EPIDEMIOLOGY

Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up

Sara Bustreo[2] · Simona Osella-Abate[1] · Paola Cassoni[1] · Michela Donadio[2] ·
Mario Airoldi[3] · Fulvia Pedani[3] · Mauro Papotti[4] · Anna Sapino[1,5] ·
Isabella Castellano[1]

Abstract Although Ki67 index suffers from poor reproducibility, it is one of the most important prognostic markers used by oncologists to select the treatment of estrogen receptor (ER) positive breast cancer patients. In this study, we aim to establish the optimal Ki67 cut-offs for stratifying patient prognosis and to create a comprehensive prognostic index for clinical applications. A mono-institutional cohort of 1.577 human epidermal growth factor receptor 2 negative/ER+ breast cancer patients having complete clinical, histological, and follow-up data was collected. The 14 and 20 % Ki67 cut-offs were correlated to disease-free interval (DFI) and disease-specific survival (DSS). To create a comprehensive prognostic index, we used independent variables selected by uni/multivariate analyses. In terms of DFI and DSS, patients bearing tumors with Ki67 < 14 % proliferation index did not differ from those with Ki67 values between 14 and 20 %. Patients with tumor with Ki67 > 20 % showed the poorest prognosis. Moreover, to tumor size, the number of metastatic lymph nodes and Ki67 > 20 % was given a score value, varying depending on definite cut-offs and used to create a prognostic index, which was applied to the population. Patients with a prognostic index ≥3 were characterized by significant risk of relapse [DFI: Hazard Ratio (HR) = 4.74, $p < 0.001$] and death (DSS: HR = 5.03, $p < 0.001$). We confirm that the 20 % Ki67 cut-off is the best to stratify high-risk patients in luminal breast cancers, and we suggest to integrate it with other prognostic factors, to better stratify patients at risk of adverse outcome.

Keywords Ki67 cut-offs · Prognostic index · Luminal breast cancer · Relapse

Abbreviations
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
DFI	Disease-free interval
DSS	Disease-specific survival
PR	Progesterone receptor
AIC	Akaike information criterion
NCCN	National comprehensive cancer network

Sara Bustreo and Simona Osella-Abate have contributed equally to this work.

✉ Isabella Castellano
  isabella.castellano@unito.it

1 Division of Pathology, Department of Medical Sciences, University of Torino, Via Santena 7, 10126 Turin, Italy

2 Department of Medical Oncology 1, Città della Salute e della Scienza Hospital, Turin, Italy

3 Department of Medical Oncology 2, Città della Salute e della Scienza Hospital, Turin, Italy

4 Department of Oncology, University of Torino, Turin, Italy

5 Pathology Unit, Fondazione del Piemonte per l'Oncologia (FPO), Candiolo Cancer Institute (IRCCS), Candiolo, Italy

Introduction

The immunohistochemical surrogate of molecular subclasses of breast cancers proposed by the Saint Gallen Consensus Meetings [1–3] is largely used to classify patients in different risk categories. However, the main drawback of this classification is the low consistency in classifying Luminal A and Luminal B/HER2-negative (HER2−) carcinomas. Actually, both of them are estrogen

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receptor positive (ER+) and HER2−, but Luminal B category should identify patients with worse prognosis, endorsed for chemotherapy in addition to hormone blockage. Therefore, to differentiate Luminal A from Luminal B/HER2− breast cancers carries important therapeutic implications. For this aim, the Saint Gallen Guidelines [1] recommend to assess the proliferation index by Ki67, a nuclear marker of cell proliferation that is expressed in all cell cycle phases, except G0 [4]. A Luminal B breast cancer should show a higher proliferation index than Luminal A; however, the Ki67 cut-off for differentiating these two categories changed over time. The 2011 Saint Gallen Consensus Meeting defined as "low proliferation" tumors with a Ki67 index <14 % [3], a cut-off established by comparison with PAM50 intrinsic multigene molecular test classification of Luminal cancers [5]. Otherwise, during the 2013 Saint Gallen Conference, the majority of panelists voted that a threshold of ≥20 % was indicative of "high" Ki67 status. At the same time, several works reported low reproducibility of Ki67 results, mainly in the subset of cancers with intermediate proliferation activity (between 15 and 30 %) [6–10]. Thus, in March 2015, during the last Saint Gallen Conference [1], the use of the median Ki67 value of local laboratory was proposed as the cut-off, and accepted by the panel of experts.

In our Institution, the median Ki67 value (tested on 547 consecutive luminal invasive breast cancers, operated between January and December 2014) is 14 %. Thus, we analyzed a large retrospective cohort of Luminal ER+/HER2− breast cancers, with long follow-up, with the following aims: (1) to establish which is the optimal Ki67 cut-off (14 vs 20 %) to stratify cases according to disease-free interval (DFI) and disease-specific survival (DSS) at 5 and 10 years, and (2) to assess the prognostic significance of intermediate Ki67 values. In addition, considering that patient prognosis depends on tumor and patient characteristics, we investigated the role of proliferation activity within a comprehensive prognostic index, which uses independent variables to stratify patients having "good" or "poor" prognosis.

Patients and methods

Study design and population

We investigated 1688 female patients with ER+/HER2− primary breast cancers who underwent surgery from June 1994 to December 2012 at the Breast Unit of the Città della Salute e della Scienza of Torino, University Hospital of Torino, Italy. Ethical approval for this study was obtained from the Ethical Committee of our Institution.

The following clinico-pathological data were obtained from clinical charts: age at diagnosis, type of surgery (conservative surgery vs radical mastectomy), therapy, type, and site of recurrence. In addition, data regarding size (<15 vs ≥15 mm), histological type and grade of tumor, nodal involvement, vascular invasion, Progesterone Receptor (PR), ER, HER2, and Ki-67 was obtained from pathological reports. In particular, for what concerns Ki67, we set cut-points at 14 % (low) and 20 % (high), and of PR at 20 % as recommended by St. Gallen experts [2, 3]. Complete clinical and histopathological data were available for 1577 out 688 patients.

Statistical analyses

Pearson's Chi-square test and Student's t test were preliminary performed to compare categorical and continuous variables, respectively, and to evaluate potential differences in the variable distribution among groups. The disease-free interval (DFI) was calculated from the date of surgical excision of the primary tumor to the date of the first relapse or last check-up. Disease-specific survival (DSS) was calculated from the surgical excision date of the primary tumor to the date of breast cancer death or last check-up. Survival distribution curves were plotted using the Kaplan–Meyer method, and the statistical comparisons were performed using the log-rank test. Cox regression analyses were carried out on DFI and DSS to calculate crude and adjusted HRs and 95 % CIs for the different study group. Cases lost to follow-up and cases with a non-breast cancer related deaths were censored at the last follow-up control. A model was created for evaluating the prognostic role of different variables. The proportional hazard assumption was assessed with the Schoenfeld residuals. This did not give reasons to suspect violation of this assumption. The nature of variables (continue/categorical) included in the model was evaluated considering literature reports and the results of the log-likelihood ratio test. Akaike information criterion (AIC) was used for model selection. All statistical tests were two sided. p values <0.05 were considered significant. Statistical analyses were performed using Stata/SE12.0 Statistical Software (STATA, College Station, TX).

Results

The characteristics of the 1688 patients with ER+/HER2− breast cancer stratified according to Ki67 cut-offs are reported in Tables 1 and 2. DFI and DSS were evaluated in patients with low (<14 %), intermediate (14–19 %) and high (≥20 %) Ki67 levels. Patients affected by cancers

Table 1 The characteristics of the 1688 patients with ER+/HER2− breast cancer stratified according to Ki67 cut-offs

	Total	Ki67 <14 %	Ki67 ≥14 %	p	Ki67 <20 %	Ki67 ≥20 %	p
Age at diagnosis							
Median, interval	62 (23–92)	62 (34–87)	62 (23–92)	0.674	62 (23–92)	61 (27–88)	0.141
LN metastatic (n) (missing 5)							
0	1133	596	537	<0.001	834	299	<0.001
1–3	405	177	228		249	156	
4–9	96	34	62		48	48	
>9	49	14	35		18	31	
Tumor size (mm) (missing 58)							
<15	912	527	385	<0.001	717	195	<0.001
≥15	718	277	441		409	309	
Tumor grade (missing 1)							
1	634	462	172	<0.001	564	70	<0.001
2	806	333	473		534	272	
3	247	27	220		53	194	
Vascular invasion (missing 13)							
No	1104	637	467	<0.001	858	246	<0.001
Si	571	178	393		283	288	
PgR% (missing 111)							
<20%	337	151	186	0.023	212	125	<0.001
≥20%	1,240	642	598		891	349	
Histotype (missing 138)							
DC	961	420	541	<0.001	608	353	<0.001
ILC	198	134	332		253	79	
IDC + ILC	157	63	94		94	63	
other	234	139	95		194	40	
Surgery (missing 5)							
Conservative	1180	608	572	<0.001	655	447	<0.001
Mastectomy	503	213	290		234	236	
Radiotherapy (missing 1)							
No	462	196	266	<0.001	282	180	<0.001
Yes	1215	624	591		862	353	
Site of progression							
Contralateral	16	7	9	0.329	8	8	0.483
Regional	51	13	38		17	34	
Distant	94	25	69		36	58	

with low and intermediate Ki67 values had similar DFI and DSS, whereas patients with high Ki67 proliferation index had lower DFI and DSS than patients in the two former groups (Log-rank test DFI $p < 0.001$; DSS $p < 0.001$), Fig. 1.

Univariate Cox analyses estimates reported in Table 2 showed that tumor size ≥15 mm, nodal involvement, high grade, and vascular invasion negatively impact on DFI and DSS, together with a high-Ki67 index. As expected, cancers having such features were treated with more aggressive surgery and radiotherapy (Table 1). PR >20 % failed to play a favorable prognostic role on DSS (Table 3).

Multivariable analyses (Table 4) were performed on 1577 patients with complete clinical and histopathological data. The proportionality assumption was satisfied both on DFI ($p = 0.6469$) and DSS ($p = 0.8008$). Tumor size ≥15 mm, nodal involvement, 20 % Ki67 cut-point maintained an unfavorable role on DFI and DSS.

To create a comprehensive index associated to "good" and "poor" prognosis and based on multivariate analysis, a score was attributed to each significant variable according to its HR. Thus, a score value of 1 was given to tumors >15 mm and tumors with Ki67 ≥ 20 %, whereas a score 0 was given to tumors ≤15 mm and tumors with Ki67 <20 %. Four score

values were used for lymph nodes (0: lymph nodes free of metastases; 1: from 1 to 3 metastatic lymph nodes, 2: from 4 to 9 metastatic lymph nodes; 3: more than 9 metastatic lymph nodes). The comprehensive prognostic index was created

Table 2 Classification on the basis of Ki67 and PgR values

ER+	Luminal A (Ki67 low, PgR high)	Luminal B (Ki67 high or PgR negative/low)
Ki67		
<14 %	642	151
≥14 %	–	784
Ki67		
<20 %	891	212
≥20 %	–	474

Ki67 ≥ 20 %	Luminal A	Luminal B
Ki67 (%)		
<14	642	151
14–19	249	61
≥20	–	474

Ki67 (%)	PgR% <20 %		PgR% ≥20 %	
	Luminal A	Luminal B	Luminal A	Luminal B
<14	–	151	642	–
14–19	–	61	249	–
≥20	–	125	0	349

using the following formula: (tumor size score value) + (number of metastatic lymph nodes score value) + (Ki67 score value), determining a range of 0–5. Kaplan Maier analysis was then carried out for each value. We found that 575 had a final index of 0; 481 of 1; 303 of 2, 147 of 3, 40 of 4, and 21 of 5. Following the performance curves, we set the index cut-off at 3, indicating <3 a good prognosis and ≥3 a poor prognosis. Patients with an index ≥3 had a significant increased risk of relapse (DFI: HR = 4.74, CI 3.46–6.51, $p < 0.001$) and of death (DSS: HR = 5.03, CI 3.19–7.94, $p < 0.001$) Fig. 2.

We then analyzed the outcome of patients according to treatment (1040 hormonal therapy alone; 451 chemotherapy followed by hormone therapy). Eighty-six patients were not treated (comorbidities, older age, therapy refused) and were thus excluded from further analyses. Treatment type did not change DFI and DSS of patients with a score <3 (good prognosis) Fig. 3. On the contrary, patients with poor prognosis (score ≥ 3) treated with hormonal therapy alone had a DSS disadvantage, compared to patients treated with adjuvant chemotherapy before hormonal treatment Fig. 4.

Discussion

The first result of our study is that Luminal breast cancer patients with low (<14 %) and intermediate (14–19 %) Ki67 have the same DFI and DSS and a better prognosis
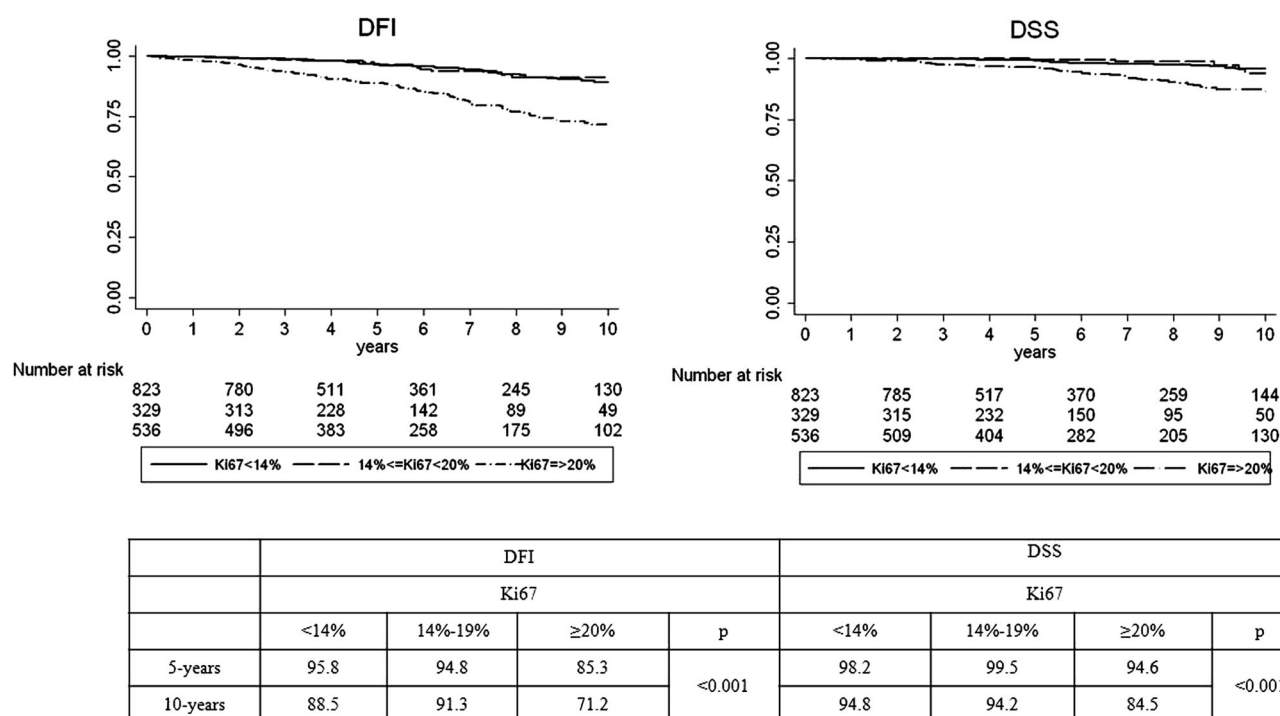

	DFI				DSS			
	Ki67				Ki67			
	<14%	14%-19%	≥20%	p	<14%	14%-19%	≥20%	p
5-years	95.8	94.8	85.3	<0.001	98.2	99.5	94.6	<0.001
10-years	88.5	91.3	71.2		94.8	94.2	84.5	

Fig. 1 DFI and DSS in low, intermediate, and high Ki67 patients

Table 3 Univariate analyses on DFI and DSS

Variable	DFI			DSS		
	HR	CI	p	HR	CI	p
Ki67 ≥ 14 %	2.33	1.65–3.29	<0.001	2.53	1.50–4.26	<0.001
Ki67 ≥ 20 %	3.24	2.35–4.45	<0.001	3.77	2.32–6.13	<0.001
Ki67 %						
<14 %	1			1		
14–20 %	0.90	0.51–1.59	0.710	0.68	0.26–1.83	0.454
≥20 %	3.14	2.21–4.47	<0.001	3.44	2.03–5.84	<0.001
PgR ≥ 20%	0.67	0.47–0.95	0.026	0.65	0.39–1.10	0.107
Mastectomy versus conservative surgery	3.70	2.71–5.07	<0.001	3.87	2.44–6.15	<0.001
LN metastatic						
N0	1					
N1	1.88	1.30–2.74	0.001	1.36	0.76–2.45	0.303
N2	4.78	3.03–7.56	<0.001	4.91	2.63–9.20	<0.001
N3	7.34	4.41–12.2	<0.001	6.75	3.32–13.7	<0.001
Tumor size ≥15	3.62	2.52–5.21	<0.001	4.24	2.45–7.35	<0.001
Tumor grade						
1	1			1		
2	1.94	1.28–2.95	0.002	1.92	1.00–3.67	0.048
3	3.95	2.53–6.16	<0.001	5.00	2.61–9.57	<0.001
Vascular invasion	2.71	1.98–3.71	<0.001	3.00	1.87–4.82	<0.001
Histotype						
IDC	1			1		
ILC	1.02	0.69–1.51	0.906	1.17	0.68–2.01	0.577
IDC + ILC	0.79	0.47–1.36	0.413	0.657	0.29–1.47	0.302
Other	0.72	0.43–1.19	0.197	0.707	0.33–1.49	0.358
Radiotherapy	0.36	0.28–0.49	<0.001	0.44	0.28–0.71	0.001

Table 4 Multivariate analyses on DFI and DSS

Variable	DFI			DSS		
	HR	CI	p	HR	CI	p
Age at diagnosis	0.99	0.97–1.00	0.355	1.00	0.98–1.03	0.549
Tumor size ≥ 15	2.06	1.35–3.14	0.001	2.78	1.42–5.41	0.003
Vascular Invasion	1.14	0.76–1.73	0.522	1.62	0.86–3.05	0.138
PgR ≥ 20 %	0.70	0.47–1.02	0.066	0.66	0.38–1.16	0.153
Metastatic LN						
N0	1			1		
N1	1.53	0.98–2.40	0.061	1.13	0.57–2.26	0.724
N2	3.03	1.69–5.44	0.000	2.89	1.29–6.47	0.010
N3	4.11	2.19–7.67	0.000	3.41	1.43–8.12	0.006
Tumor grade						
1	1			1		
2	1.15	0.71–1.88	0.569	1.10	0.51–2.36	0.806
3	1.06	0.59–1.89	0.851	1.09	0.46–2.58	0.836
Ki67 ≥ 20 %	2.35	1.56–3.55	0.000	2.37	1.26–4.47	0.008

Global test DFI $p = 0.6429$; DSS $p = 0.8008$
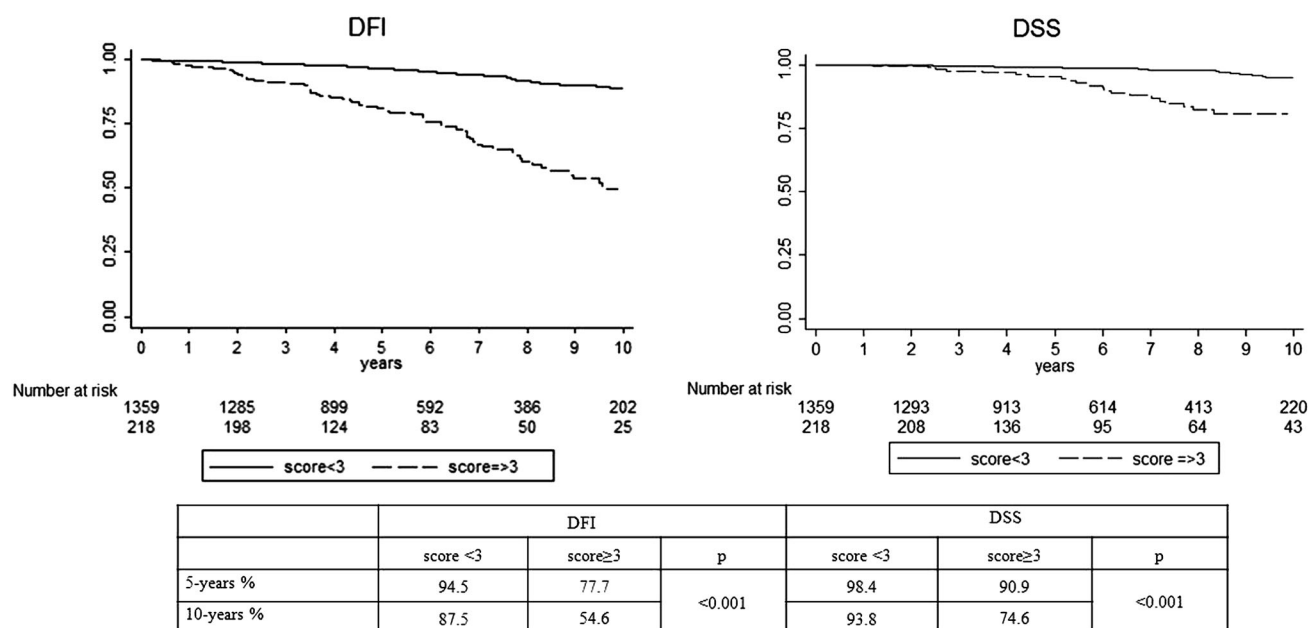
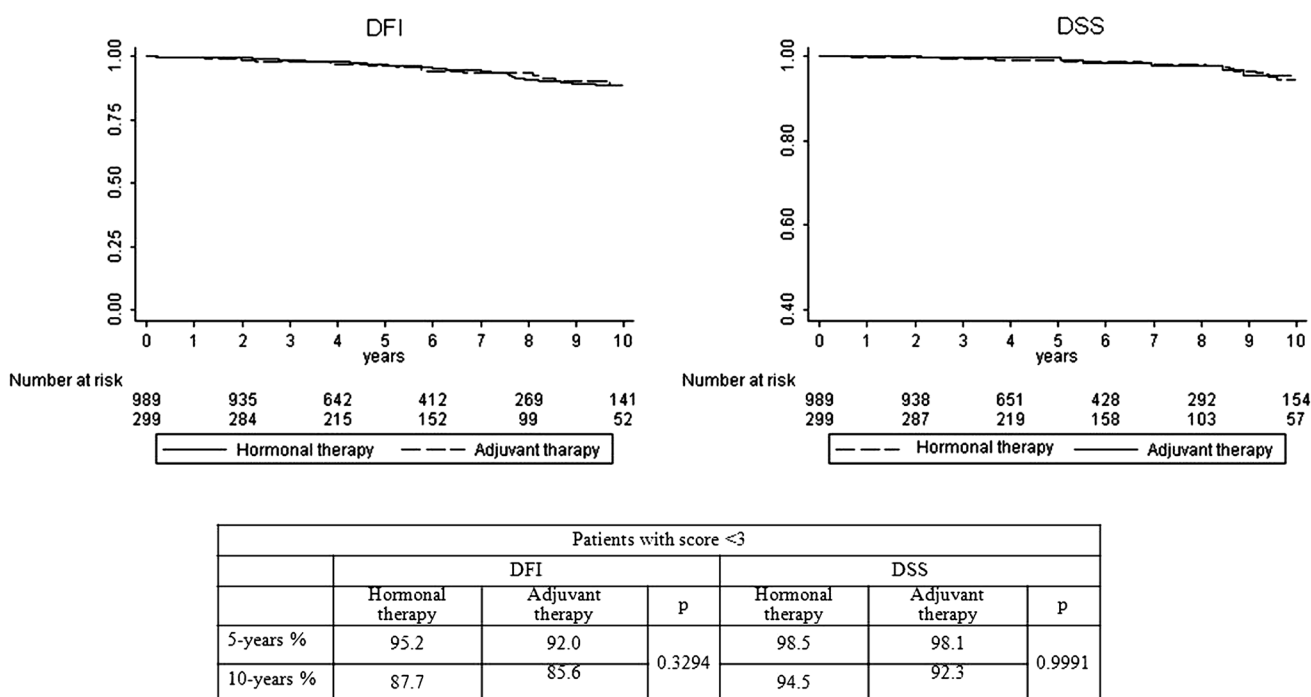Fig. 2 DFI and DSS in patients stratified on the basis of good (<3) or poor (≥3) score

	DFI			DSS		
	score <3	score≥3	p	score <3	score≥3	p
5-years %	94.5	77.7	<0.001	98.4	90.9	<0.001
10-years %	87.5	54.6		93.8	74.6	

Fig. 3 DFI and DSS in patients with good score (<3) stratified on the basis of therapy

Patients with score <3						
	DFI			DSS		
	Hormonal therapy	Adjuvant therapy	p	Hormonal therapy	Adjuvant therapy	p
5-years %	95.2	92.0	0.3294	98.5	98.1	0.9991
10-years %	87.7	85.6		94.5	92.3	

than patients with high (≥20 %) Ki67. This result confirms that, within ER+/HER2− breast cancers, the 20 % Ki67 cut-off can reliably (1) discriminate patients at low or high risk of recurrence and death, and (2) stratify patients at higher risk, eligible to adjuvant chemotherapy before hormone therapy.

The usefulness of Ki67 for decision on breast cancer management is a matter of discussion. A number of oncologists advise not to rely on Ki67 in the clinical practice, because of its low reproducibility [2–11]. Additionally, the American National Comprehensive Cancer Network (NCCN) guidelines do not provide any
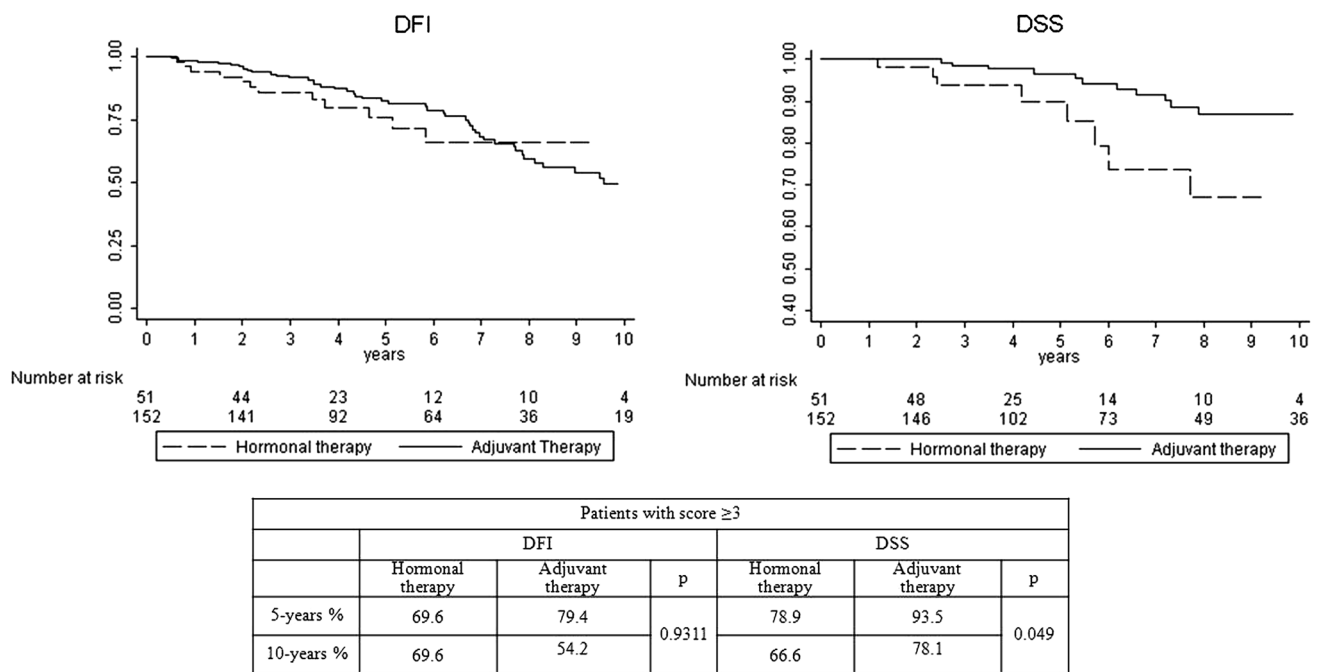
Fig. 4 DFI and DSS in patients with poor score (≥3) stratified on the basis of therapy

information regarding Ki67 immunohistochemical assessment and its role in breast cancer prognosis [12].

However, in Europe, the Saint Gallen Consensus Meeting had suggested to use Ki67 for classifying Luminal cancers since 2009. Still, uncertainty regarding the definition of highly proliferating tumors remains. In 2011, a cut-off of 14 % [3] was proposed, on the basis of the study by Cheang et al. [5], but 2 years later, it was upgraded to 20 % [2]. In the same year, Denkert et al. [13] proposed to considered Ki67 a continuum variable and suggested that the cut-points are context dependent and may change according to the prognostic or predictive role given to Ki67 itself. Thus, they suggested that the scientific community could define Ki67 cut-points depending on the study purpose [14]. To solve the literature ambiguities, we evaluated Ki67 in a subset of patients with ER+ (Luminal) breast cancer, with the aim to verify its impact on prognosis. First of all, we calculated the median value of Ki67 in our case series, as suggested by the last Saint Gallen Consensus Meeting [1] and showed that it corresponded to the value of 14 % identified by Cheang [5] as able to discriminate Luminal A from Luminal B molecular subtypes. Patients with a low (<14 %) and intermediate (14–19 %) Ki67 value showed a better DFI and DSS than those with higher Ki67 (≥20 %). As a consequence, when the purpose is to recognize high-risk patients, the 20 % cut-off is more reliable and reproducible than the 14 % cut-off. Moreover, Cserni et al. [15] showed that, in the routine practice, the proportion of Ki67 positive cells "tends to cluster around

values ending with 5 or 0," and accordingly, they proposed to select cut-off values ending with one of these two numbers.

Although we confirm that Ki67 is a reliable marker for identifying patients at low and high risk of recurrences/death, we firmly believe that Ki67 percentage has to be assessed following International Guidelines [16] and that the laboratory should be strictly subjected to local and external quality control assurance [17].

Moreover, in line with several studies suggesting that one marker is not sufficient to stratify prognosis of breast cancer patients, we designed a comprehensive prognostic index combining Ki67 with tumor size and number of metastatic lymph nodes. This index was highly predictive of DSS and DFI, at both uni- and multivariate analyses, confirming that tumor burden and proliferative index remain the most important parameters in ER+ breast cancer prognosis, as suggested by several other studies [18–22]. Actually, both tumor size and the number of positive axillary lymph nodes are traditionally accepted as important prognostic factors in breast cancer patients [23, 24], and molecular tools such as Endopredict® and PAM50® include these parameters, in association with specific gene expression, to create a recurrence score called EPclin® and Prosigna® ROR score, respectively. Since a number of genes in these assays are related to proliferative index and to ER pathway, we can reasonably suggest that the use of our prognostic index in Luminal cancer could "surrogate" the multigene prognostic test results.

In addition, we found that, within the subset of patients with a poor prognostic index (≥ 3), those treated with hormonal therapy alone showed a poorer DSS than those receiving also adjuvant chemotherapy. This result suggests that our prognostic index may be useful to identify patients that need a more aggressive treatment.

Although our study is based on a retrospective and a monocentric dataset of patients, which could represent possible biases, in our Institute, two different pathologists, skilled in breast pathology, assess Ki67 expression, and treatment is decided during multidisciplinary meetings, allowing for discussion among different clinicians.

In conclusion, we confirm the reliability of Ki67 as a prognostic marker in Luminal breast cancers, using a cut-off value of 20 %, and we stress the important role of Ki67 in the clinical management of patients. In addition, waiting for molecular test accessibility through the healthcare system, Ki67 together with tumor size and lymph nodal status may be useful to identify ER+ breast cancer patients with adverse prognostic outcome that need combined chemotherapy and hormonal therapy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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