



Association of tumor-infiltrating lymphocytes with recurrence score in hormone receptor-positive/HER2-negative breast cancer: Analysis of four prospective studies

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ABSTRACT

Background: The clinical value of tumor infiltrating lymphocytes (TILs) in hormone receptor-positive (HR+)/HER2- breast cancer (BC) may be unearthed by focusing on more biologically aggressive tumors. Here we deepen and describe the correlation between RS and TILs, proposing an immuno-genomic model for HR+ /HER2- BC. **Methods:** We enrolled T1-T3, N0-N1 BC patients with available RS® and TILs in the context of four multicenter, prospective studies. RS® and TILs were considered as continuous and categorical variables. RS® was categorized into: 0–10 (low risk), 11–25 (intermediate risk) and 26–100 (high risk); TILs were categorized into: low TILs (0–10%), intermediate TILs (11–59%) and high TILs (60–100%).

Results: 811 patients were included. RS distribution was (n = 810): low risk 22.0%, intermediate risk 61.2%, high risk 16.8%. TIL distribution was (n = 455): low TILs 84.6%, intermediate TILs 13.6% and high TILs 1.8%. A significant, weak positive, linear correlation was found between continuous TILs and RS (Pearson coefficient=0.223, p < 0.001). When considering RS and TILs categories, tumors with intermediate/high TIL levels significantly enriched the high RS subgroup (p = 0.006). This was confirmed both within Luminal A and Luminal B cohorts. Among high-RS patients, 16.7% of Luminal A and 26.7% of Luminal B tumors had intermediate/high TILs.

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Conclusions: We observed that RS® and TILs capture only slightly overlapping information on the biology of HR+ /HER2- tumor microenvironment. We demonstrated the feasibility of combining RS and TILs into a composite immuno-genomic model, which may serve the purpose of guiding and focalizing patient selection in the further development of immunotherapy strategies for Luminal-like disease.

1. Background

In the last years, the landscape of hormone receptor-positive (HR+)/HER2-negative early breast cancer (BC) has undergone deep changes, driven, among others, by the development of gene-expression assays to guide adjuvant treatment decisions. These tools have been proven to be capable of optimizing the prognostic stratification of patients with surgically resected HR+ /HER2- BC, translating into the improvement in the estimation of the possible added benefit of chemotherapy with respect to endocrine therapy alone [1]. Within this framework, among commercially available genomic assays, test Oncotype DX® (RS®) is associated with the highest quality of evidence driving a strong recommendation for its use to guide decisions on endocrine and chemotherapy for patients with Luminal-like BC both in premenopausal node-negative and post-menopausal N0-N1 patients [1]. In detail, it captures the individual tumor biology by assessing the expression of proliferation-, invasion-, HER2-, and hormone- related genes on untreated tumor samples, providing a 0–100 ranging recurrence score (RS®) [2,3]. RS® scores up to 25 have been proven to be capable of identifying post-menopausal N0-N1 patients for whom chemotherapy can be safely spared [4]. Regarding pre-menopausal node-negative patients, available evidence suggests that the RS® score cutoff for which chemotherapy is not expected to add a prognostic gain may be more conservative than in the post-menopausal setting [4]. In pre-menopausal node-positive patients, data regarding the value of test Oncotype DX® for selecting patients suitable for endocrine therapy alone is less convincing [5] and therefore not sufficient to recommend the use of test Oncotype DX® with this purpose in this patient population [1].

In the last years, the evaluation of tumor-infiltrating lymphocytes (TILs) has progressively gained consideration within the early BC landscape both in terms of prognostic stratification and neoadjuvant chemotherapy sensitiveness. Although the largest and most solid evidence regards triple-negative (TN) and HER2 + subtypes, a mounting body of data has brought out a possible relevance of TILs also in Luminal-like disease [6–10]. In this respect, available evidence suggests that the immunogenicity of HR+ /HER2- BC may rely on finer and more granular mechanisms as compared to the more aggressive BC counterparts (namely HER2 + and TN), reflecting the complex and mutual interplays between cancer cells and the immune milieu, further shaped by the influence of endocrine therapy and chemotherapy [10]. In more detail, it has been reported that within HR+ /HER2- subtype, TILs may have a differential role as a prognostic biomarker according to tumor biological features. Indeed, while available evidence is mostly polarized in suggesting a marginal clinical value of the mere quantification of TILs in terms of prognostic stratification in unselected HR+ /HER2- BC patients, a mounting body of data is consistent in highlighting a positive prognostic role of TILs in patients whose tumors exhibit features of higher biological aggressiveness [7,11], thus fostering the increasing awareness of the need for a deeper understanding of TIL role in Luminal-like BC. These data suggest that the clinical value of TILs in HR+ /HER2- BC may be unearthed by focusing on patients whose tumors exhibit features of higher biological aggressiveness.

The correlation between RS® and TILs has not been extensively investigated so far. However, we hypothesize that they might capture different aspects of HR+ /HER2- disease biology. Here, we aim to deepen and describe the correlation between RS® and TILs in a large population of patients enrolled within the Roxane [12], Breast-Dx [13], BonDx [14] and PonDx [15] multicentric studies (which have prospectively evaluated the impact of the 21-gene assay on clinical decision and

resource optimization in T1-T3, N0-N1 HR+/HER2- BC in Italy), aiming to assess the feasibility of building a composite immuno-genomic model based on the integrated evaluation of RS® and TILs.

2. Methods

2.1. Patients

Inclusion and exclusion criteria of patients enrolled in the Roxane, Breast-Dx, BonDx and PonDx studies have been detailed elsewhere [12–15]. Briefly, they represent four multicenter, prospective, real-life experiences involving several Italian Breast Units, enrolling a total of 2744 patients (female or male ≥ 18 years) with T1-T3, N0-N1 HR+ /HER2- BC, and primarily aimed at assessing the impact on adjuvant treatment decisions of the Oncotype-Dx test in a clinical practice scenario. In addition, a real-life cohort of patients undergoing the 21-gene assay in a clinical practice scenario has also been included.

For the purposes of the present study patients with unavailability of RS® and TILs were excluded.

Patients were subcategorized into Luminal A and Luminal B according to the following definition: Luminal A: Ki67 < 20% and PgR $\geq$ 20%, Luminal B: ki67 $\geq$ 20% and/or PgR < 20% [16].

3. Recurrence score

RS® was considered both as a continuous and a categorical variable, by adopting the most recently endorsed cutoffs [4,5]: 0–10 (low risk), 11–25 (intermediate risk) and 26–100 (high risk).

3.1. Pathology evaluation

Stromal TILs were assessed on hematoxylin and eosin (H&E) stained slides from treatment-naïve primary tumor surgical samples, by complying with the recommendations by the International Working Group on Immune Biomarkers [17]. TILs were considered both as a continuous and a categorical variable, by adopting the cutoff by Denkert et al [6]: low TILs (0–10%), intermediate TILs (11–59%) and high TILs (60–100%).

3.2. Statistical considerations

Statistical analyses were performed using IBM software SPSS v.24 (RRID:SCR_002865).

Descriptive statistics were performed to analyze patient demographics, clinical and pathological features. Mean, median, ranges and quartiles were computed for continuous variables. The Kolmogorov-Smirnov non parametric test was applied to assess the normal distribution of continuous variables. The distribution of not normally distributed continuous variables across subgroups was assessed by applying the Mann-Whitney and Kolmogorov-Smirnov nonparametric tests, while the Student-T test was applied to compare mean values of normally distributed variables. The Chi-squared test (χ^2) was applied to make comparisons of categorical variables across subgroups. A logistic regression model was adopted to perform multivariate analyses. The Pearson and Spearman coefficient were calculated to assess the correlation between continuous or categorical variables.

3.3. Ethical considerations

The protocols of prospective studies and real-life cohort constituting the clinical platform of the present work were approved by the respective Ethical Committees of all centers. All included patients provided written informed consent.

4. Results

4.1. Patient population

811 patients were included. The CONSORT diagram of the study is shown in Figure 1.

Main clinicopathological features are shown in Table 1. In detail, median age at diagnosis was 57 years. For patients with known menopausal status ($n = 673$), 28.1% were premenopausal and 71.9% were post-menopausal. The majority of patients presented with pT1, pN0, G2 and HER2-low tumors. Median ER, PgR and ki67 expression was 90%, 80% and 20%, respectively. 66.6% of patients exhibited a Luminal B phenotype. 70.6% of patients received adjuvant endocrine therapy alone, while 25.9% were also administered adjuvant chemotherapy. Endocrine therapy consisted of aromatase inhibitor (+/- ovarian function suppression) in 74.0% of cases. Among patients receiving also chemotherapy, in 73.3% of the cases it consisted of anthracycline+/-taxane based treatment.

Clinico-pathological features across the 5 patients' cohorts (4 multicentric studies and 1 real-life cohort) are reported in Supplementary Table 1. RS scores and TIL levels were well balanced across cohorts.

4.2. Association between RS® and clinicopathological features

RS® was available for 810 patients. Median RS® was 16 and RS® class distribution was as follows: low risk (RS® <11) 22.0% ($n = 178$), intermediate risk (RS® 11–25) 61.2% ($n = 496$), high risk 16.8% ($n = 136$).

When considering RS® as a categorical variable, we observed a significant association between high-risk RS® class and features of enhanced biological aggressiveness. In particular, high RS® levels were significantly associated with poorer tumor differentiation, lower ER and PgR expression levels, as well as higher proliferative index and higher rate of Luminal B phenotype, as compared to low or intermediate RS® classes, as shown in Table 2. In addition, we observed an enrichment for HER2-low cases of the intermediate RS® category. Finally, N1 cases enriched the low RS® category, possibly reflecting a selection bias in terms of Oncotype® Dx request by the treating physicians in the context of the observational studies serving as clinical platform for the present analysis.

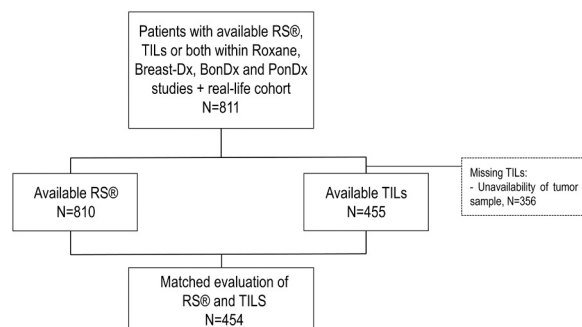


Fig. 1. CONSORT diagram of the study The flow diagram of the study shows the population of the study and the populations for which Recurrence Score, tumor infiltrating lymphocytes and their integrated evaluation were available. Abbreviations: RS®, Recurrence Score; TILs, tumor infiltrating lymphocytes; N, number.

Table 1

Main clinicopathological features of the overall population.

Total, n (%)		811 (100)
Age, median (Q1-Q3)		57.0 (48.0–66.9)
Menopausal status, n (%)*	Pre-menopausal	78 (28.1)
	Post-menopausal	197 (71.9)
AJCC pathologic stage, n(%)	1	587 (72.4)
	2	212 (26.1)
	3	5 (0.6)
	NA	7 (0.9)
Nodal status, n(%)	N0	478 (59.0)
	N1	328 (40.4)
	NA	5 (0.6)
Tumor grade, n(%)	1	51 (6.3)
	2	527 (65.0)
	3	229 (28.2)
	NA	4 (0.5)
HER2, n(%)	0	320 (39.4)
	Low	419 (51.7)
	NA	72 (8.9)
Phenotype, n(%)	Luminal-A	264 (32.5)
	Luminal-B	540 (66.6)
	NA	7 (0.9)
ER expression, median (Q1-Q3)		90.0 (90.0–95.0)
PgR expression, median (Q1-Q3)		80.0 (30.0–90.0)
Ki67 expression, median (Q1-Q3)		20.0 (15.0–30.0)
Adjuvant treatment choice, n (%)	ET alone	573 (70.6)
	CT + ET	210 (25.9)
	NA	28 (3.5)
ET type, n(%)	Tamoxifen + /- OFS	177 (21.8)
	AI + /- OFS	600 (74.0)
	NA	34 (4.2)
CT type, n(%)	Taxane-based	42 (5.2)
	Anthracycline-based	85 (10.5)
	Taxane+anthracycline-based	69 (8.5)
	Other	11 (1.3)
	NA	3 (0.3)

* percentages have been calculated by considered the total population with available menopausal status ($n = 673$)

Abbreviations: NA, not available; Q1-Q3 inter-quartile range; ET, endocrine therapy; CT, chemotherapy; AI, aromatase inhibitor; OFS, ovarian function suppression

We then developed a logistic regression model to perform a multivariate analysis, inputting variables individually significantly associated with high RS® (versus low and intermediate RS®): poorer tumor grade (OR 0.25, 95% CI 0.14–0.42, $p < 0.001$), lower ER expression (continuous, OR 0.97, 95% CI 0.95–0.99, $p < 0.001$) and PgR expression (continuous, OR 0.97, 95% CI 0.96–0.98, $p < 0.001$) as well as higher ki67 levels (continuous, OR 1.06, 95% CI 1.04–1.08, $p < 0.001$) preserved their independent association with RS®, as shown in Supplementary Figure 1.

4.3. Association between TILs and clinicopathological features

TIL evaluation was available for 455 patients. Median TIL level was 5%. 84.6% ($n = 385$), 13.6% ($n = 62$) and 1.8% ($n = 8$) had low, intermediate and high TIL levels, respectively.

When considering TILs as a categorical variable, we observed a significant association between intermediate/high TIL levels (TILs>10%) and higher proliferation index and lower PgR expression, with a borderline statistical significance for Luminal B phenotype, as summarized in Table 3.

At the multivariate analysis, only ki67 expression was found to be independently associated with TILs (continuous, OR 1.03, 95% CI 1.01–1.05, $p = 0.002$), as shown in Supplementary Figure 2.

Table 2

Main clinicopathological features according to RS classes (low RS versus intermediate RS versus high RS).

		RS low (0–10)	RS intermediate (11–25)	RS high (26–100)	Significance
Total, n (%)		178 (22.0)	496 (61.2)	136 (16.8)	
Age, median (mean)		62.0 (59.9)	55.0 (56.4)	59.0 (57.8)	0.003 * *
AJCC pathologic stage, n(%)	1	125 (70.2)	367 (74.0)	94 (69.1)	0.403
	2	51 (28.7)	120 (24.2)	41 (30.1)	
	3	2 (1.1)	3 (0.6)	0 (0)	
	NA	0 (0)	6 (1.2)	1 (0.8)	
Nodal status	N0	97 (54.5)	282 (56.8)	98 (72.0)	0.003
	N1	80 (44.9)	210 (42.3)	38 (28.0)	
	NA	1 (0.6)	4 (0.9)	0 (0)	
Tumor grade, n(%)	1	15 (8.4)	35 (7.0)	1 (0.8)	< 0.001
	2	136 (76.4)	339 (68.3)	51 (37.5)	
	3	27 (15.2)	118 (23.8)	84 (61.7)	
	NA	0 (0)	4 (0.9)	0 (0)	
HER2, n(%)	0	77 (43.2)	179 (36.1)	63 (46.3)	0.035 * **
	Low	86 (48.3)	273 (55.0)	60 (44.1)	
	NA	15 (8.5)	44 (8.9)	13 (9.6)	
Phenotype, n(%)	Luminal-A	86 (48.3)	167 (33.7)	11 (8.0)	< 0.001
	Luminal-B	90 (50.6)	324 (65.3)	125 (92.0)	
	NA	2 (1.1)	5 (1.0)	0 (0)	
ER expression, median (mean)		90.0 (91.9)	90.0 (90.1)	90.0 (86.7)	< 0.001
PgR expression, median (mean)		90.0 (81.6)	76.5 (60.8)	20 (35.1)	< 0.001
Ki67 expression, median (mean)		18.0 (19.7)	20.0 (21.1)	30.0 (31.3)	< 0.001

* percentages calculated over the total population with RS available

* * statistical significance shown only for the comparison between low RS and intermediate RS

Abbreviations: NA, not available

Table 3

Main clinicopathological features according to TIL categories (low TILs versus intermediate/high TILs).

		TILs low (0–10)	TILs intermediate/high (11–100)	Significance
Total, n (%)		385 (84.6)	70 (15.4)	
Age, median (mean)		58.8 (58.1)	56.5 (57.3)	0.292
AJCC pathologic stage, n(%)	1	272 (70.6)	60 (85.7)	NS
	2	110 (28.6)	10 (14.3)	
	3	1 (0.3)	0 (0)	
	NA	2 (0.5)	0 (0)	
Nodal status	N0	234 (60.8)	48 (68.6)	0.266
	N1	146 (37.9)	22 (31.4)	
	NA	5 (1.3)	0 (0)	
Tumor grade, n(%)	1	18 (4.7)	4 (5.7)	0.215
	2	244 (63.3)	37 (52.9)	
	3	120 (31.2)	29 (41.4)	
	NA	3 (0.8)	0 (0)	
HER2, n(%)	0	144 (37.4)	21 (30.0)	0.460
	Low	187 (48.6)	34 (48.6)	
	NA	54 (14.0)	15 (21.4)	
Phenotype, n(%)	Luminal-A	111 (28.8)	12 (17.1)	0.053
	Luminal-B	272 (70.7)	56 (80.0)	
	NA	2 (0.5)	2 (2.9)	
ER expression, median (mean)		90.0 (90.1)	90.0 (88.9)	0.180
PgR expression, median (mean)		80.0 (62.4)	75.0 (52.5)	0.018
Ki67 expression, median (mean)		20.0 (23.1)	25.0 (30.3)	< 0.001

* percentages calculated over the total population with RS available

Abbreviations: NA, not available; NS, not significant

4.4. Correlation between RS® and TILs

The matched evaluation of RS® and TILs was available for 454 cases. When considered as continuous variable, a significant, weak positive, linear correlation was found between TILs and RS® (Pearson coefficient=0.223, $p < 0.001$).

When considered as categorical variables, RS® classes and TIL categories were significantly associated with each other, as shown in Figure 2. In particular, we found that cases with intermediate/high TILs significantly enriched the high-RS® cohort (proportion of intermediate/high TILs in low/intermediate vs high RS®: 13.1% vs 25.9%, $p = 0.006$).

This phenomenon was confirmed both within Luminal A and Luminal B subgroups. In particular, the proportion of patients with

intermediate/high TILs was numerically higher among Luminal A and significantly higher among Luminal B patients in the high genomic risk group as compared to the low/intermediate genomic risk group (16.7% of Luminal A tumors and 26.7% of Luminal B tumors simultaneously exhibited intermediate/high TIL levels), as shown in Figure 3.

5. Discussion

The main aim of the present work was to investigate the correlation between RS® and TILs in pT1-T3, pN0-N1 HR+ /HER2- BC patients undergoing Oncotype Dx assay in the context of four Italian prospective multicentric studies focused on the impact of this genomic test on adjuvant clinical decisions.

We observed that RS® and TILs were significantly associated with

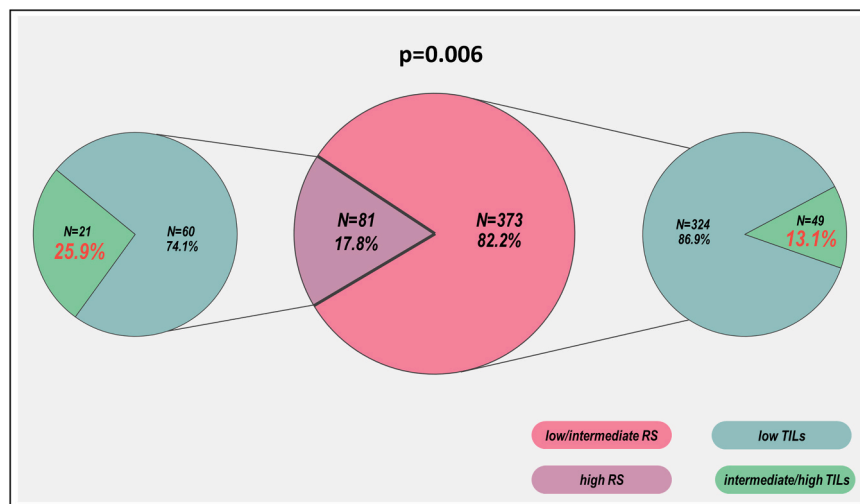


Fig. 2. Association between RS® and TILs (as categorical variables) in the total population. Visual representation of the association between categorical RS® (low/intermediate versus high) and categorical tumor-infiltrating lymphocytes (low versus intermediate/high). Abbreviations: RS®, Recurrence Score; TILs, tumor infiltrating lymphocytes.

each other, thus confirming previous reports [18,19]. When deepening the strength of this association we found a weak relationship between continuous RS® and TILs, which was consistent with results from a small retrospective study, similarly observing a weak correlation between the two biomarkers [18]. Additionally, both RS® and TILs were associated with features of enhanced biological aggressiveness. Overall, these findings suggest that TILs and RS® capture different aspects of tumor biology, which may be only sub-optimally surrogated by traditional features of increased aggressiveness. Indeed, a straightforward interpretation is that while RS® may reflect the biology underlying the tumor compartment, TILs are rather more reflective of the immune milieu. It is therefore not surprising that, albeit correlated, the strength of the association between RS® and TILs is no more than weak. Within this framework, it is interesting to note that, among genes covered by the Oncotype Dx assay, CD68 is the only one clearly associated with the immune counterpart of the tumor microenvironment. CD68, typically expressed by tumor-associated macrophages, has been consistently associated with unfavorable clinicopathological features and poorer survival outcome across several breast cancer cohorts [20–22], thus proxying an enhanced clinical aggressiveness. Moreover, several proliferation-associated genes, likewise contributing to the definition of RS®, may represent an additional element of overlap between TILs and RS®. Thus, not surprisingly, and consistently with available evidence [19], in the present study, we found a significant and independent association between higher levels of TILs and ki67 expression, which represents the most adopted surrogate for tumor proliferation.

Overall, we demonstrated the feasibility of combining information capturing different and complementary aspects of Luminal-like BC biology by assessing RS® and TILs. The adoption of this immunogenomic model enabled us to identify a subgroup of patients simultaneously exhibiting high RS® and intermediate-high TIL levels, underpinning traits of marked biological aggressiveness paralleled with features of immune activation. Importantly, the enrichment of the high-risk RS® category for tumors with higher levels of TILs was confirmed both in Luminal A and Luminal B cohorts, thus suggesting that the complexity of HR+ /HER2- BC biology might not be fully recapitulated by the currently adopted clinical surrogate for defining the “luminality”.

Although hypothesis-generating, these results may arise several orders of considerations. The major breakthroughs we have witnessed in the last years in terms of genomic stratification and treatment optimization of Luminal BC have been accompanied by a growing awareness regarding the deep and complex heterogeneity of this BC entity. Within this framework, the greatest efforts have been catalyzed upon the

possibility of safely sparing a not negligible proportion of intermediate risk patients from the exposure to cytotoxic treatments [4,5,23], alongside the investigation of escalated endocrine-based strategies for higher risk women [24,25]. In this context, although the immunogenicity of Luminal-like disease is very far from being unraveled and data regarding immunotherapy activity and efficacy have been unsatisfactory in unselected Luminal-like patients [26,27], available evidence suggest that the clinical value of immunotherapy may be unearthed by improving and optimizing HR+ /HER2- patients' selection [28–32] [NCT03725059 - press release]. In detail, although major differences in terms of chemotherapy backbone, treatment combinations and patient composition limit the value of cross-study comparisons, results from published trials of neoadjuvant immunotherapy dedicated to patients with Luminal-like early-stage disease [28–32] were all consistent in generating the urgency of deepening the role of this treatment strategy in high-risk patients with features of enhanced biological aggressiveness and immunogenicity. Our findings well fit within this scenario by putting forward the contextual evaluation of RS® and TILs as a highly reproducible and easily obtainable tool which may serve the purpose of guiding and focalizing patient selection in the further development of (chemo-)immunotherapy strategies for Luminal-like disease. Indeed, the incorporation of RS® and TILs into this proposed immune-genomic model may assist in the identification of patients simultaneously exhibiting features of high biological/clinical risk and marked immune infiltration, who may represent the ideal candidates to receive treatment escalation with chemotherapy plus immune checkpoint inhibitors in an experimental environment. In this context, research efforts are ongoing to further explore this possibility. Interestingly, it has been recently reported a significant association between increasing RS® scores and programmed cell death ligand 1 (PD-L1) positivity in a cohort of ER+ early BC patients, thus further emphasizing the interest in this topic [33].

The present study has several strengths. Firstly, its clinical platform is represented by four large multicentric prospective trials. In addition, TIL evaluation has been carried out according to a standardized methodology [17], by pathologists expert in the field of immune-oncology biomarker evaluation. Moreover, both RS® and TILs represent standardized and clinically available biomarkers, thus making our study easily reproducible in future investigations.

This work has also limitations. In particular, data regarding the composition of the immune infiltrate are missing, thus limiting the speculations that can be made regarding the immunogenicity of Luminal-like BC. In addition, the present study lacks survival data, thus

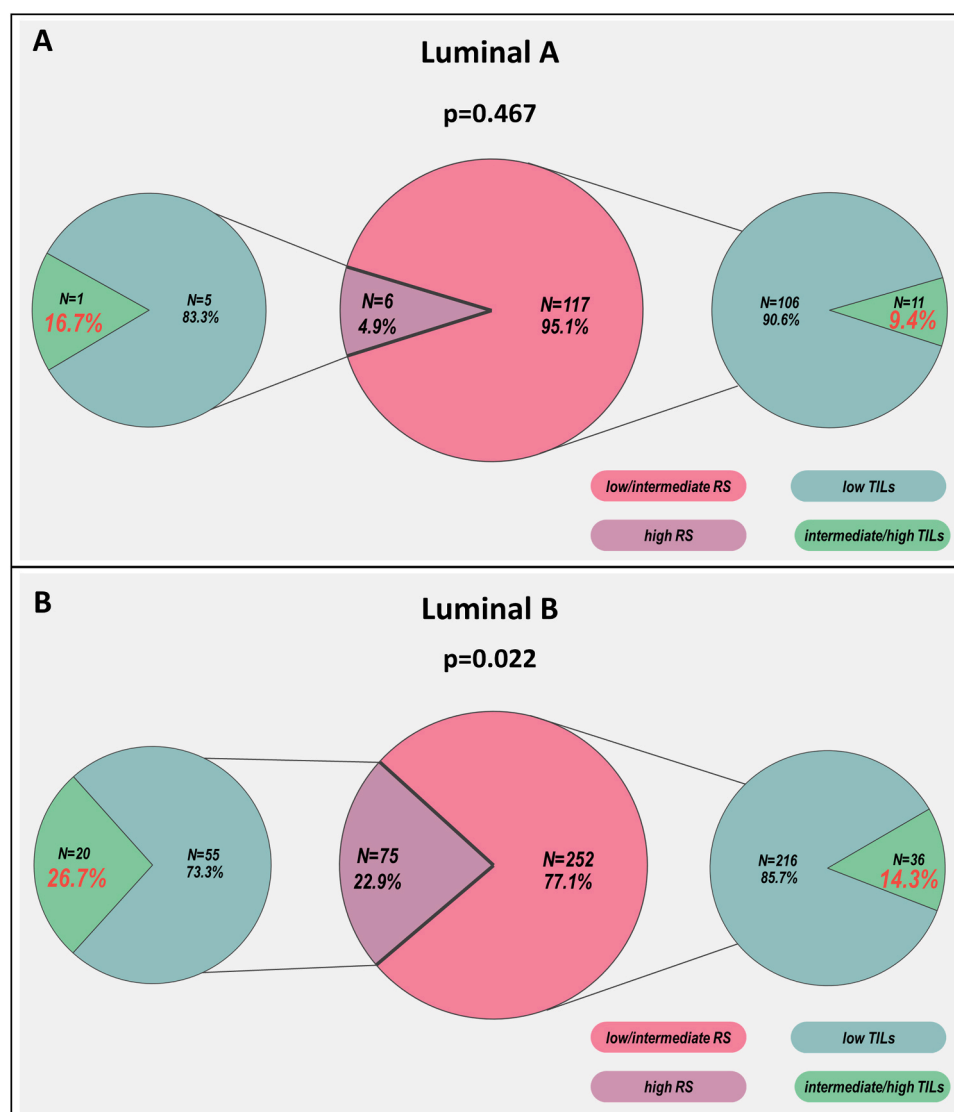


Fig. 3. Association between RS® and TILs (as categorical variables) according to tumor phenotype: A) Luminal A cohort, B) Luminal B cohort. Visual representation of the association between categorical RS® (low/intermediate versus high) and categorical tumor-infiltrating lymphocytes (low versus intermediate/high) in the Luminal A cohort (3A) and the Luminal B cohort (3B). Abbreviations: RS®, Recurrence Score; TILs, tumor infiltrating lymphocytes.

limiting the conclusions that can be drawn regarding the clinical value of adopting our immuno-genomic model for prognostication purposes. In this regard, although preliminary data suggest that the incorporation of RS® with TILs may refine the prognostic stratification of HR+ /HER2-BC patients [34], the potential clinical value of the diagnostic association between TILs and RS® into the composite immune-genomic model may subsist regardless their potential prognostic association.

In conclusion, we observed that RS® and TILs capture only slightly overlapping – and therefore complimentary - information on the biology of HR+ /HER2- tumor microenvironment. We also demonstrated the feasibility of combining these two biomarkers into a combined immuno-genomic model, which may potentially help guiding patient selection in the future development of (chemo-)immunotherapy strategies for Luminal-like disease, by allowing the identification of patients simultaneously showing features of high biological/clinical risk and enhanced immunogenicity.

CRediT authorship contribution statement

Study concept: MVD, AZ, VG, Study design: FM, MVD, AZ, VG, Data acquisition: all authors, Quality control of data and algorithms: FM,

MVD, AZ, VG, Data analysis and interpretation: FM, MVD, Statistical analysis: FM, MVD, Manuscript preparation: FM, Manuscript editing: FM, MVD, AZ, VG, Manuscript review: all authors.

Declaration of Competing Interest

FM: reports personal fees from Roche, Novartis, Gilead, Seagen, Pfizer, outside the submitted work.

MVD: reports personal fees from Eli Lilly, Exact Sciences, Novartis, Pfizer, Seagen, Gilead, MSD, AstraZeneca, Daiichi Sankyo, and Roche outside of the submitted work.

TG: reports personal fees from Gilead, Roche, outside the submitted work.

MG: Consulting/Advisor: Roche, AstraZeneca, Lilly, Daiichi Sankyo, Novartis, Pfizer, Seagen, MSD, Eisai; Honoraria: Novartis, Pfizer, Lilly, AstraZeneca, Daiichi Sankyo; Research funding to the Institution: AstraZeneca; Travel, accommodation, expenses: Lilly, Pfizer, AstraZeneca.

MM: personal fees from Accord, Gentili, Novartis, Lilly. EB: received speakers' and travels' fee from MSD, Astra-Zeneca, Pfizer, Eli-Lilly, BMS, Novartis and Roche.

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VG: reports personal fees for advisory board membership for Astra-Zeneca, Daiichi Sankyo, Eisai, Eli Lilly, Exact Sciences, Gilead, Merck Serono, MSD, Novartis, Pfizer, Olema Oncology, Pierre Fabre; personal fees as an invited speaker for AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Gilead, GSK, Novartis, Roche and Zentiva; personal fees for expert testimony for Eli Lilly.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejca.2023.113399](https://doi.org/10.1016/j.ejca.2023.113399).

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