

Immunological Assessment of Key Biomarkers in Stage III Breast Cancer

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Abstract

Objective: To investigate the predictive role of some immune parameters in progressive and diagnostic of stage III breast cancer BC patient.

Methods: The study was conducted at Specialized Oncology Center – Tikrit City, and the Oncology of Baghdad Teaching Hospital, Baghdad city, Iraq, on 80 blood samples, 50 samples from women with stage III breast cancer, and 30 samples for control group (healthy women), the age range (25–50) year. The collect serum assessed by ELIZA, and the data analyzed by Independent-Samples T test spss. While AUC is measured by MedCalc.

Results: Serum levels of all parameters were dramatically higher in patients group compared to control except in IL-23 which exhibited slight elevation statically not significant. The (AUC) for the associated ROC curve of CA15-3 (1), TGF- β (1) and IL-10 (1) were the largest. AUC is valuable diagnostic parameters to predict of progressive and diagnostic of the disease, followed by TNF- α (0.997), IL-6 (0.974), IL-17 (0.890), IL-8 (0.838), CTL-4 (0.819) and finally the lowest on was IL-23 (0.616).

Conclusion: Increasing levels of the measured parameters are an essential predictor of progressive and diagnostic of the disease.

Keywords: Breast cancer, CTLA-4, interleukins, ROC analysis

Introduction

Two million three hundred thousand women diagnosed with breast cancer and caused 670,000 deaths globally, it is common in women in 157 countries in 2022.¹ In Iraq the total number of new cases of cancer recorded in 2020 was 31,692 and represented the first level of incidence rate of the top ten cancers types.²

Cancer induces by many functional and structural changes to the immune system which regulate by interactions between diverse cells. Cancer antigen 15-3 (CA 15-3) is a glycoprotein that is formed by the top surface of epithelial ducts and mammary cells then naturally secreted into the milk. While, in abnormal breast morphology in BC (CA15-3) pours in to the blood and consider a useful variable in determining the extent of the permeate cancer cells.³

Cytotoxic T lymphocyte-associated protein-4 (CTLA-4) is an inhibitory receptor of the CD-28 immunoglobulin subfamily that is mostly originated from T cells, and used in therapeutic target in autoimmune diseases and cancer.⁴ It appears to be critical for Treg cell suppressive function in many circumstances, and it sends an inhibitory signal to cells that produced it.⁵ A large number of cytokines may be involved in the complex responding between immune system and tumour cells where this dynamic cross-talk, can either adjusted tumour growth or tumour spread and metastasis take place.⁶ They tightly control pro- and antitumour effects such as immune stimulation and inhibition, inflammation, cell damage, angiogenesis, invasion, metastasis, and cancer stem-cell-like cell maintenance.⁷ Cancer cells can produce the cytokines, which in turn can provide an environment for cancer growth. In addition, some normal cells are stimulated to produce additional cytokines, which may be associated with different types of cancer. The initiation, promotion, angiogenesis, and metastasis in tumours are substantially relevant to inflammation. Many Cytokines increasing level are associated with malignances include IL-6, IL-7, TNF- α , IL-2, IL-7, IL-8, IL-10, and TGF- β were enhanced at early-stage with cancer patients.⁸ Also, high levels of (TGF- β) and (TNF- α) are

causative inflammatory cytokines in many cancers including breast cancer.⁹

Subject and Method

Sample Collection and Study Design

The study was conducted on 80 blood samples, 50 samples from women with stage III breast cancer, and 30 samples for control group (healthy women), the age range (25–50) year. Samples were collected from the Specialized Oncology Center -Tikrit, and the Oncology Teaching Hospital -Medical City – Baghdad, during the period January 2024–April 2024. The serum samples were obtained from the collected blood after centrifugation and used for the determination of the immune parameters under investigation.

Inclusion Criteria

Patients' women with stage III breast cancer aged between 25–50 year.

Exclusion Criteria

Patients' women with benign malignant and stage I or II breast cancer.

Methods

Serum separated from five ml of venous blood samples were obtained from the patients and healthy control groups to assessed cancer antigen-15-1 (CA15-3) by using the American company Monobind, Cytotoxic T lymphocyte associated protein 4 (CTLA-4) by the American company My Biosourceb and the cytokine IL-23, IL-8, IL-6, IL-10, IL-17, TGF- β and TNF- α by the American company my Elabscience. All variables were measured by ELISA.

Statistical Analysis

Data analyzed by SPSS version 27. The differences of significant $M \pm SD$ were measured by Independent-Samples T-test,

and probability of (*P*) value < 0.05 is regarded significant. Receiver operating characteristic (ROC) the area under the curve (AUC) was assessed by MedCalc. V.20 for all parameters in this study. The (AUC) measure of how well variables can distinguish between two diagnostic groups (diseased/normal). The (AUC) score can range (0 to 1).

Results

Results displayed a significant increment in all parameters levels of patients at $P \leq 0.0001$ except in IL-23 the differences were not significant. Table 1, shows the mean \pm SD for each patient and control as follow 8.194 ± 3.212 & 53.564 ± 12.855 , 3.486 ± 1.070 & 5.096 ± 1.456 , 24.808 ± 4.050 & 52.112 ± 3.859 , 11.510 ± 3.695 & 19.600 ± 5.763 , 10.343 ± 3.189 & 11.683 ± 3.752 , 23.742 ± 3.289 & 30.069 ± 5.112 , 29.674 ± 4.993 & 47.473 ± 8.308 , 2.911 ± 0.965 & 17.492 ± 4.135 and 54.557 ± 12.550 & 114.164 ± 11.824 respectively. Also results represented in Figure 1 and 2.

Area Under the ROC Curve (AUC)

Comparing the results with ROC analysis (Figure 3), variable assessment of CA15-3 (AUC = 1, $P < 0.001$), CTL-4 (AUC = 0.819, $P < 0.001$), IL-10 (AUC = 1, $P < 0.001$), IL-17

(AUC = 0.890, $P < 0.001$), IL-23 (AUC = 0.616, $P < 0.001$), IL-8 (AUC = 0.838, $P < 0.001$), IL-6 (AUC = 0.974, $P < 0.001$), TGF- β (AUC = 1, $P < 0.001$) and TNF- α (AUC = 0.997, $P < 0.001$) were significant parameters in predicting the risk of breast cancer tumour.

Table 1. The level of CA15-3, CTLA-4, IL-10, IL-17, IL-23, IL-8, IL-6, TGF- β and TNF- α Mean \pm S.D of patients and control

Parameters	Mean \pm SD		P-Value
	Control (n = 30)	Patients (n = 50)	
CA15-3 (U/ml)	8.194 \pm 3.212	53.564 \pm 12.855	0.001***
CTL-4 (pg/ml)	3.486 \pm 1.070	5.096 \pm 1.456	0.001***
IL-10 (pg/ml)	24.808 \pm 4.050	52.112 \pm 3.859	0.001***
IL-17 (pg/ml)	11.510 \pm 3.695	19.600 \pm 5.763	0.001***
IL-23 (pg/ml)	10.343 \pm 3.189	11.683 \pm 3.752	0.106 ^{NS}
IL-8 (pg/ml)	23.742 \pm 3.289	30.069 \pm 5.112	0.001***
IL-6 (pg/ml)	29.674 \pm 4.993	47.473 \pm 8.308	0.001***
TGF- β (pg/ml)	2.911 \pm 0.965	17.492 \pm 4.135	0.001***
TNF- α (ng/ml)	54.557 \pm 12.550	114.164 \pm 11.824	0.001***

NS = Non-significant, *** High significant.

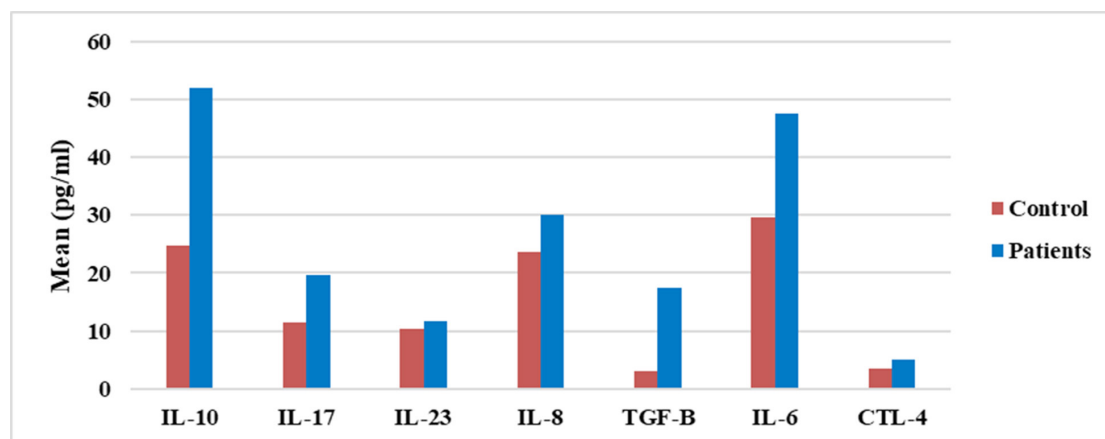


Fig. 1 Mean level of cytokine IL-10, IL-17, IL-23, IL-8, IL-6 and TGF- β in patient and control.

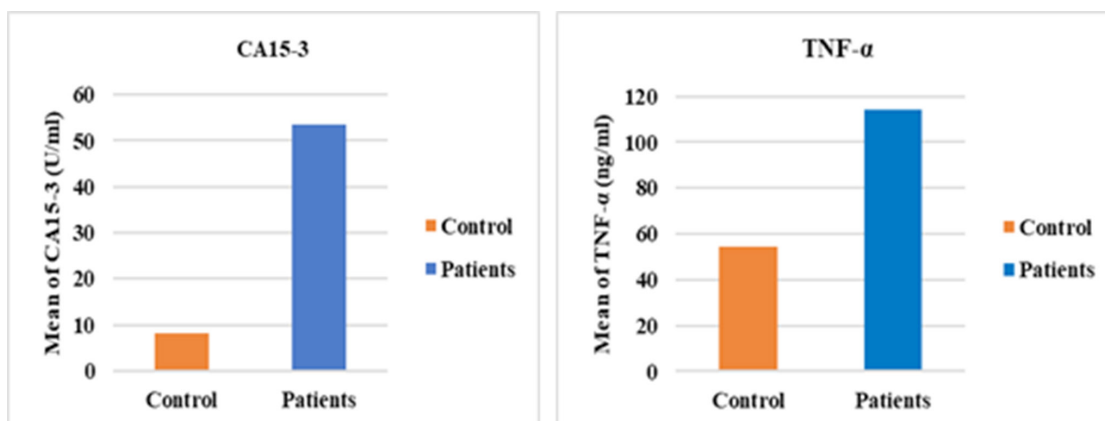


Fig. 2 Mean level of CA15-3 and TNF- α in patient and control.

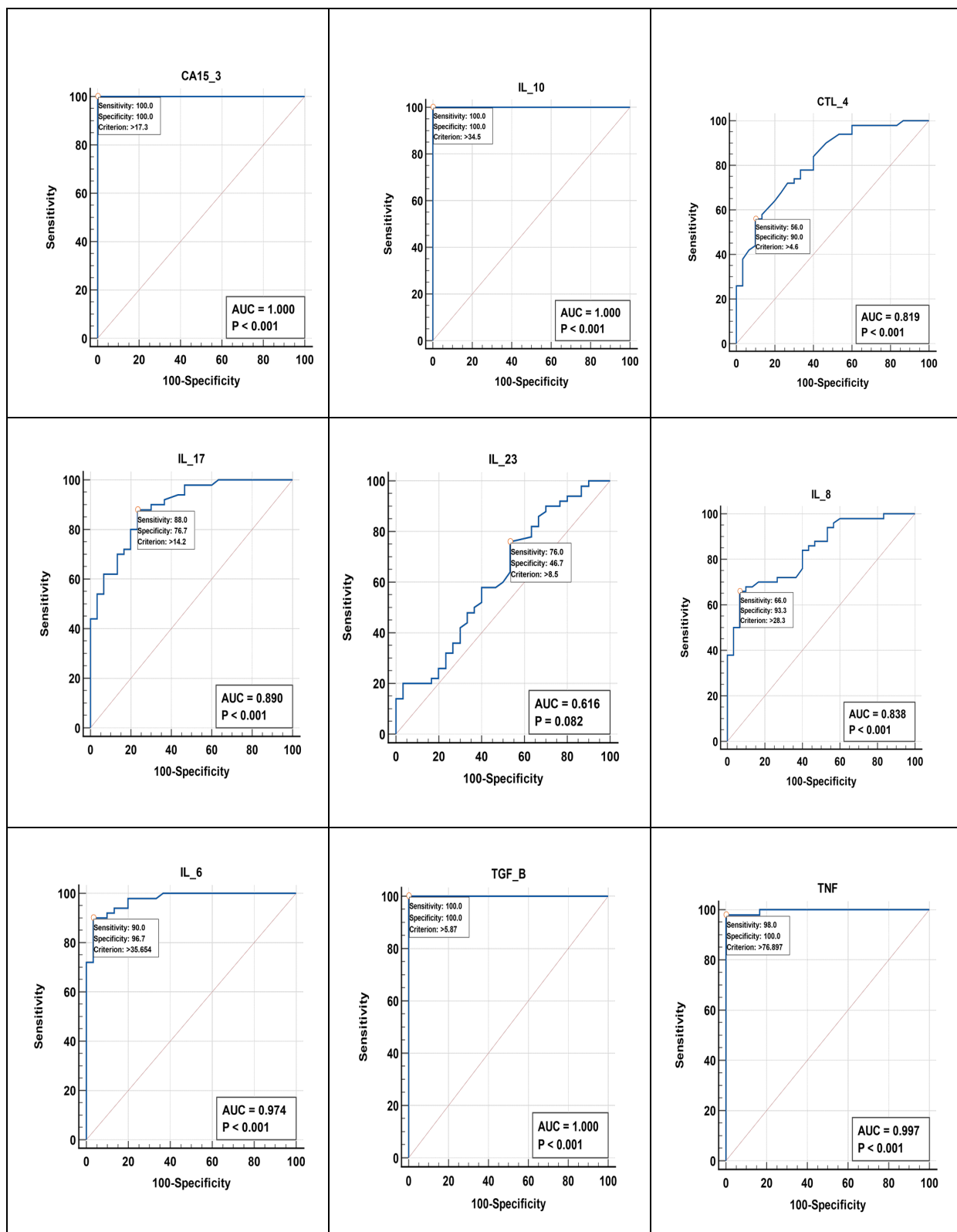


Fig. 3 Receiver operating characteristic (ROC) curve analysis AUC of all immune parameters in the studied group.

Discussion

Cytokines are highly stimulatory secretory proteins that arrange communication between cells in the immune system. They play a prominent function in the tumour microenvironment (TME) and they secreted in many types of cancer, including breast cancer.¹⁰ Different clinical and epidemiological

data suggest that cytokines are correlated with an elevated risk of cancer.¹¹

The results indicate a significant rise in all cytokines concentration, but in IL-23 the elevated was not noticeable. The study also exhibits high level of CA15-3, which is an important diagnostic tumour marker for predicting an early and advanced stages of breast cancer.¹²

Some studies have mentioned the association between CTLA-4 and some tumours, including breast cancer.¹³ It was found that CTLA-4 expressed mainly in regulatory-T cells and CD4-T cells.¹⁴ The protein level was found to be elevated in women with BC compared to healthy women.¹⁵ When anti-CTLA-4 antibodies bind with affinity to CTLA-4 molecules, leading to disruption of cell functions and thus inducing antibody-dependent cytotoxicity. All of these processes activate T cells, increase antitumour immunity, and eventually eradicate breast cancer cells. As it was found in a study conducted by Chen et al. introduced anti-CTLA-4 antibodies to tissue cultures of CTLA-4 breast cancer cells, results revealed that stem cells generate more cytokines and had a stronger ability to transfer antigens, which led to death of CTLA-4+ breast cancer cells.¹⁶ Inactivation of CTLA-4 may enhance the activity of lymphocytes which reduce the proliferation capacity of breast cancer cells.¹⁷ Even in breast cancer tissue, genes expression of CTLA-4 were elevated as compare to normal breast tissue.¹⁸ Tu Lei and his colleagues indicated that high expression of CTLA-4, Lymphocyte-activation gene 3 (LAG-3), and mucin domain-containing protein 3 (TIM-3) is a warning sign in breast cancer patients who received chemotherapy.¹⁹

Many studies have been conducted on the action of TGF- β and TNF- α in different types of cancers. Both cytokine cases in tumour microenvironment (TME) due to the inflammatory reaction.²⁰ They both have a coordinatin and antagonistic influence on tumour regulation. Inhibiting TGF- β can enhance development rate tumour, but increasing TGF- β can increase tumour aggressiveness. The microenvironment plays a role in this relationship, TGF- β acts as an anti-inflammatory factor, suppressing the formation and function of effector T cells and antigen-presenting dendritic cells also, it is controls NK cells, dendritic cells, macrophages, and granulocytes to decrement inflammation.²¹ TGF- β promotes tumour growth in normal tissue and early-stage breast cancer by causing cell-cycle arrest and death.²² It is also increases tumour growth in late-stage breast cancer by increasing metastasis, resistance to radiotherapy and chemotherapy, and reprogramming of the tumour microenvironment.²³

The inflammatory effect of TNF- α can lead to the emerging of tumours, as it has been found to play important in causing many malignant tumours and thus works to raise the level of inflammatory factors, as it plays a functional role in organizing the proliferation and invasion of divers types of cancer.²⁴

On the other hand, the current results on the concentration of cumulative cytokines (IL-6, IL-10, IL-18, IL-23, and IL-27) showed a significant elevation in the serum of studied women with BC.¹¹

It has been found that interleukins, including IL-1, IL-6, IL-10, TNF- α , MIF, and TGF- β , show a rise in tumour initiation and development. Therefore, it has a role in causing cancer. Interleukin 6 stimulates the proliferation of cancer cells by activating epithelial cells.^{25,26} A significant difference between the concentration of cytokines, such as IL-6 and IL-17 IL-1 β , IL-2 and TNF- α , between affected women and healthy control group, as their high levels may be considered a diagnostic sign of the growth of breast cancer.²⁶

The coordinated expression of IL-8 and IL-6 is critical for the growth of triple breast cancer as well as resistance to apoptosis.²⁷ Some of these cytokines, such as (IL-1, IL-6, IL-11, IL-18), and TGF stimulate BC proliferation and invasion.²⁸

Langowski et al. found in their study the role played by IL-23 in causing tumours. Where its genetic blockade it will cause increased cytotoxic T cell tumour permeation,²⁹ carcinoma cell metastas,³⁰ tumour size and stages.³¹ It was found to be important in the formation of tumours by inhibiting tumour-responsive immunity.³² IL-23 works to enhance the infiltration of macrophages and M2-type neutrophils, they overexpress and secrete pro-tumour immunosuppressive cytokines.³³ The elevated inflammatory cytokines could give new insights into effective interventions to treat breast cancer patients.³⁴

A study indicated that elevated levels of IL-10, IL-8, IL-6, may serve as a predictive marker for breast cancer diagnosis.³⁵ It was found in the research that interleukin-6 levels are increase in women with B.C compared to healthy people, as the increase is associated with a decrease in treatment response in patients, which causes death.³⁶ Also, patients who respond better to treatment have lower levels.³⁷

Regarding to the calculating an area under the curves (AUCs) by Receiver operating characteristic (ROC) analysis studies about the cytokine role as predictive factor in progression and diagnostic of BC the results were comparable to the present study. Although measurements such as positive or negative predictive values depend to a large extent on the extent of the disease's prevalence in the population studied, the sensitivity and specificity of the test are independent of the extent of the test, which allows valid conclusions to be obtained for the test's performance when using area under the curve analysis. Therefore, it was found from the results that the sensitivity and specificity were excellent for the percentage of the CA15-3. Therefore, the diagnosis of breast cancer women suffering from tumour depends mainly on measuring or the extent of the increase in the CA15-3 in women with stage III breast cancer. A study enrolled in China on benign and malignant breast tumour showed that AUC curve was 0.649, P -value < 0.05 for IL-6 and 0.656, P -value 0.016 for IL-17. Inflammatory cytokines have been associated with cancer development and may be important immunological variables for diagnosis. It was found in a study that the area under the curve was higher by 0.801, and the P -value < 0.001 . When IL-17, IL-6, and VEGF were combined together for detection, so inflammatory cytokines have a role in early detection of breast cancer.³⁸ A study conducted in patients with invasive breast cancer found that AUC value was 0.877, $P = 0.013$ and 0.854, $P = 0.001$ for IL 6 and C reactive protein (CRP) respectively for predicting recurrence-free survival.³⁹ IL-6 is a biomarker with raised sensitivity and specificity compared to CA15-3, so it may be a warning sign of disease progression.⁴⁰

Conclusion

The results of this paper revealed that patients with stage III breast express high level of inflammatory cytokine. The experimental analysis by AUC showed that serum level of all parameter even IL-23, which did not show a significant elevation in its level compared to the control group, had a prognostic value with the development and diagnostic indicator of B.C. This association need further study, and search for early and faster diagnostic indicators.

Conflict of Interest

None. ■

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