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Prognostic Value of Circulating Cytokines in Breast Cancer

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Abstract

Objectives: The aim of this study was to measure circulating cytokines (IL17, IL6, IL22, IL23 and TNFα) and to evaluate their role as markers and in prognosis in Tunisian patients with breast cancer.

Materials and methods: Our prospective study enrolled 60 untreated patients affected by breast cancer. We evaluated their levels of TNF-α and IL6 within solid-phase, two-site chemoluminescent enzyme immunemetric assay. Seric levels of IL17, IL22 and IL23 were measured by ELISA sandwich technique and results compared by chi-2 square.

Results: Our population, all females, have a mean age of 48 years, a localized disease in 75% of cases and metastatic in the resting 25%. The mean cytokines levels for IL6, IL17, TNFα, IL22 and IL23 were respectively 4.8 ± 7.2 pg/ml, 0.27 ± 0.69 pg/ml, 5.9 ± 2.2 pg/ml, 50.8 ± 34.7 pg/ml and 18 ± 30.9 pg/ml. Serum IL6 levels were significantly higher with advanced stages and particularly, metastatic stage IV and in relapsing patients. We observed also higher TNFα levels in advanced stages (III and IV) and IL22 in cases with grade III SBR. For IL23, higher levels were observed in axillary node positive cases and in patients younger than 35 years. IL17 was significantly higher with patients who relapsed.

Conclusion: Our results highlight the role of cytokines in the serum as potential prognostic biomarkers in breast cancer patients, which could contribute to tumor growth and progression. Analyzing serum cytokine levels may help to identify patients with poor prognosis who may benefit from more aggressive treatment.

Keywords: ***Cytokines; Inflammation; Breast cancer***

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Introduction

Breast cancer is a major worldwide public health problem with 1.67 million cases in 2012 and in Tunisia (with around 2500 yearly cases), representing the 1st female neoplasia [1]. Conversely to western countries where structured large scale mammography screening improving the prognosis, most of the cases in our country remain detected at late advanced and/or metastatic stages. Relapse risk after curative therapy in early breast cancer is evaluated via anatomo clinical parameters (histologic size, grade, her 2 and hormone receptor status and Ki 67 index) and more recently genomic tests. Cancer cells communicate with the host primarily via cytokines and use this communication system to shape tumor microenvironment and promote metastasis by facilitating tumor dissemination, motility and invasion [2] and could have an activating or inhibiting role according to the cytokine type. The inflammatory microenvironment could be implicated in all steps of carcinogenesis. Mantovani et al. [3] hypothesize that inflammation contributes to the development of cancer, which in turn promotes more inflammation in a "vicious cycle" in a microenvironment around the tumor. The aim of this study was to measure the concentration of circulating inflammatory cytokines (IL-6, IL17, IL22, IL23 and TNF-α) to evaluate their seric levels and to evaluate their impact on breast cancer prognosis.

Methods

Study

Our prospective study included 60 patients, all females, followed and treated for breast cancer at the department of medical oncology of the military instruction hospital of Tunis. We excluded patient with any immunologic disorders: pregnancy, patients under immune therapy and those carrying an infection.

Blood sampling and technique of analysis

Samples from venous blood samples were centrifuged and stored at -80°C until CK analysis. We calculated the circulating levels of inflammatory interleukins, by solid-phase, two-site chemi luminescent enzyme immune-metric assay (Immulite 1000, Siemens, USA) for TNF-α and IL-6 technique and enzyme-linked immunosorbent assays (ELISAs) for IL-17, IL22 an, IL23.

Clinical variables

We collected the epidemiologic and anatomo-clinical features: age, gender, underlying diseases, and outcome. We used the TNM classification. The present study was approved by the Ethic committee of Military Hospital of Tunis which is affiliated to faculty of Medicine of Tunis (FMT) (decret n° 94-1939 of 19 September 1994), all patients has signed informed consent at Oncology department of Military Hospital of Tunis.

Statistical analyses

Data were seized and analyzed by SPSS software version 22. Results were expressed as frequency and percentages for qualitative variables, while quantitative data was expressed as mean ± standard deviation. The comparison of qualitative variables between groups was performed by the non-parametric test, the chi-square test (chi 2).

The normal distribution of quantitative variables was verified by the Kolmogorov-Smirnov test or the shapiro wilk test. Student's t-test for two independent samples and analysis of variance (Anova) were used to compare the means between groups for quantitative variables that have a normal distribution. The non-parametric U-test of Mann-Whithey and H of Kruskal-Wallis was used to compare quantitative variables that have an asymmetric distribution.

The strength of association (correlation) between the quantitative variables was estimated by the "Spearman Rho" nonparametric test for those that have an asymmetric distribution and the "pearson" parametric test for variables with normal distribution. The P value <0.05 was considered statistically significant.

Results

The median age of our 60 patients (all females) was 48 years (26-72). They have a localized disease in 70% of cases and metastatic in 15 patients (25%), mostly to bones (67%) and liver (60%), while 5% of patients were relapsing after adjuvant treatment. At diagnosis, tumors are at stage I in 13%, stage II in 32%, stage III in 30% and IV in 25% Regarding-molecular subtypes, we observed 68% of ER+/PR+, 12% of ER+/PP- and ER-/PR- in 20%, while Her 2 neu gene was over expressed in 27% of patients.

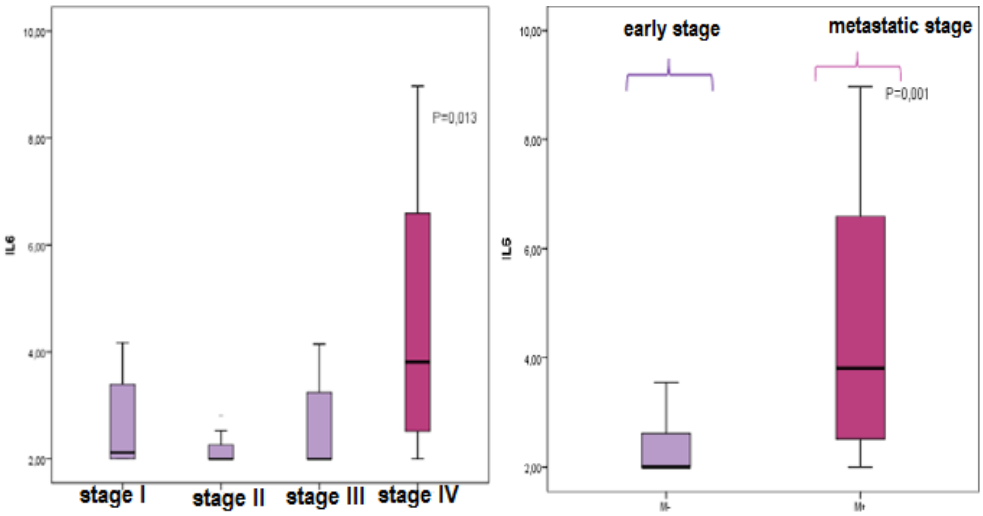

Figure 1A - 1B: Bars representing means +/- standard deviation in pg/ml showing significant differences in IL6 levels in BC patients according to clinical tumor stage (1A) and metastatic status (1B).

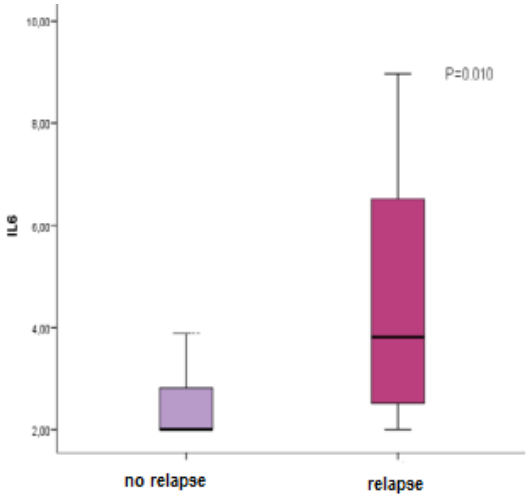

Figure 1C: Bars representing means +/- standard deviation in pg/ml showing significant differences in IL6 levels in BC patients relapsing versus those in complete remission.

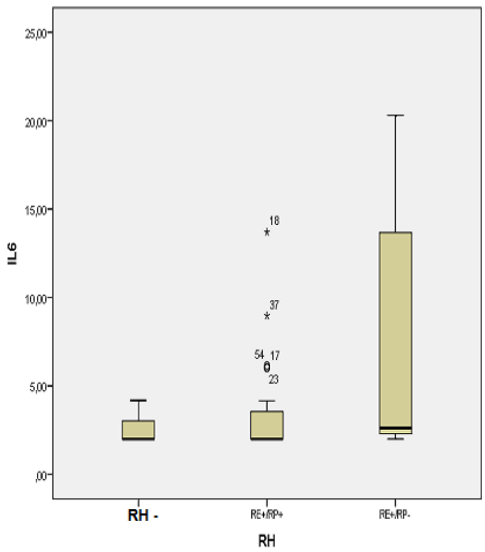

Figure 1D: Bars representing means ± standard deviation in pg/ml showing correlation between Serum IL6 levels and hormone receptor status (p=0.06).

		Mean cytokines serum level (pg/ml) [p value]				
	Total number	**IL6**	**TNFa**	**IL17**	**IL22**	**IL23**
	60pts	4.80 ± 7.26	5.93 ± 2.27	0.27 ± 0.69	50.82 ± 34.78	18.05 ± 30.91
		(2-36.80)	(4-15.30)	(0-3.62)	(26.48-199.48)	(0-200.21)
Age (years) <35	8 (13 %)	8.08 ± 12.74	4.53 ± 1.08	0.423 ± 0.66	38.1 ± 9.51	24.25 ± 16.14
>35	52 (87 %)	4.15 ± 6.04	6.06 ± 2.34	0.25 ± 0.72	52.6 ± 36.68	17.19 ± 32.45
		p=0.346	P=0.128	P=0.116	P=0.286	P=0.034
TNM Stage I	8 (13%)	3.84 ± 4.05	4.62 ± 0.80	0.01 ± 0.02	47.85 ± 33.87	9.47 ± 6.45
Stage II	19 (32%)	2.33 ± 0.95	5.26 ± 1.7	0.48 ± 1.11	50.31 ± 39.01	13.70 ± 14.95
Stage III	18 (30%)	4.30 ± 7.51	6.58 ± 2.01	0.13 ± 0.30	55.86 ± 42.38	31.10 ± 51.43
Stage IV	15 (25%)	8.43 ± 10.80	6.52 ± 3.26	0.34 ± 0.56	47.05 ± 18.39	12.50 ± 14.73
		P=0.013	P=0.098	P=0.349	P=0.655	P=0.27
Axillary node						
N0	25 (41%)	3.07 ± 2.60	5.35 ± 1.37	0.19 ± 0.81	44.76 ± 22.21	13.50 ± 12.22
N1	15 (25 %)	5.30 ± 9.21	5.93 ± 3.17	0.28 ± 0.54	57.79 ± 45.58	7.06 ± 10.69
N2 –N3	20 (34%)	6.02 ± 9.18	6.25 ± 2.43	0.37 ± 0.79	52.46 ± 40.21	20.75 ± 26.82
		P=0.963	P=0.670	P=0.632	P=0.821	P=0.046
Metastasis:						
M0	45 (75%)	3.39 ± 5.04	5.66 ± 1.85	0.25 ± 0.76	52.05 ± 38.75	19.79 ± 34.54
M1	15 (25%)	8.43 ± 10.80	6.52 ± 3.26	0.34 ± 0.56	47.04 ± 18.39	12.5 ± 14.73
		P=0.001	P=0.356	P=0.151	P=0.366	P=0.471
SBR grade :						
I+II	29 (48%)	4.43 ± 6.05	5.58 ± 2.45	0.27 ± 0.75	42.58 ± 15.96	10.53 ± 12.33
III	24 (40%)	5.72 ± 9.38	6.24 ± 2.25	0.17 ± 0.41	62.75 ± 48.85	19.08 ± 25.36
Unknown	7 (12%)	P=0.291	P=0.073	P=0.670	P=0.028	P=0.144

Table 1: Cytokines seric level rates and anatomo-clinical features.

Mean levels of cytokines IL6, IL17, TNFα, IL22 and IL23 (Table 1) were respectively 4.8 pg/ml ± 7.2 pg/ml (2-36.8); 0.27 pg/ml ± 0.69 pg/ml (0 pg/ml - 3.62 pg/ml), 5.9 pg/ml ± 2.2 pg/ml (4 pg/ml -15.3 pg/ml), 50.8 pg/ml ± 34.7 pg/ml (26.4 pg/ml - 199.4 pg/ml) and 18 pg/ml ± 30.9 pg/ml (0 pg/ml - 200.21 pg/ml).Comparisons showed that IL6 levels were slightly higher (Figure 1A) in advanced stages (p=0.013), specially metastatic with p=0.001 (Figure 1B) and in relapsed cases with p=0.010 (Figure 1C). We observed also an increase of IL6 in ER+/PR- cancers compared to ER+/PR+ and HR negative but with marginal difference at p=0.06 (Figure 1D). We didn't observe correlations of CK levels and Her 2 status, KI67 and SBR grade. High TNFα levels were also significantly associated (p=0.019) with advanced stages III-IV (Figure 2) and high rates of IL22 (p=0.028) with grade III SBR (Figure 3). Regarding - IL23, seric levels seems to be correlated with axillary node involvement (p=0.042) and age younger than 35 years (p=0/034) (Figure 4A - Figure 4B). For the last, IL17 levels were significantly higher in relapsed patients (p=0.018) after adjuvant therapy (Figure 5).

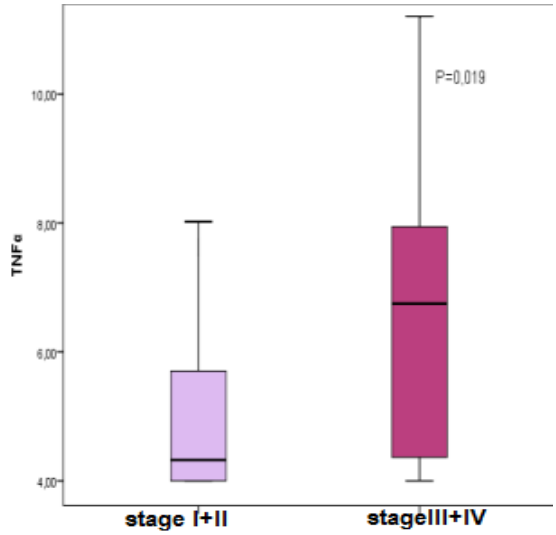

Figure 2: Bars representing means ± standard deviation in pg/ml showing significant differences in TNFa levels in BC patients with advanced stages versus those with early one.

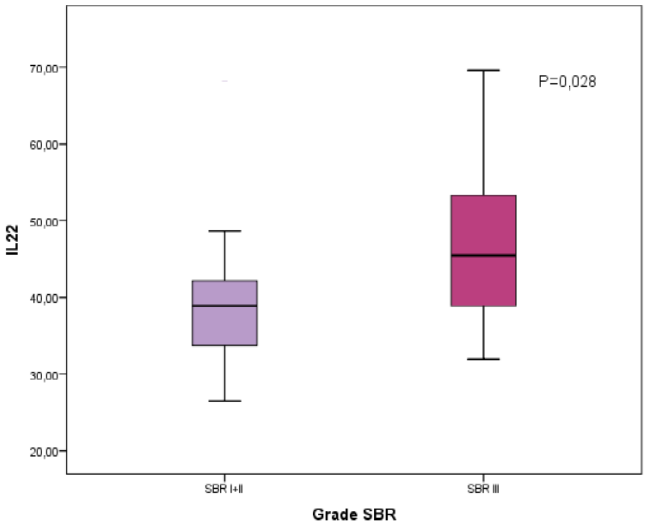

Figure 3: Bars representing means ± standard deviation in pg/ml showing significant differences in IL 22 levels according to SBR grade.

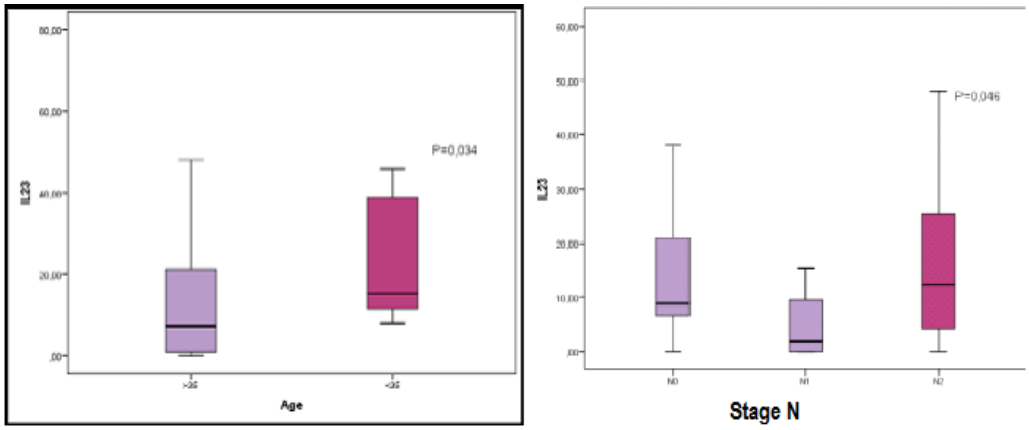

Figure 4A - Figure 4B: Bars representing means ± standard deviation in pg/ml showing significant differences in serum IL23 levels according to infiltrating lymph node (4A) and age (4B).

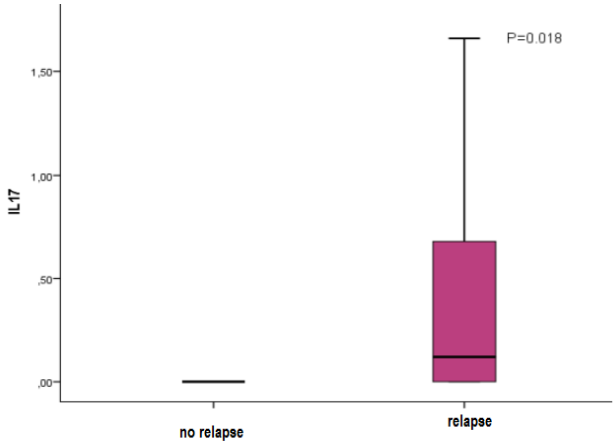

Figure 5: Bars representing means ± standard deviation in pg/ml showing significant differences in serum IL17 levels in patients with relapses versus those in complete remission.

Inter-correlations between IL6, IL17, IL22, IL23 and TNFa levels

We observed only a significant positive correlation (r = 0.462; p<0.05) between TNFa and IL22 levels (Figure 6).

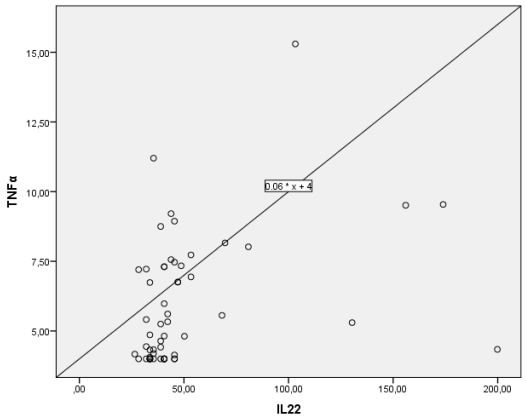

Figure 6: Correlation between IL22 and TNFa (r = 0.462) (p=0.001).

Discussion

Our prospective study including 60 Tunisian patients with breast cancer tried to evaluate the inflammation cytokines and their role in diagnosis and prognosis. As previously reported, inflammation could play a role in tumor promotion and progression, via several mechanisms such as, cell proliferation, suppression of apoptosis, enhanced angiogenesis and induced tumour immune escape [4]. Some authors suggest that pro-inflammatory mediators produced by the cancer patient's cells activate local immune networks to promote the development and growth of malignant cells by increasing their proliferation and survival as well as angiogenesis and making immune system unable to destroy tumour cells [5]. Cytokines have been measured in serum from breast cancer patients and we need to pay attention to tumor microenvironment.

TNFα is a potent immunostimulatory cytokine produced by macrophages, contributes to proinflammatory environment maintenance with local effects in the tumor microenvironment and systemic effects and high serum level were described in several cancers as well as breast cancer [6]. In our study we have found a significant correlation between high TNFα level and advanced stages in breast cancer patients. There was also a strong positive correlation with IL22 which was associated with high SBR grade. This was reported by Rui et al. [7] showing that IL22 were up regulated in the serum and tissues of BC patients and were associated with clinical stages. Therefore, IL22 and TNFα might act synergistically in the development and invasiveness of tumors.

IL6 is also a proinflammatory cytokine. Some authors suggest that IL6 linked to metastasis through increasing invasion, migration and EMT (epithelial to mesenchymal transition) [8].

Others showed that circulating levels of IL6 were higher in breast cancer patients and positively correlated with advanced stage, infiltrating node, relapsing disease and metastases and thus a negative prognosticator in BC patients [9].

In our study we found similar results, high IL6 levels were significantly correlated with clinical tumour stage, distant metastases and recurrent disease, so this cytokine may be able to serve as a biological marker of tumor metastasis and invasiveness and may help to identify patients with poor prognosis who may benefit from more aggressive therapy.

In this present work we have found also a strong correlation between high IL17 levels and relapses. In fact, several studies [10,11] have shown that IL17 produced by th17 lymphocytes and IL17R play roles in diverse cancers including some subtypes of breast cancer such as TNBC and HER2+ known related to inflammation in which 3 members of the IL17 family "IL-17A, IL-17B and IL-17E" were reported to have oncogenic effects inducing proliferation, angiogenesis, resistance to chemotherapy and escape to immune cells.

Tumor in filtrating th17 cells is a general feature of cancer, its expanding and maintain depends on IL23, Thus this cytokine could be an indirect marker of th17 cells presence. In fact, in our study we found strong correlation between high levels of IL23 and infiltrating lymph node. Moreover some others [12] had demonstrated that IL23 may have an important prognostic value in the assessment of survival of breast cancer patients, showing higher mortality in patients with more elevated levels of IL23. In our work we have found a significant higher serum levels in young patients less than 35 years without significant differences according to the biomolecular characteristics, different subtypes and presence or no of metastases. Thus we can conclude that IL23 might be a prognostic biomarker in breast cancer correlated with poor prognosis and shorter outcome.

This work was limited by small simple size and the different stages of disease (45 patients in the adjuvant setting and 15 with metastatic one). Therefore, our measurements need to be extended to find more significant results with a larger population. In addition, it is interesting to confront serum interleukins levels and interleukins gene expressing in tumor microenvironment.

Our data suggest that the introduction of circulating inflammatory mediators in breast cancers could identify individuals with high risk of recurrence independently of conventional risk predictors, and patients with high levels of proinflammatory cytokines should be monitored more closely in the follow up, regardless of their TNM status. This would be an additional tool in the decision-making process.

Further validations are needed in larges samples to investigate the clinical implication of cytokines and their correlation with prognosis in blood and tumour tissues in breast cancer patients.

Conclusion

This work had evaluated for the first time the role of IL6, IL17, IL22, IL23 and TNFα in the serum as a potential prognostic biomarkers in Tunisian breast cancer patients showing that these cytokines could be probably used to identify patients with poor prognosis needing aggressive treatment and propose them in future as a new target therapy for an anti-cancer treatment.

Ethics Committee

Local ethical committee of Military Hospital of instruction of Tunis approved the study protocol, which was in accordance with the principles of the Helsinki declaration.

Acknowledgment

We would like to thank Professor Hammouda Boussen for all his efforts in this work and all the staff of the Oncology department and the Laboratory of Immunology (Military Hospital of Tunis, Tunisia).

Notes

Ethical statement: the protocol was approved by local ethical committee of Military Hospital of instruction of Tunis, which is affiliated to faculty of Medicine of Tunis (FMT) (decret n° 94-1939 du 19 September 1994).

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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