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Original Research

Tumour-infiltrating lymphocytes add prognostic information for patients with low-risk DCIS: findings from the SweDCIS randomised radiotherapy trial

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Abstract ***Background:*** The immune microenvironment is an important modulator of tumour progression and treatment response. In invasive breast cancer, assessment of tumour-infiltrating lymphocytes (TILs) provides prognostic and predictive information. However, the clinical impact of TILs for ductal carcinoma *in situ* (DCIS) has not yet been demonstrated.
Patients and methods: *Post hoc* analysis of the SweDCIS randomised radiotherapy trial including primary DCIS cases following breast-conserving surgery. TILs were assessed on haematoxylin-eosin sections (n = 711) according to the International Immuno-Oncology Biomarker Working Group guidelines. TILs-scores were analysed as continuous and dichotomised ($\leq$5% versus >5%) variable regarding ipsilateral breast events (IBEs) as the predefined primary endpoint.
Results: Most women (61.9%) showed a TILs prevalence of $\leq$5%. High TILs-scores were associated with larger lesion size, human epidermal growth factor receptor 2 (HER2)-positivity, higher nuclear grade, and KI67-score. DCIS cases with high TILs prevalence had a significant increased cumulative IBE incidence at five years post-surgery ($TILs^{low}$-versus $TILs^{high}$ 9% versus 18%; $p < 0.001$). Among patients with HER2-negative DCIS, high TILs remained

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an independent poor prognosis marker for IBE risk in multivariable analysis with an adjusted hazard ratio of 2.41 [95%CI 1.17–4.95, p = 0.017]. Including TILs-status provided a refined stratification of patients with general low-risk DCIS (grade <3, size <25 mm, free margin). No interaction between TILs and radiotherapy benefits was detected.

Conclusion: High TILs are associated with higher IBE risk over 5-years post-surgery, particularly for HER2-negative DCIS. Our data indicate that TILs should be integrated into the clinical workup to define patients with low-risk DCIS who can omit adjuvant therapy or patients with potential benefits from immunotherapy.

1. Introduction

The incidence of ductal carcinoma *in situ* (DCIS), the precursor lesion of invasive breast cancer, has considerably increased with the introduction of mammography [1]. The majority of women with DCIS receive similar standard treatment as for early-stage invasive breast cancer with breast-conserving surgery (BCS) followed by adjuvant radiotherapy (RT) [2]. The recurrence rate of DCIS at 10 years after surgery is up to 20%, while breast cancer-specific deaths (BCSD) remain very few, with barely 3% [3]. Although studies demonstrated a 50% relative risk reduction of ipsilateral recurrences by RT at 10 years, no overall survival benefit has yet been proven, implying a possible overtreatment [4]. Clinical trials or guidelines often apply a combination of tumour size, nuclear grade and adequate surgical margins to stratify patients in low- or high-risk groups and assign RT accordingly. However, there are still no generally accepted criteria in clinical routine [5]. Thus, the identification of prognostic or treatment predictive biomarkers in DCIS is of urgent clinical need [6]. According to a meta-analysis of randomised trials, RT was effective in DCIS regardless of traditional clinicopathologic parameters [2]. Thus, it could be assumed that the underlying tumour biology had a greater effect on radiosensitivity and should be included in efforts to build predictive models.

The transition from DCIS to invasive cancer is characterised by disassembly of the myoepithelium, an activation of periductal fibroblasts along with extracellular matrix remodelling as well as a compositional and spatial reorganisation of the immune microenvironment [7–10]. Anti-tumour immunity and tumour-promoting inflammation or immunosuppression coincide along tumour progression, while microenvironmental factors affect their balance [11]. In invasive breast cancer, tumour-infiltrating lymphocytes (TILs) have recently been proven as a biomarker of both prognostic and treatment-predictive value for patients with human epidermal growth factor receptor 2 (HER2)-positive or triple-negative disease (TNBC) receiving tailored treatment [12–16]. Furthermore, growing evidence from different invasive cancer types suggests that RT benefit is the consequence of favourable immune modulations [17–19] and that RT efficiency, also in the adjuvant setting, might depend on the immune-status of the primary tumour [13,20].

The clinical relevance of TILs in DCIS, particularly in the context of RT, is, however, still largely undefined, and therefore, the purpose of the presented study was to analyse the prognostic and RT-response predictive potential of TILs in DCIS, following assessment guidelines from the International Immuno-Oncology Biomarker Working group. The adaptation of these guidelines in DCIS is still under investigation and their clinical relevance has not yet been demonstrated [21].

2. Materials and methods

2.1. Patients

The study cohort is based on the national Swedish randomised DCIS trial (SweDCIS), including 1046 women with primary DCIS diagnosed 1987–1999 [22]. All patients underwent BCS before random assignment to postoperative RT or control. Endocrine therapy was applied in about 3% of cases; the tumour-clear surgical margin was achieved in >80%. The SweDCIS trial and secondary analyses were approved by the Umeå University ethics committee (Fek1987-05-05§2, Dnr05:065M, Dnr2012-224-32M, Dnr2014-230-32M) and conducted in accordance with the Declaration of Helsinki.

FFPE tissue blocks were retrieved from 897 patients. An independent pathologist reevaluated H/E-sections and 186 cases were excluded due to the absence of DCIS foci, resulting in a final of 711 included cases (Fig. 1), with a median follow-up of 17.4 years.

2.2. Assessment of TILs and clinico-pathological characteristics

H/E-slides were scanned at 40×-magnification on the Hamamatsu Nanozoomer S60 (Hamamatsu, Hamamatsu City, Japan). Scoring was performed blinded by two independent raters (AS, VT). TILs were assessed as predefined single markers following published guidelines for DCIS from the International Immuno-Oncology Biomarker Working group [21] (Supplementary

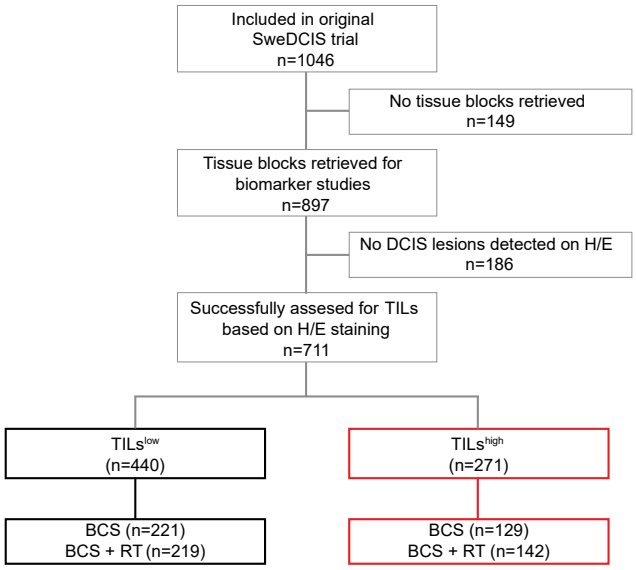

Fig. 1. CONSORT diagram. DCIS ductal carcinoma *in situ*; H/E haematoxylin/eosin; TILs tumour infiltrating lymphocytes; RT radiotherapy; BCS breast conserving surgery.

Material and Supplementary Fig. 1). TILs-scores were analysed as continuous as well as binary variables based on median dichotomisation into TILs^{low}(≤5%)- and TILs^{high}(>5%)-groups. This predefined median cut-off was applied as no validated cut-off for TILs is described for DCIS. This strategy was adapted from recent TILs studies in DCIS, also reporting a median TILs-score of 5% which was applied as cut-off [23–25].

For the assessment of additional clinico-pathological characteristics see Supplementary Material.

2.3. Statistics

The predefined primary endpoint of this retrospective study was ipsilateral breast events (IBEs) while treating the secondary endpoints distant metastasis, BCSD and any death as competing risks, and ignoring contralateral breast events.

R-Studio Version 1.0.143 was used for statistical analyses. Cumulative incidence functions, including competing risks, were generated with the *cmprsk* package, including Gray's test. Cause-specific hazard ratios (HRs) were calculated by univariable and/or multivariable Cox proportional hazards regression with Efron's method for ties and reported p-values are based on Wald test (*survival* package). The proportional hazards assumption was analysed using the Schoenfeld Residuals Test revealing a violation ($p < 0.05$) for the association of TILs with IBE. To model the estimated IBE hazard in dependency of TILs-status over time, a flexible parametric survival model (stpm2 function, *Rstpm2* package) was used. See Supplementary Material for more detailed information on the statistical analyses.

Contingency tables were evaluated by Fisher's exact test. All given 95% confidence intervals (CIs) and statistical tests were two-sided. P-values <0.05 were considered statistically significant.

3. Results

3.1. Associations between TILs and clinico–pathological factors

H/E-sections of 711 patients were successfully evaluated for TILs, representing 68% of the original SweDCIS cohort (Fig. 1). The clinico–pathological characteristics of the study cohort are summarised in Table 1; no significant differences were detected compared to the original SweDCIS cohort (Supplementary Table 1).

TILs were assessed by two independent raters; the intraclass correlation coefficient of 0.81 (95%CI 0.78–0.84) indicated an excellent agreement. The TILs-scores showed a distribution from minimum <1% to maximum 60% (Supplementary Fig. 2). Patients were dichotomised into TILs^{low} (n = 440; 61.9%) and TILs^{high} (n = 271; 38.1%) using ≤5% as predefined cut-off.

A statistically significant positive association was noted between the TILs^{high}-group and larger DCIS foci ($p = 0.023$ Fisher's exact test), high nuclear grade ($p < 0.001$), high KI67-index ($p < 0.001$), screen detection ($p = 0.010$) and HER2-positivity ($p < 0.001$), as well as PR-negativity ($p < 0.001$) (Table 1). No associations were detected for TILs-status and RT or endocrine therapy.

3.2. Prognostic impact of TILs

An increasing percentage of TILs was associated with an increased cumulative incidence of IBE (*in situ* and invasive), as the predefined primary endpoint (Supplementary Fig. 3 left panel). Analysis of the time-depended effect of the continuous TILs-score on IBE demonstrated that in univariable Cox regression the unfavourable prognosis of the patients with higher TILs infiltration was restricted to the first five years post BCS (HR_{5years} per 10% unit increments 1.29 95%CI [1.11–1.49], $p = 0.001$ Wald test; Supplementary Fig. 3 right panel and Table 2). However, the association between TILs and IBE risk did not remain significant in the multivariable analysis, including age, DCIS size, RT, detection modus, nuclear grade, KI67 and PR as confounder variables (HR_{5years} per 10% unit increments 1.13 [0.92–1.38], $p = 0.242$) (Table 2).

The dichotomisation of the patients into TILs^{low}- and TILs^{high}-groups preserved the statistically significant increased cumulative IBE incidence for patients with TILs^{high} (at 5-year 0.09 95%CI[0.06–0.12] for TILs^{low}- versus 0.18 [0.14–0.23] for TILs^{high}-group, $p < 0.001$ Gray's test) (Fig. 2A left panel), as well as the increased relative IBE risk in univariable analysis (HR 2.18 95%CI [1.44–3.31], $p < 0.001$ Wald test; Fig. 2B

Table 1
Clinico-pathological cohort characteristics and association with TILs status.

N (%)	Whole cohort	Low TILs	High TILs	p-value[g]
Total number of patients	711 (100)	440 (61.9)	271 (38.1)	
Age, years:				
<50	170 (23.9)	107 (24.3)	63 (23.2)	0.226
50–60	282 (39.7)	164 (37.3)	118 (43.5)	
>60	259 (36.4)	169 (38.4)	90 (33.3)	
Size[a], mm:				
≤15	455 (69.7)	296 (72.9)	159 (64.4)	**0.023**
>15	198 (30.3)	110 (27.1)	88 (35.6)	
Focality[a]:				
unifocal	587 (89.9)	371 (91.6)	216 (87.1)	0.081
multifocal/multicentric	66 (10.1)	34 (8.4)	32 (12.9)	
Margin status[b]:				
clear	578 (86.7)	363 (87.5)	215 (85.3)	0.481
not clear	89 (13.3)	52 (12.5)	37 (14.7)	
Laterality:				
left	365 (51.3)	237 (53.9)	128 (47.2)	0.090
right	346 (48.7)	203 (46.1)	143 (52.8)	
Screen detected[c]:				
screen	562 (79.4)	335 (76.3)	227 (84.4)	**0.010**
clinical	146 (20.6)	104 (23.7)	42 (15.6)	
Diagnosis year:				
≤1995	492 (69.2)	294 (66.8)	198 (73.1)	0.094
>1995	219 (30.8)	146 (33.2)	73 (26.9)	
Radiotherapy:				
yes	361 (50.8)	219 (49.8)	142 (52.4)	0.537
no	350 (49.2)	221 (50.2)	129 (47.6)	
Endocrine therapy:				
yes	22 (3.1)	15 (3.4)	7 (2.6)	0.658
no	689 (96.9)	425 (96.6)	264 (97.4)	
HER2[d]:				
0–2	488 (69.2)	382 (87.4)	106 (39.6)	**<0.001**
3	217 (30.8)	55 (12.6)	162 (60.4)	
Nuclear grade:				
1	218 (30.7)	194 (44.1)	24 (8.9)	**<0.001**
2	222 (31.2)	159 (36.1)	63 (23.2)	
3	271 (38.1)	87 (19.8)	184 (67.9)	
Ki67[e] %:				
<20	476 (68.6)	354 (82.3)	122 (46.2)	**<0.001**
≥20	218 (31.4)	76 (17.7)	142 (53.8)	
PR[f] %:				
<10	267 (38.3)	103 (23.8)	164 (61.9)	**<0.001**
≥10	431 (61.7)	330 (76.2)	101 (38.1)	
Endpoint	**N [5/10/20 years]**	**N [5/10/20 years]**	**N [5/10/20 years]**	-
IBE	90/141/171	40/79/98	50/62/73	-
in situ	69/85/91	33/45/49	36/40/42	
invasive	21/56/80	7/34/49	14/22/31	
CBE	23/45/82	14/29/48	9/16/34	
distant metastasis	6/15/23	1/5/9	5/10/14	
BCSD	3/13/31	0/3/13	3/10/18	
other death	14/44/129	6/24/81	8/20/48	

Significant p-values are indicated in bold.
TILs tumour infiltrating lymphocytes; HER2 human epidermal growth factor receptor 2; PR progesterone receptor; IBE ipsilateral breast event; CBE contralateral breast event; BCSD breast cancer specific death.
[a] Data missing for 58 patients.
[b] Data missing for 44 patients.
[c] Data missing for 3 patients.
[d] Data missing for 6 patients.
[e] Data missing for 17 patients.
[f] Data missing for 13 patients.
[g] Fisher's exact test for comparison of the TILs low/TILs high groups (2-sided).

Table 2
HR for ipsilateral breast events (*in situ* and invasive) within 5 years post breast-conserving surgery.

	Whole cohort		HER2-negative		HER2-positive	
Univariable analysis	**HR (95% CI); N=711**	**p-value***	**HR (95% CI); N=488**	**p-value***	**HR (95% CI); N=217**	**p-value***
TILs (per 10% increase)	1.29 (1.11–1.49)	**0.001**	1.52 (1.10–2.11)	**0.012**	1.02 (0.83–1.25)	0.873
Multivariable analysis	**HR (95% CI); N=624**	**p-value***	**HR (95% CI); N=434**	**p-value***	**HR (95% CI); N=188**	**p-value***
TILs (per 10% increase)	1.13 (0.92–1.38)	0.242	1.64 (1.13–2.39)	**0.010**	0.92 (0.72–1.19)	0.530
RT (no/yes)	0.32 (0.19–0.53)	**<0.001**	0.27 (0.12–0.59)	**0.001**	0.37 (0.19–0.73)	**0.004**
Age (per year increase)	0.99 (0.96–1.01)	0.269	0.99 (0.95–1.03)	0.580	0.98 (0.95–1.02)	0.323
Size (per mm increase)	1.02 (1.01–1.04)	**0.006**	1.04 (1.01–1.07)	**0.011**	1.02 (0.99–1.04)	0.108
Screen detected (screen/clinical)	1.49 (0.86–2.58)	0.155	1.31 (0.60–2.88)	0.497	2.19 (0.99–4.83)	0.052
Nuclear grade (1 as reference)	1	–	1	–	1	–
2	2.09 (1.04–4.22)	**0.040**	2.33 (1.06–5.15)	**0.036**	0.82 (0.16–4.24)	0.813
3	2.26 (1.05–4.85)	**0.036**	1.25 (0.42–3.70)	0.684	0.98 (0.23–4.23)	0.973
KI67 (<20%/≥20%)	1.37 (0.81–2.31)	0.236	1.19 (0.50–2.83)	0.701	1.03 (0.52–2.04)	0.941
PR (negative/positive)	1.09 (0.63–1.89)	0.748	2.03 (0.79–5.20)	0.141	1.08 (0.49–2.36)	0.853

*p-value is based on Wald test; HR is based on cause specific Cox regression model.
Significant p-values are indicated in bold.
TILs tumour infiltrating lymphocytes; HR hazard ratio; CI confidence interval; PR progesterone receptor; HER2 human epidermal growth factor receptor 2.

left panel). The association remained statistically significant in multivariable analysis (HR 1.86 [1.08–3.20], p = 0.024) (Supplementary Table 2).

Analyses for the impact of TILs on *in situ* and invasive IBE risk separately, indicated comparable prognostic effects for both IBE types (Supplementary Fig. 4).

Stratification of the patients into HER2-defined subgroups revealed that the prognostic effect of TILs was limited to HER2-negative DCIS. A statistically significant association of higher TILs-scores with increase in 5-year relative IBE risk was noted in both univariable and multivariable analysis (uni: HR per 10% unit increment 1.52 [1.10–2.11], p = 0.012; multi: 1.64 [1.13–2.39], p = 0.010) (Table 2). In accordance, comparison of the dichotomised TILs-defined patient groups indicated an increased 5-year cumulative IBE incidence (0.07 95%CI [0.05–0.11] $TILs^{low}$- versus 0.15 [0.09–0.23] $TILs^{high}$-group, p = 0.019) and relative IBE risk (uni: HR 2.09 [1.14–3.85], p = 0.017 Wald test; multi: 2.41 [1.17–4.95], p = 0.017) for the $TILs^{high}$-group (Fig. 2A and B, middle panels; Supplementary Table 2). Further subdivision of the patients with HER2-negative DCIS into PR-positive and PR-negative showed that the association of TILs with higher IBE risk was predominant among the patients with PR-positive DCIS (continuous TILs-score p = 0.010 and TIL^{high} versus $TILs^{low}$ p = 0.036, Wald test) (Supplementary Fig. 5).

For the patients with HER2-positive DCIS, neither the continuous (Table 2) nor dichotomised TILs-variable (Fig. 2A and B, right panels; Supplementary Table 2) was associated with IBE incidence and risk.

No association between TILs and contralateral breast events, distant metastasis, BCSD or any death was observed at 20 years post-surgery (Supplementary Table 3).

3.3. Combination of TILs-status and DCIS risk classification

Inclusion of the TILs-status refined commonly applied risk stratification strategies for DCIS. Among patients with low-risk DCIS (nuclear grade <3 and DCIS size <25 mm and tumour free margin), a high TILs-status was associated with a statistically significant increased 5-year cumulative IBE incidence (Fig. 3A) and relative IBE risk compared to low TILs-status (HR 2.84 95%CI [1.23–6.56], p = 0.015) (Table 3). As a consequence, patients with low-risk DCIS and a high TILs-status had a similar IBE incidence rate and risk as patients of the general high-risk DCIS group (nuclear grade = 3 and/or DCIS size ≥ 25 mm and/or no tumour free margin) (Fig. 3A and Table 3).

3.4. Association of TILs with RT benefit

Analyses for RT benefit regarding IBE were performed separately in the patient groups with $TILs^{low}$ and $TILs^{high}$-status. Both groups indicated a statistically significant reduction of the 5-year cumulative IBE incidence (Fig. 3B) as well as a relative risk reduction in the RT treatment arm (HR 0.32 95%CI[0.16–0.65], p = 0.002, in $TILs^{low}$-group and 0.31 [0.17–0.57], p < 0.001, in $TILs^{high}$-group). No significant interaction between the continuous TILs-variable and RT was detected in a Cox regression model (5-years p_{uni} = 0.531 and

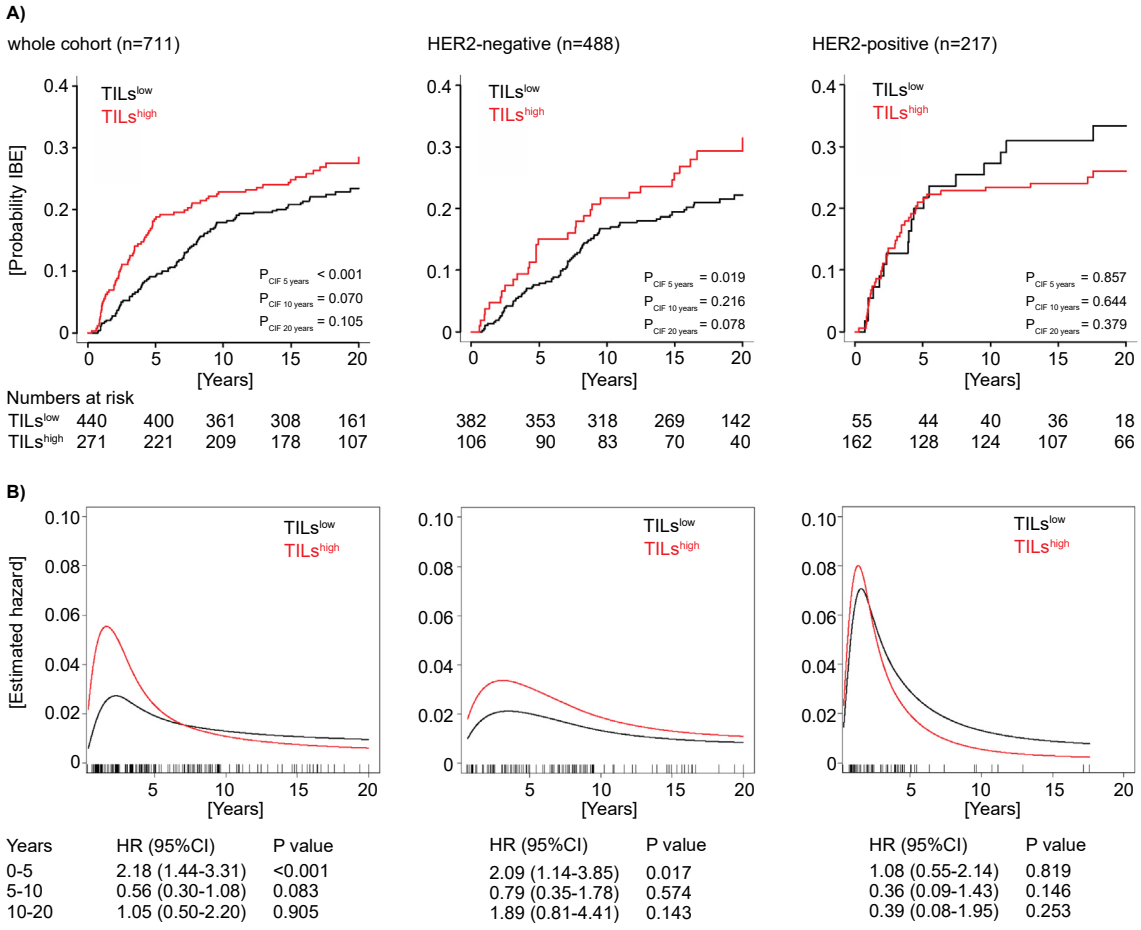

Fig. 2. (**A**) Cumulative incidence of ipsilateral breast events (*in situ* and invasive) after breast-conserving surgery for primary DCIS, in dependence on TILs status in the whole cohort (left panel), the patients with HER2-negative DCIS only (middle panel) and the patients with HER2-positive DCIS only (right panel). Distant metastases, breast-cancer specific death and any death were treated as competing risks. TIL score was dichotomised in the 'low (0–5%)' and 'high (>5%)' group based on the median score of 5%. Gray's test was applied for comparison of the cumulative incidence functions at 5, 10 and 20 years post-surgery. (**B**) Estimated hazard for ipsilateral breast recurrences (*in situ* and invasive) over time after breast-conserving surgery for primary DCIS in dependence of TILs status. Time stratified hazard ratios with low TILs group as reference are indicated for 0–5 years, 5–10 years and 10–20 years and are based on cause-specific Cox regression and p-values on Wald test. DCIS, ductal breast carcinoma *in situ*; TILs, tumour infiltrating lymphocytes; HER2, human epidermal growth factor receptor 2; IBE, ipsilateral breast events; HR, hazard ratio; CI, confidence interval.

$p_{multi} = 0.355$). Further, no interactions were noted for any of the secondary endpoints (Supplementary Table 4).

4. Discussion

The SweDCIS trial, a phase III randomised RT trial with more than 20-year follow-up [22], is one of four trials leading to the recommendation of adjuvant RT for DCIS. The presented study is a *post hoc* analysis of this trial, testing TILs as prognostic and RT predictive biomarkers. To the best of our knowledge, this represents the first randomised DCIS cohort in which the impact of TILs is investigated. We applied a standardised TILs scoring system for DCIS defined by the International Immuno-Oncology Biomarker Working Group, which is a derivative of the TILs evaluation approach for invasive breast cancer [21].

We found that a high prevalence of periductal TILs was significantly associated with an increased 5-year IBE risk after BCS for women with DCIS in univariable analysis. Interestingly, upon stratification by HER2-status, the prognostic effect of TILs with regard to IBE risk was found limited to the HER2-negative DCIS subgroup. We cannot exclude that the prognostic effect of TILs was confounded by an association between TILs and a more aggressive ER-negative phenotype, due to the lack of oestrogen receptor-alpha (ER) data for the study cohort. However, ER expression is described to positively correlate with PR and inversely correlate with nuclear grade and KI67-index [26–28]. When adjusting for these aggressiveness features in multivariable models, the prognostic effect of TILs for patients with HER2-negative DCIS remained statistically significant, suggesting TILs as an independent marker.

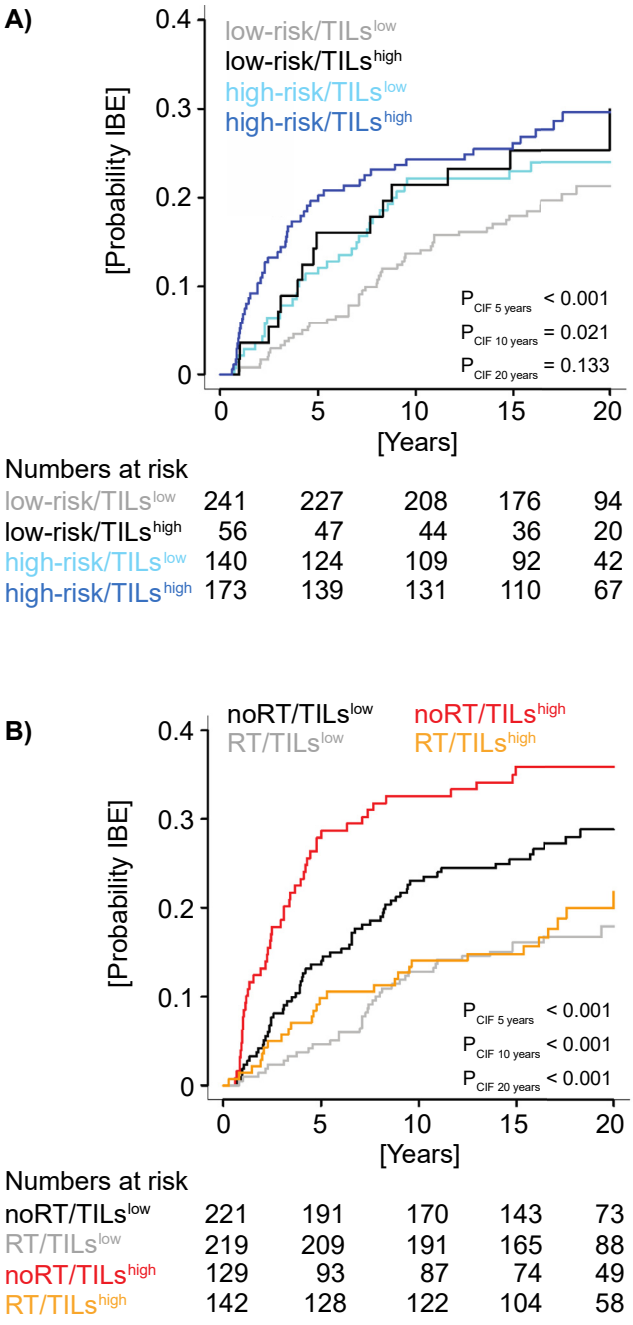

Fig. 3. (**A**) TILs status in combination with DCIS risk group (low-risk group: nuclear grade <3 and lesion size <25 mm and tumour free margin; high-risk group: all other patients). Curves indicate the cumulative incidence of ipsilateral breast events (*in situ* and invasive) in dependence on DCIS risk group and TILs status. The analysis is restricted to patients with complete data. (**B**) Radiotherapy effect in TILs defined patient groups. Curves indicate the cumulative incidence of ipsilateral breast events (*in situ* and invasive) in dependence of radiotherapy and TIL status.
Distant metastases, breast-cancer specific death and any death were treated as competing risks. Gray's test was applied for comparison of the cumulative incidence functions at 5, 10 and 20 years post breast conserving surgery. TILs, tumour infiltrating lymphocytes; RT, radiotherapy; IBE, ipsilateral breast events.

Table 3
Combination of DCIS risk score with TILs status – 5 tears post breast conserving surgery.

	Cox regression – UVA	
	HR (95% CI)	p-value*
low-risk group/TILs^{low}	1 (reference)	–
low-risk group/TILs^{high}	2.84 (1.23–6.56)	0.015
high-risk group/TILs^{low}	2.02 (0.99–4.14)	0.055
high-risk group/TILs^{high}	3.80 (2.04–7.07)	<0.001

*p-value is based on Wald test; HR is based on cause specific Cox regression model (610 patients with complete data included in the regression model).
Low-risk group is defined as nuclear grade <3 and lesion size <25 mm and tumour free margin. High-risk group includes the remaining patients. TILs score was dichotomised in "low (0–5%)" and "high (>5%)" group based on the median score of 5%.
TILs tumour infiltrating lymphocytes; HR hazard ratio; CI confidence interval; UVA univariable analysis.

Previous studies on DCIS have described similar associations between high TILs and more aggressive features, including HER2-positivity; however, analyses on the association between TILs and general IBE risk, or *in situ*/invasive IBE risk separately, were partly inconsistent [25,29–35]. Notably, most studies have evaluated TILs according to self-defined, semi-quantitative density scores or used population-based cohorts, which often do not allow a clear distinction between predictive and prognostic effects.

In invasive breast cancer, high TILs prevalence was likewise associated with the HER2-positive and partly also the TNBC histological subtype [13]. Interestingly, the prognostic impact of TILs in invasive breast cancer appears subtype specific. In HER2-positive and TNBC subtypes, high TILs were associated with a favourable prognosis, while in ER-positive/HER2-negative tumours, low TILs were associated with longer overall survival [13–16,36]. The latter observation is in accordance with our findings in HER2-negative DCIS.

Adverse prognostic effects of TILs within breast cancer subtypes and DCIS might also be caused by an impact of TILs on distinct treatment modalities. Such response-predictive effect of TILs was demonstrated for neoadjuvant chemotherapy in invasive breast cancer [37]. For stage I/II breast cancer, the effect of adjuvant RT was more pronounced in women with low TILs [13]. In our study on DCIS, the RT efficacy was, however, independent of the TILs-status.

While the strength of our study is the analysis of whole tissue sections from a randomised RT trial and the application of defined guidelines, we recognise the explorative study design and the use of PR-status as a proxy for ER data. The lack of ethnical diversity within the SweDCIS study cohort is also noted.

Importantly, our results indicate that TILs provide additional information to better define patients with DCIS of low or high recurrence risk, in a range as the Oncotype DCIS-score risk stratification [38]. This could improve current criteria for the application of adjuvant therapy and should thus be integrated with the clinical work-up. The TILs-score, as performed on H/E-sections and achieving excellent inter-rater reliability in our and other studies, could easily be applied in clinical routine. Nevertheless, it should be noted that a low risk of recurrence does not necessarily translate to a low risk of progression to invasive cancer, should curative surgery be omitted. Further efforts are required to address this relevant question. Hopefully, our work will lead to the acknowledgement of the potential importance of TILs for the risk stratification of DCIS. Presumably, this might stimulate researchers to include TILs assessment in future studies on DCIS; especially in large ongoing trials using molecular signatures such as Oncotype-DCIS-score or DCISionRT (ELISA, PREDICT trial) to define patients with low recurrence risk who could omit adjuvant RT. Assessment could also be included in active surveillance studies such as the COMET and LORD trials, as well as trials where analysis of data is in progress, such as LORIS and NSABP_B-43. If such investigations lead to the validation and refinement of our findings, TILs might become part of the standard pathology report for DCIS.

In the future, the possibility of using MRI to assess TILs represents an interesting prospect of a non-invasive approach [39]. Yet, the distinction between pure DCIS-lesions and invasive cancer by MRI remains a challenge [40].

Although the applied score as recommended by the International Immuno-Oncology Biomarker Working Group is easily applicable and standardised, it does not integrate differences in spatial immune cell patterns. While in some specimens TILs appeared as homogenous infiltration within the stroma, other cases presented with TILs remarkably concentrated around DCIS foci, either touching or as a halo-like structure. It should further be considered that TILs assessment based on H/E is only quantitative and not qualitative. Kim *et al.* [29] found that a higher infiltration of specifically $FOXP3^{+}$-TILs and PD-$L1^{+}$-immune cells in DCIS was associated with recurrence. Further work in this field is required to understand the role of different immune subsets in DCIS and possibly develop appropriate immunomodulatory therapies.

In conclusion, our study showed that TILs act as an independent prognostic marker for IBE risk in patients with HER2-negative DCIS. No association was found between TILs and RT benefits. Follow-up studies are needed to better define TILs-subsets based on immune cell markers. In combination with mechanistic studies, such data could potentially identify novel therapeutical opportunities for example immunotherapies. Finally, and importantly, our data suggest that TILs should be integrated in efforts to define patients with low-risk DCIS that can be spared adjuvant therapy.

Disclaimer

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Data availability

The clinical database of the SweDCIS study including TILs scores is available from the corresponding author on reasonable request.

Author contributions

CS, AS and FW designed the study; FW, PK, EH and TB contributed tissue and data resources; AS, VT, HO and PM contributed to data curation; CS, AS, AST, EH and AL contributed and assisted in data analysis and data presentation; all authors contributed to data interpretation; CS and AS wrote the original draft of the manuscript, all authors provided comments and adjustments for the final manuscript version; all authors approved the submission of the final manuscript.

Conflict of interest statement

CS and PM are co-owners of HistoOneAB. FW received funding for tumour collection by PreludeDX. AST, EH and PK hold research contract with PFS Genomics. CS, AST, EH and PK hold research

contracts with PreludeDx. TB is an employee of and holds ownership interest (including patents) in Prelude Corporation, reports receiving commercial research grants to Prelude Corporation from National Cancer Institute, and is a co-inventor(s) on one or more patents and patent applications licensed to or owned by Prelude Corporation. No potential conflicts of interest are disclosed by the other authors AS, VT, HO and AL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.01.016.

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