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Histological grade provides significant prognostic information in addition to breast cancer subtypes defined according to St Gallen 2013

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Running title: Histological grade is an important prognostic factor in breast cancer

Abstract

Background: The St Gallen surrogate definition of the intrinsic subtypes of breast cancer consist of five subgroups based on estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor type 2 (HER2), and Ki-67. PgR and Ki-67 are used for discriminating between the ´Luminal A-like´ and ´Luminal B-like (HER2-normal)´ subtypes. Histological grade (G) has prognostic value in breast cancer; however, its relationship to the St Gallen subtypes is not clear. Based on a previous pilot study, we hypothesized that G could be a primary discriminator for ER-positive/HER2-normal breast cancers that were G1 or G3, while Ki-67 and PgR could provide additional prognostic information specifically for patients with G2 tumors. To test this hypothesis, a larger patient cohort was examined.

Patients and methods: Six hundred seventy-one patients (≥35 years of age, pT1-2, pN0-1) with ER-positive/HER2-normal breast cancer and complete data for PgR, Ki-67, G, lymph node status, tumor size, age, and distant disease-free survival (DDFS; median follow-up 9.2 years) were included.

Results: ´Luminal A-like´ tumors were mostly G1 or G2 (90%) while ´Luminal B-like´ tumors were mostly G2 or G3 (87%) and corresponded with good and poor DDFS, respectively. In ´Luminal B-like´ tumors that were G1 (n=23), no metastasis occurred, whereas 14 out of 40 ´Luminal A-like´ tumors that were G3 metastasized. In subgroup analyses of G2 tumors, low PgR and high Ki-67 were both weakly associated to an increased risk of distant metastases, hazard ratio (HR) and 95% confidence interval (CI) 1.8 (0.95-3.4) and 1.5 (0.80-2.8), respectively.

Conclusions: Patients with ER-positive/HER2-normal/G1 breast cancer have a good prognosis, similar to that of ´Luminal A-like´, while those with ER-positive/HER2-normal/G3 breast cancer have a worse prognosis, similar to that of ´Luminal B-like´, when assessed independently of PgR and Ki-67. Therapy decisions based on Ki-67 and PgR might thus be restricted to the subgroup G2.

Introduction

Adjuvant systemic therapy has improved survival among breast cancer patients, the majority of which have estrogen receptor (ER)-positive, human epidermal growth factor receptor type 2 (HER2)-normal disease. For patients with this subtype, adjuvant endocrine therapy is usually recommended, often in combination with chemotherapy. One of the greatest challenges within this group of patients is to identify those with good prognosis for whom chemotherapy can be avoided [1]. In 2013, the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer updated their surrogate panel, based on ER, Ki-67, progesterone receptor (PgR), and HER2, for classification of the intrinsic subtypes of breast cancer

[2]. In this update, ER-positive/HER2-normal breast cancer was further divided into ´Luminal A-like´ and ´Luminal B-like (HER2 normal)´ subgroups wherein the prognosis of patients with the former is better than that for the latter. In the ´Luminal A-like´ group, adjuvant chemotherapy might thus be avoided in the absence of other negative prognostic factors.

Histological grade (G) has repeatedly been shown to be a strong and independent prognostic factor [3-5], however, in 2013 the majority of St Gallen expert panelists voted that G3 could not be used as a substitute for high Ki-67 [2]. In contrast, in a pilot study that investigated the role of G in breast cancer prognosis in addition to that afforded by the 2013 St Gallen classification system we found that in 161 premenopausal lymph node-negative patients with ER-positive/HER2-normal breast cancer, G was strongly associated with St Gallen subtypes [7]. Indeed, ´Luminal A-like´ were mostly G1 or G2, whereas ´Luminal B-like´ were usually G2 or G3 [6]. Of the cases that diverged, a follow-up period of 10 years revealed that two out of four patients with ´Luminal A-like´ G3 breast cancer developed distant metastases and hence had a prognosis more similar to that of ´Luminal B-like´ breast cancer, whereas of the three patients with ´Luminal B-like´ breast cancer that were G1, not one experienced relapse and thus their clinical outcome was more similar to that of ´Luminal A-like´ breast cancer. These results, although based on a small number of cases, suggest that, independent of PgR and Ki-67, patients with ER-positive/HER2-normal breast cancers that are G1 might have a better prognosis than those with G3.

The primary aim of the present investigation was to use independent patient series to confirm the additional prognostic value of G to that of the 2013 St Gallen surrogate

classification of ER-positive/HER2-normal breast cancer. We hypothesized that for the ER-positive/HER2-normal subgroup of patients, G would be the first discriminator for those with G1 or G3 tumors, while Ki-67 and PgR would provide additional prognostic information specifically for patients with G2 tumors. As a secondary aim, the prognostic importance of PgR and Ki-67 was evaluated in patients with G2, ER-positive/HER2-normal breast cancer.

Patients and Methods

Patients

For the primary aim, we included breast cancer patients from two randomized multicenter trials (Patient series I and II) and one additional cohort (Patient series III) (Table 1). Patients with complete information regarding follow-up, number of positive lymph nodes, tumor size, ER, PgR, HER2, Ki-67, and G were included (Figure 1). Patients with at least one of the following characteristics were excluded: ER negativity, HER2 positivity, <35 years of age, ≥4 positive lymph nodes, tumor size >50 mm. Patients with these characteristics are most likely candidates for adjuvant chemotherapy without consideration of other prognostic factors.

For the second aim, an additional 110 patients with G2 tumors were included (Patient series IV; see below). These patients were not included when addressing the primary aim as they were part of the pilot study [6].

Patient series I: (N=185). Premenopausal patients with stage II breast cancer participated in a randomized trial comparing the effect of 2 years of tamoxifen

treatment versus no adjuvant systemic treatment. The original trial included 564 patients enrolled in the South and South-East Swedish Health Care Regions between 1986 and 1991 [7].

Patient series II: (N=103). Postmenopausal patients with stage II breast cancer were enrolled, between 1983 and 1991, in a randomized trial launched by the Swedish Breast Cancer group of 2 versus 5 years of adjuvant tamoxifen treatment (Swedish Breast Cancer Cooperative Group 1996) [8]. From the original trial, paraffin embedded tumor material was collected from a subgroup of patients treated with tamoxifen for 2 years in the South Swedish Health Care Region, for comparison of cytosol and immunohistochemistry methods for the analyses of ER and PgR [9]. This subgroup was included in the present study.

Patient series III: Bone marrow metastases cohort (N=273). The purpose of the original cohort was to study the prognostic importance of the presence of cytokeratin-positive cells in the bone marrow. It included 555 patients recruited from three hospitals in the South Swedish Health Care Region between 1999 and 2003 [10].

Patient series IV: SB91b (N=110). Premenopausal, lymph node-negative women were enrolled between 1991 and 1994 in a trial administrated by the South Swedish Breast Cancer Group, for evaluation of the prognostic importance of prospectively analyzed S-phase fraction by flow cytometry [11]. The original trial included 237 patients of which 110 patients with G2 tumors were included in the present study.

Evaluation of histological grade

Histological grade of whole tissue sections was re-evaluated by breast pathologists according to Elston and Ellis [3], as previously described for patient series I–III. Patient series IV was re-evaluated by one of the authors of the present study (CWE) using the same guidelines.

Analysis of ER, PgR, Ki-67, and HER2

The expression levels of ER, PgR, Ki-67, and HER2 were evaluated on whole sections or tissue microarrays as previously described [7, 12, 13]. Two core biopsies were evaluated from each formalin-fixed, paraffin-embedded breast cancer tissue, and the one with the highest percentage of positively stained cells was chosen. All cores were 0.6 mm in diameter with the exception of those used for ER and PgR analyses in Patient material IV that were 1.0 mm in diameter.

Cut-offs: ER and PgR positivity were defined as >10% stained nuclei, high Ki-67 as >20% stained nuclei, and HER2 positivity as 3+ or amplified 2+. It should be mentioned that since ER and PgR had previously been analyzed and reported in categories (positive *vs.* negative), we could not strictly apply the cut-offs according to the St Gallen recommendations (ER positivity: $\geq$1% and high PgR: $\geq$20%). Based on our experience from one of the included cohorts (SB91B), however, only a very small percentage of the tumors would have been influenced by this difference.

The 2013 St Gallen classification of intrinsic subtypes

St Gallen classification, based on ER, PgR, Ki-67, and HER2, was used to divide ER-positive/HER2-normal breast cancer cases into two intrinsic subtypes, as follows:

´Luminal A-like´: ER-positive, PgR-positive, HER2-normal, and low Ki-67;

´Luminal B-like (HER2-normal)´: ER-positive, HER2-normal, and one or both of high Ki-67 and PgR-negative.

Statistics

Distant disease-free survival (DDFS) was chosen as the endpoint in the present study. Differences in DDFS between subgroups of patients were evaluated using Kaplan-Meier estimates and log-rank tests. All tests were stratified for patient series. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox regression, also stratified for patient series. Owing to violations of proportional hazards assumptions for most variables included in the models, the follow-up was restricted to the first 10 years after diagnosis. This action led to fewer problems with non-proportional effects, but all effects should nevertheless be interpreted as average effects over time and not as constant effect estimates valid independent of follow-up time. All analyses were carried out using Stata version 14 (StataCorp LP, College Station, TX, USA, 2015).

Results

Histological grade in ´Luminal A-like´ and ´Luminal B-like (HER2-normal)´ breast cancer

The 2013 St Gallen International Panel of Experts guidelines were used to classify breast cancers from patient series I–III. According to these guidelines, 390 (70%) of the 561 ER-positive/HER2-normal tumors were classified as ´Luminal A-like´ while the remaining 171 (30%) as ´Luminal B-like´ (Table 2). In terms of prognosis, after a median follow-up of 9.3 years for patients alive and free from distant metastases, the latter subgroup had significantly worse DDFS compared with the former (HR=1.5, 95% CI: 1.0–2.3; Figure 2a). The distribution of G in these two subgroups was also reviewed. The majority of ´Luminal A-like´ tumors were either G1 or G2 (350/390; 90%), whereas a high proportion of Luminal B-like tumors were G2 or G3 (148/171; 87%; Table 2). Notably, among the 40 patients with Luminal A-like tumors that were G3, 14 (35%) developed distant metastases during the follow-up period. In contrast, of the twenty-three patients with ´Luminal B-like´ breast cancers that were G1, none developed distant metastases during follow-up (median follow-up for these 23 patients: 9.4 years, range: 5.5–10 years). The prognostic importance of G3 in ´Luminal A-like´ and G1 in ´Luminal B-like´ breast cancer is further illustrated in Figure 3a. Because most patients with ER-positive/HER2-normal breast cancer are treated with adjuvant endocrine therapy, the prognostic value of St Gallen classification was examined in endocrine-treated patients separately (Figure 2b). Similar to the results above, DDFS was worse for patients with ´Luminal B-like´ compared with that for those with ´Luminal A-like´ breast cancers (HR=1.6, 95% CI: 0.98–2.7). Similarly, when G was also accounted for, the prognostic importance of G3 in ´Luminal A-like´ and G1 in ´Luminal B-like (HER2-normal)´ breast cancer as indicators of poor and good prognosis, respectively, was also confirmed in this subgroup of patients (Figure 3b).

To further assess prognostic factors in our study cohort, multivariable analysis was performed including G, St Gallen subtypes, tumor size, lymph node status, and patient age. Among these, only G and lymph node status were found to be significant prognostic factors (Table 3a). Similar results were obtained when patients treated with adjuvant endocrine therapy were analyzed separately (Table 3b).

PgR and Ki-67 in G2 breast cancer

Because G2 was not clearly associated with prognosis of either Luminal A-like or Luminal B-like breast cancer, PgR and Ki-67 were evaluated as possible prognostic discriminators in G2 tumors. Although both PgR negativity and high Ki-67 were associated with poor prognosis in G2 tumors, univariable analyses showed weak evidence for prognostic discrimination (PgR (negative *vs.* positive): HR=1.8, 95% CI: 0.95–3.4; Ki-67 (high *vs.* low): HR=1.5, 95% CI: 0.80–2.8; Figure 4a–b).

Discussion

In the present study, histological grade (G) added prognostic information to that obtained using the 2013 St Gallen surrogate definition for the intrinsic subtypes of breast cancer. Our findings confirm that breast cancers designated ER-positive/HER2-normal that are G1 represent a good prognosis group, with a prognosis similar to that of ´Luminal A-like´ breast cancer. In contrast, ER-positive/HER2-normal breast cancers that are G3 have worse prognosis, similar to that of ´Luminal B-like´ breast cancer. Notably, this could be ascertained

independent of Ki-67 and PgR. Moreover, these findings were essentially unchanged when the effects of G and St Gallen classification on prognosis were assessed in patients treated with adjuvant endocrine therapy alone. This therapy is generally recommended for patients with ER-positive/HER2-normal breast cancer, alone or as chemo-endocrine therapy. Based on our findings, the importance of Ki-67 and PgR could be restricted to G2 breast cancers for the discrimination between good and poor prognosis in ER-positive/HER2-normal breast cancer. Using gene expression profiling, it has previously been shown that patients with histological grade 2 tumors in a similar way could be subdivided into one group with good prognosis and one group with poor prognosis [14]. It is interesting to note that most of these genes were associated to cell cycle regulation and proliferation. The patients in our study were selected from two randomized trials and two prospectively collected cohorts, and were diagnosed between 1983 and 2003. In three of these series, the selection of patients was based on menopausal status and stage of disease. It should therefore be of value to confirm the present results in a truly populations-based series of breast cancer patients.

In our study, 10% of ´Luminal A-like´ were G3 and 13% of ´Luminal B-like´ were G1. A recent publication by Maisonneuve and co-workers obtained comparative figures of 2.5% and 4.6%, respectively [15] as did Engstrøm and colleagues, who reported 10.3% G3 in ´Luminal A-like´ and 8.0% G1 in ´Luminal B-like´ in a study of 682 patients with ER-positive/HER2-normal breast cancer [16]. The occurrence of poorly differentiated luminal A tumors (14.1%) as well as well-differentiated luminal B tumors (9.4%) has also been demonstrated in a study based on the PAM50 gene set

[17]. Although accounting for a small percentage of cases, because G3 in ´Luminal A-like´ and G1 in ´Luminal B-like´ inverted the expected prognosis dictated by the St Gallen subtypes alone, these findings could critically influence disease treatment for patients of these subgroups.

Similar to our study, Maisonneuve and colleagues suggested that G could be incorporated as a first discriminator for ER-positive/HER2-normal breast cancer, where G1 was a strong indicator for the ´Luminal A-like´ subtype and G3 for the ´Luminal B-like´. The main focus of their study was, however, to evaluate the prognostic importance of PgR and its relation to Ki-67 in the ER-positive/HER2-normal breast cancer subgroup. Both Ki-67 and PgR have been reported to be of prognostic importance for ER-positive disease in several studies [18, 19]. Indeed, based on the study of Prat and colleagues [20], PgR was introduced into the St Gallen breast cancer subtype definition in 2013. Maisonneuve and co-workers showed that the prognostic importance of PgR was restricted to the intermediate Ki-67 subgroup (14–20%), and that it did not provide any additional prognostic information for the subgroups with either low (<14%) or high ($\geq$20%) Ki-67 [16]. Subgroup analyses in our study, which was focused on G as the initial watershed, showed inconclusive results regarding the prognostic effect of Ki-67 and PgR (P=0.21 and P=0.068, respectively). The weak evidence may, however, be a power problem, since in these subgroup analyses the number of patients and events are small; 14 events in the PgR negative group (n=67) and 12 events in the high Ki-67 subgroup (n=56). In this context it should also be mentioned that the prognostic importance of considering G3 for ´Luminal A-like` tumors was based on 40 patients

with 14 events. At the 2015 St. Gallen Consensus Conference, the majority of the Panel accepted a threshold value of Ki-67 within the range 20%–29% (21). The estimated prognostic effect of Ki-67 would most likely have been slightly different for other cut-offs in this interval, but we have not explored that in the present dataset. Instead we stick to the pre-defined cut-off 20%.

One drawback with G, however, is its limited inter-observer reproducibility [22, 23]. In spite of this, it has repeatedly been shown to be a strong prognostic factor [3-5]. Furthermore, it is cheap and easily evaluated routinely in the clinical setting. Also, by using strict guidelines, the concordance between different evaluators can be improved [24]. In this context, it should be mentioned that limited inter-observer reproducibility is also a well-known problem for Ki-67 [25].

In conclusion, our findings suggest that patients above or equal to the age of 35 years at diagnosis with T1-2, N0-1, ER-positive/HER2-normal/G1 breast cancer have a prognosis similar to that of ´Luminal A-like´, without consideration of Ki-67 and PgR. For this group of patients, chemotherapy might be avoided in the absence of other adverse prognostic factors. In contrast, patients with ER-positive/HER2-normal/G3 breast cancer have a worse prognosis, similar to that of ´Luminal B-like´. Therapy decisions based on Ki-67 and PgR might thus be restricted to the ER-positive/HER2-normal/G2 subgroup of breast cancers.

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Figure Legends

Figure 1

Cohort flow diagram.

Figure 2

Distant disease-free survival (DDFS) by St Gallen subtypes, ´Luminal A-like´ and ´Luminal B-like (HER2 normal)´, for all patients (a) and for patients treated with adjuvant endocrine therapy alone (b).

Figure 3

Distant disease-free survival (DDFS) by histological grade (G) and St Gallen subtypes, ´Luminal A-like´ and ´Luminal B-like (HER2 normal)´, for all patients (a) and for patients treated with adjuvant endocrine therapy alone (b).

Figure 4

Distant disease-free survival (DDFS) in ER-positive/HER2-normal, G2 breast cancer stratified by PgR (negative *vs*. positive; a). DDFS in G2 tumors stratified by Ki-67 (high *vs*. low; b).

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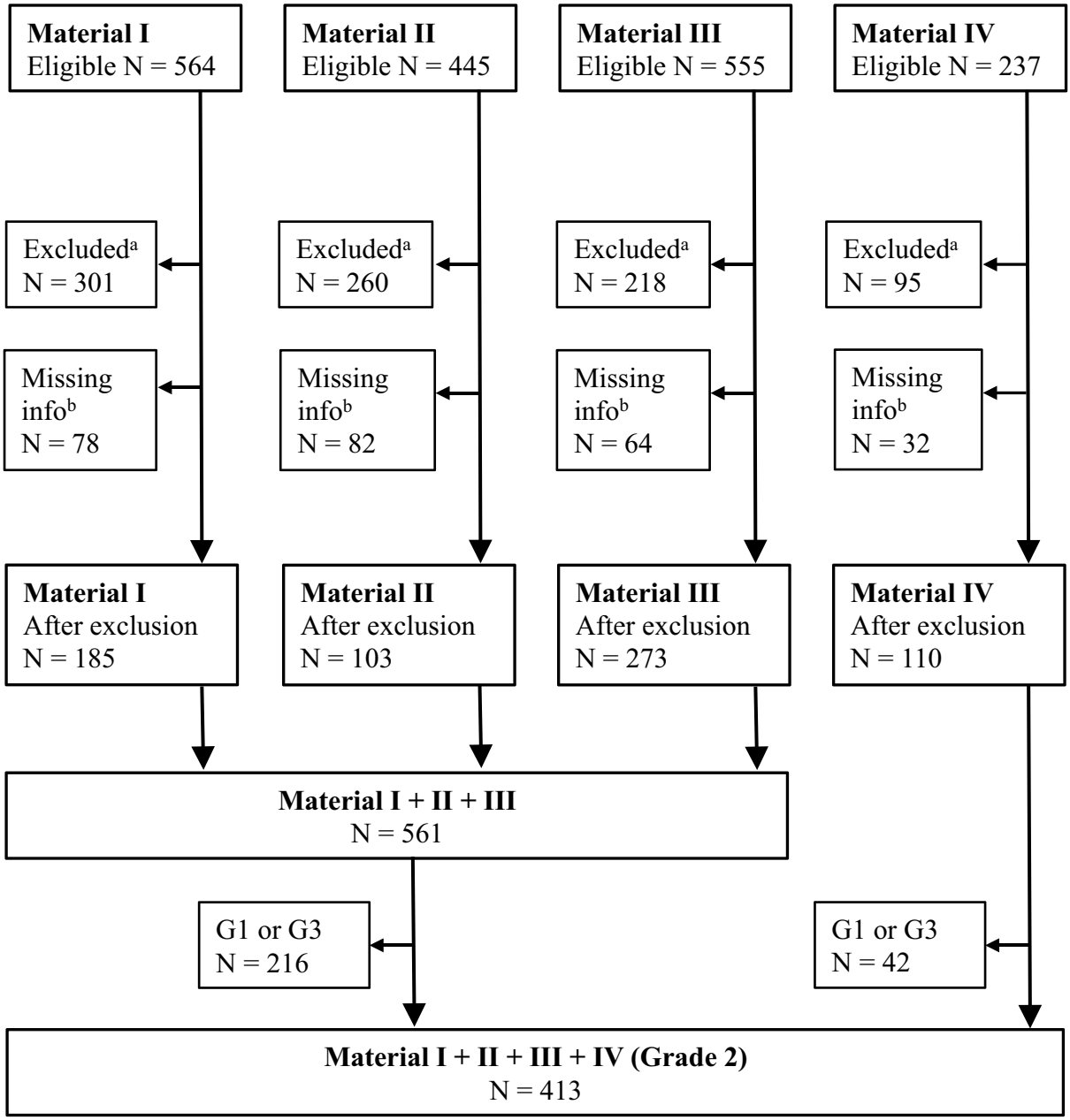
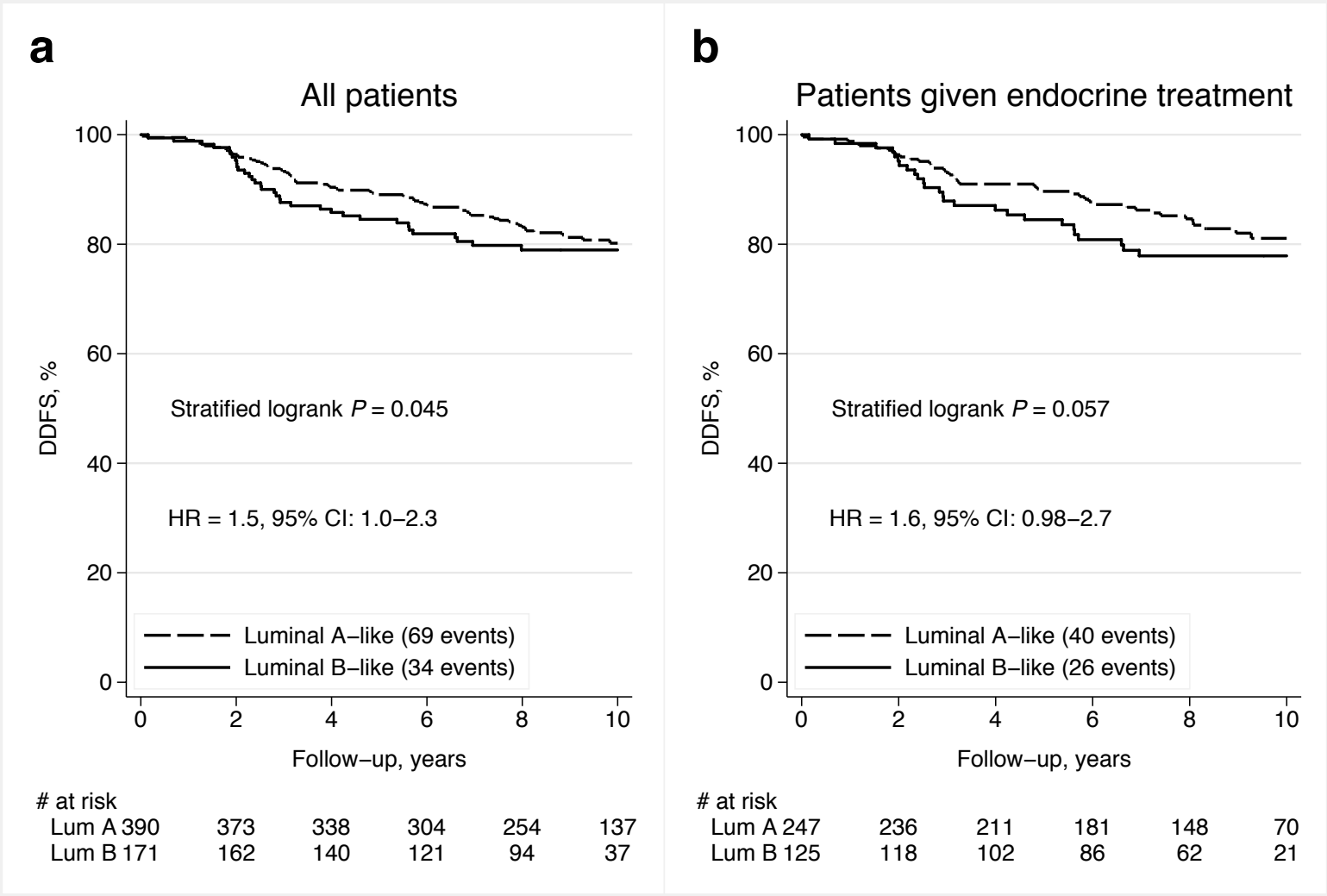
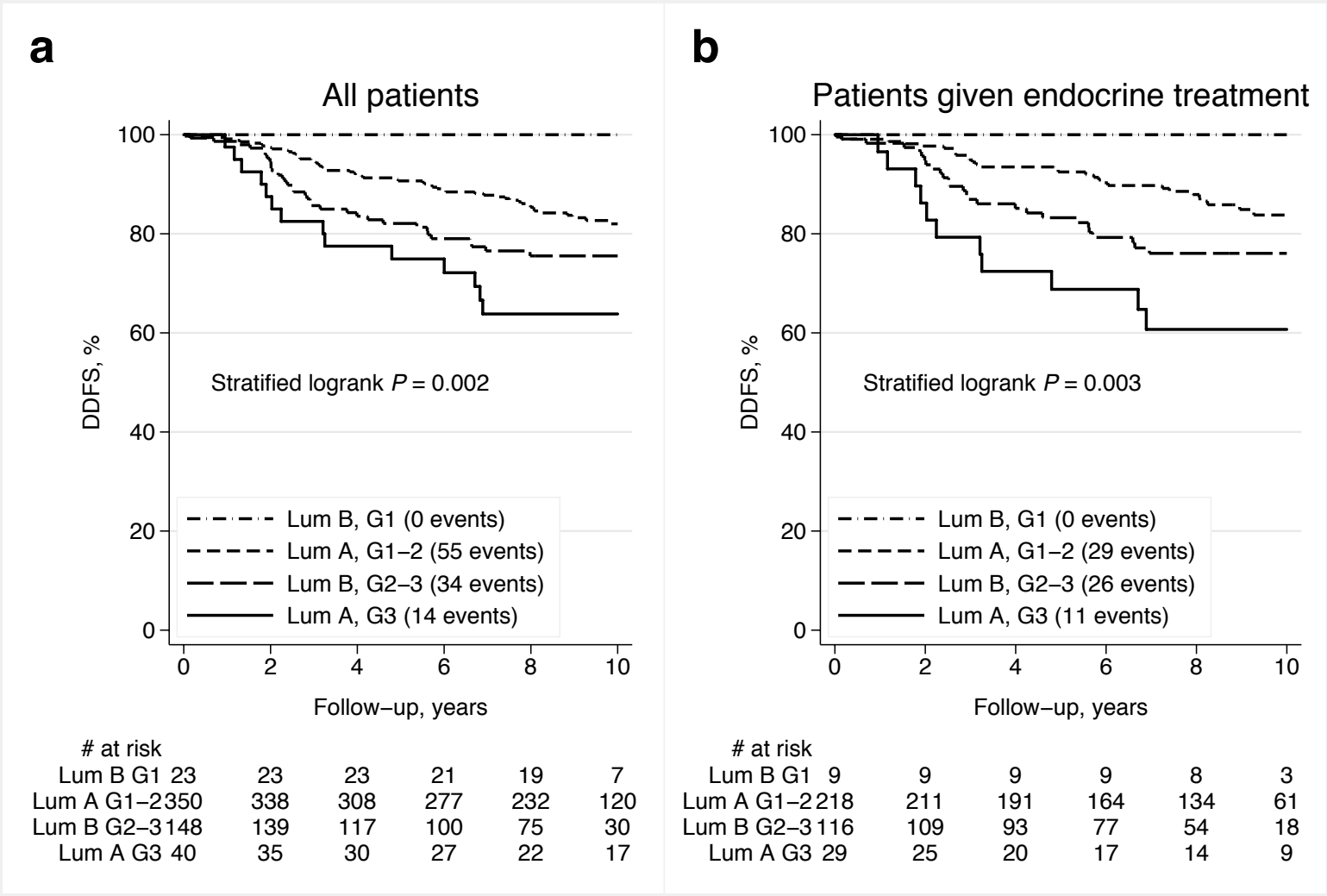


[a]Excluded= inclusion criteria's not fulfilled
[b]Missing info = missing information on inclusion variables

DDFS by St Gallen subtype



DDFS by St Gallen subtype and histological grade



DDFS in ER+/HER2–normal/G2

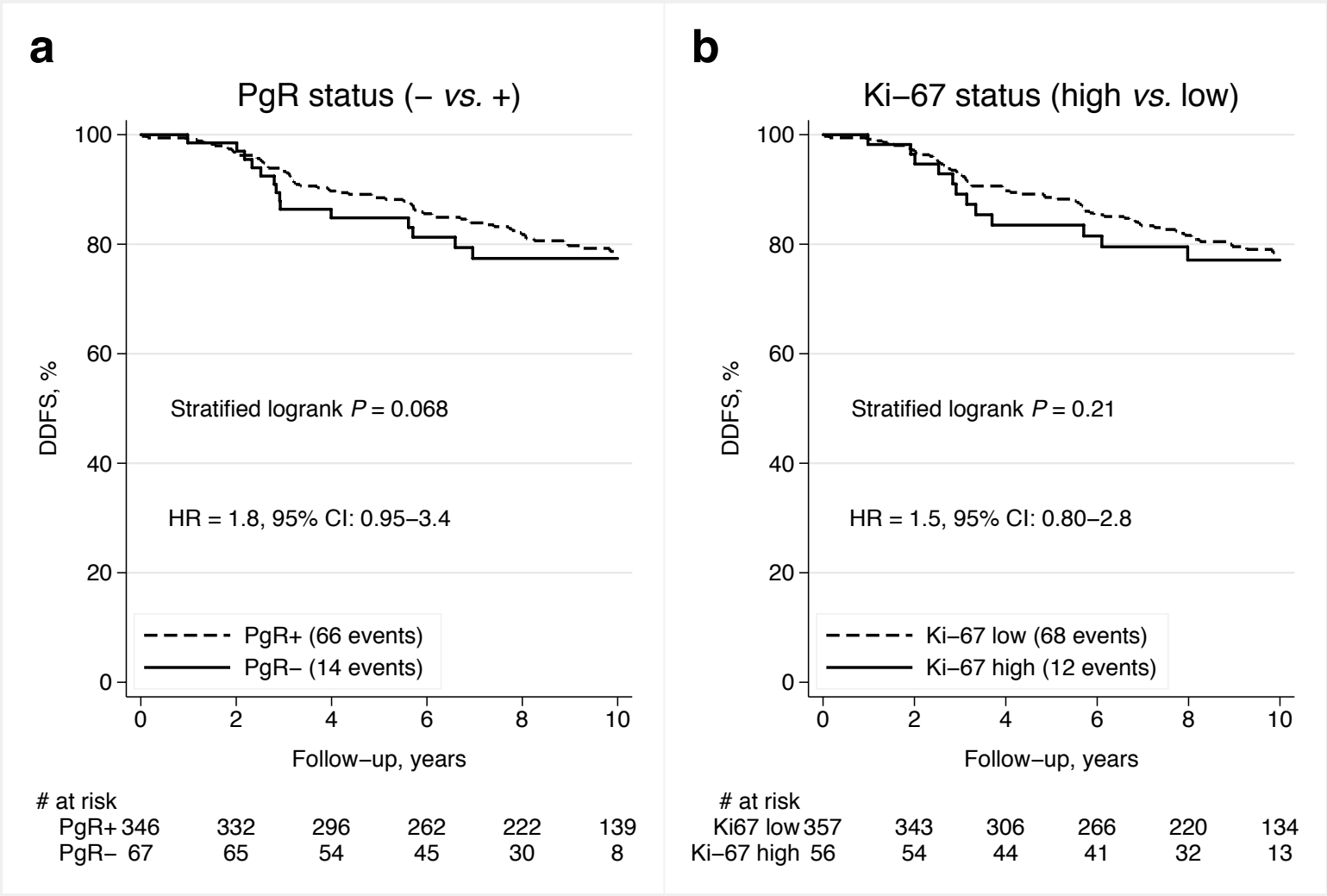


Table 1. Patient and tumor characteristics

Factor	Patient material I SB22-pre N (%)	Patient material II SB22-post N (%)	Patient material III BMM N (%)	Total I+II+III N (%)
Number of patients	185	103	273	561
Age				
Median, years	46	65	58	54
Range, years	36–56	43–81	37–88	36–88
Menopausal status				
Premenopausal	185 (100)	0	47 (17)	232 (41)
Postmenopausal	0	103 (100)	225 (83)	328 (59)
Unknown	0	0	1	1
Tumor size				
T1	79 (43)	37 (36)	206 (75)	322 (57)
T2	106 (57)	66 (64)	67 (25)	239 (43)
Median, mm	22	22	15	20
Range, mm	2–50	2–50	1–45	1–50
´Lum A-like´	151 (82)	65 (63)	174 (64)	390 (70)
´Lum B-like (HER2-neg)´	34 (18)	38 (37)	99 (36)	171 (30)
Lymph nodes				
Negative	63 (34)	36 (35)	193 (71)	292 (52)
1 Positive	56 (30)	36 (35)	50 (18)	142 (25)
2 Positive	40 (22)	19 (18)	21 (8)	80 (14)
3 Positive	26 (14)	12 (12)	9 (3)	47 (8)
ER status				
Positive	185 (100)	103 (100)	273 (100)	561 (100)
PgR status				
Negative	10 (5)	30 (29)	58 (21)	98 (17)
Positive	175 (95)	73 (71)	215 (79)	463 (83)
Histological grade				
G1	35 (19)	6 (6)	84 (31)	125 (22)
G2	104 (56)	85 (83)	156 (57)	345 (62)
G3	46 (25)	12 (12)	33 (12)	91 (16)
Ki-67				
Low	160 (86)	88 (85)	217 (79)	465 (83)
High	25 (14)	15 (15)	56 (21)	96 (17)
HER-2 status				
Negative	185 (100)	103 (100)	273 (100)	561 (100)

Adjuvant treatment				
Endocrine therapy	88 (48)	103 (100)	181[a] (66)	372 [a] (66)
Chemotherapy	0	0	9 [a] (3)	9 [a] (2)
None	97 (52)	0 (0)	89 (33)	186 (33)
DDFS 10 years				
Number of patients[b]	125 (68)	76 (74)	236 (86)	437 (78)
Median, years	10	5.7	8.9	9.3
Range, years	10–10	2.5–10	6.2–10	2.5–10

[a] Six patients received endo-chemotherapy
[b] Number of patients alive without metastasis at last follow-up (truncated at 10 years).

Table 2. Patient and tumor characteristics of ´Luminal A-like´ *vs.* ´Luminal B-like (HER2-negative)´

Factor	´Luminal A-like´ N (%)	´Luminal B-like (HER2-neg)´ N (%)
Number of patients	390	171
Material I SB22-pre	151 (38)	34 (20)
Material II SB22-post	65 (17)	38 (22)
Material III BMM	174 (45)	99 (58)
Age		
Median, years	53	58
Range, years	36–88	37–86
Menopausal status		
Premenopausal	182 (47)	50 (29)
Postmenopausal	208 (53)	120 (71)
Unknown	0	1
Tumor size		
Median, mm	19	20
Range, mm	1–50	2–45
Lymph nodes		
Negative	189 (48)	103 (60)
1 Positive	103 (26)	39 (23)
2 Positive	65 (17)	15 (9)
3 Positive	33 (8)	14 (8)
PgR status		
Negative	0 (0)	98 (57)
Positive	390 (100)	73 (43)
Histological grade		
G1	102 (26)	23 (13)
G2	248 (64)	97 (57)
G3	40 (10)	51 (30)
Ki-67		
Low	390 (100)	75 (44)
High	0 (0)	96 (56)
Adjuvant endocrine therapy		
Yes	247 (63)	125 (73)
No	143 (37)	46 (27)
Adjuvant chemotherapy		
Yes	4 (1)	5 (3)
No	386 (99)	166 (97)

Adjuvant chemo and/or endocrine therapy		
Yes	248 (64)	127 (74)
No	142 (36)	44 (26)
Events, <10 years follow-up		
Alive, no metastasis	307	130
Distant metastasis	69	34

Table 3a. Multivariable analysis of all patients (N = 561; stratified for patient material)

Factor	Hazard ratio	95% Confidence interval	*P*-value
Grade 2 *vs.* 1	2.8	1.3 – 6.0	0.006
Grade 3 *vs.* 1	4.4	2.0 – 11	<0.001
´Luminal A-like´ *vs.* ´Luminal B-like´	1.2	0.77 – 1.9	0.40
T2 *vs.* T1	1.3	0.85 – 2.0	0.22
N1 *vs.* N0	1.6	1.03 – 2.5	0.036
Age (cont.)	1.0	0.99 – 1.05	0.23

Table 3b. Multivariable analysis of patients treated with endocrine therapy (N=372; stratified for patient material)

Factor	Hazard ratio	95% Confidence interval	*P*-value
Grade 2 *vs.* 1	5.3	1.3 – 22	0.023
Grade 3 *vs.* 1	9.6	2.2 – 42	0.003
´Luminal A-like´ *vs.* ´Luminal B-like´	1.2	0.72 – 2.1	0.45
T2 *vs.* T1	1.7	0.97 – 2.8	0.066
N1 *vs.* N0	2.0	1.2 – 3.5	0.009
Age (cont.)	1.0	0.99 – 1.06	0.10