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Interleukin-33 Levels are Up-Regulated in Women with Breast Cancer and Shown to be Associated with the Triple-Negative Type

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Abstract

Breast cancer (BC) is a diverse disease with heterogeneous subgroups in pathophysiology and behavior. Recent advances in biomarker-based therapy may help improve treatment options for BC. Thus, this study aimed to assess the role of interleukin (IL)-31 and IL-33 as biomarkers associated with BC risk. Serum concentrations of IL-31 and IL-33 were quantified in women with BC (n = 77), women with benign breast lesion (BBL; n = 51), and healthy women (n = 75), using enzyme-linked immunosorbent assay kits. Compared with BBL or healthy women, IL-33 concentrations were significantly greater in BC patients and were associated with a 1.33-fold increased risk of disease and had excellent prognostic value in BC (area under the curve = 0.89). In addition, IL-33 expression was linked to triple-negative BC, distant metastasis, Tumor-Node-Metastasis (TNM) stage, status of estrogen and progesterone receptors, and human epidermal growth factor receptor 2. IL-31 concentrations showed no significant differences between BC, BBL, and healthy women, but were greater in patients with distant metastasis and those who were negative for estrogen and progesterone receptors. In conclusion, IL-33 and to a lesser extent IL-31 can be used as biomarkers associated with BC risk and to identify patients with a poor prognosis especially those with triple-negative BC. Furthermore, understanding the connection between IL-33 and BC progression may aid in the development of safe and effective therapy.

Keywords: Breast cancer; Benign breast lesion; Triple-negative tumor; Interleukin-31; Interleukin-33.

ارتفاع مستويات بين الابيضاض 33 في النساء المصابات بسرطان الثدي وارتباطها بالنوع السلبي الثلاثي

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الخلاصة

ان سرطان الثدي (BC) مرض متنوع له مجموعات فرعية غير متجانسة في الفيزيولوجيا المرضية والسلوك. قد تساعد التطورات الحديثة في العلاج القائم على العلامات الحيوية في تحسين خيارات العلاج لسرطان الثدي. كانت أهداف الدراسة هي تقييم أهمية بين الابيضاضين 31 (IL-31) و 33 (IL-33)

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كمؤشرات حيوية مرتبطة بمخاطر سرطان الثدي. تم تحديد التراكيز المصلية لكل من IL−31 و IL−33 في النساء المصابات بسرطان الثدي (BC؛ العدد = 77) والنساء المصابات بآفات الثدي الحميدة (BBL؛ العدد = 51) والنساء الأصحاء (العدد = 75) باستخدام مجموعات مقايسة الممتز المناعي المرتبطة بالإنزيم. بالمقارنة مع النساء المصابات بآفات الثدي الحميدة أو النساء الأصحاء، كانت تراكيز IL−33 أكبر بشكل ملحوظ في مرضى سرطان الثدي وكانت مرتبطة بزيادة خطر الإصابة بالمرض بمقدار 1.33 مرة وكانت لها قيمة تنبؤية ممتازة في مجموعة النساء المصابات بسرطان الثدي (المنطقة الواقعة تحت المنحنى = 0.89). بالإضافة إلى ذلك، تم ربط تعبير IL−33 بالنوع السلبي الثلاثي من سرطان الثدي، ورم خبيث بعيد، ومرحلة TNM ، وحالة مستقبلات هرمون الاستروجين والبروجسترون ، ومستقبلات عامل نمو البشرة البشري 2. لم تظهر تراكيز IL−31 أي فروق ذات دلالة إحصائية بين النساء المصابات بسرطان الثدي والنساء المصابات بآفات الثدي الحميدة والنساء الأصحاء، ولكن كانت أكبر في المرضى الذين يعانون من ورم خبيث بعيد وأولئك الذين كانت سلبية بالنسبة لمستقبلات هرمون الاستروجين والبروجسترون. في الاستنتاج، يمكن استخدام IL−33 وبدرجة أقل IL−31 كمؤشرات حيوية مرتبطة بمخاطر سرطان الثدي ولتحديد المرضى الذين يعانون من سوء التشخيص خاصة أولئك الذين يعانون من سرطان الثدي السلبي الثلاثي. علاوة على ذلك، فإن فهم العلاقة بين تقدم IL−33 وسرطان الثدي قد يساعد في تطوير علاج آمن وفعال.

1. Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, with an estimated 2.3 million new cases occurring each year [1]. Furthermore, BC is a recognizable cause of cancer-associated mortality among women with approximately 685,000 deaths in 2020 [2]. It is a highly heterogeneous malignancy that shows many phenotypic and morphological characteristics, including those related to hormone receptor status (estrogen; ER and progesterone; PR), human epidermal growth factor receptor 2 (HER2), and triple-negative BC (TNBC) [3], [4]. The expression of ER, PR, and HER2 is predominantly used to categorize BC into subtypes. This classification enhances diagnosis and improves therapy choice including hormonal therapy and HER2-targeted therapy [5]. The most aggressive subtype of BC is the TNBC, which is connected with the worst prognosis, the highest frequency of metastasis, and the lowest survival rate [6].

Although conventional multimodal therapy has increased survival rates for BC patients, more research is still needed to identify novel prognostic markers and potential therapeutic targets. In 1863, Virchow discovered the presence of leukocytes in cancerous tissues and was the first to postulate a link between the development of cancer and inflammation [7]. Inflammatory reactions are mediated and regulated by cytokines, which are soluble low molecular weight glycoproteins, through interactions with their receptors [8]. Although the interplay between inflammation and cancer progression has not been fully understood, the tumor microenvironment has been shown to harbour a delicate network of cytokines and immune cells expressing cytokine receptors. Although the etiopathogenic relationship between inflammation and cancer is not fully understood, it has been revealed that infiltrating inflammatory cells within the tumor microenvironment express different cytokines with different functions. Some of these cytokines, such as interleukin (IL)-6, exert pro-oncogenic activities, others, such as IL-2 and IL-12, suppress carcinogenesis, and others, such as IL-23, play both roles [9], [10]. Both benign and malignant breast diseases are also predominantly associated with inflammation, and there is some accumulating evidence suggesting that cytokines are involved in regulating BC induction and protection as well as promoting metastasis [11]–[13]. Therefore, tracking and emphasizing the molecules released from the immune system during cancer progression, such as cytokines, might be a good option to understand the pathogenesis of BC [14].

IL-31 and IL-33 are two novel cytokines that were recently recognized to have pathophysiological roles in tumorigenesis, and their potential as prognostic factors and targets for therapy has been proposed [15], [16]. IL-31 is a member of the IL-6 family of cytokines that was discovered in 2004 and is produced by T helper (h) 2 cells and immature dendritic cells [17]. Although the physiological effects of IL-31 are not well defined, its role in controlling signals that regulate many biological functions has been described in various human inflammatory states. Besides, IL-31 is involved in the induction of pro-inflammatory cytokines, cell proliferation, regulation, and tissue homeostasis [18]. In the context of cancer, IL-31 has been recognized to have antiangiogenic effects and immune-modulating functions in BC have also been described, but the evidence is not conclusive [19].

IL-33, a member of the IL-1 family of cytokines, is produced by epithelial and endothelial cells, as well as various types of immune cells, including macrophages and dendritic cells [20]. Since IL-33 is a ligand for the ST2/T1 receptor, the primary targets of IL-33 are ST2-expressing cells such as Th2 cells, regulatory T cells, and macrophages [21]. Depending on the type of triggered cells, microenvironment, and costimulatory molecules, IL-33 can act as pro- or anti-inflammatory cytokine [22]. Thus, IL-33 has been connected to the pathogenesis of a number of inflammation-related disorders, including atopic dermatitis and rheumatoid arthritis [23], [24]. In cancer, IL-33 has been shown to have dual effects. In one side IL-33 can act as a pro-tumorigenic cytokine and in the other side it may act as an anti-tumorigenic cytokine depending on the inflammatory environment and tumor type [16]. IL-33 has also been linked to the etiopathogenesis of BC, particularly positive hormone receptor tumors while there is no clear evidence linking IL-33 to TNBC [3], [25].

It is generally recognized that early diagnosis, prognosis and treatment are the most effective ways to increase the survival rate in patients with BC [1]. This progress would be possible through the use of potential biomarkers, such as cytokines, as diagnostic and prognostic markers for BC. Thus, the objectives of this study were to evaluate the role of IL-31 and IL-33 in the etiopathogenesis of benign and malignant breast tumors and their potential utility as reliable biomarkers associated with BC risk. The relationship between these cytokines and other BC features, including the clinical stage and certain immunohistochemical markers, was also examined.

2. Materials and Methods

2.1 Subjects

A study was conducted on 77 women with BC (mean age ± standard deviation [SD] = 49.1 ± 11.7 years), 51 women with benign breast lesions (BBL; 31.9 ± 9.5 years), and 75 healthy women (CTRL; 31.6 ± 6.7 years). Patients were referred to private clinics for gynecology during December 2022-March 2023. BC cases were diagnosed and confirmed by oncologists by performing the Triple Assessment Technique which included a physical breast examination, radiological imaging (ultrasonography and/or mammography), and fine needle aspiration cytology. Based on the histological analysis, BBLs were classified as fibroadenomas, and all BC tumors as invasive ductal carcinomas (IDC). The clinical stage of BC was assessed using the TNM (Tumor-Node-Metastasis) classification system. The TNM system is determined on the basis of three tumor assessments: size of the primary tumor (T), regional lymph nodes (N), and distant metastasis (M) [26]. Four clinical stages (I, II, III, and IV) were defined as a result of performing this system. The patients with BC and BBL were newly diagnosed and did not receive any therapy. The CTRL group included women who had no cancer, BC or BBL and no history of these diseases in their sisters or mothers. All participants provided written consent after the approval of the Research Ethics Committee of the College of Science, University of Baghdad (Reference: CSEC/1122/0145 on November 28, 2022)

2.2 Immunohistochemical assay

Immunohistochemical analysis of ER, PR, and HER2 was performed on paraffin-embedded breast tumor sections. Expression of these markers was assessed qualitatively using commercial kits following instructions of manufacturer (Dako, Denmark).

2.3 IL-31 and IL-33 immunoassay

To determine concentrations of IL-31 and IL-33 levels, 5 mL blood was collected and left to coagulate at 20-25 °C (room temperature) for 30 minutes, and then serum was separated by centrifugation (2,000 × g for 10 minutes) and stored at -20 °C until use. Serum concentrations of IL-31 and IL-33 were quantified using the human mini ABTS ELISA development kits following the manufacturer's instructions (Catalog Number: 900-M347 and 900-M398, respectively; PeproTech, UK). An ELISA reader (HumaReader HS, Germany) was used to measure the absorbance at a wavelength of 450 nm. Calculations were based on a standard curve that was plotted against the concentrations of the standards by tabulating in EXCEL sheet the absorbance of the pre-determined standards for each marker. Then an equation that fits the curve was created and used to obtain the level of the unknown samples.

2.4 Statistical analysis

GraphPad Prism version 9.4.1 (San Diego, CA, USA) was employed to carry out statistical analyses. Study variables subject to a normal distribution (parametric variables) were presented as mean ± standard deviation (SD). IL-31 and Il-33 concentrations were presented by median and interquartile range (IQR: 25-75) after normality testing of the original data (non-parametric variables). Statistical significance between each two groups was assessed using one-way analysis of variance test or Mann–Whitney U test for parametric and non-parametric variables, respectively. The diagnostic performance of IL-31 and IL-33 was evaluated by performing receiver-operating- characteristic (ROC) curve analysis. The analysis outcome was presented by the area under the curve (AUC) with its 95% confidence interval (CI), as well as the cut-off value (optimized with Youden index; YI), sensitivity, and specificity. Odds ratio (OR) and 95% CI were used to evaluate association with disease risk by multinomial logistic regression analysis. Significance was set at a value of probability (p) < 0.05.

3. Results

3.1 Baseline characteristics of BC patients

The characteristic features of patients with BC are illustrated in Table 1. With respect to tumor size, patients with T1-T2 were the most commonly observed (84.4%). In addition, 72.7% and 79.2% of patients showed regional lymph node involvement and distant metastasis, respectively. TNM staging revealed that 44.2% of patients were categorized as I-II and 55.8% as III-IV. Immunohistochemical examination of tumor sections showed that 66.2, 62.3, and 41.6% were positive for ER, PR, and HER2, respectively. Besides, nine patients (11.7%) were TNBC according to histochemical analysis of ER, PR, and HER2. When the patient's age was stratified according to these characteristics, there was no significant difference in each stratum (Table 1).

Table 1: Baseline characteristics of breast cancer patients

Characteristic	BC; n = 77			
	n	%	Mean age ± SD	*p*-value
Tumor size				
T1-T2	65	84.4	48.9 ± 11.7	0.787
T3-T4	12	15.6	49.9 ± 12.4	
Regional lymph node involvement				
Presence	56	72.7	49.2 ± 12.2	0.908
Absence	21	27.3	48.8 ± 10.6	
Distant metastasis				
Presence	61	79.2	49.0 ± 12.3	0.944
Absence	16	20.8	49.3 ± 9.7	
TNM stage				
I-II	34	44.2	46.5 ± 11.0	0.085
III-IV	43	55.8	51.1 ± 12.0	
Estrogen receptor				
Positive	51	66.2	48.0 ± 11.8	0.286
Negative	26	33.8	51.1 ± 11.5	
Progesterone receptor				
Positive	48	62.3	47.7 ± 11.3	0.181
Negative	29	37.7	51.4 ± 12.3	
Human epidermal growth factor receptor 2				
Positive	32	41.6	51.2 ± 13.2	0.19
Negative	45	58.4	47.6 ± 10.5	
Triple-negative BC				
Presence	9	11.7	48.0 ± 8.1	0.774
Absence	68	88.3	49.2 ± 12.2	

BC: Breast cancer; SD: Standard deviation; *p*: Probability of one-way analysis of variance test.

3.2 IL-31 and IL-33 concentrations

Median serum concentrations of IL-31 showed no significant differences between BC, BBL, and CTRL (16.45, 16.77, and 16.71 pg/mL, respectively; $p > 0.05$). On the contrary, median concentrations of IL-33 were significantly greater in patients with BC compared to BBL or CTRL (49.30 *vs.* 35.94 and 33.03 pg/mL, respectively; $p < 0.001$). IL-33 concentrations also tended to be greater in BBL than in CTRL but the difference was not significant ($p > 0.05$) (Figure 1).

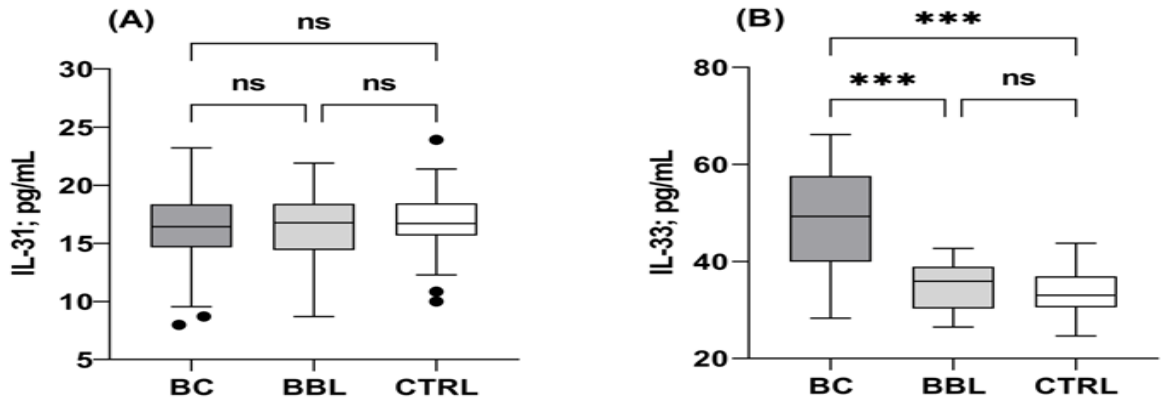

Figure 1: Serum levels (median [interquartile range; IQR]) of IL-31 (**A**: 16.45 [14.69-18.20], 16.77 [14.43-18.40], and 16.71 [15.67-18.46] pg/mL, respectively) and IL-33 (**B**: 49.30 [40.34-57.35], 35.94 [30.30-38.93], and 33.03 [30.54-36.94] pg/mL, respectively) in breast cancer (BC), benign breast lesion (BBL), and control (CTRL) groups. Horizontal line inside

box represents median. Whiskers represent IQR. Black circles represent outliers. Mann-Whitney U test was used to assess significant differences between medians (***$p < 0.001$; ns: $p > 0.05$). IL-31 levels showed no significant differences between BC, BBL and CTRL groups. IL-33 levels were significantly elevated in BC patients compared to BBL or CTRL groups.

3.3 ROC curve analysis

ROC curve analysis was conducted to assess how well IL-31 and IL-33 differentiate BC and BBL from CTRL. According to the ROC analysis of IL-31, the AUC and 95% CI for BC against CTRL were 0.57 and 0.47-0.66, respectively, whereas these values were 0.55 and 0.44-0.66 for BBL versus CTRL. For IL-33, BC against CTRL had an AUC and 95% CI of 0.89 and 0.84-0.94, respectively, whereas BBL versus CTRL had values of 0.57 and 0.47-0.68, respectively. Statistically, all analyses mentioned were not significant except IL-33 in BC against CTRL. The ROC curve analysis of IL-33 in BC revealed that serum IL-33 concentrations at an optimal cut-off value of 37.98 pg/mL (YI = 0.58) recorded a sensitivity of 78.7% and a specificity of 79.2%, respectively (Figure 2 and Table 2). These observations imply that IL-33 can distinguish BC patients from CTRL in a manner that is significant.

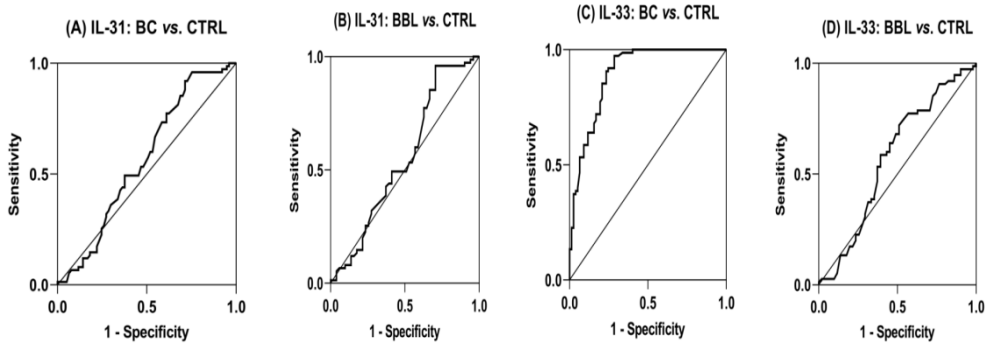

Figure 2: Receiver operating characteristic (ROC) curve analysis of IL-31 and IL-33 in breast cancer (BC) and benign breast lesion (BBL) versus controls (CTRL). (A) IL-31: BC *vs*. CTRL [AUC = 0.57; 95% CI = 0.47-0.66; $p = 0.163$]; (B) IL-31: BBL *vs*. CTRL [AUC = 0.55; 95% CI = 0.44-0.66; $p = 0.329$]; (C) IL-33: BC *vs*. CTRL [AUC = 0.89; 95% CI = 0.84-0.94; $p < 0.001$]; (D) IL-33: BBL *vs*. CTRL [AUC = 0.57; 95% CI = 0.47-0.68; $p = 0.163$]. AUC: Area under the curve; CI: Confidence interval; *p*: Probability.

Table 2: Receiver operating characteristic (ROC) curve analysis of IL-31 and IL-33 in breast cancer and benign breast lesion *versus* controls

Group	IL-31; pg/mL				IL-33; pg/mL			
	Cut-off value	YI	Sensitivity;%	Specificity;%	Cut-off value	YI	Sensitivity;%	Specificity;%
BC	16.68	0.07	53.3	53.2	37.98	0.58	78.7	79.2
BBL	16.84	0.002	49.3	50.9	34.03	0.12	58.7	58.8

BC: Breast cancer; BBL: Benign breast lesion; YI: Youden index.

3.4 Regression analysis

The outcomes of the age-adjusted multinomial logistic regression analysis of IL-31 and IL-33 as predictors of the outcome variables BC and BBL, as well as the computed OR and 95% CI, are shown in Table 3. Only IL-33 was significantly associated with the risk of BC with an OR of 1.33 and a 95% CI of 1.19-1.48 ($p < 0.001$).

Table 3: Age-adjusted multinomial logistic regression analysis of IL-31 and IL-33 in breast cancer and benign breast lesion *versus* controls

Group	IL-31; pg/mL			IL-33; pg/mL		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
BC	0.89	0.76-1.04	0.135	1.33	1.19-1.48	< **0.001**
BBL	0.91	0.80-1.04	0.179	1.06	0.98-1.14	0.139

BC: Breast cancer; BBL: Benign breast lesion; OR: Odds ratio (the reference category was controls); CI: Confidence interval; *p*: Two-tailed probability (Significant *p*-value is indicated in bold).

3.5 Stratification of IL-31 and IL-33 concentrations by BC characteristics

Serum IL-31 and IL-33 concentrations were inspected in BC patients stratified by various characteristics of disease (Table 4). First, we tested how IL-31 and IL-33 are related to tumor growth. The concentrations of IL-31 and IL-33 in patients with the tumor size T1-T2 were 15.93 and 46.56 pg/mL, respectively, while in patients with the tumor size T3-T4, these concentrations increased to 18.30 and 54.12 pg/mL, respectively, but the differences did not approach a significant level ($p = 0.083$ in the case of IL-31 and $p = 0.068$ in the case of IL-33). Second, regional lymph node involvement is a factor that might be affected by IL-31 or IL-33 in BC. In fact, patients had similar concentrations of IL-31 in the presence and absence of regional lymph node involvement (16.19 and 16.71 pg/mL, respectively; $p = 0.56$), while IL-33 concentrations were slightly increased without a significant difference (47.93 *vs*. 52.79 pg/mL, respectively; $p = 0.427$). Third, how IL-31 and IL-33 relate to distant metastases was investigated. IL-31 and IL-33 concentrations were 16.90 and 53.20 pg/mL, respectively when distant metastasis was present and reduced substantially to 14.59 and 36.48 pg/mL, respectively when distant metastasis was not present ($p < 0.001$) (Table 4).

The TNM stage was then investigated to determine whether it was a factor that may be connected to IL-31 or IL-33. Patients with the TNM stages I-II and III-IV expressed approximate concentrations of IL-31 (15.86 and 16.71 pg/mL, respectively; $p = 0.33$). However, patients with the TNM stages III and IV expressed considerably more IL-33 than those with stages I and II (55.03 pg/ml *vs*. 41.71 pg/mL; $p < 0.001$). After that, the relationship between ER, PR, and HER2 and IL-31 or IL-33 was examined. Concentrations of IL-31 and IL-33 were found to be considerably lower in BC patients with ER-positive (15.80 and 43.08 pg/mL respectively) than in BC patients with ER-negative (17.78 and 57.35 pg/ml; $p = 0.022$, and < 0.001 respectively). Concentrations of IL-31 and IL-33 were also significantly lower ($p = 0.004$, and < 0.001 respectively) in BC patients with PR-positive (15.60 pg/ml and 43.08 pg/mL, respectively) than in BC patients with PR-negative (18.53 and 55.53 pg/mL, respectively). IL-31 concentrations were similar in positive and negative HER2 with 16.19 and 16.45 pg/mL, respectively ($p = 0.45$). Conversely, patients with HER2 negative status had significantly higher concentrations of IL-33 (54.03 pg/mL) compared to those with HER2 positive status (44.82 pg/ml; $p = 0.042$). We additionally examined the relationship between the concentrations of IL-31 or IL-33 and TNBC. Women with TNBC exhibited significantly higher concentrations of IL-33 (62.67 pg/mL) compared to patients without TNBC (46.56 pg/mL; $p < 0.001$). In contrast, no statistically significant variations were recorded in IL-31 levels ($p = 0.28$) (Table 4).

Table 4: IL-31 and IL-33 levels stratified by characteristics of breast cancer.

Characteristic; median (IQR)	BC; n = 77	
	IL-31; pg/mL	IL-33; pg/mL
Tumor size		
T1-T2	15.93 (14.63-17.62)	46.56 (38.60-57.35)
T3-T4	18.30 (16.67-19.66)	54.12 (48.80-57.85)
p-value	0.083	0.068
Regional lymph node involvement		
Presence	16.19 (14.66-19.37)	47.93 (40.34-56.90)
Absence	16.71 (14.69-17.49)	52.79 (38.60-60.59)
p-value	0.56	0.427
Distant metastasis		
Presence	16.90 (15.34-19.11)	53.20 (43.08-58.35)
Absence	14.59 (13.29-15.31)	36.48 (33.86-43.08)
p-value	**< 0.001**	**< 0.001**
TNM stage		
I-II	15.86 (14.69-17.49)	41.71 (36.85-44.07)
III-IV	16.71 (14.63-19.11)	55.03 (49.30-59.26)
p-value	0.33	**< 0.001**
Estrogen receptor		
Positive	15.80 (14.43-17.42)	43.08 (36.85-55.03)
Negative	17.78 (15.93-19.11)	57.35 (52.79-60.67)
p-value	**0.022**	**< 0.001**
Progesterone receptor		
Positive	15.60 (14.46-17.03)	43.08 (36.48-52.54)
Negative	18.53 (15.93-19.70)	55.53 (53.12-60.26)
p-value	**0.004**	**< 0.001**
Human epidermal growth factor receptor 2		
Positive	16.19 (13.81-18.66)	44.82 (36.48-54.12)
Negative	16.45 (15.21-17.81)	54.03 (42.08-58.35)
p-value	0.457	**0.042**
Triple-negative BC		
Presence	17.29 (16.45-19.11)	62.67 (58.35-64.49)
Absence	16.02 (14.56-17.94)	46.56 (38.60-55.53)
p-value	0.284 (1.0)	**< 0.001**

BC: Breast cancer; IQR: Interquartile range; *p*: Probability of Mann-Whitney U test (significant *p*-value is indicated in bold).

4. Discussion

Cytokines have been given a key role in the normal homeostasis of cells and tissues as well as in the development and progression of pathogenic illnesses, particularly BC [10], [12]. Although recent studies provided insightful evidence on the involvement of cytokines in the development and progression of BC [7], several interleukins involved in BC pathology still require additional research to fully understand their role and explore their potential prognostic applications in BC. In the current study, we examined serum concentrations of IL-31 and IL-33 in two groups of breast disease, BBL and BC. No association was found between the studied interleukins and BBL. However, BC was strongly correlated with elevated serum concentrations of IL-33, which may be related to the disease's etiopathogenesis. In this context, IL-33 achieved excellent discrimination between BC and CTRL (AUC = 0.89) and

associated with a 1.33-fold increased risk of BC. The fact that BC patients have higher blood concentrations of IL-33 can be attributed to the fact that cancer cells can produce a number of cytokines, including IL-33 [16]. These findings are consistent with those of previous studies. It was discovered that compared to healthy people, BC patients had significantly higher blood concentrations of IL-33 coincided with elevated levels of factors involved in promoting angiogenesis and tumor progression such as metalloproteinase-11, vascular endothelial growth factor, and platelet-derived growth factor-C [27]. It has also shown that the combination of IL-33 and ST2 increased the intra-tumoral quantity of immune-suppressing lymphoid cells in a mouse model, which in turn promoted BC growth and metastasis [28]. Furthermore, BC patients had almost two times the amount of IL-33 in their blood than people with BBL. Besides, BC tissues showed higher expression of IL-33 compared to normal breast tissues from the same patient [22]. These outcomes imply that IL-33 may exert a significant functional role in the onset of BC and may act as a reliable biomarker for predicting the development and progression of BC. The association between IL-33 and poor prognosis of other cancers provided further evidence for a contribution of this cytokine to tumor aggressiveness and poor prognosis [29].

Our findings indicate that some clinical features of BC, including distant metastasis, TNM stage, ER, PR and HER2 receptor status, and TNBC, may be etiologically related to the concentrations of IL-31 and IL-33, while other features, such as tumor size and local lymph node involvement, may not be related. Although the study demonstrated that IL-31 concentrations showed no significant differences between BC patients and CTRL, presence of distant metastasis and a negative expression of ER and PR were associated with higher amounts of IL-31. This observation may link IL-31 to the promotion of these clinical features in BC. Since it is responsible for 90% of BC fatalities, distant metastasis is an important factor to take into account while analyzing the pathophysiology of the BC illness [30]. In addition, the state of ER and PR has also been identified as a critical element in the pathophysiology of BC [6].

Data from this study revealed that in women with BC, the serum concentrations of IL-33 were related to the status of ER, PR, and HER2 as well as the TNM stage, distant metastases, and TNBC. IL-33 levels were observed to be significantly lower in BC patients who were ER-positive and PR-positive than in BC patients who were ER-negative and PR-negative. In contrast, patients with HER2 negative status exhibited considerably greater IL-33 concentrations than those with HER2 positive status. Our findings somewhat agree with those of Liu and co-workers who demonstrate that HER2-negative BC tissue has a higher expression of IL-33 than normal breast tissue from the same patient [22]. The present study also revealed a very important finding that elevated concentrations of IL-33 were associated with TNBC. Although this observation was based on only nine patients and there is no evidence in the literature to establish a role for IL-33 in TNBC, it may frame the involvement of IL-33 in the pathophysiology of this type of BC and the interest as a potential therapeutic target. TNBC is the most aggressive type of BC, has a poor prognosis, and is not treatable by endocrine or anti-HER-2 therapy [31]. Systemic chemotherapy is likely to be the only therapeutic option in TNBC, but it has been suggested that application of immune checkpoint inhibitors, for example the IL-33/ST2 pathway, may revolutionize immunotherapy for BC and its triple-negative subtype [32].

The small number of included subjects, in particular TNBC cases, was a major limitation of the study. Besides, anthropological, demographic, and lifestyle-related risk factors for BC were not been determined.

5. Conclusion

Serum IL-31 concentrations showed no significant differences between BC, BBL, and CTRL, but may be related to distant metastasis and the expression pattern of ER and PR. Whereas, IL-33 concentrations were significantly up-regulated in women with BC, and its significance as a biomarker associated with disease risk was proposed. In addition, IL-33 was linked to distant metastasis, TNM stage, expression pattern of ER, PR, and HER2, and TNBC.

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Conflict of interest

The authors declare that there were no conflicts of interest.

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