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Patient- and tumour-related variables that are associated with the outcome of the MammaPrint

a registry-based analysis

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Abstract

Background: The MammaPrint can have added value for early stage breast cancer patients at clinical intermediate risk for recurrence, in whom on the basis of clinical variables cannot be determined if chemotherapy is beneficial. Given the fact that the MammaPrint is used within a very broad patient group, we aimed to identify patient-and tumour-related variables that are associated with the outcome of the MammaPrint, with the ultimate aim to identify specific subgroups within the early stage breast cancer patients at clinical intermediate risk, in whom the MammaPrint may be omitted because their genomic risk outcome can be predicted.

Methods: This study included all operated women younger than 70 years who where diagnosed with ER positive, HER2 negative breast cancer between 2011 and 2017, that had the following characteristics: either pT2N0, grade 1 or pT1N0, grade 2 and >34 years or pN1, grade 1 or 2 and >34 years. This population was divided in two cohorts, cohort one included patients who did not receive the MammaPrint and cohort two included patients who did receive the MammaPrint. The split sample method was used, 80% of cohort two was used to fit a multivariable logistic regression model to identify variables associated with the genomic high or genomic low risk outcome of the MammaPrint. The other 20% was used for internal validation. The area under the receiver operating characteristic curve, sensitivity, specificity, positive predictive value and negative predictive value were used to assess discriminative power and the Hosmer-lemeshow and the Brier test were used to assess calibration. Two-sample proportion tests were used to compare the likelihood of receiving chemotherapy treatment between cohort one and cohort two.

Results: PR status, differentiation grade, histological type and detection through screening were significantly associated with the MammaPrint outcome. The ROC curve (0.62) of this model showed a poor discriminative power. The Hosmer-lemeshow and Brier test showed good calibration for the validation model. Patients with a probability <10% of a genomic high risk (MammaPrint) outcome show a lower percentage chemotherapy (13.3%) when they received the MammaPrint, compared to 33.7% of the patients not receiving the MammaPrint. On the contrary, of the patients with a probability of 40-50%, 35.7% had chemotherapy when they received the MammaPrint, compared to 19.7% when they did not receive the MammaPrint.

Conclusion*:* This study shows that clinicopathological variables alone cannot accurately predict the outcome of the MammaPrint within the early stage breast cancer patients at clinical intermediate risk. In addition, some results may indicate possible overtreatment with adjuvant chemotherapy in some patient groups and undertreatment in other patient groups within these early stage breast cancer patients. This confirms that the MammaPrint is an important additional test for early stage breast cancer patients at clinical intermediate risk, to be able to make well informed treatment decisions.

Key words: MammaPrint, breast cancer, prediction model, chemotherapy, clinicopathological variables, gene expression profile.

Abbreviations: HER2, human epidermal growth factor receptor 2; ER status, estrogen receptor status; PR status, progesterone status; DCIS, ductal carcinoma in situ; GEP, gene expression profile; AUC, area under the curve; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value.

Introduction

The administration of adjuvant chemotherapy has improved the prognosis for early stage breast cancer patients (1,2). Looking at the Dutch guidelines from 1990 until 2012, the patient group eligible for adjuvant chemotherapy has been gradually expanded (3). The patients with ER positive early stage breast cancer are at risk of overtreatment with adjuvant chemotherapy (4). Some patients of this group have, based on their tumour biology, a lower risk of recurrence and their tumours are less sensitive to chemotherapy (5). Adjuvant chemotherapy treatment is highly effective, but possible adverse events could have a huge impact on a patients' daily life (6).

The MammaPrint is a gene expression profile (GEP) that is used complementary to the clinical variables. The clinical intermediate patient group could benefit from this additional test, because the advice of undergoing chemotherapy may depend on the genomic high or genomic low risk outcome of the MammaPrint (4). The Microarray in Node-Negative Disease May Avoid Chemotherapy Trail (MINDACT) provides prospective evidence that the MammaPrint has added value for clinical practice, especially for the early stage breast cancer patients at clinical high (according to Adjuvant! Online) and genomic low risk. The results for this particular group showed that they can avoid possible adverse events from chemotherapy by omitting this treatment at the cost of a possible increased risk of 1.5 percent point higher at a distant metastasis at 5 years (7). In addition, omitting chemotherapy saves costs, not only of the treatment itself but also the treatment costs of possible adverse events as well as costs for workplace absenteeism (8).

Groenendijk et al. stated that in the Netherlands between 2013-2016 the MammaPrint was performed in a much larger patient group than recommended by the ASCO guidelines. This was the result of the absence of a clearly indicated patient group (9). The current Dutch guideline (2018) describes two patient groups, one group includes patients with an indication for a GEP and one group includes patients that may have an indication for a GEP (10). A total of 52 panel members from the St. Gallen International Breast Cancer Consensus Conference in 2017 agreed that a GEP offers information that can guide the decision on adjuvant chemotherapy in node negative cases, but there was considerable discussion concerning the indication for a GEP (11). This shows that there is no overall agreement which exact patients belong to the clinical intermediate patient group, which leads to the use of the MammaPrint in a broader patient group than intended. A clearly defined clinical intermediate group would decrease the use of the MammaPrint within the patient groups that are clearly designated to the clinical high or clinical low risk patient group. A clearly defined clinical intermediate group will most likely lead to more accurate estimations about the benefits and risk of recurrence when omitting chemotherapy. The MINDACT used all early stage breast cancer patients and could not state with certainty that the early stage breast cancer patients of clinical high and genomic low risk would benefit from omitting chemotherapy or that it would lead to an unacceptable increased risk of distant metastasis at 5 years (7). This current uncertainty about the risk of recurrence with omitting chemotherapy is the reason for the Dutch Health Institute to doubt the safety of adding the MammaPrint to the standard procedure. Therefore their recommendation to the Dutch health insurance companies is to not reimburse the MammaPrint from the basic insurance packages (12). Alongside this, the clinicians and patients still emphasize that the MammaPrint is an important tool for the shared decision-making process (13).

This study aims to identify patient- and tumour-related variables that are associated with the outcome of the MammaPrint in a registry-based analysis, with the ultimate aim to identify specific subgroups within the early stage breast cancer patients at clinical intermediate risk, in whom the MammaPrint may be omitted because their genomic risk outcome can be predicted.

Material and Methods

Study Population

The women included in this study were all diagnosed with invasive breast cancer of clinical intermediate risk. To select the intermediate risk group the indications described by Kuijer, et al. were used (14). All the patients had an ER positive, HER2 negative invasive carcinoma, were younger than 70 years and had a tumour of either pT2N0, grade 1 or pT1N0, grade 2 and >34 years or pN1, grade 1 or 2 and >34 years. Data from January 2011 until December 2017 was obtained from the Netherlands Cancer Registry (NCR). Since 1989 the NCR registers all demographic and clinicopathological information from all cancer patients in the Netherlands (www.cijfersoverkanker.nl) (15,16). From this registry all relevant patient-, tumour-, and

treatment-related characteristics were abstracted. Patients were excluded in case the breast cancer was not surgically treated, when presence of metastasis was confirmed or when they were diagnosed in a foreign country. Patients were also excluded when they only received the oncotype test or when they received the MammaPrint but where the outcome was unknown. Patients with an unknown pathological tumour stage and an unknown pathological lymph node stage were excluded, except for the patients that received neoadjuvant therapy as their primary treatment. For these patients the clinical tumour and lymph node stages were used instead. All remaining women were included in this study and divided in two cohorts. Cohort one included patients who did not receive the MammaPrint. The second cohort included patients who received the MammaPrint. Patients within cohort two were divided on the basis of their genomic risk (MammaPrint) outcome, cohort 2A includes genomic low risk patients and 2B genomic high risk patients.

Statistical Analyses

Percentages were used to describe the differences between cohort one and cohort two (patients who did not receive the MammaPrint and patients who received the MammaPrint). To compare the baseline characteristics of group 2A and 2B (genomic low and genomic high risk), a two sample t-test was used for the continuous variables and the Pearson's chi-squared test for the categorical variables. The adherence to the advised treatment (on the basis of their genomic outcome) according to the guidelines was shown in the variable adjuvant therapy.

To identify variables associated with the MammaPrint outcome, a multivariable logistic regression was used on the observations of cohort two (patients who received the MammaPrint). To be able to test the internal validity the method of a split sample was used. Eighty percent of cohort two was randomly selected and used for the logistic regressions and the other twenty percent was used for the internal validation of the model. The variables were selected on the basis of clinical reasoning and were: age, multifocality, presence of a DCIS component, most extensive surgery, differentiation grade, PR status, detection through screening, performance status, cancer history, menopausal status, social economic status, sublocalization, histological type, pathological tumour stage, pathological node stage, clinical tumour and clinical nodal stage (both in case of neoadjuvant therapy), and radicality of the last surgery. The best prediction model was derived through backward variable selection based on likelihood ratio tests.

Predicted probabilities were used to identify different patient groups with their associated average probability of genomic high risk outcome. A receiver operating characteristic (ROC) curve was used to test the discriminative power of the model. Based on the classifying system described by Safari et al., the area under the receiver operating characteristic curve can be interpreted as: 0.9 - 1 = excellent; 0.8 - 0.9 = good; 0.7 - 0.8 = fair; 0.6 - 0.7 = poor and 0.5 - 0.6 = fail (17). The Hosmer-Lemeshow test and the Brier score were used to assess calibration of the developed model as well as the validation model. A non-significant p-value of the Hosmer-Lemeshow test indicates good calibration and a Brier score of 0 shows a perfect calibration and a score of ≥0.25 is undesirable (18,19). Calibration plots were made to make the differences between observed and expected probabilities visible. To determine if identified patient groups can omit the MammaPrint the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for several cut-off values.

Within the identified patient groups a two-sample proportion test was used to compare the chemotherapy treatments given to patients who did not receive the MammaPrint (cohort one) and patients who did receive the MammaPrint (cohort two). Differences were considered to be significant in case of a p-value <0.05. STATA version 14.2 was used for the statistical analyses.

Results

Patient characteristics

The final study population consisted of 10,811 patients: 7,732 in cohort one and 3,079 in cohort two. Of the patients in cohort two, 2,170 had a genomic low risk outcome (cohort 2A) and 909 had a genomic high risk outcome (cohort 2B). Figure 1 presents the patient selection and division as described in the methods. Patients who received the MammaPrint (cohort two), were generally younger, had a higher socioeconomic status, more often had a ductal tumour, a grade 2 tumour, a unifocal tumour and presence of a DCIS component as compared to patients who did not receive the MammaPrint (cohort one). In addition, these patients more often received adjuvant systemic therapy (92%) as compared to patients not receiving the

MammaPrint (86%). Patients with a genomic high risk outcome more often had a tumour of the ductal or other type (compared to lobular or mixed ductal lobular), differentiation grade 2, a negative PR status and were detected less often through screening, compared to patients with a genomic low risk outcome. Furthermore the genomic high risk patients more often received systemic therapy, 76% received either adjuvant chemotherapy or both adjuvant hormonal-and chemotherapy, compared to 3% of the genomic low risk patients. Baseline characteristics are shown in Table 1.

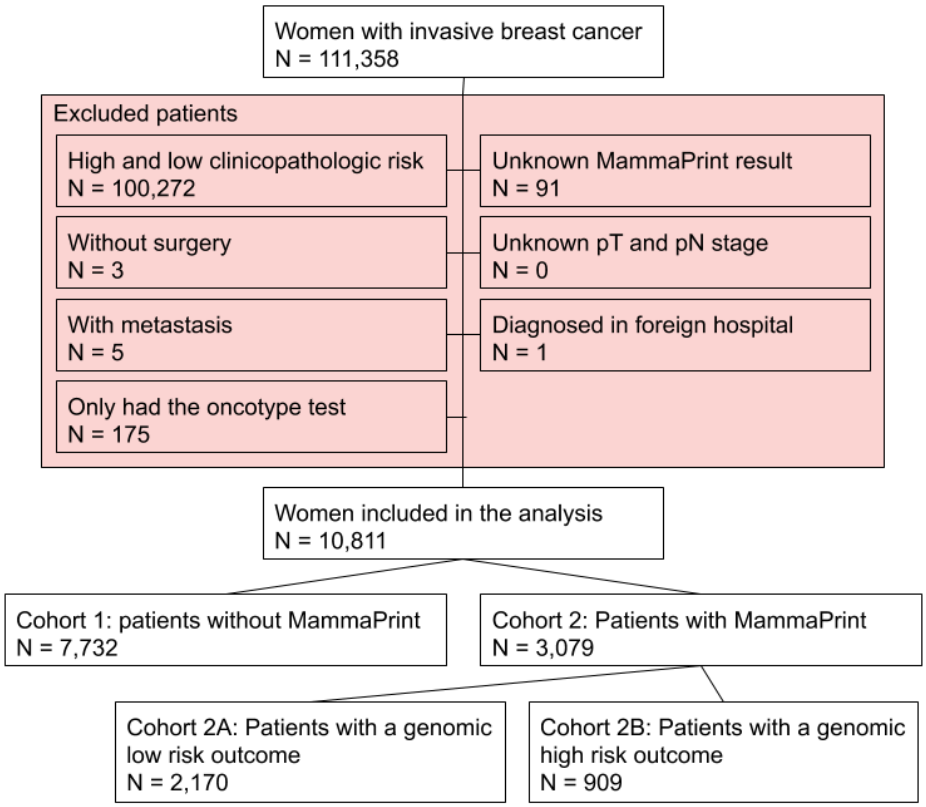

Figure 1: Flowchart of included patients and the division different cohorts.

Table 1: Baseline characteristics

	Cohort 1 Patients without MammaPrint N = 7,732, N(%)	Cohort 2 Patients with MammaPrint N = 3,079, N(%)	Cohort 2A Genomic low risk N = 2,170, N(%)	Cohort 2B Genomic high risk N = 909, N(%)	P-value
Patient characteristics					
Age in years, mean (SD)	57.6 (8.4)	55.5 (7.8)	55.4 (7.8)	55.6 (8.0)	0.67
Age category					0.29
≤ 29	1 (<0.1)	-	-	-	
≥30 & ≤39	162 (2.1)	64 (2.1)	43 (2.0)	21 (2.3)	
≥40 & ≤49	1,305 (16.9)	682 (22.1)	480 (22.1)	202 (22.2)	
≥50 & ≤59	2,554 (33.0)	1,252 (40.7)	904 (41.7)	348 (38.3)	
≥60 & ≤69	3,710 (48.0)	1,081 (35.1)	743 (34.2)	338 (37.2)	
Incidence year					0.037
2011	1,322 (17.1)	131 (4.3)	98 (4.5)	33 (3.6)	
2012	1,276 (16.5)	153 (5.0)	101 (4.7)	52 (5.7)	
2013	1,049 (13.6)	477 (15.5)	336 (15.5)	141 (15.5)	
2014	887 (11.5)	609 (19.8)	420 (19.4)	189 (20.8)	
2015	993 (12.8)	593 (19.3)	395 (18.2)	198 (21.8)	
2016	1,027 (13.3)	662 (21.5)	494 (22.8)	168 (18.5)	
2017	1,178 (15.2)	454 (14.7)	326 (15.0)	128 (14.1)	

SES					0.49
Low	2,183 (28.2)	761 (24.7)	525 (24.2)	236 (26.0)	
Medium	3,222 (41.7)	1,297 (42.1)	914 (42.1)	383 (42.1)	
High	2,327 (30.1)	1,021 (33.2)	731 (33.7)	290 (31.9)	
Menopausal stage					<0.001
pre-menopausal	1,305 (16.9)	798 (25.9)	565 (26.0)	233 (25.6)	
post-menopausal (because of chemo)	92 (1.2)	9 (0.3)	-	9 (1.0)	
peri menopausal	412 (5.3)	261 (8.5)	200 (9.2)	61 (6.7)	
post-menopausal (>55 years status unknown)	4,956 (64.1)	1,801 (58.5)	1,252 (57.7)	549 (60.4)	
Unknown (<55 years status unknown)	812 (10.5)	210 (6.8)	153 (7.1)	57 (6.3)	
Unknown	155 (2.0)	-	-	-	
Tumour characteristics					
Sublocalization					0.40
Outer quadrants	3,647 (47.2)	1,441 (46.8)	1,031 (47.5)	410 (45.1)	
Inner quadrants	1,597 (20.7)	646 (21.0)	443 (20.4)	203 (22.3)	
Central parts	539 (7.0)	214 (7.0)	155 (7.1)	59 (6.5)	
Overlapping lesions	1,841 (23.8)	739 (24.0)	511 (23.5)	228 (25.1)	
Unknown	108 (1.4)	39 (1.3)	30 (1.4)	9 (1.0)	
Histological type					<0.001
Ductal	5,662 (73.2)	2,628 (85.4)	1,825 (84.1)	803 (88.3)	
Lobular	1,534 (19.8)	283 (9.2)	226 (10.4)	57 (6.3)	
Mixed ductal lobular	335 (4.3)	109 (3.5)	89 (4.1)	20 (2.2)	
Other	201 (2.6)	59 (1.9)	30 (1.4)	29 (3.2)	
Differentiation grade					<0.001
1	1,311 (17.0)	357 (11.6)	308 (14.2)	49 (5.4)	
2	6,421 (83.0)	2,722 (88.4)	1,862 (85.8)	860 (94.6)	
*Tumour stage**					<0.001
1	6,559 (84.8)	2,807 (91.2)	1,945 (89.6)	862 (94.8)	
2	1,036 (13.4)	265 (8.6)	220 (10.1)	45 (5.0)	
3	107 (1.4)	4 (0.1)	3 (0.1)	1 (0.1)	
4	27 (0.3)	3 (0.1)	2 (0.1)	1 (0.1)	
IS	1 (<0.1)	-	-	-	
Unknown	2 (<0.1)	-	-	-	
*Nodal stage***					0.015
0	6,424 (83.1)	2,665 (86.6)	1,859 (85.7)	806 (88.7)	
1	1,296 (16.8)	412 (13.4)	311 (14.3)	101 (11.1)	
2	3 (<0.1)	1 (<0.1)	-	1 (0.1)	
3	6 (0.1)	-	-	-	
Unknown	3 (<0.1)	1 (<0.1)	-	1 (0.1)	
DCIS component					0.92
No	3,969 (51.3)	1,364 (44.3)	960 (44.2)	404 (44.4)	
Yes	3,648 (47.2)	1,715 (55.7)	1,210 (55.8)	505 (55.6)	
Unknown	115 (1.5)	-	-	-	
Multifocal					0.67
No	6,437 (83.3)	2,674 (86.8)	1,889 (87.1)	785 (86.4)	
Yes	1,277 (16.5)	398 (12.9)	277 (12.8)	121 (13.3)	
Unknown	18 (0.2)	7 (0.2)	4 (0.2)	3 (0.3)	
PR status					<0.001
Negative	1,163 (15.0)	457 (14.8)	251 (11.6)	206 (22.7)	
Positive	6,555 (84.8)	2,621 (85.1)	1,919 (88.4)	702 (77.2)	
Unknown	14 (0.2)	1 (<0.1)	-	1 (0.1)	
Radicality last surgery					0.64
Both invasive and DCIS radical	7,070 (91.4)	2,834 (92.0)	1,999 (92.1)	835 (91.9)	
Invasive radical, DCIS focal irradical	192 (2.5)	72 (2.3)	49 (2.3)	23 (2.5)	

Invasive radical, DCIS irradical	14 (0.2)	6 (0.2)	4 (0.2)	2 (0.2)	
Invasive focal irradical, DCIS radical	332 (4.3)	134 (4.4)	98 (4.5)	36 (4.0)	
Both invasive and DCIS focal irradical	24 (0.3)	8 (0.3)	4 (0.2)	4 (0.4)	
Invasive focal irradical, DCIS irradical	1 (<0.1)	1 (<0.1)	-	1 (0.1)	
Irradical and irrelevant	27 (0.3)	12 (0.4)	7 (0.3)	5 (0.6)	
Invasive radical, DCIS radicality unclear	2 (<0.1)	1 (<0.1)	1 (<0.1)	-	
Unknown	70 (0.9)	11 (0.4)	8 (0.4)	3 (0.3)	
Detection through screening					0.040
No	3,787 (49.0)	1,599 (51.9)	1,101 (50.7)	498 (54.8)	
Yes***	3,839 (49.6)	1,480 (48.1)	1,069 (49.3)	411 (45.2)	
Unknown	106 (1.4)	-	-	-	
Most extensive surgery					0.31
Breast-conserving	5,422 (70.1)	2,387 (77.5)	1,693 (78.0)	694 (76.3)	
Mastectomy	2,310 (29.9)	692 (22.5)	477 (22.0)	215 (23.7)	
Adjuvant therapy					<0.001
No adjuvant	1,087 (14.1)	232 (7.5)	203 (9.4)	29 (3.2)	
Only hormonal therapy	4,838 (62.6)	2,099 (68.2)	1,909 (88.0)	190 (20.9)	
Only chemotherapy	83 (1.1)	32 (1.0)	4 (0.2)	28 (3.1)	
Both hormonal and chemo	1,724 (22.3)	716 (23.3)	54 (2.5)	662 (72.8)	
Neoadjuvant therapy					<0.001
No neoadjuvant therapy	7,138 (92.3)	3,016 (98.0)	2,130 (98.2)	886 (97.5)	
Only hormonal therapy	106 (1.4)	38 (1.2)	32 (1.5)	6 (0.7)	
Only chemotherapy	441 (5.7)	21 (0.7)	7 (0.3)	14 (1.5)	
Both hormonal and chemo	47 (0.6)	4 (0.1)	1 (<0.1)	3 (0.3)	
Hospital type					0.86
General	3,226 (41.7)	1,341 (43.6)	950 (43.8)	391 (43.0)	
STZ	3,918 (50.7)	1,620 (52.6)	1,139 (52.5)	481 (52.9)	
Academic	588 (7.6)	118 (3.8)	81 (3.7)	37 (4.1)	

**Tumour stage = pathological tumour stage and clinical tumour stage in case of neoadjuvant*
***Nodal stage = pathological nodal stage and clinical nodal stage in case of neoadjuvant*
****Including three patients from the high risk screening program*
Abbreviations: SES = social economic status; General = general hospital; STZ= cooperating top clinical hospitals; Academic = academic hospitals.

Variables associated with the results of the MammaPrint

With the use of the split sample method (80%), the total number of patients used for the development of the model was 2,463. The multivariable logistic regression analysis show four variables that were significantly and independently associated with the MammaPrint outcome: PR status, differentiation grade, detection through screening and histological type. The odds ratios of each variable, including the 95% confidence intervals, are shown in Table 2.

Table 2: Variables associated with the MammaPrint outcome

MammaPrint		OR	95% Conf. Interval		P-value
PR status:	Negative	1			
	Positive	0.45	0.35	0.57	<0.001
Differentiation grade:	One	1			
	Two	2.96	2.08	4.21	<0.001
Detection through screening:	No	1			
	Yes	0.77	0.65	0.93	0.005
Histological type:	Ductal	1			
	Lobular	0.57	0.41	0.81	0.001
	Mixed ductal lobular	0.46	0.25	0.82	0.009
	Other	2.64	1.45	4.81	0.002
Constant		0.35	0.24	0.53	<0.001

Based on these four categorical variables, 32 unique patient groups with different probabilities of a genomic high risk outcome can be identified, which are shown in Table 3. Dividing the patients into these 32 groups on the basis of the probabilities leads to large differences in group size. Some of these 32 groups are small, which may lead to large uncertainties about their corresponding probability. Four groups have a low probability (<10%) of a genomic high risk outcome and there are no groups with a probability above 73% of a genomic high risk outcome. The identified groups with a probability of less than 10% all had a positive PR status, a differentiation grade 1 and a histological type of either lobular or mixed ductal lobular.

Table 3: Probabilities of high genomic risk outcome for 32 identified patient groups

	Characteristics				Probability		MammaPrint outcome	
Identified groups	Histological type	PR status	Differentiation grade	Detection through screening	Decimal	%	Low genomic risk, N (%)	High genomic risk, N (%)
1	3	P	1	1	0.05	5.3	3 (100)	0 (0)
2	2	P	1	1	0.07	6.6	7 (87.5)	1 (12.5)
3	3	P	1	0	0.07	6.8	5 (83.3)	1 (16.7)
4	2	P	1	0	0.08	8.4	11 (84.6)	2 (15.4)
5	1	P	1	1	0.11	10.9	73 (86.9)	11 (13.1)
6	3	N	1	1	0.11	11.1	2 (100)	0 (0)
7	2	N	1	1	0.14	13.6	3 (100)	0 (0)
8	1	P	1	0	0.14	13.7	110 (85.3)	19 (14.7)
9	3	N	1	0	0.14	13.9	1 (100)	0 (0)
10	3	P	2	1	0.14	14.2	26 (92.9)	2 (7.1)
11	2	N	1	0	0.17	16.9	1 (100)	0 (0)
12	2	P	2	1	0.17	17.3	64 (82.1)	14 (18.0)
13	3	P	2	0	0.18	17.7	22 (71.0)	9 (29.0)
14	2	P	2	0	0.21	21.3	62 (76.5)	19 (23.5)
15	1	N	1	1	0.21	21.5	15 (88.2)	2 (11.8)
16	4	P	1	1	0.24	24.5	2 (66.7)	1 (33.3)
17	1	N	1	0	0.26	26.1	11 (91.7)	1 (8.3)
18	1	P	2	1	0.27	26.7	545 (72.7)	205 (27.3)
19	3	N	2	1	0.27	27.0	4 (80.0)	1 (20.0)
20	4	P	1	0	0.30	29.5	5 (71.4)	2 (28.6)
21	2	N	2	1	0.32	31.8	21 (72.4)	8 (27.6)
22	1	P	2	0	0.32	32.0	592 (69.9)	255 (30.1)
23	3	N	2	0	0.32	32.4	6 (85.7)	1 (14.3)
24	2	N	2	0	0.38	37.6	10 (76.9)	3 (23.1)
25	4	N	1	1	0.42	42.0	1 (100)	0 (0)
26	1	N	2	1	0.45	44.8	84 (53.9)	72 (46.2)
27	4	N	1	0	0.48	48.3	0 (0)	0 (0)
28	4	P	2	1	0.49	49.0	9 (64.3)	5 (35.7)
29	1	N	2	0	0.51	51.2	45 (40.5)	66 (59.5)
30	4	P	2	0	0.55	55.4	4 (23.5)	13 (76.5)
31	4	N	2	1	0.68	68.1	3 (100)	0 (0)
32	4	N	2	0	0.73	73.4	0 (0)	2 (100)

Histological type: 1 = ductal, 2 = lobular, 3 = mixed ductal lobular, 4 = other
PR status: P = positive, N = negative
Detection through screening: 1 = yes, 0 = no
= groups with a low probability (<10%) of a genomic high risk outcome

Validation of the model

The developed model showed an area under the ROC curve of 0.62 (95% CI 0.60 – 0.64). A figure of the ROC curve can be found in the Appendix. The Hosmer-Lemeshow test was nonsignificant (P = 0.29) when predicted risks were divided in six equal groups and the Brier score was 0.20. The validation model included 616 patients and demonstrated good calibration with a Hosmer-Lemeshow test that was nonsignificant (P = 0.70) when divided in six equal groups and had a Brier score of 0.20. The area under the ROC curve was 0.65 (95% CI 0.61 – 0.69). Calibration plots of the developed model as well as the validation model are shown in Figure 2. The patients are divided into six groups of equal size (developed model N = 410 and validation model N = 103) on the basis of their predicted risk. The validation model shows an overall good calibration with a broader confidence interval for the two groups with the highest predicted risk.

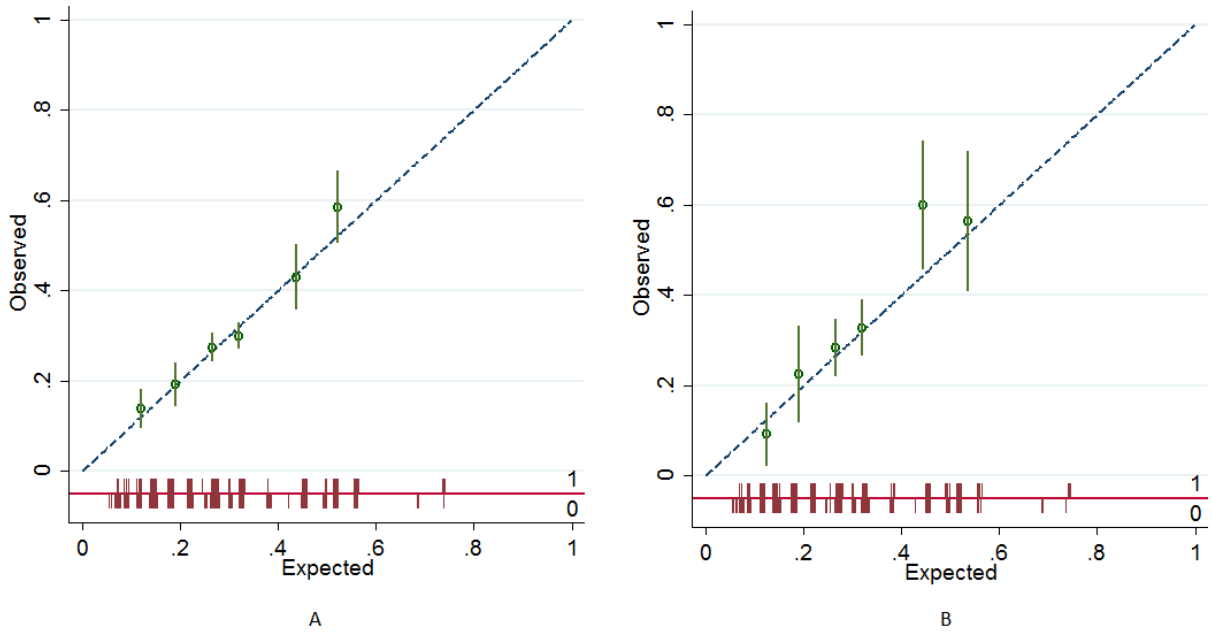

Figure 2: calibration plots of the developed model (A) and the validation model (B), observed versus expected predicted outcomes. The predicted risk is divided in to six equal groups (Hosmer-lemeshow).
Dotted line = reference line; Circles = groups; Vertical line = 95% confidence interval; Spike plot = distribution of genomic high risk outcome (1) and genomic low risk outcome (0)

Lower probabilities

The statistical measures that show the ability to predict the outcome of the MammaPrint with the use of different cut-off values is presented in Table 4. The sensitivity is the highest (99.4%) for a cut-off value of 10% and the specificity is the highest (91.1%) for a cut-off value of 35%. The highest area under the ROC curve (0.6) is found for the cut-off value of 25%.

Table 4: Statistical measures of the lower probabilities

Probability cut-off value	N below cut-off value (%) (Total N = 2,463)	Low genomic risk (N)	High genomic risk (N)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Area under the ROC Curve
35%	2,145 (87.1)	1,591	554	22.52	91.07	50.79	74.17	0.57
30%	1,262 (51.2)	972	290	59.44	55.64	35.42	77.02	0.59
25%	488 (19.8)	407	81	88.67	23.30	32.12	83.40	0.60
20%	387 (15.7)	328	59	91.75	18.78	31.61	84.75	0.58
15%	277 (11.2)	241	36	94.97	13.80	31.08	87.00	0.55
10%	30 (1.2)	26	4	99.44	1.49	29.24	86.67	0.58

Abbreviations: Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV= negative predictive value.

Difference in chemotherapy treatment between cohort 1 and 2

Over the years the percentages of the patients that did not receive chemotherapy in cohort one (patients who did not receive the MammaPrint) increases from 59.3% in 2011 to 86.8% in 2017, this is shown in Table 5. Within cohort two (patients who did receive the MammaPrint) the percentages that receive chemotherapy varies over the years but stays below 34.0%.

Table 5: Percentages chemotherapy over the years.

	Cohort 1 (without MammaPrint)			Cohort 2 (with MammaPrint)		
Year	Total N	Chemotherapy N, (%)	No chemotherapy N, (%)	Total N	Chemotherapy N, (%)	No chemotherapy N, (%)
2011	1322	538 (40.7)	784 (59.3)	102	14 (13.7)	88 (86.3)
2012	1276	467 (36.6)	809 (63.4)	136	45 (33.1)	91 (66.9)
2013	1049	338 (32.2)	711 (67.8)	393	108 (27.2)	285 (72.5)
2014	887	281 (31.7)	606 (68.3)	477	104 (21.8)	373 (78.2)
2015	993	332 (33.4)	661 (66.6)	482	144 (29.9)	338 (70.1)
2016	1027	176 (17.1)	851 (82.9)	512	105 (20.5)	407 (79.5)
2017	1178	155 (13.2)	1023 (86.8)	361	85 (23.5)	276 (76.5)

We used the 32 identified patient groups to look at the difference in chemotherapy treatment between patients who did not receive the MammaPrint and patients who received the MammaPrint (cohort one and two respectively). Table 6 shows the percentages chemotherapy of both cohorts. Within the group of patients with the lowest probability (5.3%), 12% of the patients who did not receive the MammaPrint got chemotherapy, compared to 0% of the patients who received the MammaPrint. Of the patient group with the highest probability (73%), 54.5% of the patients who did not receive the MammaPrint got chemotherapy, compared to 100% of the patients who received the MammaPrint. Both these lowest and highest probability groups have a small sample size. A visible representation of Table 6 can be found in the appendix.

Table 6: Cohort 1 and 2 divided in the 32 identified groups, and presenting the percentages of chemotherapy.

Group	Probability of genomic high risk outcome	Cohort 1 (N=7,609) Patients without MammaPrint		Cohort 2 (N=2,462) Patients with MammaPrint		Two-sample proportion test	
	%	N(%)	Chemo %	N(%)	Chemo %	Difference	p-value
1	5.3	25 (0.3)	12.0	3 (0.1)	0	0.12	0.525
2	6.6	53 (0.7)	34.0	8 (0.3)	0	0.34	0.050
3	6.8	18 (0.2)	50.0	6 (0.2)	16.7	0.33	0.151
4	8.4	85 (1.1)	36.5	13 (0.5)	23.1	0.13	0.345
5	10.9	360 (4.7)	22.8	84 (3.4)	9.5	0.13	0.007
6	11.1	3 (0.0)	0	2 (0.1)	0	-	-
7	13.6	19 (0.3)	31.6	3 (0.1)	0	0.32	0.254
8	13.7	495 (6.5)	39.0	129 (5.2)	15.5	0.23	<0.001
9	13.9	1 (0.0)	100	1 (0.0)	0	-	-
10	14.2	120 (1.6)	18.3	28 (1.1)	3.6	0.15	0.052
11	16.9	35 (0.5)	28.6	1 (0.0)	0	0.29	0.529
12	17.3	527 (6.9)	14.8	78 (3.2)	14.1	0.01	0.871
13	17.7	122 (1.6)	36.9	31 (1.3)	35.5	0.01	0.885
14	21.3	541 (7.1)	35.9	81 (3.3)	17.3	0.19	0.001
15	21.5	58 (0.7)	20.7	17 (0.7)	11.8	0.09	0.406
16	24.5	31 (0.4)	22.6	3 (0.1)	33.3	-0.11	0.675
17	26.1	51 (0.7)	25.5	12 (0.5)	8.3	0.17	0.198
18	26.7	2,065 (27.1)	20.8	750 (30.5)	22.8	-0.02	0.246
19	27.0	20 (0.3)	20.0	5 (0.2)	40.0	-0.20	0.349
20	29.5	51 (0.7)	29.4	7 (0.3)	28.6	0.01	0.963

21	31.8	151 (2.0)	19.2	29 (1.2)	17.2	0.02	0.805
22	32.0	1,884 (24.8)	43.3	847 (34.4)	26.4	0.17	<0.001
23	32.4	15 (0.2)	6.7	7 (0.3)	14.3	-0.08	0.563
24	37.6	105 (1.4)	21.0	13 (0.5)	23.1	-0.02	0.860
25	42.0	5 (0.1)	0	1 (0.0)	0	-	-
26	44.8	336 (4.4)	21.7	156 (6.3)	37.2	-0.15	<0.001
27	48.3	6 (0.1)	50.0	0 (0.0)	0	-	-
28	49.0	50 (0.7)	4.0	14 (0.6)	21.4	-0.17	0.032
29	51.2	319 (4.2)	40.4	111 (4.5)	46.0	-0.06	0.311
30	55.4	39 (0.5)	20.5	17 (0.7)	52.9	-0.32	0.015
31	68.1	8 (0.1)	12.5	3 (0.1)	0	0.13	0.521
32	73.4	11 (0.1)	54.5	2 (0.1)	100.0	-0.45	0.224

Some of the group sizes of the 32 identified groups are small, therefore we combined the groups, with approximately the same probability at a genomic high risk outcome, into 7 groups. This new classification is presented in Figure 3. Within patients who had a low (<10%) probability of a genomic high risk outcome, 33.7% of the patients who did not receive the MammaPrint received chemotherapy compared to 13.3% of the patients who received the MammaPrint. In the 10-20% probability group the percentages were 26.0% (without MammaPrint) and 14.3% (with the MammaPrint). The patients with a probability between 20-30% have approximately the same percentage (23%) of chemotherapy whether they received the MammaPrint or they did not. The group of patients with a probability between 30-40% showed a similar difference as the first two groups, 40.3% received chemo when they did not receive the MammaPrint and 26% when they received the MammaPrint. On the other hand the patient groups with a probability between 40-50% and 50-60% showed lower percentages when they did not receive the MammaPrint, 19.7% and 38.3% respectively, compared to 35.7% and 46.9% when they received the MammaPrint.

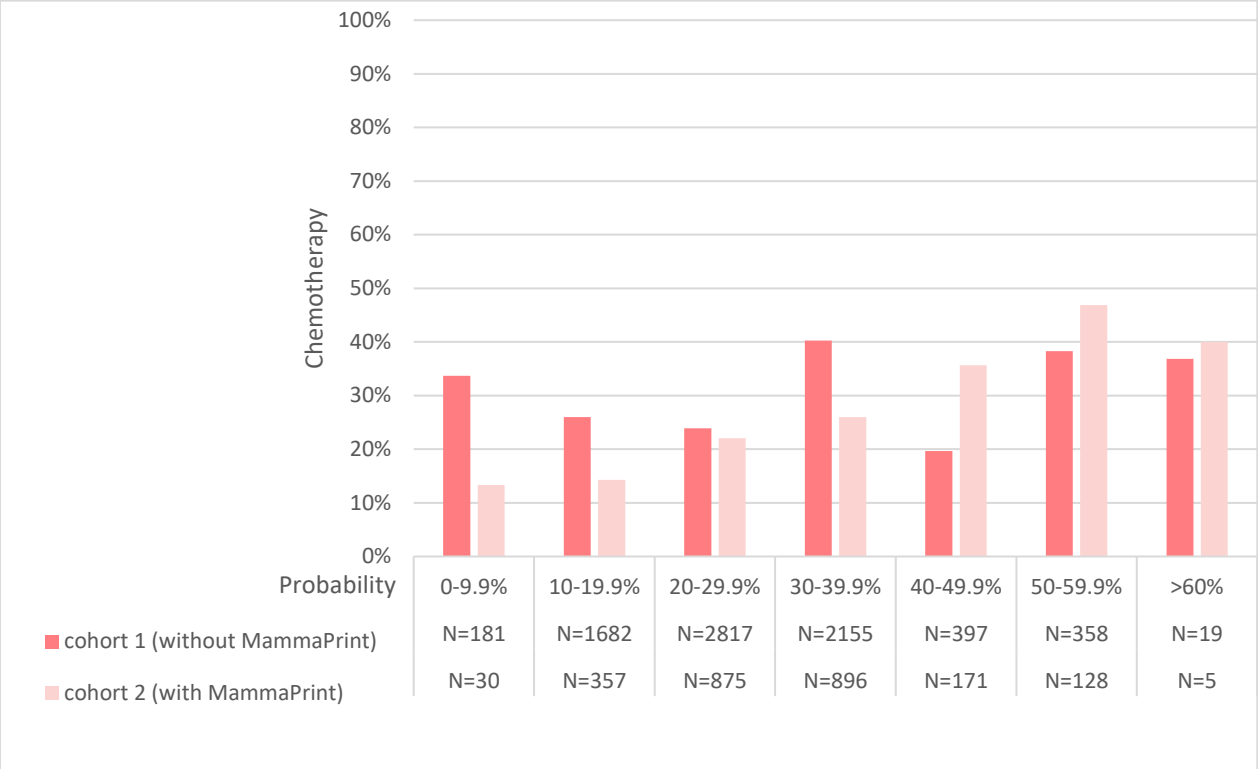

Figure 3: Percentage chemotherapy per probability group.

Discussion

In this retrospective registry-based study, we identified four clinicopathological variables that were significantly and independently associated with the outcome of the MammaPrint within the early stage breast cancer patients at clinical intermediate risk: PR status, differentiation grade, histological type and detection through screening. The variables PR status and differentiation grade have the highest influence on the probability. The broad confidence interval of histological type category “other” can be explained by the small sample size.

The study of Groenendijk, et al. also identified four clinicopathological variables associated with the outcome of the MammaPrint: ER status, PR status, HER2 status and histological type (9). Their aim was to evaluate the characteristics of early stage breast cancer that had been selected for the MammaPrint and to correlate genomic risk stratification with individual clinicopathological parameters and clinical risk as assessed by Adjuvant! Online and PREDICT. They included a broader patient selection: patients with early stage breast cancer, who underwent surgery between 2013 and 2016 and for which the MammaPrint outcome was reported. Their study confirms that patients with a negative ER status and positive HER2 status almost all have a genomic high risk outcome, and belong to the clinical high risk patient group, which is also mentioned by Mook, et al. (20). Groenendijk, et al. proposed three risk profiles; low risk (ER+/HER2-/grade1), intermediate risk (ER+/HER2-/grade2) and high risk (ER+/HER2-/grade3 or HER2+ or ER-). Of the low risk patients 13.4% had a high genomic risk outcome and 80.6% of the high risk patients had a high genomic risk outcome (9). Our study has added value because it gives specific information about the patients at clinical intermediate risk, this is the patient group where controversy exists about the benefit of chemotherapy. The added value of adjuvant therapy outside of this specific patient group can be determined on the basis of the known clinical variables.

In our study we could identify 32 patient groups. There is no patient group with a probability above 73%, this can be explained by the fact that we only included the clinical intermediate risk patient group, the patients with a high probability are presumably already in the clinical high risk patient group on the basis of their clinicopathological characteristics. We have identified patient groups where the MammaPrint may be unnecessary because of the low probability of a genomic high risk outcome. The statistical performance measures resulting from the use of different low probability cut-off values show that none of the cut-off values have a large area under the ROC curve. The negative predictive value is the highest (87%) for the cut-off value of 15%. If it is decided that the patient groups with a probability below 15% are not eligible for the MammaPrint, there will be approximately 13 out of a 100 patients who are mistakenly declined the MammaPrint. These patients wrongly omit chemotherapy which might lead to a higher risk at distant metastasis and with that a considerable chance of dying. On the positive side 87 patients will not receive an unnecessary MammaPrint, which saves approximately €2,675 per person (21). Another positive point is the shortened time of uncertainty for the patients, they do not have to wait on the MammaPrint outcome. It is arbitrary if the evidence for a cut-off point is convincing enough. The article of Van ‘t Veer, et al. described setting a threshold at a maximum of 10% misclassification (22). If we use this same threshold ($\leq 10\%$) and we also take the poor area under the ROC curve into account, we cannot indicate a cut-off value where the MammaPrint has no added value for the patients.

Our developed model as a whole showed an area under the ROC curve of 0.62 which is considered poor (17,23). This means that on the basis of clinicopathological variables we cannot predict the outcome of the MammaPrint with high accuracy. The Hosmer-lemeshow showed a nonsignificant result, which indicated that the observed and expected number of patients with a genomic high and a genomic low risk outcome were not significantly different, showing a good internal validation.

Recently, a study was published that examined if clinicopathological variables could be used to predict the Oncotype DX outcome. Five variables were used: tumour size, tumour grade, PR status, histological type and age. This model showed an area under the curve of 0.81, which indicates a good discriminative model (24). The differences in their and our model may be explained by the larger sample size (65,754 patients compared to our 2,462 patients), the inclusion of a broader patient group and the 0-100 scale outcome of the Oncotype DX which can be predicted with greater precision than the dichotomous MammaPrint outcome. The Oncotype DX model shows a good calibration in the low and high probabilities but the patients with a probability between approximately 0.45 and 0.80 show more deviation. This indicates that also within the study of Orucevic, there are patients where the Oncotype DX test likely has added value (24).

In the second part of the analysis of this present study we compared how many patients received chemotherapy in cohort one (who did not receive the MammaPrint) with the amount of patients that received chemotherapy in cohort two (who did receive the MammaPrint). When we combined the groups with approximately the same probabilities we saw that out of these seven groups the four groups with the lowest probabilities showed that patients who did not receive the MammaPrint more often received chemotherapy compared to patients who received the MammaPrint. The three groups with the highest probabilities showed that patients who did not receive the MammaPrint less often receive chemotherapy compared to the patients who received the MammaPrint. These results indicate possible overtreatment within the patients with a low probability and undertreatment in the patients with a relatively high probability when the MammaPrint is not used. This underlines the need for the MammaPrint, especially in the groups with the lower probabilities (<10%).

One of the strengths of this study is the assessment of only the patients of clinical intermediate risk, who are the patients eligible for the MammaPrint because of the uncertainty about the benefit of chemotherapy. Since the group of patients who received the MammaPrint was much larger, we additionally performed the logistic regression analysis on all patients receiving the MammaPrint, without any predefined selection. These analyses identified the same prognostic variables as presented in this study (data not shown). Another strength is that we specifically chose the indication of clinical intermediate patients defined by Kuijer, et al, they came to this indication though throughout research of the literature as well as assessing data about the use of the MammaPrint in practice. Other sources only broadly describe which patients they find eligible for the MammaPrint without being specific. Like the St. Gallen International Breast Cancer Consensus Conference, who mention node-negative patients without specifying differentiation grades or pathological tumour characteristics (11). One more strength is the fact that the data is from the population-based comprehensive NCR, which includes all Dutch patients as well as a lot of patient- and tumour characteristics expressing daily life practice.

This study has some limitations. One may be missing clinicopathological variables that could potentially be associated with the MammaPrint outcome, but are not registered. For example the Body Mass Index (BMI) and lymphovascular invasion. Kaviani, et al. found that obesity is associated with more advanced breast cancer diseases (25). The presence of lymphovascular invasion was associated with a higher mortality rate, described by Lee, et al. and Song, et al (26,27). Another limitation is the absence of external validation of the model, due to lack of excess to another database including the same variables. The last limitation may be that patient preference could have influenced a specific part of the analysis: the percentages of chemotherapy in cohort one and cohort two, for example when they insist on chemotherapy.

The amount of chemotherapy treatments is decreasing over the years. The main reasons are the growing understanding of breast cancer biology and the ability to better identify patients that most likely benefit from the various combinations of treatments (28). Another reason is mentioned by Kurian, et al., they stated that the fear of overtreatment causes clinicians to be more reluctant about subscribing chemotherapy (29). Our data also shows a decrease in chemotherapy treatments, the percentage of patients who did not receive the MammaPrint and did not receive chemotherapy increased from 59.3% in 2011 to 86.8% in 2017. If we look at the adherence to the advised treatment (on the basis of the genomic outcome) according to the guidelines, we saw that the adherence is higher for the patients with a genomic low risk outcome (97%) compared to the adherence to the advised treatment for the patients with a genomic high risk outcome (76%). This higher adherence to a low risk outcome was also found by Kuijer, et al, they found indications that clinicians are considerable reluctance to administrate chemotherapy and that that Dutch clinicians tend to use GEP merely for a substantiated decision to omit chemotherapy (30). It is important that omitting chemotherapy is properly substantiated with evidence-based information. Our study provides important information that contributes to more personalized care for early stage breast cancer patients that will lead to the necessary treatment.

In conclusion, this study shows that within early stage breast cancer patients at clinical intermediate risk clinicopathological variables alone cannot predict the outcome of the MammaPrint. In addition, some results may indicate possible overtreatment with adjuvant chemotherapy in some patient groups and undertreatment in other patient groups within these early stage breast cancer patients. This confirms that the MammaPrint is an important additional test for early stage breast cancer patients at clinical intermediate risk, to be able to make a well informed treatment decision.

References

1. Clarke M, Collins R, Davies C, Godwin J, Gray R, Peto R. Polychemotherapy for early breast cancer: An overview of the randomised trials. Lancet. 1998; 352(9132): p. 930-942.

2. Abe O, Abe R, Enomoto K, Kikuchi K, Koyama H, Masuda H, et al. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet. 2005; 365(9472): p. 1687-1717.

3. Verschoor AMF, Kuijer A, Verloop J, Van Gils CH, Sonke GS, Jager A, et al. Adjuvant systemic therapy in early breast cancer: impact of guideline changes and clinicopathological factors associated with nonadherence at a nation-wide level. Breast Cancer Res. Treat. 2016; 159(2): p. 357-365.

4. Van De Vijver MJ, He YD, Van 'T Veer LJ, Dai H, Hart AAM, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002; 347(25): p. 1999-2009.

5. Straver ME, Glas AM, Hannemann J, Wesseling J, Van De Vijver MJ, Rutgers EJT, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. Breast Cancer Res. Treat. 2010; 119(3): p. 551-558.

6. Hassett MJ, O'Malley AJ, Pakes JR, Newhouse JP, Earle CC. Frequency and cost of chemotherapy-related serious adverse effects in a population sample of women with breast cancer. J Natl Cancer Inst. 2006; 98(16): p. 1108-1117.

7. Cardoso F, Van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, et al. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med. 2016; 375(8): p. 717-729.

8. Drolet M, Maunsell E, Mondor M, Brisson C, Brisson J, Mâsse B, et al. Work absence after breast cancer diagnosis: A population-based study. CMAJ. 2005; 173(7): p. 765-769.

9. Groenendijk FH, Jager A, Cardoso F, Van Deurzen CHM. A nationwide registry-based cohort study of the MammaPrint genomic risk classifier in invasive breast cancer. The Breast. 2018; 38: p. 125-131.

10. Oncoline. Richtlijn borstkanker. [Online].: Integraal kankercentrum Nederland (IKNL); 2017 [cited 2019 Maart 21]. Available from: https://www.oncoline.nl/borstkanker.

11. Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsky P, Loibl S, et al. De-escalating and escalating treatments for early-stage breast cancer: The St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol. 2017; 28(8): p. 1700-1712.

12. Zorginstituut Nederland. Standpunt Zvw: MammaPrint bij vrouwen met vroeg stadium borstkanker. , Oncologie; 2018.

13. Van Diest P, Van Den Bongard D, Evers P, Hugen P, Smidt M, Sonke G. borstkanker. [Online].; 2018 [cited 2019 maart 21]. Available from: https://borstkanker.nl/nl/nieuws/mammaprint-niet-vergoed-in-de-basisverzekering.

14. Kuijer A, Van Bommel ACM, Drukker CA, Van der Heiden-Van Der Loo M, Smorenburg CH, Westenend PJ, et al. Using a gene expression signature when controversy exists regarding the indication for adjuvant systemic treatment reduces the proportion of patients receiving adjuvant chemotherapy: A nationwide study. Genet. Med. 2016; 18(7): p. 720-726.

15. Van der Sanden GAC, Coebergh JWW, Schouten LJ, Visser O, Van Leeuwen FE. Cancer incidence in the Netherlands in 1989 and 1990: First results of the nationwide Netherlands cancer registry. Eur J Cancer. 1995; 31(11): p. 1822-1829.

16. IKNL. Cijfers over kanker. [Online].; 2011-2019 [cited 2019 April 8]. Available from: www.cijfersoverkanker.nl.

17. Safari S, Baratloo A, Elfil M, Negida A. Evidence based emergency medicine; Part 5 receiver operating curve and area under the curve. Emergency. 2016; 4(2): p. 111-113.

18. Gerds TA, Cai T, Schumacher M. The performance of risk prediction models. Biom J. 2008; 50(4): p. 457-479.

19. Janes H, Pepe MS, Gu W. Assessing the value of risk predictions by using risk stratification tables. Ann Intern Med. 2008; 149(10): p. 751-760.

20. Mook S, Schmidt MK, Viale G, Pruneri G, Eekhout I, Floore A, et al. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. Breast Cancer Res. Treat. 2009; 116(2): p. 295-302.

21. Retèl VP, Joore MA, Drukker CA, Bueno-De-Mesquita JM, Knauer M, Van Tinteren H, et al. Prospective cost-effectiveness analysis of genomic profiling in breast cancer. Eur J Cancer. 2013; 49(18): p. 3773-3779.

22. Van 't Veer LJ, Dai H, Van de Vijver MJ, He YD, Hart AAM, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002; 415(6871): p. 530-536.

23. Janssens ACJW, Moonesinghe R, Yang Q, Steyerberg EW, Van Duijn CM, Khoury MJ. The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. Genet. Med. 2007; 9(8): p. 528-535.

24. Orucevic A, Bell JL, King M, McNabb AP, Heidel RE. Nomogram update based on TAILORx clinical trail results - Oncotype DX breast cancer recurrence score can be predicted using clinicopathologic data. The Breast. 2019; 46: p. 116-125.

25. Kaviani A, Neishaboury M, Mohammadzadeh N, Ansari-Damavandi M, Jamei K. Effects of obesity on presentation of breast cancer, lymph node metastasis and patient survival: A retrospective review. Asian Pac J of Cancer Prev. 2013; 14(4): p. 2225-2229.

26. Lee AHS, Pinder SE, Macmillan RD, Mitchell M, Ellis IO, Elston CW, et al. Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. Eur J Cancer. 2006; 42(3): p. 357-362.

27. Song YJ, Shin SH, Cho JS, Parl MH, Yoon JH, Jegal YJ. The role of lymphovascular invasion as a prognostic factor in patients with lymph node-positive operable invasive breast cancer. J Breast Cancer. 2011; 14(3): p. 198-203.

28. Tao JJ, Visvanathan K, Wolff AC. Long term side effects of adjuvant chemotherapy in patients with early breast cancer. The Breast. 2015; 24: p. 149-153.

29. Kurian AW, Bondarenko I, Jagsi R, Friese CR, McLeod MC, Hawley ST, et al. Recent trends in chemotherapy use and oncologists' treatment recommendations for early-stage breast cancer. J Natl Cancer Inst. 2018; 110(5): p. 493-500.

30. Kuijer A, Drukker CA, Elias SG, Smorenburg CH, Rutgers EJT, Siesling S, et al. Changes over time in the impact of gene-expression profiles on the administration of adjuvant chemotherapy in estrogen receptor positive early stage breast cancer patients: A nationwide study. Int J Cancer. 2016; 139(4): p. 769-775.

Appendix

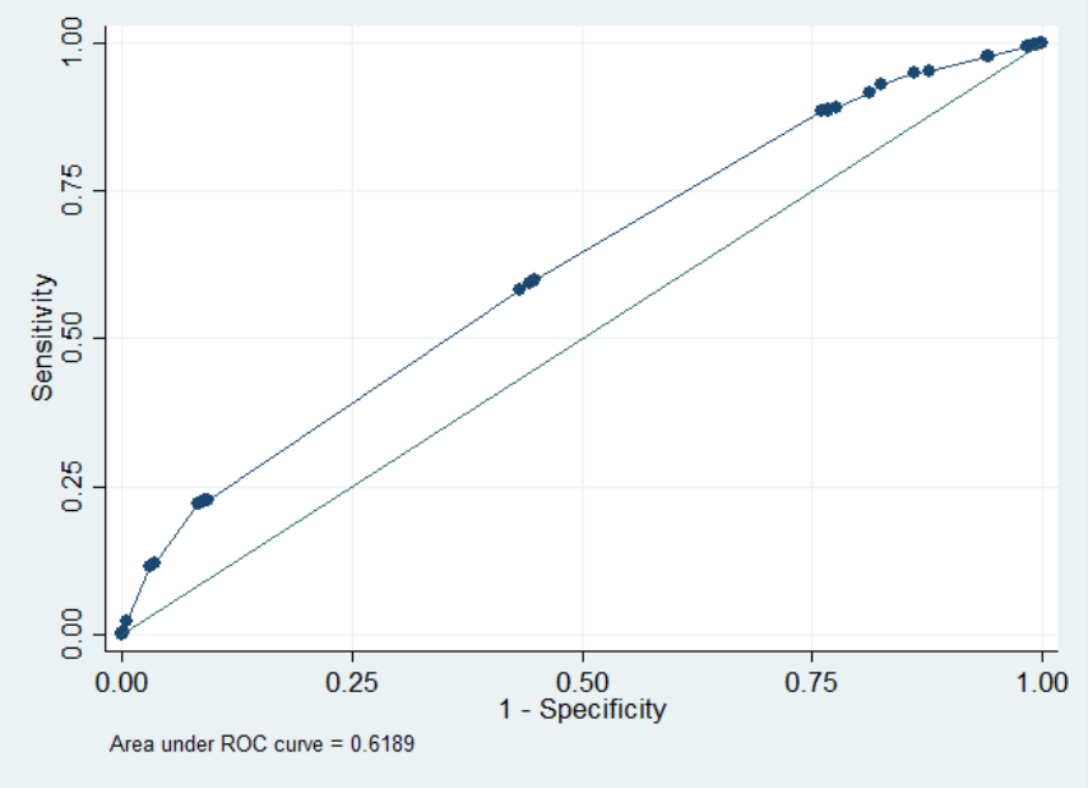

Figure 1: Area under ROC curve of the developed model

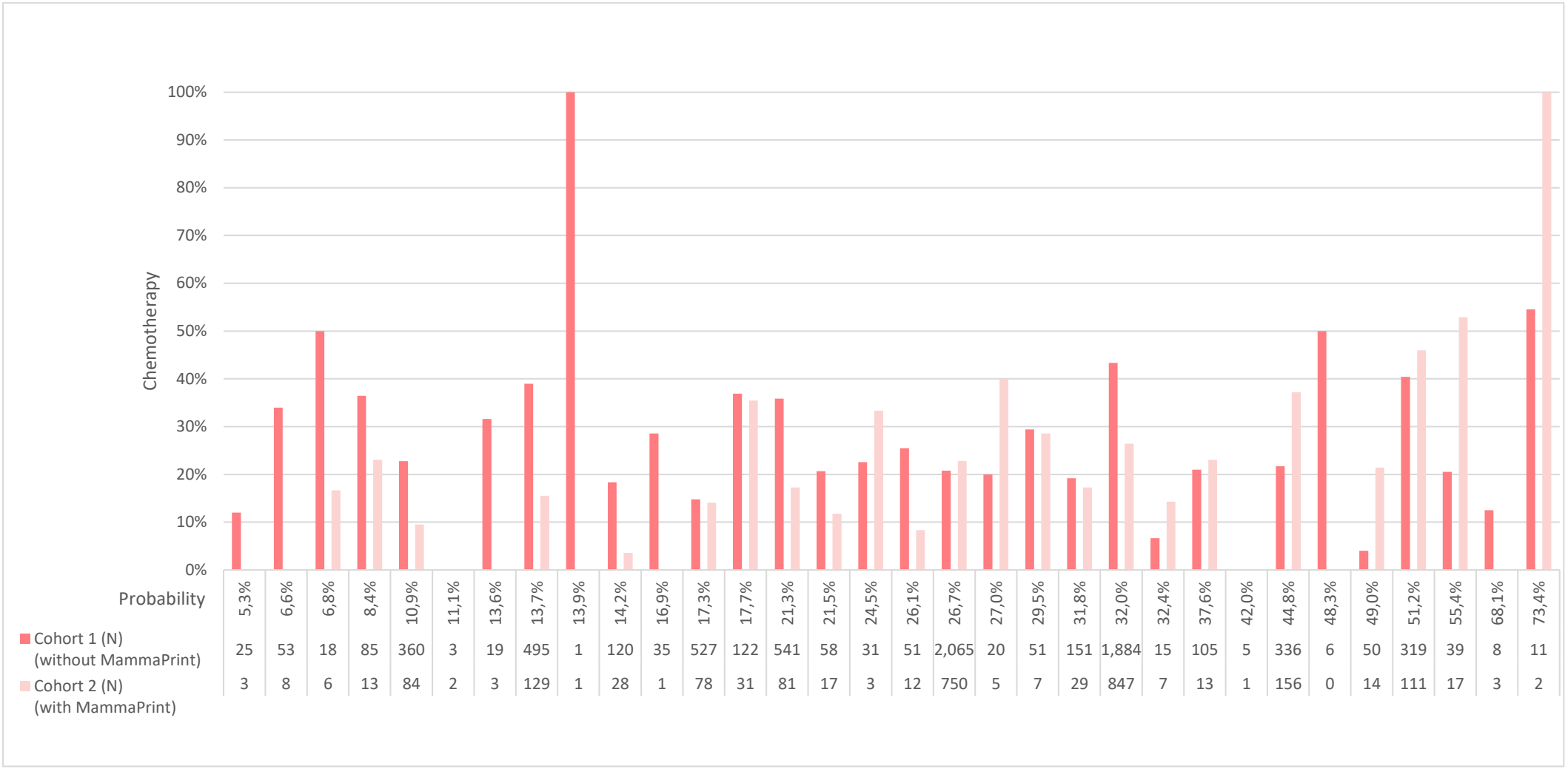

Figure 2: Percentage chemotherapy per identified group

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