

RESEARCH ARTICLE

GATA-3 expression in breast cancer is related to intratumoral M2 macrophage infiltration and tumor differentiation

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Data Availability Statement: The data underlying the results presented in the study are available via the corresponding author, and from the Department of Molecular Medicine and Surgery, Karolinska Institutet (<https://ki.se/en/mmk/departament-of-molecular-medicine-and-surgery>). No information about patient ID will be distributed due to patient confidentiality reasons. Data are available for researchers who meet the criteria for access to confidential data. All data requests must be approved by the Swedish National Ethics

Abstract

Accumulating evidence indicates that tumor-associated macrophages promote tumor progression and that high macrophage infiltration is correlated with advanced tumor stages and poor prognosis in breast cancer. GATA binding protein 3 (GATA-3) is a differentiation marker related to differentiated states in breast cancer. In this study, we explore how the extent of MI relates to GATA-3 expression, hormonal status, and the differentiation grade of breast cancer. To examine breast cancer in early development, we selected 83 patients that were treated with radical breast-conserving surgery (R0), without lymph node metastases (N0) or distant metastases (M0), with and without postoperative radiotherapy. Immunostaining of M2-macrophage-specific antigen CD163 was used to detect tumor-associated macrophages, and macrophage infiltration was estimated semi-quantitatively into no/low, moderate, and high infiltration. The macrophage infiltration was compared to GATA-3, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67 expression in cancer cells. GATA-3 expression is associated with ER and PR expression but inversely correlated to macrophage infiltration and Nottingham histologic grade. High macrophage infiltration in advanced tumor grade was associated with low GATA-3 expression. The disease-free survival is inversely related to Nottingham histologic grade in patients having tumors with no/low macrophage infiltration, a difference that is not found in patients with moderate/high macrophage infiltration. These findings indicate that macrophage infiltration might impact the differentiation, malignant behavior, and prognosis of breast cancer, regardless of the morphological and hormonal states of the cancer cells in the primary tumor.

Introduction

Differentiation is inversely correlated to malignancy and the metastatic potential of solid tumors. Neoplasia with a high degree of differentiation morphologically resembles their native

committee (<https://etikprovningensmyndigheten.se/en/>).

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tissue, whereas those with low differentiation lose their structural organization with reduced cohesiveness. Tumor growth and invasion are complicated multifactorial, but debated mechanisms involving interaction between cancer cells and the host tissue microenvironment [1]. Many solid tumors exhibit phenotypic heterogeneity, consisting of multiclonal tumor cells with different functional states. Besides tumor cells, solid tumors are comprised of stroma cells, such as infiltrating immune cells, which collectively determine the malignant behavior of cancer. Moreover, heterogeneity in cancer cell states and tumor microenvironment can possess drug sensitivities, resulting in challenges for therapeutic and prognostic assessment of solid tumors [1, 2].

Breast cancer (BC) arises from multipotent BC progenitor or stem cells yielding various tumor morphologies [3]. Nottingham histologic grade (NHG) is a scoring system used to assess the morphological differentiation in BC. It is based on tumor growth pattern and degree of differentiation, reflecting the similarities of the BC to normal breast epithelial cells. GATA binding protein 3 (GATA-3) is a transcription factor involved in critical steps of differentiation and morphogenesis of several cell types, including mammary epithelial cells, T-helper cells, and nephritic ductal cells [4]. In mammary tissue, GATA-3 is associated with estrogen receptor (ER) expression in the luminal epithelial cells and is highly expressed in well-differentiated BC [5]. The loss of GATA-3 expression results in decreased ER expression and decreases proportionally the more the BC loses its luminal differentiation. Moreover, higher expression of GATA-3 predicts a better BC prognosis and response for hormonal treatment [6, 7].

Macrophages are a heterogeneous population of innate immune cells originating from blood monocytes. In response to different stimuli in the tissue microenvironment, macrophages exhibit two polarization states, M1 and M2. The M1 macrophages are pro-inflammatory and possess microbicidal/tumoricidal activity. The M2 macrophages have an immunosuppressive phenotype, release anti-inflammatory cytokines, and enhance angiogenesis and tissue repair. Tumor-associated macrophages (TAMs) represent the M2 phenotype and constitute a main component of the microenvironment in several solid tumors, enhancing tumor progression, angiogenesis, and chemoresistance [8–11]. In BC, high macrophage infiltration (MI) is associated with poor prognostic phenotype, such as high histological grade, low ER status, and increased Ki-67 proliferation index [12, 13]. In a previous study, we reported significant differences in MI among the intrinsic subtypes of pathologic pT1-T2 BC. The ER expression by BC cells was inversely related to MI [14].

Understanding BC phenotypic differentiation is essential for histological classification and facilitates accurate disease behaviour, prognosis, and management prediction. In this study, we investigate the expression of GATA-3, as a mammary differentiation marker, in relation to M2 macrophage infiltration and differentiation estimated as NHG in BC.

Materials and methods

Patient material and study design

We retrospectively obtained data on all patients ($n = 1164$) with BC with isolated ipsilateral local recurrence (ILR) during 1983–2008 from the breast cancer registry of the south-eastern region of Sweden. For comparison, we selected an age-matched patient cohort ($n = 1164$) treated during the same period and without ILR. To examine a homogeneous group of BCs in early development, only patients with radically removed tumors (R0), without lymph node metastases (N0), or distant metastases (M0) were selected. This retrospective design enabled including patients not offered postoperative radiotherapy, as it was fully implemented in clinical routine in the early 1990s [15]. To explore possible associations between radiotherapy, MI, functional and phenotypic differentiation, and GATA-3 expression in BC tissue, 50% of

selected cases were treated with postoperative radiotherapy (RT). A detailed description of the entire patient group and the selection procedure is described in a flow chart attached as a supplement document [S1 Fig](#). Tumor histology was reviewed by an experienced pathologist (SG), and formalin-fixed paraffin-embedded tissue blocks with invasive BC were chosen for tissue microarray and constructed using two tissue cores (diameter 0.6 mm). Eighty-three patient samples were included in the total. Ethical approval from the Regional Ethics Committee in Linköping was obtained according to Swedish Biobank Law (reference number: 2010/311–31).

Immunostaining and evaluation

Five-micrometer sections were obtained from formalin-fixed paraffin-embedded TMA tumor specimens. The sections were de-paraffinized in xylene and hydrated in a series of graded alcohols, pre-treated with Heat Induced Epitope Retrieval and TrisEthylenediaminetetraacetic acid (EDTA) buffer (1 mM, pH 9, 20/5/20 min; Decloaking Chamber NxGen, Biocare Medical), and stained for M2 macrophage-specific marker CD163 (anti-human, monoclonal antibody, clone 10D6, Novocastra, Leica) and GATA-3 (anti-human, monoclonal antibody, clone L50-823, ThermoFisher Scientific). Staining for estrogen receptor (ER; clone SP1, Ventana Roche), progesterone receptor (PR; clone 1E2, Ventana Roche), Ki-67 (clone MIB-1, Dako Agilent), and human epidermal growth factor receptor 2 (HER2; clone 4B5, Ventana Roche) was done according to clinical laboratory standards. All slides were scanned to digital images using the Hamamatsu NanoZoomer XL (Visiopharm LRI AB). Evaluation of immunostaining was performed by ImageScope viewing software (Leica Biosystems).

Two experienced pathologists (SG and HO) evaluated all immunostaining, blinded to patient characteristics and outcomes. The fraction of GATA-3-positive cancer cells was calculated based on a count of 200 tumor cells in each TMA core. TAM infiltration was evaluated semi-quantitatively into three grades: no/low, moderate, or high ([Fig 1](#)). The expression of Ki-67, ER, PR, and HER-2 in cancer cells was evaluated according to ESMO guidelines (2019) [16].

Statistical analysis

SPSS statistics software, version 28 (IBM Corporation, USA), was used for the statistical analyses. MI was evaluated in relation to clinicopathologic data using Pearson's chi-square test. For continuous data, one-way analysis of variance (ANOVA) was used with a post-hoc Bonferroni's test for comparing the means between several than two variables. Mann-Whitney test for comparing the means between two variables. Survival rates were estimated according to Kaplan Meier based on disease-free survival (DFS). The statistical significance of differences between survival rates was determined by the log-rank test. For all analyses, $p < 0.05$ (double-sided) was considered statistically significant. A heat map, created by excel software (Office 365), was used to assess the multidimensional association of NHG with MI and proportion rates of cancer cells expressing GATA-3, ER, PR and Ki-67.

Results

GATA-3 expression

In total, 83 patients were included in this study. Patient characteristics are summarised in [Table 1](#). GATA-3 was expressed in 78 (94%) cases, of which 67 (84%) patients had tumors where GATA-3 expression was present in $>60\%$ of tumor cells ([Fig 2A](#)). No difference in GATA-3 expression was found between NHG1 and NHG2, with GATA-3 mean expression rates of 92% (\pm SD 17.5) and 85% (\pm SD 25), respectively. The mean GATA-3 index in NHG3 tumors was 60% (\pm SD 42), which was significantly lower compared to NHG1 ($p = 0.002$) and

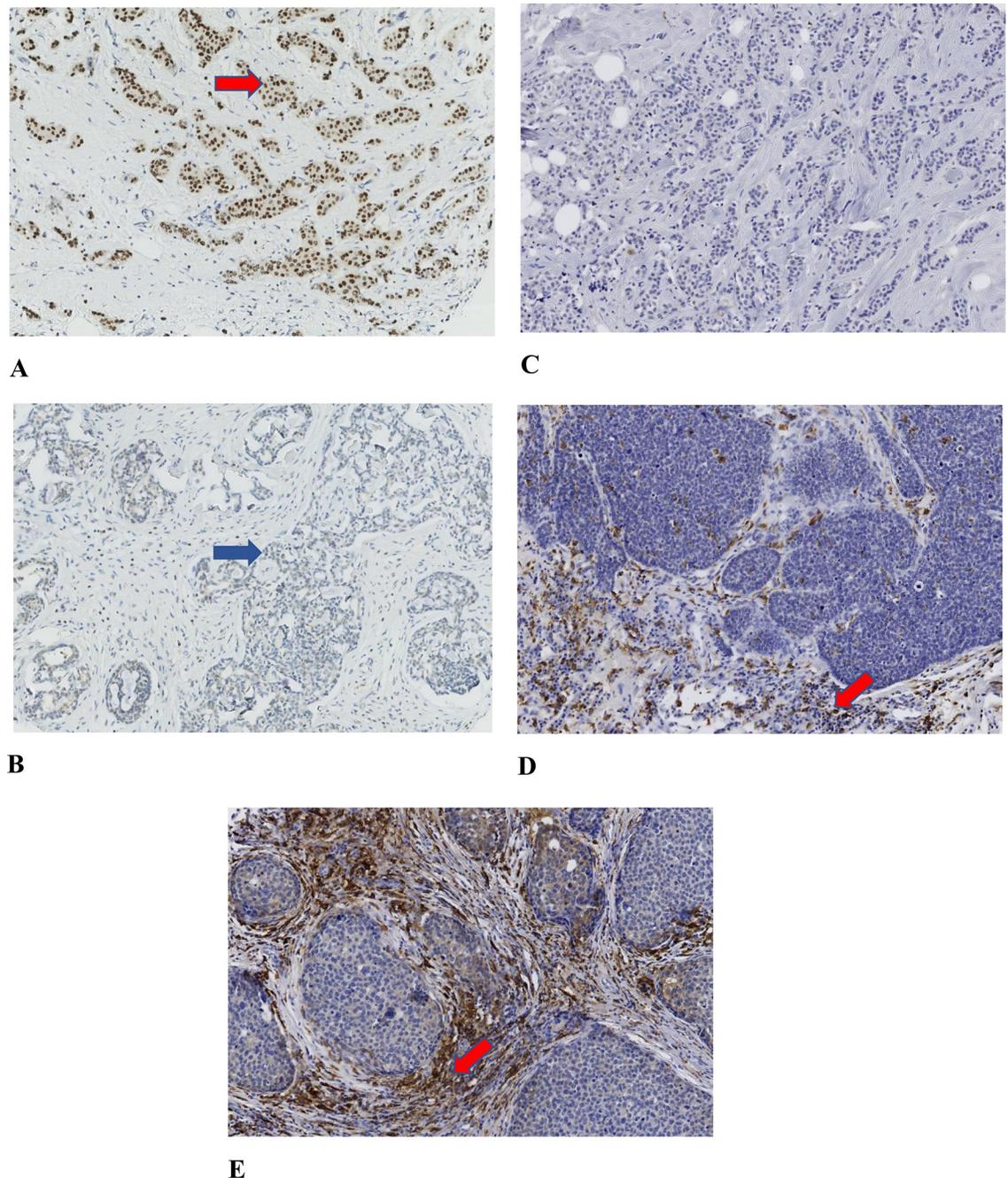


Fig 1. Breast cancer immunohistochemistry images. Image A shows a tumor with GATA-3 positive cancer cells (nuclear staining marked with a red arrow). Image B illustrates a GATA-3 negative tumor where cell nuclei (blue arrow) show no GATA-3 staining. Images C-E show immunohistochemistry for M2-macrophage specific marker CD163 used for detecting M2-macrophages (red arrow) and scoring macrophage infiltration. Images C, D, and E illustrate No/low, moderate, and high macrophage infiltration, respectively.

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NHG2 ($p = 0.006$) tumors (Fig 3A). GATA-3 expression was significantly lower in ER-negative (mean 16%, \pm SD 29) compared to ER-positive (mean 92%, \pm SD 12) tumors ($p < 0.001$). The corresponding rates in PR negative vs. positive tumors were 55% (\pm SD 43) and 91% (\pm SD 15), respectively ($p < 0.001$) (Fig 2B and 2C). GATA-3 expression in triple-negative tumors (mean

Table 1. Patient characteristics.

Variables	N (%)
Age groups (years)	
≤40	15 (18)
41–50	18 (22)
51–60	17 (20)
61–70	15 (18)
≥70	18 (22)
Pathologic T-stage	
pT1	70 (84)
pT2	13 (16)
Nottingham grade	
NHG 1	20 (24)
NHG 2	38 (46)
NHG 3	25 (30)
ER-status	
Negative	14 (21)
Positive	66 (79)
Missing data	3
PR-status	
Negative	27 (33)
Positive	55 (67)
Missing data	1
HER2-status	
Negative	73 (92)
Positive	6 (8)
Missing data	4
Ki-67-expression	
<14%	45 (63)
≥14%	27 (37)
Missing data	11
Postoperative radiotherapy	
No	42 (51)
Yes	41 (49)
Local recurrence	
No	44 (53)
Yes	39 (47)
Macrophage infiltration	
No/Low	41 (50.6)
Moderate	28 (34.6)
High	12 (14.8)
Missing data	2
Intrinsic subtypes	
Luminal A	49 (62.8)
Luminal B HER-2 negative	12 (15.4)
Luminal B HER-2 positive	5 (6.4)
Non-Luminal	1 (1.3)
Triple-negative	11 (14.1)

Abbreviations: Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2).

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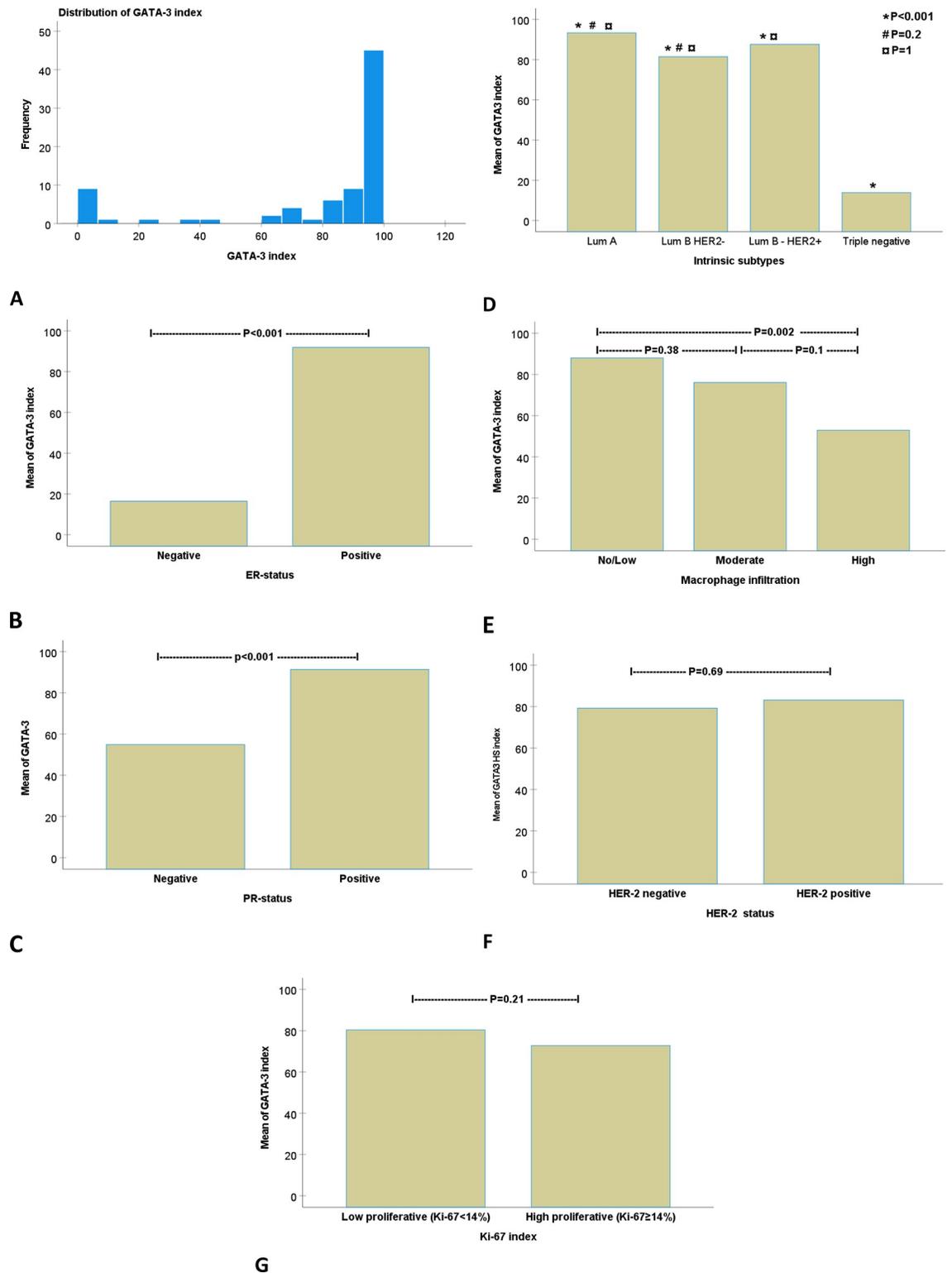


Fig 2. The relation of GATA-3 indexes to macrophage infiltration and functional differentiation of breast cancer. Panel A illustrates the distribution of GATA-3 indexes. Panels B-G show comparisons of the GATA-3 index in relation to ER status, PR status, intrinsic subtypes, macrophage infiltration, HER-2 status, and Ki-67 index, respectively. For continuous data, one-way analysis of variance (ANOVA) was used with a post-hoc Bonferroni's test for comparing the means between several than two variables. Mann-Whitney test for comparing the means between two variables.

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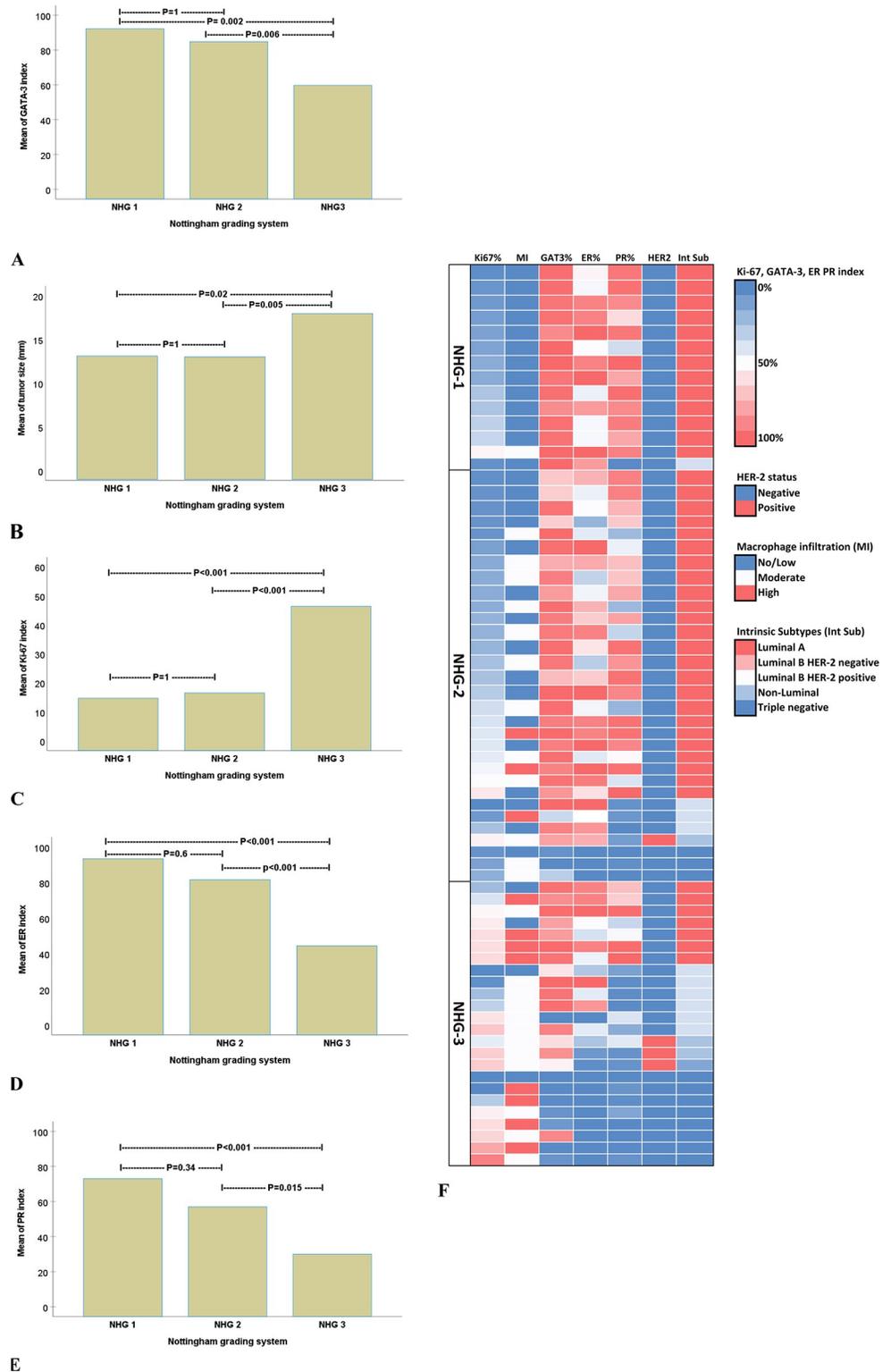


Fig 3. ANOVA analysis followed by Bonferroni's post hoc test comparing breast cancer Nottingham Histologic Grade (NHG) in relation to (A) GATA-3 index, (B) tumor size, (C) Ki-67 index, (D) ER index and (E) PR index. (F) Heat map of breast cancer NHG visualizes how segregated the distribution of macrophage infiltration, intrinsic subtypes, HER2 status, GATA3, ER, and PR indexes are between the NHG1, NHG2, and NHG3 tumors, respectively.

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14%, \pm SD 27) was significantly less than in Luminal A (mean 93%, \pm SD 9), Luminal B HER-2 negative (mean 81%, \pm SD 31) and Luminal B HER-2 positive (mean 87%, \pm SD 13) tumors ($p < 0.001$). No differences in GATA-3 expression were found between luminal A and B tumors (Fig 2D).

GATA-3 expression was inversely correlated to MI. The mean index of GATA-3 in tumors with No/Low, moderate, and high MI were 88% (\pm SD 22), 76% (\pm SD 35), and 53% (\pm SD 44), respectively. The expression of GATA-3 in tumors with high MI was significantly lower than in tumors with no/low MI ($p = 0.002$). The differences in GATA-3 expression were not statistically significant comparing tumors with high vs. moderate ($p = 0.1$) MI and no/low vs. moderate ($p = 0.38$) MI (Fig 2E). No differences in GATA-3 expression were found in relation to the expression of Ki-67 nor HER-2 status (Fig 2F and 2G).

NHG in relation to hormonal status

NHG1 and NHG2 tumors had similar sizes (mean 13 mm) and were significantly smaller than NHG3 tumors (mean 18 mm). There were no differences in Ki-67 index between NHG1 (15%) and NHG2 (16.5%) tumors ($p = 1$), whereas NHG3 tumors exhibited higher Ki-67 index (46%) compared to NHG1 ($p < 0.001$) and NHG2 ($p < 0.001$) tumors (Fig 3B and 3C).

The expression rates of ER were significantly lower in NHG3 tumors (mean index 45%) compared to NHG1 (mean index 93%, $p < 0.0001$) and NHG2 (mean index 62%, $p < 0.001$). The corresponding rates for PR expression in NHG1, NHG2, and NHG3 tumors were 73%, 57%, and 30%. PR expression in NHG3 tumors is significantly lower than in NHG1 ($p < 0.001$) and NHG2 ($p = 0.015$) tumors. No differences in ER and PR expression were found between NHG1 and NHG2 tumors (Fig 3D and 3E). HER-2 was almost exclusively expressed in NHG3 tumors. HER-2 expression was found in only one case with an NHG2 tumor. No HER-2 expression was found in NHG1 tumors.

The heat map shows that ER and PR decrease gradually in relation to NHG, of which the lowest ER and PR index rates occur in NHG 3 tumors. However, low ER and PR indices occur also in NHG1 and NHG2 tumors. The intrinsic subtypes among NHG 1–3 tumors were distinctly distributed, where NHG1 and NHG2 tumors had virtually exclusively luminal phenotypes, while NHG3 tumors exhibited non-luminal and triple-negative phenotypes (Fig 3F).

NHG in relation to MI and Ki-67 index

Macrophage infiltration was inversely correlated to the differentiation grade of BC. Out of 14 tumors with high MI, 8 (67%) had NHG3. The corresponding rates for tumors with no/low and moderate MI were 4 out of 41 (10%) and 13 out of 28 (46%), respectively. Inversely, NHG1 and NHG2 were found in 17 (41%) and 20 (49%) tumors with no/low MI ($p < 0.001$) (Table 2).

NHG3 was more common (17 out of 27, 63%) in high proliferative tumors compared to those with low proliferative tumors (Ki-67 < 14%) (8 out of 45, 18%). The corresponding rates for high and low proliferative tumors in relation to NHG1 are 2 (7%) and (29%), respectively ($p < 0.001$). As was known before, NHG was related to age ($p = 0.024$), T-stage ($p = 0.023$), and intrinsic subtypes ($p = 0.004$) (Table 2).

Macrophage infiltration in relation to Ki-67, ER, and PR status

The Ki-67 index was proportional to MI, with a mean index of 6.6%, 18%, and 20.6% in no/low, moderate, and high MI, respectively. Ki-67 was significantly lower in tumors with no/low MI compared to those with moderate ($p < 0.001$) and high ($p = 0.003$) MI. There was no difference in the Ki-67 index between tumors with moderate and high MI (Fig 4A).

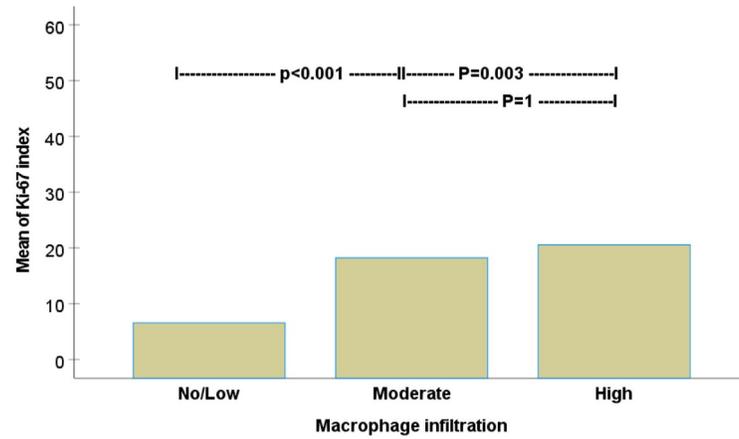
Table 2. Univariate analysis comparing Nottingham Histologic Grade (NHG) in relation to macrophage infiltration and clinical data in breast cancer.

	Nottingham grade			p
	NHG1 n (%)	NHG2 n (%)	NHG3 n (%)	
Age groups (years)				
≤40	4 (20)	4 (10.5)	7 (28)	
41–50	3 (15)	9 (24)	6 (24)	
51–60	8 (30)	10 (26)	1 (4)	
61–70	5 (25)	9 (24)	1 (4)	
≥70	2 (10)	6 (16)	10 (40)	0.024
Pathologic T-stage				
pT1	19 (95)	34 (90)	17 (68)	
pT2	1 (5)	4 (10)	8 (32)	0.023
Macrophage infiltration				
No/Low	17 (85)	20 (56)	4 (16)	
Moderate	2 (10)	13 (36)	13 (52)	
High	1 (5)	3 (8)	8 (32)	<0.001
ER-status				
Negative	0 (0)	3 (9)	11 (44)	
Positive	20 (100)	32 (91)	14 (56)	<0.001
PR-status				
Negative	3 (15)	9 (24)	15 (60)	
Positive	17 (85)	28 (76)	10 (40)	0.002
HER2-status				
Negative	18 (100)	34 (92)	21 (88)	
Positive	0 (0)	3 (8)	3 (12)	0.3
Ki-67 index				
<14%	13 (87)	24 (75)	8 (32)	
≥14%	2 (13)	8 (25)	17 (68)	<0.001
Local recurrence				
No	12 (60)	21 (55)	11 (44)	
Yes	8 (40)	17 (45)	14 (56)	0.5
Intrinsic Subtypes				
Luminal A	16 (89)	26 (72.2)	7 (29.2)	
Luminal B Her-2 negative	2 (11)	4 (11.1)	6 (25)	
Luminal B Her-2 positive	0 (0)	3 (8.3)	2 (8.3)	
Non-Luminal	0 (0)	0 (0)	1 (4.2)	
Triple-negative	0 (0)	3 (8.3)	8 (33.3)	0.004

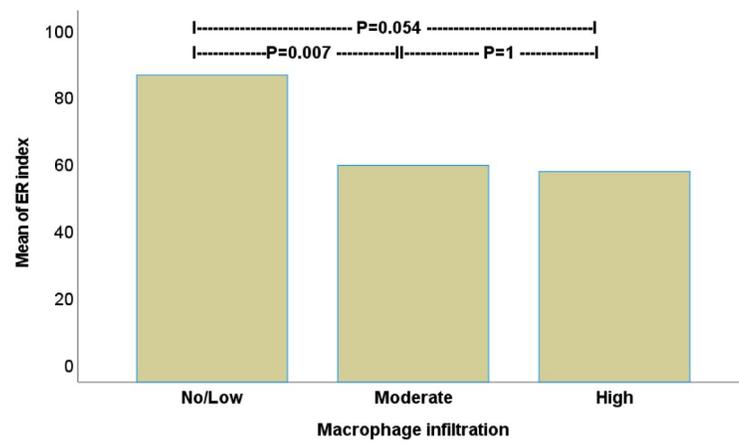
Abbreviations: Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2).

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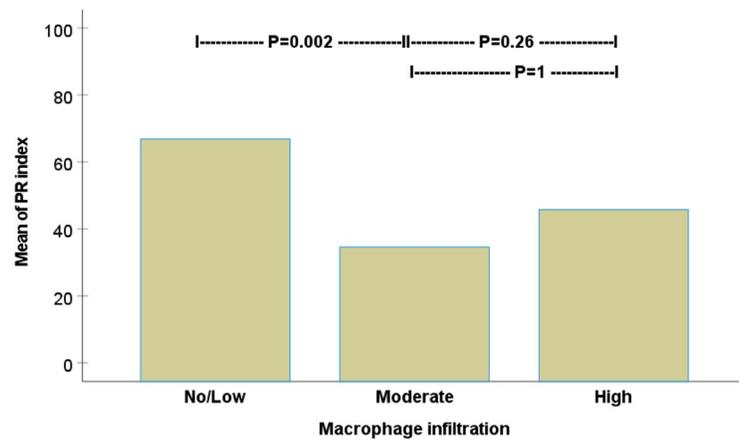
Tumors with no/low MI exhibited a mean ER index of 86%, higher than tumors with moderate (59%, $P = 0.007$) and high (57%, $p = 0.054$). The corresponding rates for PR index in tumors with no/low, moderate, and high MI were 66%, 35%, and 46%, respectively. The PR was significantly higher in tumors with no/low compared to moderate MI ($p = 0.002$). No differences in PR index were found between tumors with moderate and high MI ($p = 1$) nor between no/low and high MI ($p = 0.26$) (Fig 4B and 4C).



A



B



C

Fig 4. ANOVA analysis followed by Bonferroni's post hoc test comparing macrophage infiltration in breast cancer in relation to (A) Ki-67, (B) ER, and (C) PR indexes.

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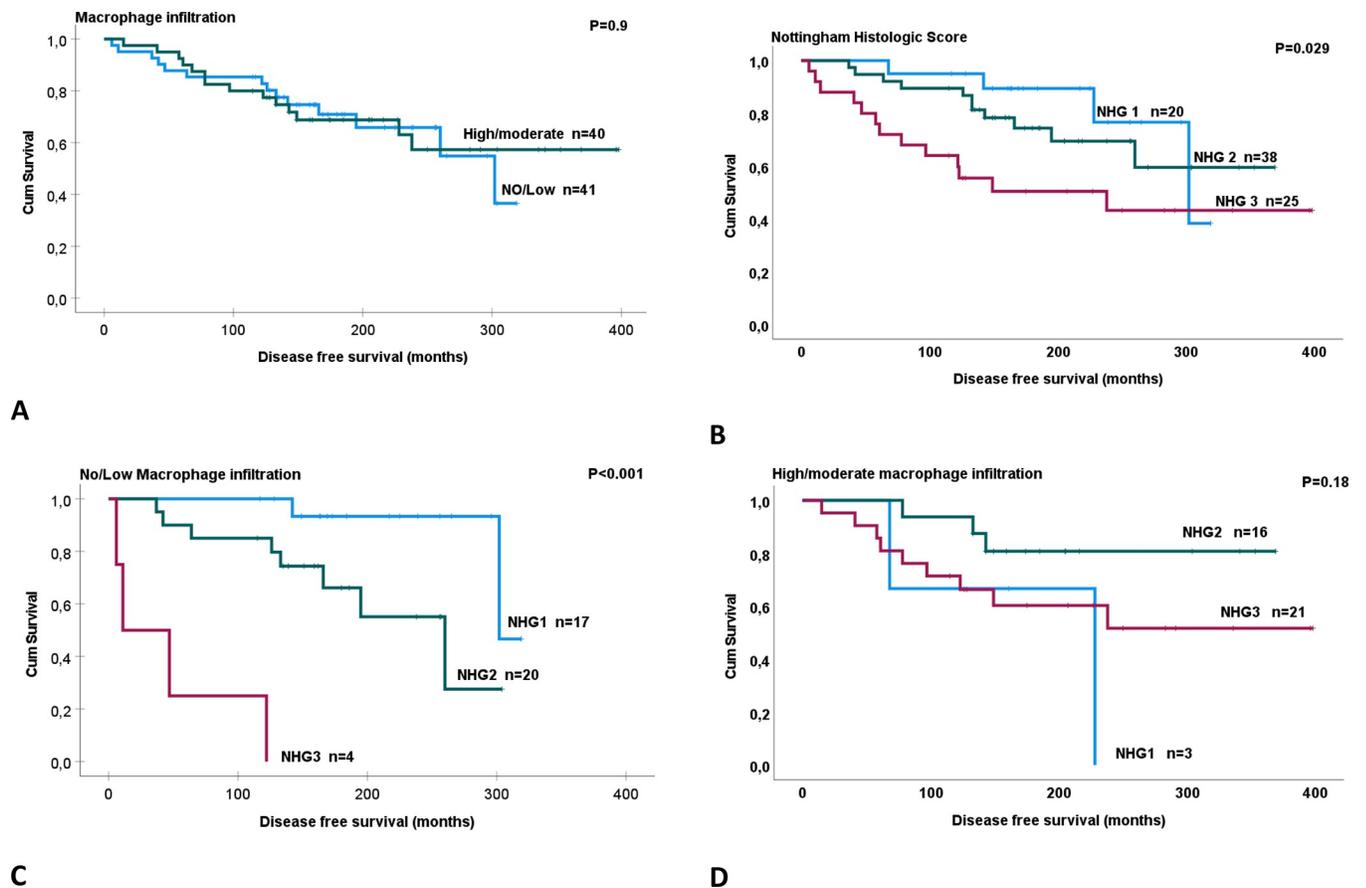


Fig 5. Kaplan-Meier curves demonstrating disease-free survival (DFS) in relation to (A) Nottingham Histologic Grade (NHG) and (B) macrophage infiltration for 83 patients with non-metastasized pT1-pT2 breast cancers treated with breast conserving surgery. In subgroup analysis, panels C and D show DFS in relation to NHG in patients having tumors with No/low and High/moderate macrophage infiltration, respectively. The comparison in this survival analysis is estimated according to the log-rank (Mantel-Cox) test.

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The prognostic impact of MI in relation to NHG

Since tumors with moderate and high MI show similar differences in relation to ER and PR status, Ki-67 index, and NHG, we chose to merge these two groups into a new group named moderate/high. To explore the prognostic impact of MI and in relation to NHG, we initially examined the DFS in relation to each of these variables individually. There was no difference in DFS between patients with no/low (236 months) and moderate/high (283 months) MI ($p = 0.9$) (Fig 5A). As expected, DFS decreased proportionally to NHG per se with DFS rates of 278, 282, and 232 months for patients with NHG1, NHG2, and NHG3, respectively ($P = 0.029$) (Fig 5B).

Patients with no/low MI tumors also had reduced DFS in relation to NHG with DFS rates of 299, 211, and 45 months for NHG1, NHG2, and NHG3 tumors, respectively ($p < 0.001$) (Fig 5C). Interestingly, we found the opposite trend of DFS in patients with moderate/high MI, although it was not statistically significant. DFS in the latter group was 175, 321, and 258 months for NHG1, NHG2, and NHG3 tumors, respectively ($p = 0.18$) (Fig 5D).

Discussion

In this study, we investigate how the presence of M2 macrophages affects the hormonal status, GATA-3 expression, and differentiation states in selected patients with pT1-T2 BCs. We found that tumors with poorly differentiated states exhibit high MI and low GATA-3 expression. GATA-3 expression was associated with ER and PR expression but inversely correlated to MI. To our knowledge, this is the first study exploring the relationship between GATA-3, MI, and NHG in BC.

Tumors evolve by clonal selection of mutated cell populations with unconstrained proliferation and invasion ability. The mechanisms of invasive cancer cell selection and the geno- and phenotypes required for metastasis in tumor biology are still unclear. However, it is now widely accepted that tumor morphology and the dominance of the invasive phenotypes largely depend on selection by the host tissue microenvironment [1, 2, 17]. Macrophages are actively recruited by tumor cells, and once in the tumors, TAMs support an immunosuppressive microenvironment, promoting angiogenesis and the metastatic potential of cancer cells. For example, TAMs suppress anti-tumor immunity by inhibiting the programmed death-1 (PD-1) T cells and their ligands, PD-L1 and PD-L2 [18, 19]. In an *in vivo* mouse model, DeNardo et al. reported that inhibition of macrophage recruitment with CSF1 receptor antagonists in combination with chemotherapy (paclitaxel) reduced the growth of the primary BCs and pulmonary metastasis [20]. In a similar experimental study, Yang et al. show that the depletion of macrophages will reduce breast cancer stem cells and decreases tumorigenicity and metastasis of mammary tissue [21]. In addition to promoting tumor progression, macrophages promote endocrine resistance through, e.g., downregulation of ER and PR and activation of the PI3K/Akt/ mTOR pathway [22, 23]. In the current study, M2-specific MI is inversely correlated to decreased BC cell expression of ER- and PR and is proportional to tumor cell proliferation detected as Ki-67 index. Moreover, high MI was related to a significant decrease in GATA-3 expression and related to advanced tumor grade indicating that increased MI is associated with poorly differentiated states in BC. Together with comprehensive experimental observation [17, 18, 22–25], these findings suggest that the malignant potential of BC and probably its response to hormonal therapy does not depend solely on the biology of the tumor cells themselves. Hence, M2 macrophages are likely a crucial player in BC pathophysiology. From the clinical perspective, this is an important mechanism, as inhibition of MI may tentatively improve the prognostic assessment and potentiate the oncological treatment of BC.

In normal development, cell differentiation is a process by which proliferating cells gradually acquire tissue-specific function by changing phenotype. During carcinogenesis, tumor cells lose tissue-specific markers developing a de-differentiated state with increased proliferative capacity and plasticity. The morphology of BC cells is determined by the degree of their differentiation. However, the differentiation process in BC cells is not unidirectional towards differentiated luminal epithelial cells, as this lineage-specific differentiation is frequently altered or reversed [26, 27].

Comprehensive evidence indicates that GATA-3 is essential in the morphogenesis and regulation of mammary epithelial differentiation [28], constituting a valuable tissue-specific marker for confirming the epithelial or mesenchymal origin of tumors [28–30]. GATA-3 is associated with ER expression and a better response to hormonal treatment. However, the prognostic value of GATA-3 is still discussed [31–33].

Even if GATA-3 is associated with the differentiation of BC, few studies have explored the correlation between GATA-3 expression and BC differentiation. In all these studies, different approaches were used to evaluate positivity and determine cut-off rates for GATA-3 expression [25, 34–36]. Min et al. was the only group that used receiver operating characteristics to

estimate the optimal cut-off rate (5% with an area under cover of 0.566) of GATA-3 [37]. These studies did not explore the extent of GATA-3 expression, i.e., the proportion of GATA-3 positive tumor cells in relation to the NHG. In this study, we avoided determining a cut-off rate nor estimating the prognostic value of GATA-3 as the primary endpoint because all BC tumor stages are not represented in this patient material (only pT1-2 tumors). Dichotomization is commonly used in clinical studies, but it is precarious to use this approach and split the continuous value of a marker when examining its correlation to other tumor variables [38]. Here we show that GATA-3 expression was proportional to ER and PR expression, consistent with observations reported in several previous studies [5, 7, 28, 31, 39]. GATA-3 expression was significantly lower in NHG3 compared to NHG1 and NHG2 tumors. Interestingly, there was no difference in GATA-3 expression between NHG1 and NHG2 tumors.

This study does not reveal the reason why NHG1 and NHG2 tumors exhibit a similar extent of GATA-3 expression. Tentatively, this similarity may be due to several factors, such as variations of GATA-3 expression in relation to individual assessment variables included in the NHG criteria (tubule formation, nuclear pleomorphism, and mitotic activity) or that de facto that NHG1 and NHG2 tumors show similar GATA-3 expression. For many years NHG has been an established BC scoring system and has proven to be an independent prognostic factor [40]. However, it is proposed that the mitotic index per se [41] better reflects BC malignant behaviour, as with the mitotic index is possible to divide the BC patients into groups with different prognoses [42, 43]. Hence, it should be logical that the expression of a biological marker, such as GATA-3, varies in relation to the assessment criteria included in the NHG classification. Further studies with larger patient samples and all BC tumor stages are needed to explore this topic.

As discussed previously, macrophages promote breast cancer progression, and that increased MI is related to advanced tumor stages and poor prognosis [12–14]. To the best of our knowledge, MI is not investigated in relation to GATA-3 expression nor its prognostic impact in association with NHG. In the current study, high MI was correlated to low GATA-3 expression and low differentiated states, NHG3. The MI itself was not related to the DSF. However, the DFS decreased inversely in relation to NHG in patients having tumors with no/low MI. Interestingly, no significant differences in DFS were found in relation to NHG in patients having tumors with moderate/high MI, indicating that MI influences the impact of NHG on DSF.

Conclusion

This study provides further insight into the biological role and clinical significance of MI and GATA-3 in pT1-T2 BC. Macrophage infiltration is associated with differentiation states, hormonal status, and GATA-3 expression in BC. Moreover, the DFS analysed in relation to NHG is influenced by the extent of MI. These observations indicate that the extent of MI in the tumor microenvironment might impact the prognosis and treatment outcomes in BC, regardless of the morphological and hormonal states of the tumor cells.

Supporting information

S1 Fig. Flow chart showing the selection of 83 breast cancer patients treated with breast-conserving surgery. All patients had no lymph node or distant metastasis at the time of surgery, and the tumors were completely removed.

(DOCX)

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