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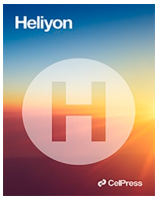

Research article

Inverse correlation between the amounts of lymphocytic infiltrate and stroma in breast carcinoma

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ABSTRACT

Background: Previous breast carcinoma studies focused on the evaluation of tumour-infiltrating lymphocytes (TILs) or of tumoural stroma via the tumour stroma ratio (TSR). Few studies assessed peritumoural lymphocytes and almost no studies investigated a possible relationship between lymphocytes and stroma. This prompted us to evaluate the amount of tumour cells, intra- and peritumoural lymphocytes, and stroma in breast cancer to support the hypothesis that the stroma may block the infiltration of lymphocytes inside the tumour.
Methods: We collected a retrospective series of 158 breast cancers (<25 mm). In addition to standard TILs and TSR evaluations, we assessed the percentages of tumour cells, stromal myofibroblasts, intra- and peritumoural lymphocytes on full-section tumours with haematoxylin and eosin and immunohistochemical staining.
Results: We showed significant negative correlations between the amounts of stroma and both intra- and peritumoural lymphocyte percentages. Considering the estrogen receptor positive invasive breast cancer of no special type cases, we showed that TSR had a positive prognostic value with an optimal threshold of 10 %.
Conclusions: This study is one of the first to show inverse correlations between tumoural stroma amount and intra- and peritumoural lymphocyte percentages, which supports the hypothesis that tumoural stroma can prevent the recruitment of lymphocytes around and within the tumour.

1. Introduction

Many studies are focused on the evaluation of tumour-infiltrating lymphocytes (TILs) in breast cancer, especially in triple-negative breast cancer (TBNC), but less for other types of cancer [1]. So far, we know that the TILs level depends on the histological subtypes [2–4].

From a clinical point of view, higher TILs levels are associated with higher tumour grades, higher Ki67 index and young patient age

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Abbreviations

TILs	tumour-infiltrating lymphocytes
TNBC	triple-negative breast cancer
ER+	estrogen receptor positive
OS	overall survival
DFS	disease-free survival
ERBC	estrogen receptor positive breast cancer
ILC	invasive lobular carcinoma
TSR	tumour stroma ratio
RFS	relapse-free survival
TMA	tissue microarrays
FFPE	Formalin-Fixed Paraffin-Embedded
PR	progesterone receptor
HER2	Human epidermal growth factor receptor 2
ISH	in situ hybridization
IBC-NST	invasive breast cancer of no special type
BCMF	invasive breast cancer of no special type with medullary features
NA	not applicable
CIS	carcinoma in situ
IHC	immunohistochemistry
CK	cytokeratin
SMA	smooth muscle actin
HE	haematoxylin and eosin
TumCellHE	percentage of tumour cells in the tumour area evaluated on the HE slide
StromaHE	percentage of stroma in the tumour area evaluated on the HE slide
Intra_Ly	percentage of lymphocytes located within the tumour area evaluated on the HE slide
TumCellCK	percentage of cells expressing cytokeratin AE1AE3 in the tumour area
StromaSMA	percentage of SMA-positive stroma in the tumour area
Peri_Ly	percentage of lymphocytes in the peritumoural area evaluated on the HE slide
IQ	interquartile
CAFs	cancer-associated fibroblasts

[4–10]. Conversely, an estrogen receptor positive (ER+) status is associated with lower intratumoural lymphocyte levels [9].

Concerning the prognostic value of TILs, studies show that a higher TILs level in TNBC is associated with a better overall survival (OS), a better disease-free survival (DFS) and can predict a pathological complete response [2,11–13]. Conversely, a lower TILs level is associated with worse prognosis [1,11,12]. The opposite effect is observed with the estrogen receptor positive breast cancer (ERBC), for which a higher TILs level decreases OS [5,12] and DFS [14]. Nevertheless, if the patient is treated with chemotherapy, a higher TILs level provides a better DFS [7]. Finally, invasive lobular carcinoma (ILC) also shows a lower OS and DFS with an increase in TILs level [5,10,15].

Nevertheless, all these studies use different methods of assessing TILs. In fact, some studies use the Salgado's 2014 guideline, assessing stromal TILs [16], others assess TILs according to Hendry's 2017 guideline, evaluating separately stromal TILs and TILs directly interacting with carcinoma cells with no intervening stroma [17], others still just examine the presence or absence of lymphocytes [3]. In addition, studies use different thresholds to determine high or low TILs levels making comparisons difficult. In this present study, we evaluated lymphocytes in a more global approach, taking into account the whole tumour zone (including tumour stroma and tumour nests). In this article, we also assessed peritumoural lymphocytes, which are still not widely investigated in the literature. In addition, for a more comprehensive and accurate spatial analysis of tumor components and their relationship, all evaluations were performed on full-section tumours, whereas most studies rely on tissue microarrays (TMAs).

Another parameter studied in breast cancer is the amount of stroma within the tumour, which also influences the prognosis according to histological subtypes. The most common method of assessing the stroma in a tumour is the "tumour stroma ratio" (TSR) [18], a stroma-high tumour being defined as having more than 50 % stroma (and therefore having a low TSR) [18]. The amount of stroma also varies with histological subtypes [3]. From a prognostic point of view, in TNBC, stroma-high would involve a shorter relapse-free survival (RFS) and OS [1,19–21]. In contrast, for the ERBC, a higher amount of stroma is associated with a longer OS [19,23] and RFS [23]. Forsare et al. and Millar et al. demonstrate a decrease of stroma amount with an increase of tumour grade [19,24] but the opposite is found by Catteau et al. [25] in ERBC. Finally, ILC with stroma-high tumours have lower tumour grades and are associated with longer breast cancer specific survival [24].

The goal of the present study is to evaluate the amount of tumour cells, intra- and peritumoural lymphocytes, and the amount of stroma in different subtypes of breast carcinomas, studies mainly concerning TNBC. The relationship between stroma and lymphocytes is currently poorly studied [4,19]. We hypothesise that the stroma could constitute a physical barrier blocking the infiltration of

lymphocytes inside the tumour, leading to an inverse relationship between intratumoural lymphocyte and stroma amounts, with a possible prognostic impact. We believe that a large amount of stroma and/or a low amount of lymphocytes could be a factor of worse prognosis. We also analysed whether peritumoural lymphocyte assessment brings additional information, as this parameter is also not widely covered in the literature.

2. Materials and methods

2.1. Clinical series

We collected a retrospective series of breast carcinomas from women who underwent surgery from 1996 to 2002 without neo-adjuvant treatment, at Erasme University Hospital (Brussels, Belgium), and for which a representative Formalin-Fixed Paraffin-Embedded (FFPE) tumour block is available (biobank of the pathology laboratory B2009/002, B2018/007). A selection of tumours smaller than or equal to 25 mm was performed to obtain a whole tumour section on a single slide, reducing the series to 158 cases. Table 1 describes clinical and histological data. Tumour grade was defined according to the Nottingham system. pTNM was revised according to the 8th UICC edition (UICC, John Wiley & Sons 2017). Lymph node, ER and progesterone receptor (PR) expressions were classified into two categories (positive or negative). Human epidermal growth factor receptor 2 (HER2) scores of 0, 1 and 2 without in situ hybridization (ISH) amplification were classified as negative. HER2 scores of 2 with ISH amplification and 3 were classified as positive. RFS is defined as the duration between the date of relapse and the date of diagnosis.

2.2. Immunohistochemistry

For each case, immunohistochemistry (IHC) targeting cytokeratin (CK) AE1AE3 (1:100 dilution; clone AE1/AE3; Agilent®) and smooth muscle actin (SMA) (1:200 dilution; clone 1A4; Cell Marque®) was used to stain on serial slides tumour cells and stromal

Table 1
Description of the clinical series for all cases and for invasive breast cancer of no special type (IBC-NST).

		All cases		IBC-NST[a]	
Age (158; 129)[b]	Mean (min-max)	58	(32–92)	59	(32–92)
Size (mm) (158; 129)	Mean (min-max)	14	[2–25]	14	[2–25]
Ki67 (%) (9; 71)	Mean (min-max)	28	(2–90)	22	(2–80)
		N	**%**	**N**	**%**
Recurrence (158; 129)	Yes	22	13.9	22	17.1
	No	136	86.1	107	82.9
Histologic type (158)	IBC-NST	129	81.6	NA	
	ILC	11	7		
	BCMF	16	10.1		
	Other[c]	2	1.3		
pT (158; 106)	T1a	3	2.2	3	2.8
	T1b	38	28.6	33	31.1
	T1c	92	69.2	70	66.1
Multifocality (158; 129)	Yes	23	85.4	22	17.1
	No	135	14.6	107	82.9
Associated CIS (158; 129)	Yes	118	74.7	110	85.3
	No	40	25.3	19	14.7
Vascular involvment (158; 129)	Yes	10	6.3	7	5.4
	No	148	93.7	122	94.6
Mitotic count (144; 118)	1	53	36.8	43	36.4
	2	44	30.6	43	36.4
	3	47	32.6	32	27.2
Tumour grade (155; 129)	1	36	23.2	33	25.6
	2	61	39.4	53	41.1
	3	58	37.4	43	33.3
Lymph Node Status (152; 123)	N0	107	70.4	84	68.3
	N+	45	29.6	39	31.7
ER status (153; 125)	Positive	129	84.3	115	92
	Negative	24	15.7	10	8
PR status (153; 125)	Positive	115	75.2	105	84
	Negative	38	24.8	20	16
HER2 status (147; 120)	Positive	22	15	19	15.8
	Negative	125	85	101	84.2

[a] Invasive breast cancer of no special type (IBC-NST) is the majority histological type in our series.
[b] (All cases; IBC-NST numbers).
[c] The histological type labelled "other" refers to mixed and cribriform type. Abbreviations: IBC-NST: invasive breast cancer of no special type. ILC: invasive lobular carcinoma. BCMF: invasive breast cancer of no special type with medullary features. NA: not applicable. CIS: carcinoma in situ. ER: estrogen receptor. PR: progesterone receptor. HER2: human epidermal growth factor receptor 2.

myofibroblasts respectively. Eleven cases were removed due to lack of tumour cells on the slides (total n = 147).

2.3. Semi-quantitative assessment of tumour components

Each haematoxylin and eosin (HE) and IHC slides were assessed by two pathologists (CV and XC); the two results were then averaged for data analysis. For each evaluation, the cases with a difference greater than 15 % between the two evaluators were reviewed together to reach a consensus value, as previously described [15].

The different tumour components were assessed as follows (see Fig. 1A to C). First, inside the tumour area, percentages of tumour cells (TumCellHE), stroma (StromaHE) and lymphocytes were evaluated on each HE slide. These lymphocytes located within the tumour area, either in the tumoural stroma or between the tumour cells, were referred to as intratumoural lymphocytes (Intra_Ly). Second, within the tumour area, the percentage of immunostained area was assessed for each IHC slide (TumCellCK, StromaSMA). Third, the lymphocyte percentage was also assessed in the peritumoural area on the HE slide (Peri_Ly). Fourth, TILs evaluation was performed on HE slides as previously described by Salgado et al., in 2014 [16]. Finally, the TSR was evaluated as previously described by Mesker et al. [18]. The precise methodology is described in Table 2 and Supplementary Fig. S1.

2.4. Statistical analysis

The statistical analysis was performed using Statistica® software (StatSoft, Tulsa, USA). The chi-square and Fisher exact tests were used to analyse the associations between categorical variables after checking the application conditions. The Mann-Whitney test and Kruskal-Wallis test (and associated post hoc tests) were applied for the comparison of independent groups of numerical data. The Spearman test was used to analyse non-parametric correlation (r_s) between pairs of numerical variables. Survival data were subjected to Kaplan-Meier analysis with the log-rank test for categorical variables or monovariate Cox regression analysis for quantitative ones. Multivariate Cox regression analysis was also applied by combining variables for which monovariate analyses showed a potential impact (i.e. with $p < 0.10$).

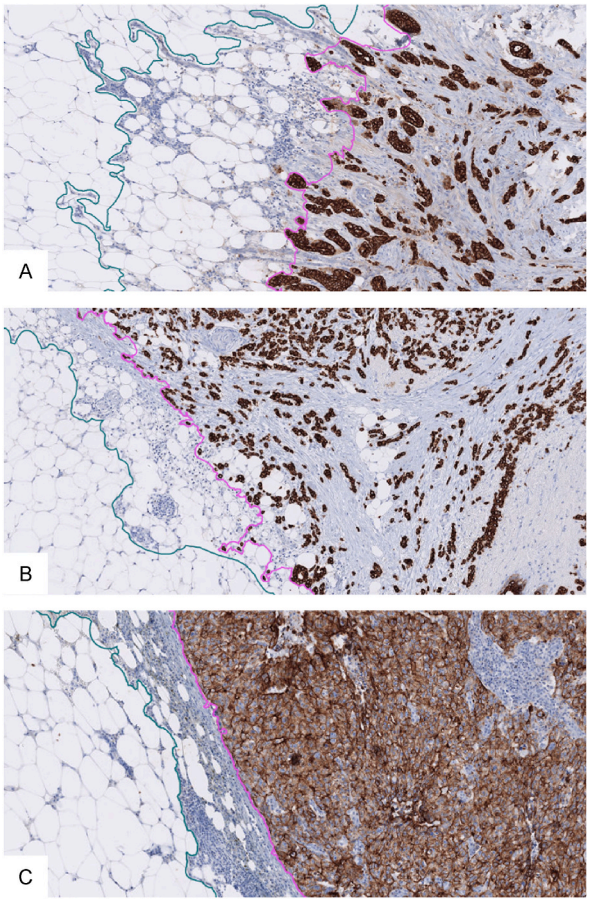

Fig. 1. Illustration of tumoural (pink line) and peritumoural (green line) delimitations in CK AE1AE3 slides from the three histological subtypes. A: IBC-NST; B: ILC; C: BCMF; ×5 magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Definition of the tumour components.

Tumour components	Abbreviations	Definitions
Tumoural cell percentage	TumCellHE	Percentage of tumour cells in the tumour area evaluated on the HE slide
	TumCellCK	Percentage of cells expressing CK AE1AE3 in the tumour area
Stroma percentage	StromaHE	Percentage of stroma in the tumour area evaluated on the HE slide
	StromaSMA	Percentage of SMA-positive stroma in the tumour area
Intratumoural lymphocyte percentage	Intra_Ly	Percentage of lymphocytes located within the tumour area evaluated on the HE slide, either in the tumoural stroma or between the tumour cells
Peritumoural lymphocyte percentage	Peri_Ly	Percentage of lymphocytes in the area just outside the tumour borders, evaluated on the HE slide
Tumour-infiltrating lymphocytes	TILs	Percentage of intratumoural stromal area occupied by lymphocytes and plasma cells evaluated on the HE slide, as previously described by Salgado et al., in 2014 [16] (so excluding lymphocytes between or in contact with tumour cells)
Tumour stroma ratio	TSR	On a tumour slide, the area containing the most stroma, still with tumour cells at all sides of the field, is microscopically determined (10× objective) and tumour percentage evaluated per increments of 10 %, as previously described by Mesker et al. [18]

Abbreviations: HE: haematoxylin and eosin. CK: cytokeratin. SMA: smooth muscle actin.

3. Results

3.1. Relationships between clinical variables and tumour components

The distribution of cases according to clinical variables is summarised in Table 1. Supplementary Table S1 summarises the statistical relationships evidenced for the complete series between clinical variables and tumour components. The most significant associations ($p < 0.01$) are as follows.

An increase in tumour grade was associated with increases in the percentages of tumoural cells, intratumoural lymphocytes and TILs, as well as a decrease in the stroma percentage. The cases with high mitotic index were associated with increases in the percentages of tumoural cells, intra- and peritumoural lymphocytes, as well as a decrease in the stroma percentage. In addition, we observed positive correlations between the Ki67 index and the percentages of tumoural cells, intra- and peritumoural lymphocytes and TILs, as well as a negative correlation with the stroma percentage.

The cases expressing ER and/or PR showed higher stroma and SMA percentages, and lower intra- and peritumoural lymphocyte percentages.

For the cases associated with carcinoma in situ (CIS), we observed higher stroma and SMA percentages and lower peritumoural lymphocyte percentages.

For TSR only, the cases with high mitotic index were associated with higher TSR, while cases associated with CIS were associated with lower TSR. There was also a positive correlation between the Ki67 index and TSR.

The same analysis was also conducted on the invasive breast cancer of no special type (IBC-NST) only, with results quite similar to those obtained with the complete series (see Supplementary Table S2). The most notable differences are the lack of significant

Table 3
Comparison of the tumour component characteristics between histological subtypes.

	IBC-NST (n = 120)		ILC (n = 11)		BCMF (n = 14)		p-value[a]
	Median (IQ)		Median (IQ)		Median (IQ)		
TumCellHE	40	(25–50)	25	(15–35)	50	(25–65)	0.0326
TumCellK	40	(25–48)	30	(15–38)	46.5	(25–63)	0.0617
StromaHE	56.5	(42.5–70.5)	70	(60–82)	11.5	(5–20)	$<10^{-5}$
StromaSMA	22	(10–33)	15	(10–31)	4	(1–12)	0.0003
Intra_Ly	3	(1–5)	2	(2–4)	38.5	(25–50)	$<10^{-5}$
Peri_Ly	8	(2–39)	2	(2–4)	94	(85–98)	$<10^{-5}$
TILs	3.5	(2–8)	3	(1–5)	77.5	(57–82)	$<10^{-5}$
	(n = 118)		**(n = 9)**		**(n = 14)**		
TSR	15	(8–30)	20	(5–40)	70	(40–90)	0.0002

Abbreviations: IBC-NST: invasive breast cancer of no special type. ILC: invasive lobular carcinoma. BCMF: invasive breast cancer of no special type with medullary features. IQ: interquartile. TumCellHE: percentage of tumour cells in the tumour area evaluated on the HE slide. TumCellCK: percentage of cells expressing cytokeratin AE1AE3 in the tumour area. StromaHE: percentage of stroma in the tumour area evaluated on the HE slide. StromaSMA: percentage of SMA-positive stroma in the tumour area. Intra_Ly: percentage of lymphocytes located within the tumour area evaluated on the HE slide. Peri_Ly: percentage of lymphocytes in the peritumoural area evaluated on the HE slide. TILs: tumour-infiltrating lymphocytes. TSR: tumour stroma ratio.

[a] The p-values result from Kruskal-Wallis tests.

variations concerning TSR in general and CIS and PR status.

3.2. Variations in tumour components between the histological subtypes

The tumour component characteristics of the three most frequent histological subtypes in our series are compared in Table 3. All these characteristics varied highly significantly ($p < 0.001$) between these subtypes, except for the tumoural cell percentages.

As detailed in Fig. 2A to C, we observed the highest stroma percentage in ILC, followed by IBC-NST and invasive breast cancer of no

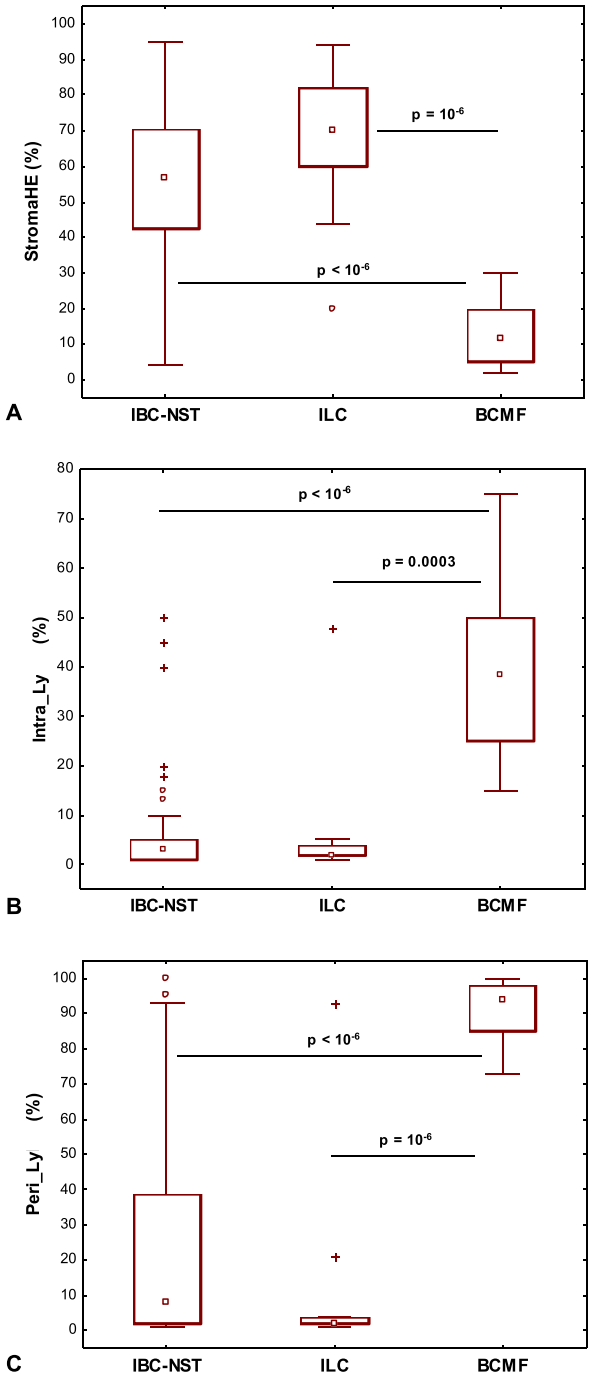

Fig. 2. Box plots of the significant distribution of different tumour components according to histological subtypes. (A) Stroma, (B) intratumoural and (C) peritumoural lymphocyte percentages. The small square is the median, the box locates the interquartile range, the whiskers indicate the non-outlier minimum and maximum, and single dots and crosses show outliers. The (significant) p-values result from Kruskal-Wallis post-hoc tests.

special type with medullary features (BCMF), and the highest intra- and peritumoural lymphocyte percentages in BCMF, followed by IBC-NST and ILC. TILs showed the same trend as intratumoural lymphocytes (see Table 3). The SMA percentage was higher in IBC-NST, followed by ILC and BCMF (see Fig. 3).

3.3. Relationships between the stroma and lymphocyte components

In the complete series, there was a negative correlation between the stroma and intratumoural lymphocyte percentages ($r_s = -0.54$; $p < 10^{-7}$, see Fig. 4A). A more than 40 % stroma was strongly associated with intratumoural lymphocyte percentage less than or equal to 10 % (Fisher test: $p < 10^{-5}$). There were also similar negative correlations between stroma and TILs ($r_s = -0.62$; $p < 10^{-7}$), with a strong association between a stroma of more than 40 % and a TILs less than or equal to 10 % (Fisher test: $p < 10^{-5}$). However, in any case, a stroma below 40 % was not associated with a specific range of intratumoural lymphocyte percentage or TILs.

There were also significant negative correlations between the SMA percentage and either the intratumoural lymphocyte percentage or TILs ($-0.25 \leq r_s \leq -0.23$; $0.0020 \leq p \leq 0.0045$).

These inversely proportional relationships can be seen in the three main histological subtypes in Supplementary Figs. S2–S5.

When limited to IBC-NST alone, significant negative correlations were also observed between the stroma percentage and either the intratumoural lymphocyte percentage ($r_s = -0.42$; $p < 10^{-5}$) or TILs ($r_s = -0.51$; $p < 10^{-7}$), but without finding significant correlations involving the SMA percentage.

In the complete series, there was also a negative correlation between the stroma and peritumoural lymphocyte percentages ($r_s = -0.64$; $p < 10^{-7}$, see Fig. 4B). We observed that a stroma of more than 40 % was strongly associated with a peritumoural lymphocyte percentage of less than 50 %, and conversely (Fisher test: $p < 10^{-6}$), with a different distribution according to the histological subtype (see Fig. 4B). While the BCMF showed a stroma lower than 40 % combined with a peritumoural lymphocyte percentage higher than 70 %, the ILC had a stroma higher than 40 % combined with a peritumoural lymphocyte percentage lower than 25 %. There was no significant relationship between SMA percentage and peritumoural lymphocyte percentage.

For the IBC-NST group only, we observed similar negative correlations between the stroma and peritumoural lymphocyte percentages ($r_s = -0.54$; $p < 10^{-7}$), but without finding significant correlations involving the SMA percentage.

We observed positive correlations between TSR and the different lymphocyte components. However, compared to the above results, these correlations remained relatively weak ($0.29 < r_s < 0.46$ for the complete series and $0.20 < r_s < 0.39$ for the IBC-NST) and TSR showed no informative threshold value.

3.4. Survival analysis

Out of 158 cases, only 22 patients had a recurrence. All of these recurrences were from IBC-NST cases, which also included a large majority of ER + cases with better prognosis in terms of RFS ($p = 0.002$, see Supplementary Fig. S6). We therefore focused the survival

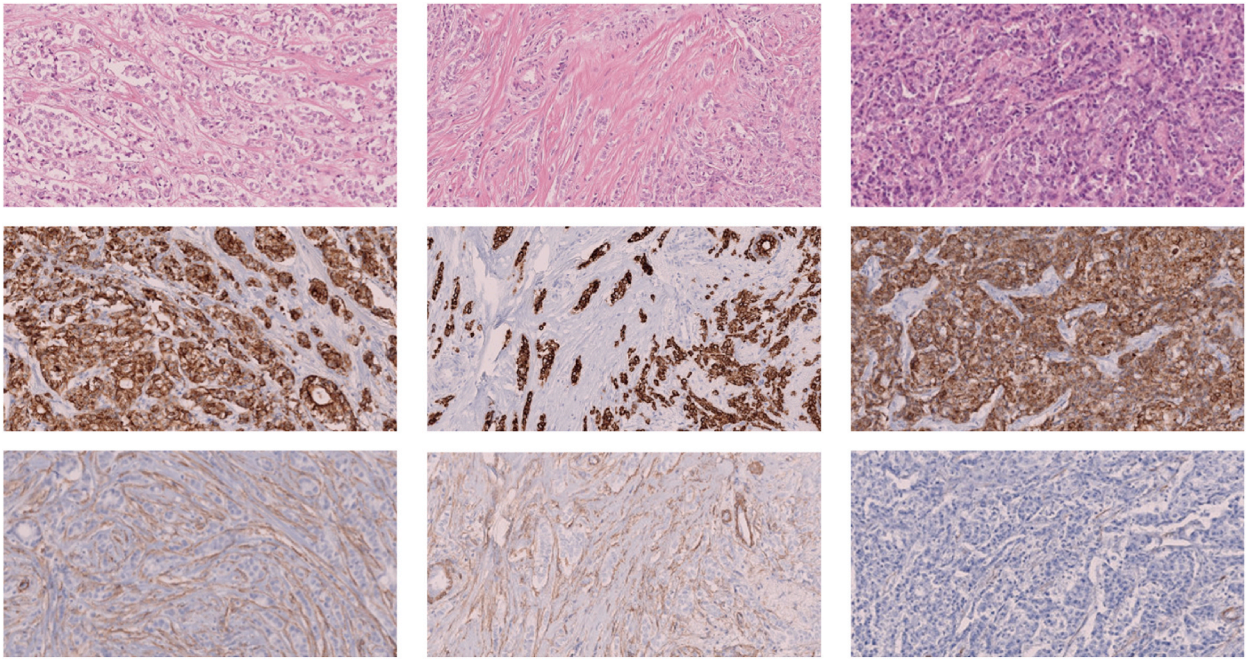

Fig. 3. Overview of the three histological subtypes with their respective immunohistochemistry. First column: IBC-NST; second column: ILC; third column: BCMF. First line: HE; second line: CK AE1AE3; third line: SMA. (×5).

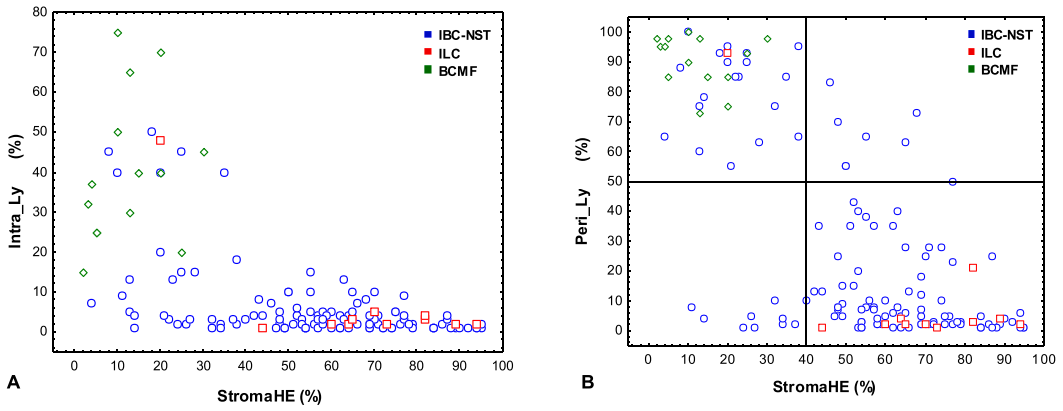

Fig. 4. Distribution of intra- and peritumoural lymphocytes according to StromaHE **A.** Distribution of Intra_Ly according to StromaHE. The histological subtypes are identified by means of different symbols. A StromaHE of more than 40 % is associated with Intra_Ly less than or equal to 10 %. **B.** Distribution of Peri_Ly according to StromaHE. The histological subtypes are identified by means of different symbols. A StromaHE of more than 40 % is associated with Peri_Ly of less than 50 %, and conversely. There is also a different distribution according to the histological subtype. Concentration of ILC in the lower-right corner of the graph, and BCMF in the upper-left corner of the graph.

analyses on this ER + IBC-NST subgroup.

Monovariate RFS analyses highlighted TSR as a positive prognostic factor, with an optimal threshold of 10 % (see Fig. 5), whereas the other tumour characteristics had no potential prognostic values ($p > 0.1$). To note that age was the only clinical variable with a significant impact ($p = 0.003$).

Table 4 shows the multivariate RFS model combining age and TSR>10 %.

4. Discussion

In this study we focused on the relation between the tumoural components (epithelium and stroma) and the lymphocytic infiltrate in and at the periphery of the tumour in different histological subtypes. We decided in this study to assess lymphocytes in a more global way, the aim being to relate the percentage of lymphocytes to the percentage of stroma. For intratumoural lymphocytes, we considered those located in tumoural stroma and in tumoural nests. For a better comparison with the other studies, we also evaluated the TILs according to Salgado's guideline 2014 [16], which only analysed stromal TILs. We did not evaluate TILs interacting with carcinoma cells separately, like in Hendry's guideline 2017, given the absence of any clear additional prognostic value in comparison to stromal TILs [17].

Only a few studies investigated possible relationships between stroma and lymphocytes. Gujam et al. (for TNBC) and Lee et al. (histological subtype unspecified) showed that a tumour with a lot of stroma contains fewer TILs, notably less than 10 % for Millar et al. [4,19,26]. Olsson et al. showed that aggressive TNBC are characterised by less stroma and at least 1 % of lymphocytes [3]. In line with these data, we found a negative correlation between stroma and intratumoural lymphocyte percentages, and showed that a percentage of stroma of more than 40 % was associated with an intratumoural lymphocyte percentage less than or equal to 10 %. This result could

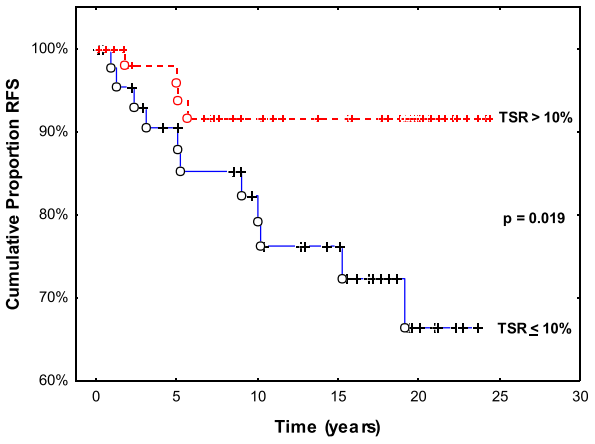

Fig. 5. Relapse-free survival according to the TSR values for the ER + IBC-NST. TSR is a positive prognostic factor with an optimal threshold of 10 %.

Table 4
Multivariate analysis combining age and TSR>10 % on the ER + IBC-NST cases.

n = 104	Model p-value = 0.0037		
	HR	p-value	Confidence interval
Age	1.0503	0.0166	(1.0090, 1.0933)
TSR>10 %	0.3429	0.0714	(0.1071, 1.0976)

Abbreviations: TSR: tumor stroma ratio. ER+: estrogen receptor positive. IBC-NST: invasive breast cancer of no special type.

support our hypothesis that the stroma acts as a physical barrier to lymphocyte penetration into the tumor. We also observed that the relationship was valid regardless of the histological subtype. Nevertheless, this hypothesis could be demonstrated by further fundamental studies (e.g., using mouse models).

We also observed a (weaker) negative correlation between the percentage of myofibroblastic stromal reaction (highlighted by SMA in our study), on the one hand, and the intratumoural lymphocyte percentage or TILs, on the other. The stromal cells expressing SMA are considered as a subtype of cancer-associated fibroblasts (CAFs). The literature shows an important effect of the tumour stroma on proliferation, progression and invasiveness of breast cancer [25,27,28]. Indeed, CAFs are implicated in the secretion of regulatory factors reducing apoptosis, promoting angiogenesis and remodelling the extracellular matrix [20,29,30]. Catteau et al. showed that high grade breast tumours were associated with denser myofibroblastic tumour stroma [25]. Wu et al. also found that stromal perivascular-like-cells were associated with exclusion of lymphocytes [31], which can be related to the inverse correlation we highlighted between intratumoural lymphocyte and stroma percentages. As the stroma appears to act as a barrier to lymphocytes, it may inhibit an adequate immune response; therefore, CAFs expressing SMA could be interesting therapeutic targets. Some molecules with such targets are promising [30] and would perhaps weaken the stromal barrier, allowing lymphocytes to infiltrate the tumour.

To the best of our knowledge, we are the first to show a strong relationship between peritumoural lymphocyte and stroma percentages. We showed a negative correlation between them for both the complete series and the IBC-NST group. For this latter, more than 40 % of stroma was strongly associated with less than 50 % of peritumoural lymphocytes, and conversely. Interestingly, the ILC cases consistently showed a stroma percentage higher than 40 % and a peritumoural lymphocyte percentage lower than 25 %, whereas the BCMF cases were characterised by less than 40 % of stroma and at least 70 % of peritumoural lymphocytes. These results suggest that the stroma might also influence the peritumoural space. The comparison with the literature is difficult because few studies investigate peritumoural lymphocytes, or do not analyse them in relation to clinical data. However, our results are in line with those of Zhang et al. [32], even though they studied the peritumoural lymphocytes in the form of tertiary lymphoid structure. Interestingly, we demonstrated that the peritumoural lymphocyte percentage was positively correlated with the Ki67 percentage, and that lower peritumoural lymphocyte percentages were associated with ER+ and PR + statutes. We further showed that higher peritumoural lymphocyte percentages were associated with higher tumour grades and higher amounts of mitoses.

Concerning relations between the lymphocyte percentage and other clinical and morphological criteria, we found that tumour grade and the Ki67 index were positively correlated with the intratumoural lymphocyte percentages. We also showed that a higher TILs level was associated with a higher tumour grade, as already established by others [5,7–10].

As shown by Criscitiello et al. [7], higher TILs levels were also associated with higher Ki67 percentages. We found that ER + status was associated with lower intratumoural lymphocyte percentages (but not with lower TILs levels). We also established new associations between a lower intratumoural lymphocyte percentage and either a lower amount of mitosis or a PR + status. The latter was also associated with lower TILs levels.

Concerning relations between the stroma percentage and other clinical and morphological criteria, we found new associations, such as between a higher stroma percentage and ER + or PR + statutes, and between a lower stroma percentage and a higher number of mitosis. We also established that ILC has the highest stroma percentage, followed by IBC-NST and BCMF, as in literature [3]. On all histological subtypes, a higher stroma percentage was associated with a lower tumour grade, as previously shown [3,19,24]. We also found that a higher stroma percentage was associated with a lower Ki67 percentage, as highlighted by Forsare et al. [24].

Concerning TSR, it had significant associations with CIS, ER expression, mitoses and Ki67. The TSR>10 % tended to be observed in younger patients, as already known [19,26]. Authors identified additional associations between a lower TSR and invaded lymph nodes and HER2+ status [26].

From a prognostic point of view, we focused our analysis on ER + IBC-NST and identified TSR as a positive prognostic factor, with an optimal threshold of 10 %. This result is consistent with the study of Roeke et al. [34] who showed, for TNBC and ER + IBC-NST, that a higher TSR was associated with better OS and RFS. In contrast, few studies focusing on ER + IBC-NST demonstrated the opposite [19,23,24]. It should be noted that different studies involving TNBC showed that a lower TSR was associated with a poor OS and RFS [21,26,35]. In particular, Vangangelt et al. showed that the combination of a lower TSR and few lymphocytes is associated with worse prognosis [35]. Millar et al. also showed that high TSR/high TILs provide better prognosis that low TSR/low TILs [19]. Our results agree with both, but indirectly via the positive correlation between TSR and lymphocyte components. These data could be explained by our hypothesis regarding the role of the stroma as a physical barrier, blocking the infiltration of lymphocytes inside the tumour and thus preventing an optimal anti-tumour response. This hypothesis should still be verified for other histological subtypes. In summary, although our prognostic study focuses on ER + IBC-NST, we obtained results in line with the current literature on TNBC.

One of the study's strengths is the choice to assess the different components on whole tumour sections, which contrasts with other articles on this topic that are often based on TMAs. Our approach allowed a more complete spatial analysis of tumour and peritumoural

components and the relation between them. Indeed, Olsson et al. [3] report that TMA taken from the centre of the tumour could reduce the percentage of lymphocytes effectively present. In addition, Reznitsky et al. 's study [33] show an unacceptable inter-observer disagreement between pathologists evaluating TILs in the same breast cancer TMA, and an unacceptable disagreement between the evaluations carried out on a TMA and on the matched full section. The two other strengths are the study of the relationship between the stroma and lymphocytes, not only intratumoural but also peritumoural.

However, these data need confirmation in larger cohorts of patients in a multicenter prospective study, including a wider range of histological subtypes and tumor sizes, and must also be validated by experimental models.

5. Conclusions

To the best of our knowledge, this study is one of the first to show, on full-section tumours, an inverse correlation between tumoural stroma percentages and intra- and peritumoural lymphocyte percentages, which supports the hypothesis that the stroma could prevent the recruitment of lymphocytes around and within the tumour. This fact would contribute to a worse prognosis. Larger clinical and experimental studies on mechanisms of stromal change are needed and may potentially lead to novel treatment strategies.

CRediT authorship contribution statement

Camille Verocq: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jean-Christophe Noël:** Resources, Investigation. **Manon Charry:** Resources. **Egor Zindy:** Software, Data curation. **Sandrine Rorive:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Isabelle Salmon:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Christine Decaestecker:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xavier Catteau:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

Ethics

Patients at admission to Erasme Hospital received the information that the patient's surplus biological material can be used for research, unless they don't consent (opt out). The document has been established by the local Ethics Committee of Erasme Hospital and is in accordance with Belgian and International law (Helsinki declaration). The present study was approved by the Ethics Committee of Erasme Hospital (Brussels, Belgium; approval no. SRB2022089 - P2022/213).

Data and code availability

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40295.

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