tissue bank of the Netherlands Cancer Institute [18]. The validation cohort consisted of 295 women, aged < 53 years, with stage I or II breast cancer and tumours ≤ 5 cm. The cohort included 61 patients who were also included in the development sample [19]. The study measured the 70-gene panel in these patients and evaluated the prognostic power of the tool at predicting distant metastasis. Regression analysis showed that the assay was a strong independent factor in predicting distant metastasis.

MammaPrint is only licensed for use in the adjuvant setting, although one study [20] looked at its ability to analyse chemosensitivity in the neoadjuvant setting. Tumour biopsies were taken prior to treatment from 171 patients treated at the Netherlands Cancer Institute. Of the samples, 144 (86%) were deemed at high risk of recurrence based on the MammaPrint score. The results showed that those shown to have a poor prognosis by MammaPrint were more likely to achieve a pathological complete response (pCR), compared to those shown to have a good prognosis. Within the group of patients at high risk of recurrence, 29 (20%) achieved pCR, compared to none of the patients at low risk of recurrence. The study suggests that MammaPrint could be utilised in core needle biopsy (CNB) samples, to help predict those patients who may benefit from neoadjuvant therapy.

Mayordomo et al. [21] have also presented the results of a study investigating the use of MammaPrint in samples obtained from CNB. A total of 52 patients who had stage II or III breast cancer and planned to have neoadjuvant chemotherapy were included in the study. MammaPrint was successfully applied to 34 CNBs. Of these, three (9%) were assigned to the low risk category for recurrence and 31 cases were predicted to be high risk. The study concluded that MammaPrint could be conducted in the majority of CNB specimens. This study has a small sample size and is not specific to older women; however, along with the study by Straver et al. [20], it provides promising results which suggest that MammaPrint could potentially be applied to CNB samples prior to decision of primary treatment.

MammaPrint is also the only tool which has been tested in vacuum-assisted biopsy (VAB) specimens. This common technique provides more tissue than standard CNB, but less tissue than SE. A study by Osaki et al. [22] studied 67 patients with early-stage breast cancer. For each patient, five to 10 biopsies were taken, two of which were used for the analysis. MammaPrint was successfully performed on 50 of the samples and defined 22 cases (44%) as low risk and 28 cases (56%) as high risk. Information about the age of the patients was not provided.

Validation of the Tool in Older Women

Since the initial data from the Netherlands, there have been further validation studies, but studies including older women are still lacking.

The Microarray In Node-negative Disease may Avoid ChemoTherapy (MINDACT) trial includes patients up to 70 years of age [23]. The MINDACT trial is the first randomised controlled trial using a tumour-profiling test in clinical practice; others have been retrospective in nature.

The MINDACT multicentre trial recruited 6693 patients from 112 institutions across nine European countries [23]. The median age of the patients was 55 years (range 23–71). The study compared the patient's genomic risk (using MammaPrint) with their clinical risk (using Adjuvant! Online). Women with both high genomic and high clinical risk received chemotherapy. The primary goal was to assess the prognosis of those with low genomic and high clinical risk who did not receive chemotherapy. A total of 1550 (23.2%) women were in this category; 1000 of these patients

(64.5%) were aged 50–70 and 16 women (1%) were aged > 70 years. At 5-year median follow-up, there was no significant difference the in rate of survival between those who received chemotherapy and those who did not; however, the study was not sufficiently powered to test this. The trial is ongoing. The results so far have led the American Society of Clinical Oncologists (ASCO) to include MammaPrint in their guidelines. They recommend the use of MammaPrint for those with early breast cancer deemed at high clinical risk of recurrence, to identify patients with a good prognosis irrespective of adjuvant chemotherapy [21, 24].

Summary

The ongoing MINDACT trial shows the potential of MammaPrint as a predictive tool of response to chemotherapy as adjuvant therapy. The inclusion of some older women is a positive start, however not enough to enable results of this study to be applicable to older women in general. Further studies are needed to assess the usefulness of MammaPrint in women ≥ 65 years of age.

Oncotype DX

Clinical Utility

Oncotype DX is a 21-gene assay that assesses the expression of five reference genes (beta-actin [ACTB], GAPDH, GUS, RPLPO, and TFRC) and 16 cancer-related genes (Ki67, STK15, survivin or BIRC5, CCNB1 or cyclin B1, MYBL2, GRB7, HER2, ER, progesterone receptor [PgR], BCL2, SCUBE2, MMP11 or stromelysin 3, CTSL2 or cathepsin L2, GSTM1, CD68, and BAG1). The test is available for women with stage I or II, ER-positive, HER2-negative breast cancer with up to three positive lymph nodes [25]. The assay uses an algorithm to derive a recurrence score (RS), which is a continuous score from 0 to 100, with a higher number indicating a greater chance of recurrence at 10 years [26]. The tool has also been approved for use in predicting the likely benefit of adjuvant chemotherapy and remains the only assay to date proven to be useful in this context [26].

Currently, Oncotype DX is only available from a central laboratory in California, USA, and samples obtained from surgery must be sent to this laboratory for testing.

In the UK, NICE has approved Oncotype DX for use in patients with ER-positive, HER2-negative, lymph node-negative breast cancer, where the patient had an intermediate risk of disease with the PREDICT or Nottingham Prognostic Index tools [26].

Development of the Tool

The assay was developed in 2004 by Genomic Health (Redwood, CA) and has been available in the UK since 2007 [25]. Initially, 250 potential genes were analysed in three independent clinical studies involving a total of 447 patients with early invasive breast cancer and the relationship between gene expression and recurrence measured. Statistical analysis selected the final 16 genes and five reference genes as the minimum panel to obtain predictive information regarding recurrence [27].

Validation of the Tool

The primary validation study was a retrospective analysis using the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 and B-20 trial [27] data. The B-14 was a randomised trial comparing outcomes for patients after surgery to 5 years of tamoxifen or 5 years of placebo. The B-20 trial was later created to investigate adding chemotherapy to tamoxifen in node-negative patients who had ER-positive tumours [28].

Paik et al. [27] validated the prognostic utility of Oncotype DX using the B-14 trial, which comprised 668 patients. The levels of the genes and subsequent recurrence score determined a risk group for each patient: low, intermediate or high. No age range is given in this validation study, but 301 (45%) were aged > 60 years. The team showed that Oncotype DX score could predict recurrence, independent of age and tumour size.

A subsequent validation by the same team was performed in the B-20 trial [29]. The specific aim of this validation study was to understand the ability of Oncotype DX to predict benefit from chemotherapy. A total of 651 women were assessed, 227 of whom were randomly assigned

tamoxifen and 424 tamoxifen plus chemotherapy. The study showed that patients who were in the high-risk group as determined by Oncotype DX had a significant benefit from chemotherapy. Subgroup analysis by age was not performed in this study; however, 196 (30%) of the participants were aged > 60 years.

The tool's performance has been explored in the neoadjuvant setting. Three of four studies with this aim have measured Oncotype DX in pretreatment CNB specimens. All of the studies have demonstrated that pCR was only found in patients with a high RS (> 25) [30–33]. Although not formally validated for this purpose, Oncotype DX may be able to help predict response to neoadjuvant therapy.

Most recently, in response to the COVID-19 pandemic, Oncotype DX has been extensively tested in over 900,000 CNB specimens. The study concluded that Oncotype DX can be reliably performed on CNB with consistent results to those achieved in surgical samples [34].

Validation of the Tool in Older Women

Since initial validation, there has been an abundance of studies incorporating Oncotype DX. Of note is the Southwest Oncology Group (SWOG) 8814 study [35], which tested Oncotype DX in a cohort of 367 postmenopausal women with ER-positive, node-positive breast cancer. The age range of participants was 42–-81 years, and 108 (29.4%) were aged ≥ 65 years. The results suggest that Oncotype DX predicted a benefit of chemotherapy, even in patients with node-positive disease. Unfortunately, subgroup analysis by age was not performed.

The Trial Assigning Individualized Options for Treatment (TAILORx) study was developed to look at women with an intermediate-risk RS (score 11–25) and whether they would benefit from chemotherapy [36]. This is a prospective trial involving 10,273 women with ER-positive, HER2-negative, node-negative breast cancer. The women were divided into a low-risk group (score below 10, who were given adjuvant endocrine therapy only), a high-risk group (score above 26, who received chemoendocrine therapy) and those with intermediate risk (score of 11–25). The intermediate group in total comprised 6711 who were randomised to receive either chemoendocrine therapy or endocrine therapy. The age range of participants was 23–75 years, but in the intermediate-risk cohort only 291 (4.3%) were aged > 70 years. The 9-year rate of distant recurrence in the intermediate group was 4%, irrespective of chemotherapy use. The study concluded that Oncotype DX might be particularly useful for predicting women who do not need adjuvant chemotherapy [36].

Considering the results from TAILORx, ASCO updated their guidelines for Oncotype DX in 2019 [37]. They now recommend Oncotype DX for use in ER-positive, HER-2 negative, lymph node-negative breast cancer to guide adjuvant therapy. Unlike NICE in the UK, the guidelines are age-specific. ASCO recommends that those over 50 years of age with an Oncotype DX RS of < 26 or under 50 years of age with a score of < 16 should be offered endocrine therapy alone, on the basis that there is no benefit to chemotherapy. Women with an RS score > 30 should be offered chemoendocrine therapy. Finally, women in the 26–30 RS score category may be offered chemoendocrine therapy; however, this recommendation is not as strong [30–33].

Summary

To date, validation studies of Oncotype DX have been restricted to younger women or have included only a limited number of older women. The RxPONDER (Rx for Positive Node, Endocrine Responsive Breast Cancer) trial [38] is an ongoing multicentre, phase III prospective study. It aims to include 9400 women with hormone receptor-positive, HER2-negative breast cancer, with 1–3 positive nodes and deemed at low risk of recurrence by Oncotype DX. The study is randomising patients to the use of adjuvant endocrine alone or endocrine plus chemotherapy. The trial has no age limitations and therefore may provide valuable data on the use of Oncotype Dx in older women.

Mammostrat

Clinical Utility

The Mammostrat assay measures five biomarkers by immunohistochemistry: p53, HTF9C,

CEACAM5, NDRG1, SLC7A5 [39] on surgical specimens. The assay can be performed in patients with early (stage I or II), ER-positive, node-negative invasive breast cancer. The total scores are assigned to a low, moderate or high category of risk of 10-year recurrence. The Mammostrat score should then be used as a guide to inform clinicians on the possible risk and aid decisions regarding adjuvant treatment.

Mammostrat requires central processing at a laboratory in the USA [26]. It is not recommended for use by NICE in the UK or by ASCO [40].

Development of the Tool

Mammostrat was developed in 2006 at the Comprehensive Cancer Institute of Huntsville, Alabama, USA [41]. The discovery cohort comprised 466 patients with primary invasive breast cancer presenting to the unit. The age range of the patients was not disclosed; however, 327 (71%) were aged > 50 years and 135 (29%) < 50 years. Over 140 biomarkers were stained on the tumour samples, and Cox proportional hazards analysis identified the minimum panel of reagents to be able to predict risk of recurrence at 5 years.

Validation of the Tool

The test was validated on two cohorts [41]. The first cohort consisted of 229 patients with primary invasive breast cancer who presented to the Cleveland Clinic Foundation between 1995 and 1996. Of the cohort, 225 (75%) of patients were aged > 50 years; the age range of patients is not provided. Follow-up data was available for up to 5 years.

The second cohort consisted of 344 patients who presented to Vancouver General Hospital between 1974 and 1995 [42]. The age range of this cohort is also not provided. Follow-up data was available for up to 11.7 years.

In both these cohorts, Kaplan–Meier estimates of recurrence based on the Mammostrat model distinguished patients with poor outcomes following surgery [41].

There is no evidence for using Mammostrat outside of this setting at the present time.

Validation of the Tool in Older Women

The prognostic power of Mammostrat was further validated in a study involving 711 tamoxifen-treated patients with ER-positive and node-negative tumours combined from the NSABP B-14 and B-20 studies, one of which was the same trial used to validate Oncotype DX, as described above. In the B-14 study population, 1804 out of 2615 (69%) patients were > 50 years of age, and in the B-20 clinical study 420 out of 771 patients (55%) were in this age group [43]. The upper age limit of patients included in these studies is not clear. Analysis of the Mammostrat model was significantly associated with risk of recurrence in ER-positive, node-negative tumours, irrespective of the age of the patient.

This study further looked at the benefit of adjuvant chemotherapy in patients from the B-20 study population who were already receiving adjuvant tamoxifen. Specifically, in patients aged ≥ 60 years, Mammostrat categorised 22% of patients as high risk of recurrence and predicted that there was a 21% decrease in recurrence rate if these patients received adjuvant chemotherapy in addition to tamoxifen. The age stratification was not prespecified in the study, and more evidence is needed to confirm these findings, but these results suggest that Mammostrat may be useful for older patients [39]

Bartlett et al. [44] performed Mammostrat in 4598 patients from the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial [45], a multinational randomised trial comparing exemestane alone or following tamoxifen for 5 years as adjuvant therapy in postmenopausal patients with early PgR/ER-positive invasive breast cancer. The TEAM trial included women with a median age of 64 years (range 34–96). Mammostrat accurately predicted distant recurrence-free survival (DRFS) for patients treated with exemestane and patients treated with tamoxifen followed by exemestane, irrespective of nodal status and chemotherapy. The ability of this test to provide additional outcome data after treatment is evidence for its use in risk stratification of ER-positive tumours in postmenopausal patients receiving an aromatase inhibitor [44].

Summary
The evidence for use of Mammostrat in older women is growing. A breakdown of the results by age from the TEAM trial data would be helpful.

Prosigna/PAM50

Clinical Utility
The Prosigna assay (based on the PAM50 signature) measures 58 genes (50 cancer-related genes and eight controls) and classifies a patient's tumour into a subtype based on the signature (luminal A, luminal B, HER-2 enriched or basal-like) [46]. The test is designed for postmenopausal women with early, ER-positive, HER2-negative breast cancer with up to three positive lymph nodes, receiving 5 years of hormonal therapy [14]. The results of the test provide a risk of recurrence after 10 years (from diagnosis), in the form of a risk of recurrence (ROR) score from 1 to 100. A score of 100 indicates the highest risk of recurrence. This score can further categorise patients into low, intermediate or high risk based on nodal status [47, 48]. A low risk score indicates < 10% predicted risk of distant recurrence, intermediate score indicates 10–20% and high risk indicates more than 20% risk. These scores can be used to aid the clinician's decision-making about whether to use adjuvant chemotherapy in a patient [49].

Prosigna is recommended by NICE for guiding the use of adjuvant therapy in ER-positive, HER2-negative, node-negative breast cancer [26]. The test is also recommended in the same context by ASCO, in conjunction with other clinicopathological variables [40]

Development of the Tool
The tool was developed in 2009 using tumour tissue obtained from multiple centres across the USA [50]. An expanded gene set of over 1900 genes was measured in 189 breast tumour samples and profiled by cluster analysis. The mean age of the sample group was 58 years (standard deviation 15 years, age range not provided). A minimised gene set was derived to select the 50 most distinguishing genes, which were correlated with standard clinical variables and time to relapse. Test sets from 761 patients who did not receive any systemic therapy and 133 who received neoadjuvant chemotherapy were evaluated for concordance [50].

Validation of the Tool
Dowsett et al. [51] compared the PAM50 risk of recurrence score with that of Oncotype DX. mRNA was taken from 1017 patients (mean age 64.4 years, standard deviation 8.3 years, age range not available) with ER-positive early breast cancer enrolled in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial [52], and concordance tests were used to assess the prognostic information provided by each of the scores. The ATAC trial was a randomised trial to compare anastrozole with tamoxifen for the adjuvant treatment of early breast cancer in postmenopausal women [53]. Dowsett et al. [51] showed that PAM50 provided more prognostic information in endocrine-treated patients with ER-positive, node-negative disease than Oncotype DX. Furthermore, PAM50 could differentiate more clearly between patients in intermediate- and high-risk groups.

A recent study has looked at the performance of PAM50 in the neoadjuvant setting using CNB [54]. The study evaluated the feasibility of using the assay on CNB samples before assessing the response to neoadjuvant chemotherapy in HR-positive, HER2-negative tumour samples. The study population comprised 95 patients, resulting in 122 formalin-fixed paraffin-embedded (FFPE) tumour blocks; information about the patient's age was not provided. The correlation between paired surgical resection and CNB samples was high ($r > 0.90$). This study demonstrated that PAM50 could be measured accurately in CNB specimens.

Validation of the Tool in Older Women
In 2014, PAM50 was validated on samples from 1478 postmenopausal women with ER-positive early breast cancer [55]. These samples were drawn from the archived Austrian Breast and Colorectal Cancer Study Group (ABCSG) 8 cohort. In this trial, postmenopausal patients were randomly selected to receive tamoxifen for

either 5 or 2 years followed by anastrozole for 3 years. The median age of patients was 64 years (range 41.4–80). None of the patients in this test set received adjuvant chemotherapy. The study concluded that the test was suitable for predicting the risk of recurrence (after 10 years) in postmenopausal women with ER-positive tumours. The risk of recurrence at 10 years was < 3.5% in the low-risk category, a finding that could help guide adjuvant chemotherapy.

Summary

PAM50 has been specifically validated in women up to the age of 80 years and is recommended for use in postmenopausal women.

EndoPredict

Clinical Utility

EndoPredict is a genomic test comprising the measurement of 12 genes: eight cancer-related (BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP, and STC2), three reference genes CALM2, OAZ1, and RPL37A) and one DNA reference gene. The test is designed for women with early, ER-positive, HER2-negative breast cancer with up to three positive lymph nodes. Measurement of these genes generates an EPclin score, which estimates the risk of metastasis within 10 years, assuming 5 years of adjuvant endocrine therapy. A score of 0–5 indicates low risk (less than 10% risk of metastasis in 10 years) and > 5–15 indicates high risk of recurrence [15], which can be used when considering adjuvant treatment.

Development of the Tool

EndoPredict was developed in Austria in 2011 from samples of 964 ER-positive HER2-negative tumours in patients treated with surgery followed by adjuvant tamoxifen [56]. Patient samples were collected from two centres. The median age of this cohort was 65.8 years (range 40.0–93.5). RNA levels of the genes of interest were assessed by reverse transcriptase polymerase chain reaction (PCR) and generated a score, along with additional clinicopathological characteristics such as tumour size and nodal status, to discriminate patients into high or low risk of recurrence.

NICE and ASCO recommend EndoPredict as an option when considering adjuvant chemotherapy in ER-positive, HER2-negative, node-negative patients [26, 40].

Validation of the Tool

The test was independently validated on two cohorts of patients enrolled into two randomised trials at the ABCSG, the ABCSG-6 [57] (n = 378) and ABCSG-8 [58] trials (described above) (n = 1324) [56].

The ABCSG-6 was a phase III trial in postmenopausal women comparing adjuvant tamoxifen alone for 5 years with tamoxifen and aminoglutethimide for the first 2 years. The median age of this group was 65.6 years (range 43.7–80.7). In the ABCSG-8 group, postmenopausal patients were randomly selected to receive tamoxifen for either 5 or 2 years, followed by anastrozole for 3 years. The median age for this group was 63.4 years (range 41.5–80.5).

The EPclin score was calculated and evaluated using multivariate Cox analysis, and the risk score was found to be an independent predictor of distant recurrence in both cohorts.

Mueller et al. [59] specifically compared EndoPredict performance between CNB and surgical specimens. The study population comprised 40 patients with ER-positive, HER-2 negative breast cancer. No information about the patients' ages is provided. Each patient gave two samples, one CNB and one surgical resection, and the samples were compared. The RNA yield was considerably lower for the CNB but enough to measure an assay in all samples. The EndoPredict score was highly correlated (p: 0.92) in the paired samples. These results suggest that EndoPredict could be used in samples from CNB, but more evidence would need to be gathered from a larger sample.

EndoPredict has also been evaluated for its prediction of response to neoadjuvant chemotherapy [60]. Bertucci et al. analysed data from 553 ER-positive, HER2-negative tumour samples treated with neoadjuvant therapy, and looked at the EPclin prediction of pCR. They tested EndoPredict on surgical specimens, following neoadjuvant treatment. The overall pCR rate was 12%. EndoPredict was associated with a

pCR rate in 7% of the low-risk group and 17% of the high-risk group. Further evidence is needed to validate EndoPredict in this setting.

Validation of the Tool in Older Women

Further validation of EndoPredict was performed in a cohort of patients from the phase III, randomised Group Español de Investigación en Cáncer de Mama (GEICAM) 9906 trial looking at postmenopausal women treated with adjuvant chemotherapy and endocrine therapy [61]. The patients were randomised to receive either six cycles of fluorouracil, epirubicin and cyclophosphamide (FEC) or four cycles of FEC and then eight weekly courses of paclitaxel (FEC-P). Those with HR-positive tumours also received endocrine therapy. The cohort consisted of 555 patients with ER-positive, HER2-negative, node-positive breast cancer. The study included 250 (45%) patients aged < 50 years and 305 (55%) aged ≥ 50 years. The study showed EndoPredict to be an independent prognostic marker of recurrence at 10 years.

Summary

There is evidence to support the use of EndoPredict in older women up to the age of 80 years. Subgroup analysis by age in the ABCSG trials would be helpful to determine the exact number of older women included.

IHC4

Clinical Utility

Immunohistochemical 4 (IHC4) is a model incorporating four immunohistochemical markers: ER, PgR, HER2 and ki67. The model is designed for use in early breast cancer patients receiving surgery followed by 5 years of adjuvant endocrine therapy, and predicts the risk of distant recurrence after 10 years [46].

Following on from IHC4, the IHC4+C prognostic tool has been developed [62]. The IHC4+C score incorporates the four markers along with the clinicopathological (+C) factors of nodal burden, tumour grade and size, patient age, and treatment with AI or tamoxifen [63] The tool calculates a risk of recurrence score, with low risk indicating less than 10% chance of distant recurrence within 10 years, intermediate risk 10–20%, and high risk $> 20\%$. This score can be used by clinicians to aid in decisions about whether a patient can forgo adjuvant therapy.

In the most recent NICE guidance, IHC4+C was not recommended to be used when considering adjuvant chemotherapy decisions, due to uncertain analytical validity of the test [52]. This decision was bolstered by the fact that patients in the trials had not undergone adjuvant chemotherapy [62]. In the UK, the IHC4+C test is currently performed only at the Royal Marsden Hospital; the average turnaround time for the test is 1 week.

ASCO does not recommend the use of IHC4 [40]

Development of the Tool

The tool was developed in 2011 at the Wolfson Institute of Preventative Medicine, London, UK. The four markers were measured on patients included in the translational arm of the ATAC cohort [62], as previously described. For the purposes of testing IHC4, 1225 patients with ER-positive breast cancer, and sufficient good-quality tissue for IHC testing were selected from the ATAC cohort. The median age of the cohort selected was 64 years (range 57–70). A prognostic model to estimate recurrence was developed based on the measurement of the four markers in this cohort.

Validation of the Tool

The tool was first compared with the Oncotype DX Recurrence Score, which was also tested on the ATAC cohort. IHC4 was found to provide similar prognostic information [64].

The model was initially validated in a cohort of 786 patients treated in Nottingham between 1990 and 1998 [63, 65]. The Nottingham cohort had a median age of 55 years (range 48–63) and was not restricted by menopausal status. All patients had ER-positive tumours and received either tamoxifen or no treatment as adjuvant therapy. The IHC4 score was highly significantly prognostic for outcome in this cohort.

The IHC4+C score was developed in retrospective analysis of the ATAC cohort, once the

independent prognostic power of IHC4 had been established in endocrine patients [53].

The IHC4 tool was then further validated in the TEAM trial [64]. IHC4 was quantified on 2919 of the TEAM samples (as previously described). The breakdown by age is not published for this subset, nor are the criteria for selection. The results showed the IHC4 algorithm to be effective in predicting residual risk following endocrine therapy in postmenopausal patients.

A recent study showed the potential to use IHC4 on core needle biopsy (CNB) specimens, as an alternative to surgical excision (SE) sections [64]. Core biopsy samples from 108 patients diagnosed with invasive breast cancer were retrieved. None of the patients had been treated with neoadjuvant therapy. The mean age of participants in the study was 49 years (range 29–82). Paired CNB and SE samples were evaluated for IHC4 scores, and the two were analysed for concordance.

The results of the study showed that CNB had moderate concordance with SE scores and therefore could be used for IHC4 testing, provided an adequate amount of CNB tissue was available. This is an exciting possibility, which would allow use of IHC4 in the neoadjuvant and primary settings. One potential drawback as noted by this study was that the total length of malignant tissue obtained was inversely proportional to age, and increased atrophy was seen in older patients; therefore, greater care would be needed in obtaining a sample in this manner. The study noted that in general, low concordance was related to a low number of cores obtained; however, the number of cores did not differ between younger and older patients. It was specifically tumour tissue atrophy that seemed to contribute to this factor. This suggests a possible disadvantage for older women if CNB were to become the mainstay for obtaining tumour samples for profiling.

Validation of the Tool in Older Women

Although initially designed based on a cohort of younger women, validation of IHC4 using TEAM trial data has included some older women, although the exact number is unclear. Based on this, further validation of IHC4 is needed to determine its value in older women. Furthermore, IHC4 is based on the prediction of recurrence following adjuvant therapy and is only validated in this setting.

Summary

The analytical validity for IHC4 is currently scant and there is very little evidence for its use in older women, although the findings relating to tissue quality in older women from the CNB study present an interesting concept [64].

CONCLUSIONS AND FUTURE DIRECTIONS

Summary of Existing Tools

Oncotype DX stands out as the only tool validated for both predictive and prognostic utility. It is the most used tool in the USA and has the largest body of evidence in terms of trials conducted and research undertaken [13, 66]. The large-scale TAILORx study [36] was promising in its recruitment of older women, but in the intermediate-risk group the study focused on, only 4.3% of the women were over 70 years old. RxPONDER [41] may shed further light, as there is no age limit, but the results remain to be seen. As the oldest tool, MammaPrint also has bountiful evidence and scope for use as both a predictive and prognostic tool. However, in the ongoing MINDACT trial [23, 27], only 1% of the recruits are over 70 years. It was also validated on younger women of < 53 years [18].

Many of the key trials concerning Mammostrat only provide age information of categories of < 50 or > 50 years. There is overall less evidence for Mammostrat. The TEAM trial [45] (also concerning IHC4) includes patients up to the age of 96, but further breakdown by age has not been provided, which makes the results difficult to analyse in this way.

PAM50 is specifically recommended for postmenopausal women only, which implies it may be preferential to an older group. In addition, unlike the other tests, its classification by subtype may pose an advantage in terms of tumour heterogeneity by age. However, since its

validation there has been no contribution to the evidence for its use in older women.

EndoPredict has evidence to support its use in patients up to the age of 80 years, but like PAM50 has not been included in trials concerning older women since its inception. The GEICAM 9906 trial [61] includes 55% who are over 50 years of age, but further breakdown is not provided.

The newest tool, IHC4, is also included in the TEAM trial, but as previously mentioned, information about age is lacking.

Current Issues with the Use of Tumour Profiling Tools in Older Women

Age Range

From this review, it is clear that overall there is a lack of evidence specific to older women, which is echoed in clinical trials of new therapies and research in general. This is partly due to lack of recruitment of older women, and where older women are recruited, numbers are few. This may be due to failure of inclusion by the researchers or lack of willingness to participate by the patient due to competing comorbidities or concern of the impact on treatment or quality of life.

Where older women are included, often analysis by age is not done, or a breakdown of results by age not given. This makes it difficult to interpret the results in this specific cohort.

Setting

Current tools available are focused mainly on ER-positive tumours and considering adjuvant treatment decision-making, especially chemotherapy. Although the majority of breast cancers in older women are ER-positive, there is an important group of patients who are ER-negative. Especially if the patient is unfit for or declines surgery, we need to know what alternative treatments are available to them, as PET is not recommended.

Sample Required

The tumour profiling tools were initially developed and validated on surgical excisions. More recent studies have enabled testing on CNB specimens [67]

The use of core needle biopsy presents a degree of uncertainty, and some studies have shown discordance between different markers, particularly PgR when it comes to CNB [31, 32]. As of 2016, Oncotype DX had been performed 600,000 times and in CNB 20% of the time [32]. The evidence is unclear, and although all of the tools (except Mammostrat) have been tested in CNB samples, most studies of this kind involve small sample sizes. This is an important area for future research.

VAB is also an emerging tool, although the evidence for its use in combination with the genomic tools is scant; only MammaPrint [22] had been tested in a small population for feasibility in this source.

Cost Effectiveness

As the only validated predictive tool, Oncotype DX is used worldwide, and its cost effectiveness has been considered widely. NICE's estimated cost for the test was £2850 [26], the most expensive of all the tools considered here. The possibility has been raised of reducing costs by using traditional pathological tests as a way of screening for Oncotype DX requirement. This is currently recommended by NICE with the use of PREDICT or NPI. Gage et al. [68] analysed information about 1268 breast cancer patients from the University of Texas MD Anderson Cancer Center database. The mean age of the group was 54.8 years. The aim of the study was to conclude whether using a pathologic-genomic algorithm could avoid the use of Oncotype DX in low- and high-risk categories. The team compared the Oncotype DX RS scores with the Anne Arundel Medical Center (AAMC) model predictions, which use standard pathology data. Five-year distant recurrence rates were shown to be similar in the AAMC low-risk group (2.7%, $n = 322$) and the RS low-risk (< 18 RS) group (3.4%, $n = 703$). The high-risk group was also well correlated: AAMC (22.8%, $n = 230$) and the Oncotype DX RS > 30 (23.0%, n = 141). The algorithm predicted that risk of metastasis was 3.3% for the low-risk group ($n = 739$) and 24.2% for the high-risk group ($n = 272$). In theory, by using this algorithm and reserving

genomic testing for the intermediate-risk group, 44% of the patients (n = 522) could have avoided the Oncotype DX testing.

Tumour profiling tests are expensive to conduct. NICE's Economic Analysis Group estimated the costs to be as follows: MammaPrint (£2326), Oncotype DX (£2580), Mammostrat (£1135), EndoPredict (£1500), Prosigna (£1970) and IHC4 (£203) [26]. In light of this, NICE recommends reserving the tools for patients in an intermediate-risk category as predicted by a traditional non-genomic tool. Perhaps a more relevant consideration for older patients is cost analysis in terms of quality-adjusted life-years.

Limitations of the Current Study

Many of the salient studies did not share information about results by age, or specifics about numbers of participants in older age categories. This means that interpretation of these studies has been somewhat limited. Due to variations in healthcare systems and regulatory bodies worldwide, the use of the tools in different countries can be difficult to interpret.

Future Directions

In summary, the evidence for use of the tools discussed in this article specific to older women with primary breast cancer is lacking. These tools are most often derived from and validated in cohorts of patients consisting entirely of younger women, or including a small number of older women. The tools are mainly focused on predicting the benefit of adjuvant therapy after surgery, and therefore cannot provide any useful information in the cohort of older women who have PET.

If it were possible to provide a breakdown by age of the current available studies, this would greatly add to the evidence base for use of these tools in older women.

There needs to be greater recognition and focus specifically on the older population when it comes to validating existing tools and in the development of new tumour-based tools. A lack of information means that categories such as postmenopausal and age > 50 years are falsely attributed to the evidence base for older women. The biology of breast cancer in older women has been shown to have different biological features compared to their younger counterparts; in the era of personalised treatments, their unique tumour biology can no longer be ignored.

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