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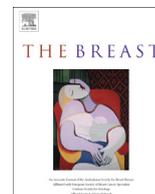
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Original article

European inter-institutional impact study of MammaPrint



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ABSTRACT

Aim: To measure the impact of MammaPrint on adjuvant treatment decisions and to analyze the agreement in treatment decisions between hospitals from 4 European countries for the same patient cohort.

Methods: Breast cancer patients were prospectively enrolled and MammaPrint was assessed. Patients' clinical data without and then with MammaPrint results were sent to the different multidisciplinary teams and treatment advice was provided for each patient.

Results: Using MammaPrint, chemotherapy treatment advice for ER+/HER2- breast cancer patients was changed in 37% of patients by the Dutch, 24% by the Belgian, 28% by the Italian and 35% by the Spanish teams. MammaPrint increased the inter-institutional agreement in treatment advice (chemotherapy or no chemotherapy) from 51% to 75%.

Conclusion: The results of this study indicate that MammaPrint impacts adjuvant chemotherapy recommendation. MammaPrint can decrease inter-institutional and inter-country variability in adjuvant treatment advice for breast cancer patients.

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Introduction

Breast cancer is the second most common cancer in Europe (data from 40 countries), comprising 13.1% of all cancers [1]. In 2008, the estimated age-adjusted annual incidence of breast cancer in Europe was 88.4/100,000 and the mortality rate was 24.3/100,000 [1]. Breast cancer is the main cause of cancer death in women in Europe [2] and worldwide [3]. The incidence of breast cancer is increasing due to a number of factors, including improved diagnosis as a result of the expanded use of mammographic screening, an aging population [4], postmenopausal hormone replacement therapy [4,5], obesity [4,6] and alcohol [7] and tobacco consumption [8,9].

Systemic adjuvant treatment can decrease the rate of recurrence and improve survival in patients with early-stage resected breast cancer [10]. Adjuvant treatment includes chemotherapy, hormonal therapy, combined chemotherapy plus hormonal therapy and targeted therapy such as trastuzumab [10]. Treatment recommendations are based on the patient's risk of recurrence, the benefits and potential adverse effects of therapy and the patient's preference. Factors that affect the risk of tumor recurrence include the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) overexpression status, tumor size and grade, the presence of lymphovascular invasion, lymph node metastasis and the proliferation index [11].

The 70-gene tumor expression profile MammaPrint classifies patients as Low Risk or High Risk for the development of distant metastases. MammaPrint has been shown to be a powerful predictor of disease outcome in breast cancer in a number of studies [12–18]. The 2013 St Gallen International Expert Consensus on the primary therapy of early breast cancer include gene-expression signatures as an indicator for adjuvant therapy [19]. In addition,

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the use of MammaPrint to obtain additional prognostic and/or predictive information for risk assessment and to predict the benefit of adjuvant chemotherapy is included in the European Society for Medical Oncology (ESMO) practice guidelines for primary breast cancer [2]. Inter-institutional differences in the interpretation of prognostic and predictive factors and in the quality of immunohistochemistry (IHC) analysis [20–23] can substantially affect the adjuvant treatment advice provided, resulting in the risk of potential over or under treatment [24]. The introduction of a centralized multi-gene assay could potentially lead to more formalized and standardized treatment recommendations throughout Europe.

In the prospective multi-center microarray prognostics in breast cancer (RASTER) study the clinical impact on adjuvant systemic therapy decision making was assessed [12]. A considerable discrepancy in risk estimations among different clinicopathologic guidelines and MammaPrint was observed. The addition of MammaPrint to standard clinicopathological factors led to a change in adjuvant systemic treatment advice in 19% of patients [12]. After a median follow-up of 61.6 months, 15% (33/219) of MammaPrint Low Risk patients received adjuvant chemotherapy versus 81% (169/208) of MammaPrint High Risk patients. In a follow-up analysis, the 5-year distant-recurrence-free-interval probabilities for MammaPrint Low Risk ($n = 219$) and High Risk ($n = 208$) patients were 97% and 92%, respectively [18]. The decision not to use adjuvant chemotherapy based on a Low-Risk MammaPrint result, did not appear to affect patient outcomes.

The current study measured the impact of MammaPrint on adjuvant treatment decisions in four European countries and analyzed the agreement in adjuvant treatment decisions between four European hospitals for the same patient cohort.

Patients and methods

Study design

Eligible patients were consecutively enrolled from three hospitals in the Netherlands, Belgium and Italy. Clinicopathological data was assembled and MammaPrint assessed. Patients' clinical data without the MammaPrint results from one country were sent to multidisciplinary teams at two hospitals in the other two countries (e.g. data from the Netherlands was sent to Belgium and Italy) plus a fourth hospital in Spain and adjuvant treatment advice was provided for each patient. Subsequently, patients' clinical data including the MammaPrint results were sent to the same multidisciplinary teams and adjuvant treatment advice was again provided for each patient.

The impact of MammaPrint on chemotherapy treatment advice was assessed for each hospital. Furthermore the inter-institutional agreement which is defined as the agreement in adjuvant treatment decisions between hospitals for the same patient cohort.

Study objectives

To investigate the impact of MammaPrint on adjuvant chemotherapy treatment advice as determined by the multidisciplinary teams at four European hospitals and to assess the agreement in adjuvant treatment decisions for the same patient cohort between European hospitals.

Patients

Women (aged 18–70 years) with histologically proven, operable, unilateral, invasive breast cancer and sentinel node or axillary

clearance (T1–3, N0–1, M0) and a successful MammaPrint were eligible for inclusion. Written informed consent was provided by all patients prior to their participation in the study.

MammaPrint

MammaPrint was performed on fresh tumor samples obtained from surgical specimens (minimum 3 mm³ tumor tissue) or core needle biopsies (two cores of tumor tissue from a 14-gauge or one core from a 10–12-gauge needle). Microarray analysis for obtaining the profiles was performed at the centralized Agendia Laboratories blinded for clinical and pathological data [25].

ER, progesterone receptor (PR) and HER2 IHC/fluorescence in situ hybridization (FISH) assessments

IHC/FISH assessments were performed according to local standards at each institution. The threshold for ER and PR was set at 1% positive staining. HER2 3+ was considered to be positive with a threshold of $\geq 10\%$ positive staining. HER2 2+ cases were assessed by FISH.

Results

MammaPrint results were prospectively collected from 194 patients in the Netherlands ($n = 66$), Belgium ($n = 92$) and Italy ($n = 36$). Patients' clinical characteristics are shown in Table 1. The majority of patients (86%) were ER+ and HER2– (88%). 85 (44%) patients were MammaPrint Low Risk and 109 (56%) were MammaPrint High Risk. The decision by the multidisciplinary teams whether or not to advise adjuvant chemotherapy, without and then with the MammaPrint results, is shown in Table 2. Without knowing the MammaPrint results 97/128 (76%) of patients were advised adjuvant chemotherapy by the Dutch team. After knowing the MammaPrint results there was a 14% decrease in adjuvant chemotherapy treatment advice (79/128 62%). Overall the treatment advice was changed for 33% of patients by the Dutch team. Both without and with knowing the MammaPrint results 44/102 (43%) of patients were advised adjuvant chemotherapy by the Belgian team. However, for 22% of patients the treatment advice was changed because of MammaPrint (equal number of patients changed from chemotherapy to no chemotherapy and vice versa). Without knowing the MammaPrint results 94/158 (59%) of patients were advised adjuvant chemotherapy by the Italian team. After knowing the MammaPrint results, there was an increase of 13% in adjuvant chemotherapy treatment advice (115/158 73%). Overall the treatment advice was changed for 28% of patients by the Italian team. Without knowing the MammaPrint result 117/194 (60%) of patients were advised adjuvant chemotherapy by the Spanish team. After knowing the MammaPrint results, there was a decrease of 2% in adjuvant chemotherapy treatment advice (114/194 59%). Overall the treatment advice was changed for 27% of patients by the Spanish team.

A subgroup analysis in patients with ER+/HER2– breast cancer indicated that adjuvant chemotherapy treatment advice after disclosure of the MammaPrint results was changed for 37% of patients by the Dutch team (a 21% reduction in advising chemotherapy), for 24% of patients by the Belgian team (equal number of patients changed from chemotherapy to no chemotherapy and vice versa), for 28% of patients by the Italian team (a 14% increase in advising chemotherapy) and for 35% of patients by the Spanish team (a 2% reduction in advising chemotherapy) (Table 3). Overall, MammaPrint increased the inter-institutional agreement in adjuvant chemotherapy/no chemotherapy treatment advice for patients from 57% to 79% (Fig. 1A) and in patients with ER+/HER2– breast

Table 1
Patient clinical characteristics.

	Netherlands (n = 66)	Belgium (n = 92)	Italy (n = 36)	Total (N = 194)
Median age, years, (range)	55 (23–70)	55 (27–70)	59 (26–68)	56 (25–69)
Tumor size, n				
<1 cm	1	8	5	14
1–<2 cm	31	47	27	105
2–5 cm	33	34	2	69
>5 cm	0	3	1	4
Unknown	1	0	1	2
Grade, n				
I	20	23	1	44
II	29	50	21	100
III	17	19	14	50
ER (IHC), n				
Positive	54	81	32	167
Negative	12	11	4	27
HER2 (IHC), n				
Positive	8	15	1	24
Negative	58	77	35	170
ER–/PR–/HER2–, n	7	8	4	19
Lymph nodes, n				
0	37	65	27	129
1–3	29	27	7	63
Unknown	0	0	2	2
Chemotherapy, n				
Yes	48	38	17	103
No	18	54	19	91
Hormonal therapy, n				
Yes	42	79	13	134
No	24	13	23	60
MammaPrint, n				
Low Risk	32	33	20	85
High Risk	34	59	16	109

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PR, progesterone receptor.

cancer, MammaPrint increased the inter-institutional agreement from 51% to 75% (Fig. 1B).

Adjuvant chemotherapy advice in ER+/HER2– breast cancer was also analyzed by subgroups including age (<40, 40–55 and >55 years of age), tumor size (<1, 1–2, and >2 cm), tumor grade (grade 1–3), and nodal status (N0 and N1). In all subgroups analyzed, a higher proportion of MammaPrint High Risk patients were advised chemotherapy compared with MammaPrint Low Risk patients (Fig. 2). MammaPrint reduced chemotherapy recommendation in the following subgroups: <40 and 40–55 years, grade 3, >2 cm and N1. Chemotherapy recommendation was equal (≤5%) in the following subgroups: >55 years, grade 1 and 2, 1–2 cm, and N0. MammaPrint increased chemotherapy recommendation in <1 cm. In all subgroups analyzed the proportion of High Risk patients

Table 2
Impact of MammaPrint on chemotherapy treatment advice: overall population.

Multi-disciplinary team	Without MammaPrint	With MammaPrint		Impact (%)
		CT advised	CT not advised	
The Netherlands (n = 128)	CT advised CT not advised	67 12	30 19	42/128 (33%)
Belgium (n = 102)	CT advised CT not advised	35 11	11 45	22/102 (22%)
Italy (n = 158)	CT advised CT not advised	84 31	10 33	41/158 (26%)
Spain (n = 194)	CT advised CT not advised	89 25	28 52	53/194 (27%)
Overall (n = 582)	CT advised CT not advised	275 79	79 149	158/582 (27%)

CT, chemotherapy.

Table 3
Impact of MammaPrint on chemotherapy treatment advice: ER+/HER2– subset.

Multi-disciplinary team	Without MammaPrint	With MammaPrint		Impact (%)
		CT advised	CT not advised	
The Netherlands (n = 100)	CT advised CT not advised	44 8	29 19	37/100 (37%)
Belgium (n = 82)	CT advised CT not advised	17 10	10 45	20/82 (24%)
Italy (n = 120)	CT advised CT not advised	54 25	8 33	22/120 (28%)
Spain (n = 151)	CT advised CT not advised	46 25	28 52	53/151 (35%)
Overall (n = 453)	CT advised CT not advised	161 68	75 149	143/453 (32%)

CT, chemotherapy.

versus Low Risk patients who were recommended chemotherapy increased with MammaPrint.

In patients with HER2+ breast cancer, adjuvant chemotherapy/no chemotherapy treatment advice with the MammaPrint results was changed for 25% of patients by the Dutch team, for 11% of patients by the Belgian team, for 35% of patients by the Italian team and for none of the patients by the Spanish team. In patients with ER– breast cancer, adjuvant chemotherapy/no chemotherapy treatment advice with the MammaPrint results was changed for 7% of patients by the Dutch team, for 13% of patients by the Belgian team, for 4% of patients by the Italian team and for none of the patients by the Spanish team.

Discussion

This is the first pan-European study on the impact of MammaPrint on clinical decision making for patients with breast cancer. The results demonstrate the high variability in adjuvant treatment strategies for breast cancer between multidisciplinary teams at four hospitals in four European countries based on traditional patient- and tumor-related parameters. In similar populations of patients the advice for adjuvant chemotherapy differs markedly across 4 European countries ranging from 45% in Belgium to 76% in the Netherlands. Using MammaPrint would increase the agreement in adjuvant treatment decisions between European hospitals for the same patient cohort from 57% to 79%. The decision whether or not to advise chemotherapy after disclosure of the MammaPrint results was changed for 22% to 33% of patients by the multidisciplinary teams of the 4 European hospitals. In patients with ER+/HER2– breast cancer, the adjuvant chemotherapy treatment advice provided after disclosure of the MammaPrint results was changed for 24% to 37% of patients leading to an increased inter-institutional agreement (51% to 75%).

Most patients with MammaPrint High Risk results were recommended adjuvant chemotherapy, whereas less than 20% of patients with MammaPrint Low Risk results were recommended chemotherapy (except for patients <40 years, grade 3 and N1).

The differences in adjuvant treatment strategies for breast cancer between the countries are most likely due to the differing recommendations in the national guidelines of the Netherlands [26], Belgium [27,28], Italy [29] and Spain [30]. The national guidelines vary in their selection criteria of which patients should receive adjuvant chemotherapy. In Belgium and Spain, MammaPrint only altered chemotherapy treatment advice for ER+/HER2– patients. In the Netherlands, according to the CBO guidelines [26], ER– and HER2+ patients with small tumors (≤1 cm) do not receive adjuvant chemotherapy. MammaPrint had an impact on the treatment advice given to these patients. In Italy, MammaPrint did not alter the treatment advice for ER– patients (only one of 23 ER–

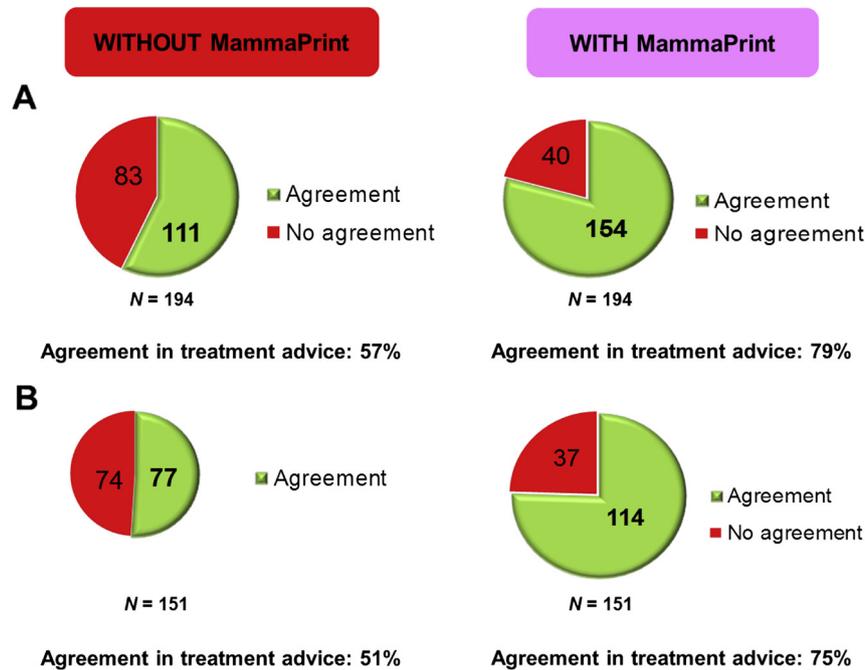


Fig. 1. Inter-institutional agreement in treatment advice for the same patient cohort all patients (A) and for patients with ER+/HER2- breast cancer (B).

patients changed from chemotherapy to no chemotherapy) but in the subset of HER2+ patients, treatment was changed as a result of MammaPrint in 35% of patients. In Spain, all HER2+ patients receive chemotherapy; therefore MammaPrint had no impact on the treatment advice provided for these patients.

Our results on the impact of MammaPrint on adjuvant treatment decisions are similar to those of a Spanish study that investigated the impact of the 21-gene tumor expression profile Oncotype DX on adjuvant treatment recommendations in 107 women with ER + node-negative breast cancer [31]. In this study, the use of Oncotype DX changed the recommended treatment in 34 of 107 (32%) women. The initial recommendation was changed from chemotherapy plus hormonal therapy to hormonal therapy in 22 patients and from hormonal to chemotherapy plus hormonal therapy in 12 patients.

The variation in treatment advice between institutions and countries in our study is similar to that reported by Bueno-de-Mesquita et al. in a retrospective review of two patient cohorts from 18 hospitals in the Netherlands [23]. They reported a significant difference in grade, ER status, HER2 status and clinicopathological risk between hospitals due to inter-observer variation in the pathological examination and a resultant difference in the adjuvant treatment advice provided [24]. If clinical risk had been assessed according to Dutch guidelines or Adjuvant! Online, 15% or 8% of patients, respectively, would have been assigned to a different clinical risk group and thus received different adjuvant treatment advice.

The ideal molecular test should be reproducible and reliable, should have data that are validated both for prognostic and predictive power, should be independent of receptors status and treatment compliance and must provide a definite result [32].

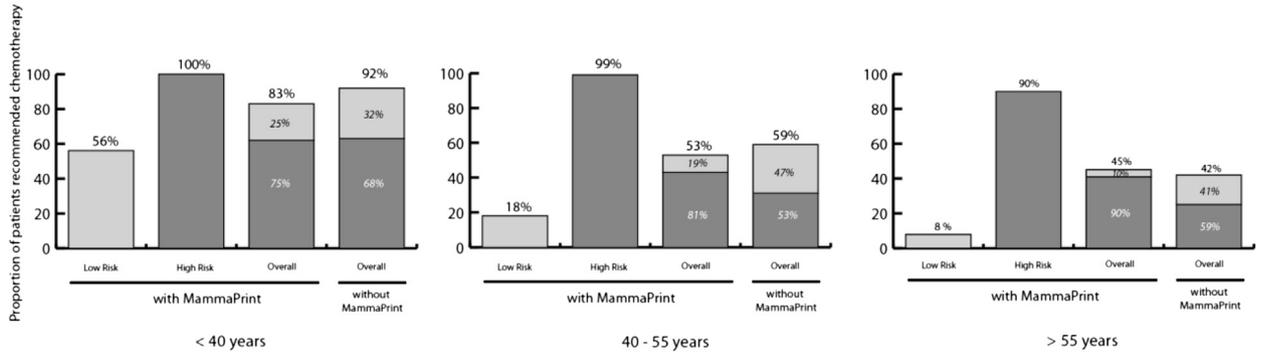
The prognostic power of MammaPrint has been confirmed in multiple studies [12–18]. The predictive value of MammaPrint for chemotherapy benefit in addition to endocrine therapy has been analyzed from pooled study series [33]. For patients ($n = 252$) who were identified MammaPrint Low Risk, receiving endocrine treatment alone and endocrine after chemotherapy was statistically insignificant ($p = 0.200$). By contrast, 289 patients who were

identified as MammaPrint High Risk benefitted from the addition of chemotherapy to endocrine treatment ($p < 0.010$). The absolute benefit was 12% [33]. MammaPrint was also tested in the neo-adjuvant setting as a predictive test for chemotherapy [34]. Of the 167 patients, 23 (14%) were MammaPrint Low Risk, but none showed pathologic complete remission (pCR) in the breast and axilla. By contrast, among 144 (86%) patients who were MammaPrint High Risk, 29 (20%) achieved pCR. MammaPrint High Risk was significantly associated with pCR ($p = 0.015$). Thus, a pCR is unlikely to be achieved in patients with tumors that have a Low Risk MammaPrint, whereas patients with High Risk tumors are sensitive to chemotherapy [34].

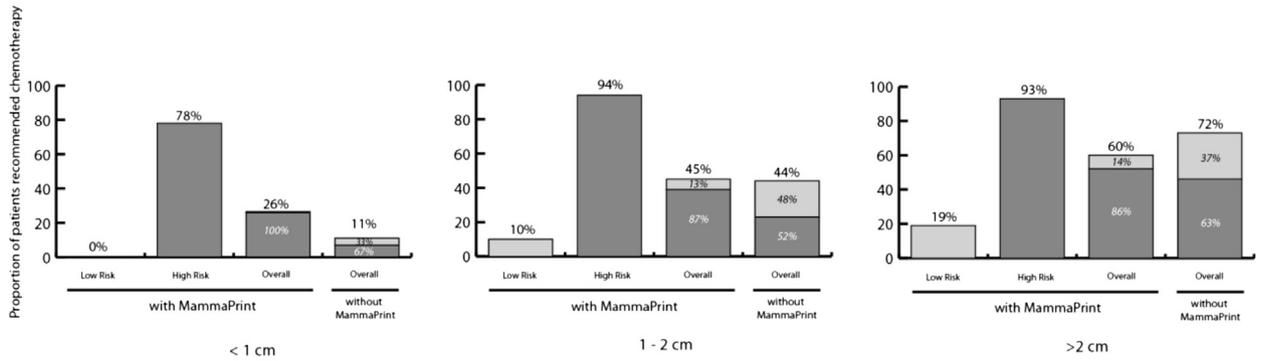
MammaPrint has been shown to be a cost-effective strategy to guide adjuvant chemotherapy treatment in a number of studies using a Markov model [35–37]. Using a model with three health states; Chen et al. compared the cost effectiveness of MammaPrint and Adjuvant! Online [35]. In the base-case model, MammaPrint reclassified 29% of patients and resulted in 10% more patients avoiding chemotherapy. Compared with Adjuvant! Online, MammaPrint was associated with a higher total cost per patient of \$1,440 and increased life expectancy by 0.14 year or 0.15 quality-adjusted life-year (QALY). The incremental cost-effectiveness ratio was approximately \$10,000 per life-year or QALY, which is within the range generally considered to be cost-effective for a diagnostic or therapeutic intervention. In a comparison of MammaPrint with the St Gallen guidelines and Adjuvant! Online for early breast cancer, quality-adjusted survival was found to be longer with MammaPrint (12.44 versus 12.20 and 11.24 QALYs, respectively), which had the highest probability of being cost-effective for a willingness to pay for a QALY $> \text{€}4,600$, based on costs per QALY [36]. Compared with Oncotype DX, MammaPrint was more cost effective at a threshold willingness-to-pay of \$50,000 per QALY and was associated with a per-patient cost of \$21,598 for a gain of 7.461 QALYs versus a per-patient cost of \$27,882 for a gain of 7.364 QALYs with Oncotype DX [37].

Although outcome data of the randomized prospective MIND-ACT study has not been published MammaPrint and Oncotype DX have been included in the St. Gallen and ESMO clinical practice guidelines to gain additional prognostic and/or predictive

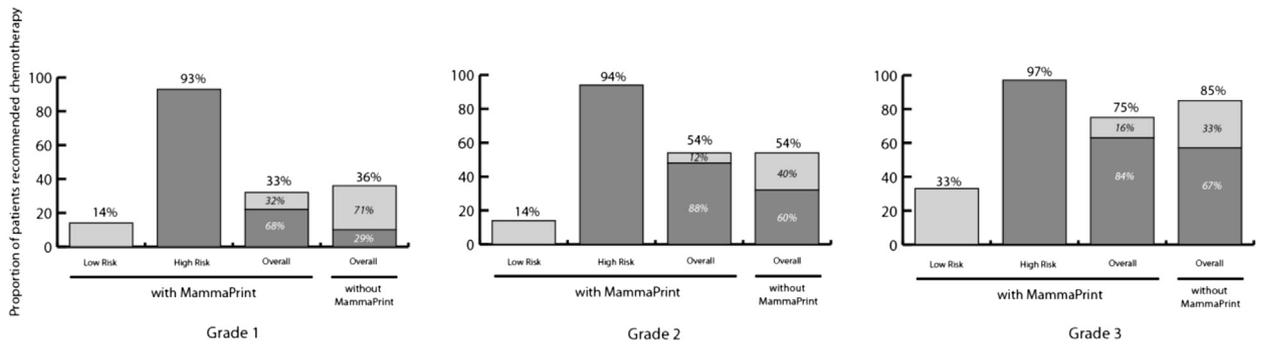
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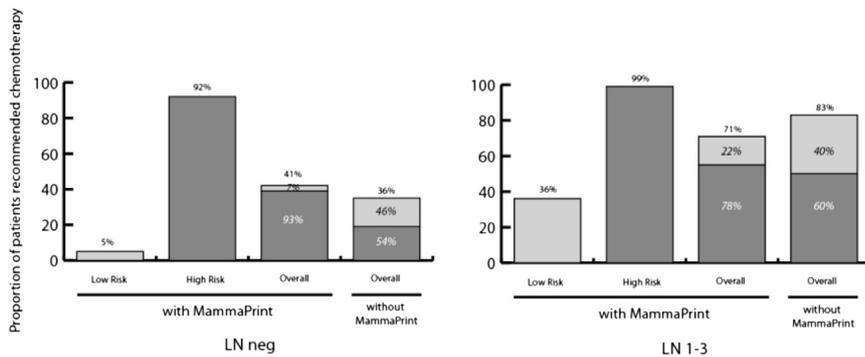


Fig. 2. Proportions of patients receiving chemotherapy by MammaPrint category and age group (A), tumor size (B), grade (C), and nodal status (D).

information to complement pathology assessment and to predict response to adjuvant chemotherapy, in particular in patients with ER+ and HER2– early breast cancer [2,19].

In conclusion, the use of MammaPrint to classify patients as Low or High Risk increased the inter-institutional agreement for adjuvant chemotherapy treatment advice from 57% to 79% for the overall patient population and from 51% to 75% for patients with ER+/HER2– breast cancer. The impact of MammaPrint on the change in adjuvant treatment advice for patients with ER+/HER2– breast cancer, the patient group with the highest MammaPrint clinical utility, ranged from 24% to 37% in this European study. MammaPrint can decrease the inter-institutional and inter-country variability in the adjuvant treatment advice provided to female patients with breast cancer.

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Conflict of interest statement

F. de Snoo, L. Stork-Sloots and L. Dekker-Vroling are employees of Agendia NV. The other authors have no conflicts of interest to disclose.

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