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Adjuvant Chemotherapy De-Escalation with Genomic Assay Protocol in Patients with Early Breast Cancer: A Single-Centre Prospective Cohort Study

Redução de Quimioterapia Adjuvante com Utilização de Teste Genómico em Doentes com Carcinoma da Mama Localizado: Estudo de Coorte Prospetivo Unicêntrico

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ABSTRACT

Introduction: Genomic assays are useful tools for tailoring adjuvant treatment in early breast cancer. We aimed to analyse the role of an institutional protocol of a genomic assay for chemotherapy de-escalation.

Methods: Prospective cohort study of all consecutive women diagnosed with hormone receptor-positive and human epidermal growth factor receptor 2-negative early breast cancer, tested with the 21-gene Recurrence Score (RS) assay from August 2015 to July 2018 at a Portuguese cancer centre. For being tested, patients should meet at least one of the pre-defined inclusion criteria: i) luminal A-like, pT2pN0; ii) luminal A-like, 1 – 3 positive nodes and comorbidities with higher risk of chemotherapy-induced toxicity; iii) pT1-2pN0, progesterone receptor ≤ 20% and/or Ki67 14% – 40%. Adjuvant treatment was de-escalated to isolated endocrine therapy if RS was less than 18. We measured the reduction in chemotherapy prescribing and its clinical impact, the RS association with pathologic features, and the protocol feasibility.

Results: We tested 154 women with a median age of 61 years old (range: 25 – 79), 69% postmenopausal. Tumours were mainly pT1 (55%), pN0 (82%), invasive ductal (73%), G2 (86%), luminal B-like (69%) and stage IA (85%). We obtained a RS less than 18 in 60% of women, with an overall adjuvant chemotherapy reduction of 65%. Seven (95% confidence interval: 5 – 10) patients needed to be screened with the 21-gene RS assay to prevent one clinically relevant adverse event during the first six months of adjuvant treatment. Considering the currently used RS cut-off, only 9% of node-negative and 11% of node-positive patients had RS over 25. We found no relevant associations between RS and pathologic features. The protocol was feasible and did not compromise the adequate timing for adjuvant treatment.

Conclusion: These criteria allowed the de-escalation of adjuvant systemic treatment in at least six out of ten women.
Keywords: Antineoplastic Agents, Hormonal; Breast Neoplasms; Chemotherapy, Adjuvant; Gene Expression Profiling; Precision Medicine

RESUMO

Introdução: As análises genómicas têm personalizado o tratamento adjuvante em cancro de mama localizado. O objetivo deste estudo foi avaliar o impacto de um protocolo institucional de análise genómica para de-escalação de quimioterapia.

Métodos: Estudo de coorte prospetivo de todos os casos consecutivos de carcinoma da mama localizado com expressão positiva de receptores hormonais e sem sobre-expressão de *human epidermal growth factor receptor* 2, submetidos a um teste de quantificação de expressão de 21 genes para avaliação de *score* de recorrência (RS) entre agosto de 2015 e julho de 2018 num centro oncológico português. Para serem testadas, as doentes teriam de cumprir pelo menos um dos seguintes critérios de inclusão: i) *luminal* A-*like*, pT2pN0; ii) *luminal* A-*like*, 1 – 3 gânglios positivos e comorbilidades que constituam um maior risco para toxicidade induzida por quimioterapia; iii) pT1-2pN0, PR ≤ 20% ou Ki67 14% – 40%. O tratamento adjuvante foi de-escalado para hormonoterapia isolada quando o RS foi inferior a 18. Foi medida a taxa de redução de prescrição de quimioterapia e o seu impacto clínico, a associação do RS com características patológicas e a exequilidade do protocolo.

Resultados: Testámos 154 mulheres com mediana de idade de 61 anos (mínimo – máximo: 25 – 79), 69% pós-menopáusicas. Os tumores eram maioritariamente pT1 (55%), pN0 (82%), subtipo ductal invasivo (73%), G2 (86%), *luminal* B-*like* (69%) e estadio IA (85%). Obtivemos RS inferior a 18 em 60% das mulheres, com uma taxa de redução global de quimioterapia adjuvante de 65%. Esta análise genómica preveniu um evento adverso clinicamente relevante durante os primeiros seis meses de tratamento adjuvante por cada sete (intervalo de confiança 95%: 5 – 10) mulheres testadas. Considerando o *cut-off* mais recente para o RS, apenas 9% tiveram RS superior a 25, sendo que 11% das doentes com doença ganglionar teve RS superior a 25. Não houve correlação relevante entre RS e características anatomopatológicas. O protocolo não comprometeu o início atempado do tratamento adjuvante.

Conclusão: Este protocolo evitou a exposição a quimioterapia em pelo menos seis em cada dez mulheres.
Palavras-chave: Antineoplásicos Hormonais; Medicina de Precisão; Neoplasias da Mama; Perfilação da Expressão Génica; Quimioterapia Adjuvante

INTRODUCTION

Breast cancer is the most frequent malignancy and the leading cause of cancer related mortality in women.[1] About two-thirds of breast cancer cases are hormone receptor-positive and human epidermal growth factor receptor 2-negative (HR+/HER2-).[2] Immunohistochemistry allows to define a surrogate of two molecular subclasses of HR+/HER2- tumours, luminal A-like and luminal B-like, the latter when progesterone receptor (PR) expression < 20% and/or Ki67 ≥ 20%.[3] However, the clinicopathologic features do not accurately distinguish the patients who benefit from

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adjuvant chemotherapy.

The OncotypeDx™ Recurrence Score (RS) is the result of a genomic assay of a panel of 21 genes developed to analyse the tumour and to assess gene activity through RNA expression profiling. RS was initially divided into three groups based on retrospective analyses of prospective trials: i) low-risk of recurrence, RS < 18; ii) intermediate-risk, RS 18 – 30; iii) high-risk, RS ≥ 31.[4] OncotypeDx was later prospectively validated in two trials that recruited women with HR+/HER- early breast cancer: i) TAILORx for node-negative,[5] ii) RxPONDER for node-positive.[6] The TAILORx trial divided patients with node-negative HR+/HER2- disease into three categories of recurrence risk: low-risk (RS 0 – 10); medium-risk (RS 11 – 25) and high-risk (RS 26 – 100). Patients with RS 0 – 10 were spared from adjuvant chemotherapy and had similar outcomes of recurrence at five years.[7] The TAILORx analysis at nine years of follow-up showed that endocrine therapy was not inferior to chemoendocrine therapy in the group of women with RS 11 – 25. However, younger women (aged under 50 years old) with RS > 15 benefited from chemotherapy with a significantly lower risk of recurrence. These findings can be the consequence of estrogen deprivation from the chemotherapy-induced failure of ovarian function, since only 13% of the patients aged under 50 years old and treated with isolated endocrine therapy were given concomitant ovarian suppression.[5]

The RxPONDER trial[6] randomised patients with RS ≤ 25 to receive endocrine therapy alone or chemoendocrine therapy, and showed no benefit with the addition of chemotherapy in postmenopausal women with 1 – 3 positive nodes. In premenopausal women, chemotherapy was protective, with a five-year invasive disease-free survival of 89.0% in the endocrine therapy group and 93.9% in patients receiving chemoendocrine therapy (HR 0.60; 95% CI, 0.43 to 0.83), with a similar benefit in distant relapse-free survival (HR 0.58; 95% CI 0.39 to 0.87). Contrary to TAILORx, the RxPONDER trial failed to demonstrate the predictive value of RS for chemotherapy benefit in node-positive breast cancer. However, both studies agreed with the lack of chemotherapy benefit in postmenopausal women with zero to three positive nodes HR+/HER2- breast cancer and RS ≤ 25. Regarding premenopausal patients, women with node-negative disease can be spared from chemotherapy if RS < 15, but node involvement in premenopausal patients is associated with a chemotherapy benefit that is independent of RS.

Associations between the clinicopathologic features and RS have been explored, namely histology and Ki67. Some authors consider that well-differentiated tumours with favourable histologic subtypes might not need to be tested, since these features are associated with lower RS.[8,9] On the other hand, Ki67 expression has been reported as a strong individual prognostic factor,[10] with correlation with RS.[11] However, contradictory data supports no correlation of RS with Ki67 and conventional prognostic markers.[12]

The aim of this study was to evaluate the proportion of early breast cancer cases with adjuvant treatment de-escalation using a 21-gene RS assay protocol at a Portuguese cancer centre. As secondary objectives, we explored the clinical impact and the feasibility of this protocol. As exploratory objectives, we interpreted the results according to the TAILORx and the RxPONDER trials and measured the relationship between RS and histology or Ki67.

METHODS

Study design and setting

Prospective cohort study of all consecutive women diagnosed with stage I-II, HR+/HER2- invasive breast cancer, who performed tumour analysis with the 21-gene RS assay at Instituto Português de Oncologia de Lisboa Francisco Gentil, from August 2015 to July 2018. This is one of the largest cancer centres in Portugal, serving a geographical area of about four million inhabitants, and receiving nearly 14 000 new cancer cases per year. Of these, around 800 are newly diagnosed breast cancer cases, and about 60% receive chemotherapy. The manuscript was prepared according to The Strengthening the Reporting of Observational Studies in Epidemiology statement.[13]

Data source and ethical considerations

We used anonymous data prospectively collected from the electronic health records since protocol implementation. This institutional guideline protocol was reviewed and approved by the Ethics Committee of Instituto Português de Oncologia de Lisboa Francisco Gentil. Informed consent was not required due to the nature of the study (observational study in an academic hospital). We excluded all patients whenever refusal to participate with clinical data for investigational purposes was written in medical records.

Cohort

From August 2015, it was prospectively defined according to the institutional protocol that the 21-gene RS assay would be available for women with HR+/HER2- early breast cancer and at least one of the following criteria: i) Luminal A-like, pT2pN0; ii) Luminal A-like, 1 – 3 involved axillary nodes and comorbidities or performance status (PS) that put patients at high risk of chemotherapy-induced toxicity; iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 25%. Women included with more than one inclusion criterion were analysed separately. The upper Ki67 cut-off for inclusion was revised to 40% in April 2017, after an interim analysis (Martins-Branco, oral communication). All patients were

discussed at the multidisciplinary team meeting and the test was requested either at that time or later during the first appointment with the medical oncologist. The estimated time from sample shipment to the result was seven to 10 days (central laboratory). The period of patient inclusion for this analysis was closed in July 2018, when the final results of TAILORx trial were published,[5] which led to modifications of the RS cut-offs for adjuvant treatment recommendations. All tumour samples were locally reviewed by a single pathologist.

Outcomes

The study's primary outcome was the impact of the institutional protocol on the adjuvant treatment decision – proportion of patients with adjuvant treatment de-escalation: overall, per protocol, and by inclusion criterion. At the time of institutional protocol implementation, the treatment recommendations were: i) RS < 18 – isolated adjuvant ET; ii) RS ≥ 18 – adjuvant chemoendocrine therapy. All these patients would have been previously proposed for adjuvant chemoendocrine therapy according to the prior institutional treatment protocol.

As secondary clinical outcomes, we reported the cumulative incidence of recurrence and mortality until March 15th, 2022, clinically relevant adverse events (CRAE) occurring during the first six months of adjuvant treatment (unscheduled medical visits, hospital admissions, grade 3 febrile neutropenia as defined by Common Terminology Criteria for Adverse Events Version 5.0[14] – absolute neutrophil count < 1000/mm^3 with a single temperature of > 38.3˚C or a sustained temperature of ≥ 38˚C for more than one hour – and treatment discontinuation), and the number need to screen (NNS) - referring to the number of patients that need to be screened with 21-gene RS assay to prevent one CRAE. We also evaluated the feasibility of this genomic assay protocol.

As exploratory analyses we 1) interpreted the RS results according to the subsequently published TAILORx/RxPONDER data,[5,6] 2) measured the association of RS with histologic subtype and grade, and 3) tested the correlation of RS and Ki67 for the whole cohort and for the node-negative patients from inclusion criterion iii).

Statistical analysis

We performed a descriptive analysis using median and range for quantitative variables and absolute and relative frequencies for categorical variables. The cumulative incidence of adjuvant chemotherapy de-escalation was assessed in the whole cohort and by inclusion criteria subgroup. The 95% confidence intervals (95% CI) for proportions were estimated using the binomial 'exact' method.[15] The NNS was calculated using the formula NNS = NNT/prevalence,[16] with NNT representing the number of patients that need to be de-escalated to prevent one patient from having at least one CRAE (NNT = 1/ARR). Calculations considered the absolute risk reduction (ARR) in the proportion of patients with at least one CRAE resulting from de-escalating adjuvant chemoendocrine therapy to isolated endocrine therapy due to RS < 18, and the prevalence of RS < 18 observed in our sample. The associations of histologic subtype and grade with RS cut-offs (≥ 18 *vs* < 18, and > 25 *vs* ≤ 25) were evaluated with the chi-squared test or Fisher's exact test as appropriate, and the correlation between Ki67 and RS was tested with Pearson correlation coefficient. We used R and significance level of 5%.

RESULTS

Cohort characteristics

We included 154 women, 23 (15%) by criterion i) luminal A-like pT2pN0, seven (5%) by criterion ii) luminal A-like, 1 – 3 positive nodes, with comorbidities/PS that confer higher risk for chemotherapy-induced toxicity, and 110 (71%) by criterion iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 40%. Fourteen patients (9%) were included with more than one criterion [Appendix 1, S1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

The median age was 61 years (range, 25 – 79), 69% were postmenopausal, and tumours were mostly pT1 (55%), pN0 (82%), invasive ductal (73%), grade 2 (86%), luminal B-like (69%), stage IA (85%), and with a median Ki67 of 20% (1% – 40%). Surgery was lumpectomy plus sentinel lymph node biopsy in 104 cases (67%), mastectomy plus sentinel lymph node biopsy in 31 (20%), mastectomy plus axillary lymph node dissection in 15 (10%) and lumpectomy with axillary lymph node dissection in four (3%) (Table 1).

Recurrence score and impact on the adjuvant treatment decision

We obtained RS < 18 in 60% (95% CI: 52% – 68%; 93/154), ≥ 18 in 38% (30% – 47%, 59/154) and undetermined in 1% (0.2% – 5.0%, 2/154). In patients with luminal A-like, pT2pN0 tumours (n = 23) only two had RS ≥ 18 (RS = 19 and 20). From luminal A-like node-positive patients included due to comorbidities or PS that conferred higher risk for chemotherapy-induced toxicity (n = 7), two had RS ≥ 18 (RS = 19 and 20). Regarding patients included by PR ≤ 20% and/or Ki67 14% – 40% (n = 110), 49 (45%) presented RS ≥ 18. Out of the 14 included with more than one criterion, six patients (43%) had a RS ≥ 18 (Fig. 1).

We found an overall adjuvant treatment de-escalation of 65% (57% – 72%, 100/154), 58% (50% – 66%, 90/154) per protocol (Table 2). Patients with RS < 18 (n = 93), as per protocol, were discussed for treatment de-escalation for isolated adjuvant endocrine therapy with or without

Table 1 – Cohort baseline characteristics

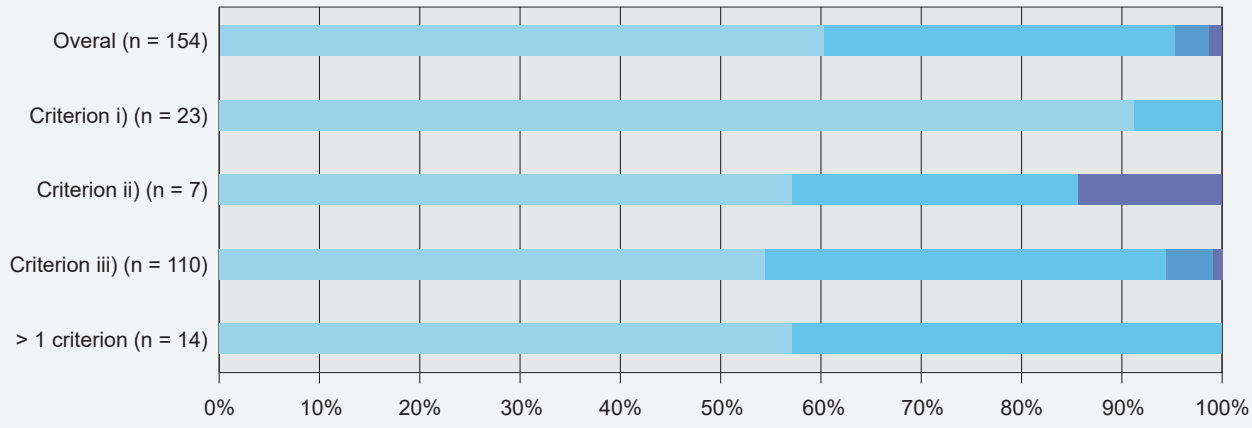
		n = 154
Age	Median (range)	61 (25 – 79)
	≤ 50 years (%)	43 (28)
Menopausal status, n (%)	Pre-	48 (31)
	Post-	106 (69)
Surgery, n (%)	Lumpectomy + SLNB	104 (67)
	Lumpectomy + ALND	4 (3)
	Mastectomy + SLNB	31 (20)
	Mastectomy + ALND	15 (10)
pT, n (%)	1	85 (55)
	2	69 (45)
pN, n (%)	0	126 (82)
	(1 – 3gg)	28 (18)
Histologic subtype, n (%)	Invasive ductal carcinoma	112 (73)
	Invasive lobular carcinoma	20 (13)
	Invasive carcinoma with ductal and lobular features	10 (6)
	Mixed ductal and mucinous carcinoma	8 (5)
	Carcinoma with invasive papillary component	2 (1)
	Carcinoma with neuroendocrine component	1 (< 1)
	Tubular/cribriform carcinoma	1 (< 1)
Histologic grade, n (%)	1	18 (12)
	2	132 (86)
	3	4 (3)
Luminal, n (%)	A-like	48 (31)
	B-like	106 (69)
Ki67, median (range)		20 (1 – 40)
AJCC/TNM staging, 8th edition, n (%)	IA	131 (85)
	IB	12 (8)
	IIA	10 (6)
	IIB	1 (< 1)

ALND: axillary lymph node dissection; SLNB: sentinel lymph node biopsy

adjuvant radiotherapy. However, three patients (3%) were proposed for adjuvant chemoendocrine therapy. The remaining 90 patients (97%) were proposed for isolated adjuvant endocrine therapy as per protocol, mostly with aromatase inhibitor (62%, 56/90). Of patients with RS ≥ 18 (n = 59), 51 patients (86%) were proposed for adjuvant chemotherapy as per protocol, mostly with a taxane-based regimen (63%, 32/51). Eight patients with RS ≥ 18 (14%, 8/59), were reconsidered for isolated endocrine therapy. The two patients with an undetermined RS result were proposed for isolated endocrine therapy due to a delay longer than three months from surgery date, after two attempts of genomic assay (Table 2).

Cohort follow-up: clinical outcomes

With a median follow-up of 51 months (range: 25 – 77), there were two local (1%) and six distant recurrences (4%). The local recurrences occurred 33 months after lumpectomy and sentinel lymph node biopsy in a patient with RS = 7 who refused to receive adjuvant radiotherapy and tamoxifen, and 25 months after lumpectomy and sentinel lymph node biopsy in a premenopausal, node-negative woman with RS = 19 who suspended adjuvant chemotherapy after a hypersensitivity reaction to taxane during the first cycle. The distant recurrences occurred in women with RS = 10, 14, 25, 28, 29, and 44, with a median of 31 months (18 – 38) until the distant recurrences observed. Except from the postmenopausal node-negative patient with RS = 25,



	> 1 criterion (n = 14)	Criterion iii) (n = 110)	Criterion ii) (n = 7)	Criterion i) (n = 23)	Overal (n = 154)
RS < 18	8	60	4	21	93
RS 18 – 30	6	44	2	2	54
RS ≥ 31	0	5	0	0	5
RS und.	0	1	1	0	2

RS < 18 RS 18 – 30 RS ≥ 31 RS und.

Figure 1 – Recurrence score (RS) distribution in the whole cohort and by institutional 21-gene assay protocol inclusion criterion. Inclusion criteria: i) Luminal A-like, pT2pN0; ii) Luminal A-like, with 1 – 3 involved axillary nodes and presence of comorbidities or performance status that constitute a higher risk for chemotherapy-induced toxicity; iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 25%.

who received isolated endocrine therapy after TAILORx data publication,[5] all the other patients received adjuvant chemotherapy. The woman with RS = 10, was proposed for adjuvant chemoendocrine therapy due to a diagnosis of supraclavicular node-positive disease after initial staging, surgery, and genomic assay, while the woman with RS = 14 was given adjuvant chemoendocrine therapy by patient-clinician joint decision. There was one case of contralateral breast cancer in a postmenopausal women treated with isolated endocrine therapy after a RS = 12 (1%). There were five deaths (3%), two due to breast cancer progressive disease and three from a non-breast cancer cause. Regarding CRAE during the first six months of adjuvant therapy, chemotherapy was associated with higher rate of patients with unscheduled medical visits (31%, 17/54 *vs* 5%, 5/100). From patients who attended unscheduled medical visits, those receiving adjuvant chemotherapy showed a trend for more visits (median 2, range 1 – 6 *vs* 1, 1 – 1). Patients receiving isolated endocrine therapy did not have any hospital admission during the first six months of adjuvant treatment, while 13% (7/54) of patients receiving chemotherapy experienced a hospital admission with a median duration of eight days (range: 1 – 18). The rate of patients with grade 3 febrile neutropenia under chemotherapy was 17% (9/54). Chemotherapy was discontinued due to docetaxel adverse events in 9% (5/54), in two of them due to infusion reactions. Overall, 35% (19/54) of the patients treated with chemotherapy experienced at least one CRAE, compared with 5% (5/100) of those treated with isolated endocrine therapy, which represents an absolute risk reduction of 30% (95% CI: 17% – 44%) [Appendix 1, S2 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

Considering the 30% absolute risk reduction of CRAE observed in the de-escalated patients treated with isolated endocrine therapy, and that by our sample inclusion criteria 60% of tested patients would be de-escalated to isolated endocrine therapy as a result of RS < 18, seven (95% CI: 5 – 10) patients needed to be screened with the 21-gene RS assay to prevent one CRAE during the first six months of adjuvant treatment.

Protocol feasibility

There was an increase in the number of tests requested per trimester across the study period, mainly after revision of the protocol criteria in April 2017. This number ranged from five (February – April 2016 - third trimester of the protocol) to 23 (February – April 2018 - second last trimester of the protocol) [Appendix 1, S3 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

Regarding the compliance with inclusion criteria, we

found 15 protocol deviations (10%) with the inclusion of node-positive patients by criterion iii). The median time from sample shipment to the central laboratory to 21-gene RS assay feedback was eight (range: 3 – 27) days. The proportion of cases that needed to repeat the assay was 4% (6/154), and the result was undetermined due to insufficient sample in 1% (2/154). As described above and shown in Table 2, there were 11 protocol deviations (7%) concerning the multidisciplinary team meeting proposal for adjuvant treatment.

Interpretation of RS according to TAILORx/RxPONDER data

From the 126 node-negative patients tested, 38% (48/126) had a RS ≥ 18, but only 9% (11/126) had RS > 25 [Appendix 1, S4A (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)] and all of them were given adjuvant chemoendocrine therapy. Within the 79 patients aged > 50 years with RS 0 – 25, 32% (25/79) had a RS 18 – 25 and of these 80% (20/25) were given chemoendocrine therapy as per protocol. On the other hand, from the 19 patients aged ≤ 50 years with RS > 15, 32% (6/19) had a RS 16 – 17 and were given isolated endocrine therapy as per protocol. Considering our protocol inclusion criteria, only criterion i) – luminal A-like pT2pN0 – did not register any case of RS > 25 (S4A). However, within this subgroup there were two out of ten patients aged ≤ 50 years who had a RS > 15 (S4C). From the 28 node-positive patients tested, 39% (11/28) had RS ≥ 18, but only 11% (3/28) had RS > 25. The three were postmenopausal and were given adjuvant chemoendocrine therapy. Among the 20 postmenopausal patients with RS 0 – 25, 25% (5/20) had a RS 18 – 25, and of these, four were given adjuvant chemoendocrine therapy, as per protocol. Four out of the eight premenopausal patients were given isolated endocrine therapy [Appendix 1, S5 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)]. According to the current

Table 2 – Multidisciplinary team (MDT) proposal and adjuvant treatment starting choice

Recurrence Score	MDT proposal	Adjuvant treatment - starting choice	
Low (< 18) (n = 93)	Endocrine therapy (n = 90) – per protocol	Aromatase inhibitor (n = 56)	Letrozole (n = 56)
		Tamoxifen (n = 34)	
	Chemoendocrine therapy (n = 3)	Anthracycline and taxane based (n = 2)	FEC3 + paclitaxel (n = 2)
		Taxane-based (n = 1)	TC4 (n = 1)
Intermediate/high (≥ 18) (n = 59)	Chemoendocrine therapy (n = 51) – per protocol	Taxane-based (n = 32)	TC4 (n = 31)
			TC6 (n = 1)
		Anthracycline and taxane based (n = 16)	FEC-D (n = 10)
			FEC3 + paclitaxel (n = 6)
		Anthracycline based (n = 3)	FEC6 (n = 2)
			AC4 (n = 1)
	Endocrine therapy (n = 8)	Aromatase inhibitor (n = 7)	Letrozole (n = 6)
			Exemestane (n = 1)
		Tamoxifen (n = 1)	
Undetermined (n = 2)	Endocrine therapy (n = 2)	Aromatase inhibitor (n = 2)	Letrozole (n = 2)

AC4: four cycles, three weekly, of doxorubicin 60 mg/m² IV and cyclophosphamide 600 mg/m² IV; FEC3: three cycles, three weekly, of fluorouracil 500 mg/m² IV, epirubicin 100 mg/m² IV and cyclophosphamide 500 mg/m² IV – two patients were given reduced dose of epirubicin – 75 mg/m² IV per cycle; FEC6: six cycles, three weekly, of fluorouracil 500 mg/m² IV, epirubicin 100 mg/m² IV and cyclophosphamide 500 mg/m² IV; FEC-D: FEC3 followed by three cycles, three weekly, of docetaxel 100 mg/m² IV; Paclitaxel: nine to 12 cycles, weekly, 80 mg/m² IV; TC4: four cycles, three weekly, of docetaxel 75 mg/m² IV and cyclophosphamide 600 mg/m² IV; TC6: six cycles, three weekly, of docetaxel 75 mg/m² IV and cyclophosphamide 600 mg/m² IV

guidance for tailoring adjuvant treatment, the inclusion criteria of this institutional protocol identified 76% (96/126) of the node-negative patients and 57% (16/28) of node-positive patients with a RS compatible with adjuvant treatment de-escalation and with indication for treatment with isolated endocrine therapy. This would mean an overall treatment de-escalation of 73% (112/154) with the currently used RS cut-offs.

Relationship between RS and histology or Ki67

There were no associations between RS categories and histologic subtype or grade [Appendix 1, S6 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)]. There was no RS > 25 for grade 1 tumours (n = 18), but within the six cases of grade 1 tumours with RS > 15, five were aged ≤ 50 years, and three were node-positive with RS ≥ 18 (RS 18 and 22).

There was a statistically significant weak correlation (r = 0.22, 95% CI: 0.07 – 0.37) between Ki67 and RS for the whole cohort, but no correlation could be demonstrated for the subgroup of node-negative patients from inclusion criterion iii) [Appendix 1, S7 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

DISCUSSION

This study found that the use of the selected clinicopathologic inclusion criteria for 21-gene RS assay was associated with decreased chemotherapy exposure in at least six out of each 10 tested women. This protocol avoided unnecessary treatment toxicity by preventing one clinically relevant adverse event by every seven women that undergo testing. Another important finding was that all women older than 50 years and included in our study due to pT2pN0 tumours with PR > 20% and Ki67 < 14% presented a RS ≤ 25. Although stronger evidence should be used to support this decision, this finding suggests that this latter subgroup could be eventually spared from adjuvant chemotherapy without the use of this genomic assay.

An accurate selection of patients with uncertain benefit from adjuvant chemotherapy, but a higher likelihood of low RS, might be the key to increase the cost-effectiveness of this tool that has already been demonstrated in other countries.[17–20] This was the reason to select more restrictive inclusion criteria than those considered eligible for genomic assay: HR+/HER2-, pT1 – 2, 0 – 3 nodes.[21] Therefore, we did not include patients with pT1pN0 tumours with PR > 20% and Ki67 below 14%, for whom local and international guidelines do not recommend adjuvant chemotherapy.[22,23] On the other hand, we also did not include patients with higher-risk tumours who have indication to receive adjuvant chemotherapy, such as luminal B-like node-negative with Ki67 > 40% or luminal B-like with 1 – 3 positive nodes.[22,23] By excluding these cases, the eligibility was restricted to women with tumours belonging to a shorter 'grey area' of risk, to whom adjuvant chemotherapy would have being prescribed as per local standards. This resulted in a higher proportion of treatment de-escalation than those reported in real-word studies from other European countries.[24,25]

Considering the results from the TAILORx trial, that prospectively validated the increase of the cut-off for adjuvant chemotherapy in node-negative disease to RS > 25,[5] the reported percentage of patients spared from adjuvant chemotherapy with this protocol would be even higher (76%), even though still slightly above than what was more recently reported in another European country.[26] Twenty additional women could have been de-escalated for isolated adjuvant endocrine therapy, while only six women with ≤ 50 years with a RS 16 – 17 could have had benefitted from adjuvant chemotherapy.

Regarding node-positive disease and considering the new data from the RxPONDER trial,[6] we should consider that all premenopausal women with node-positive disease may benefit from adjuvant chemotherapy, regardless of RS. Thus, in premenopausal women, the use of OncotypeDx should be restricted to node-negative patients. However, the selection of node-positive postmenopausal women in this cohort was compatible with a high de-escalation proportion (80%), with only three cases of RS > 25. Therefore, the use of this genomic assay should be extended to more postmenopausal women with luminal A-like tumours and 1 – 3 positive nodes.

Our study did not find a significant association of RS with either histologic subtype or grade, as suggested by larger studies.[8,9] Indeed, our data suggests that selected well-differentiated tumours in women with aged under 50 years old, or node-positive disease might still benefit from the genomic assay. Despite the weak correlation in the whole cohort, we found no correlation between Ki67 and RS in patients with node-negative and Ki67 14% – 40%. Therefore, our data do not allow drawing any conclusions on to what extent histology features or Ki67 could be used to redefine eligibility criteria for the use of this genomic assay. These uncertainties reinforce the utility of this tool in tailoring the adjuvant treatment.

Importantly, the use of this genomic assay protocol did not compromise the adequate timing for adjuvant chemotherapy, with low proportion of undetermined RS. We reported an increasing number of tests per trimester as clinicians were recognising the clinical usefulness of this tool, which, along with the pre-defined restrictive inclusion criteria, might explain the inclusion of only 9% of all stage I-II HR+/HER2- breast cancer cases treated in the institution during the period of the study.

This study has some methodological limitations, such as using data from a single-centre and employing an observational design, which limit both external and internal validity of the results. Moreover, the sample size limits the statistical power to explore associations or correlations between RS and clinicopathologic features. The 10% rate of eligibility protocol deviations, derived from the inclusion of node-positive patients with more aggressive tumours than permitted by the protocol, might have generated bias towards a lower overall rate of adjuvant treatment de-escalation. On the other hand, the 7% rate of protocol deviations related to adjuvant treatment recommendations was in part influenced by the shift in the cut-off for adjuvant chemotherapy after the publication of the results from the TAILORx trial,[5] and did not lead to disease recurrence due to potential undertreatment. Despite these limitations, and to the best of our knowledge, this is the first report of a wide institutional protocol use of this 21-gene RS assay in a Portuguese public hospital, providing guidance on how to potentiate the use of a limited resource in this setting.

CONCLUSION

In the era of personalised medicine, as genomic assays are becoming widespread in developed countries, it is important that clinicians can recognise which patients may benefit the most from them. This study identified inclusion criteria for performing genomic assay in women with HR+/HER2- early breast cancer leading to de-escalation of adjuvant systemic treatment in at least six out of ten women undergoing testing, and prevention of one clinically relevant adverse event in one out of seven women undergoing testing. This assay is replicable in real-world settings and does not considerably delay the appropriate timing for adjuvant systemic treatment.

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PRESENTATIONS AND AWARDS

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DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

DMB: Data curation; formal analysis; investigation; methodology; project administration; resources; validation; visualization; writing of the draft.

SCF: Data curation; methodology; validation; visualization; writing of the draft; manuscript review and editing.

EG: Conceptualization; investigation; methodology; resources; validation; visualization; manuscript review and editing.

SA: Conceptualization; data curation; investigation; methodology; resources; validation; visualization; manuscript review and editing.

SE: Formal analysis; methodology; resources; validation; visualization; manuscript review and editing.

MB: Conceptualization; data curation; investigation; methodology; project administration; resources; validation; visualization; manuscript review and editing.

AM: Conceptualization; investigation; methodology; project administration; resources; supervision; validation; visualization; manuscript review and editing.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare that they followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

DMB declares honoraria from Daiichi Sankyo, Novartis, Merck Sharp & Dohme, Janssen, Pfizer, Angelini, and AstraZeneca; meeting/travel grants from Novartis, Merck Sharp & Dohme, LEO Farmacêuticos, Ipsen, and Janssen; and institutional grants from Novartis and F. Hoffmann-La Roche Ltd (all outside the submitted work).

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