CLEC4E: A NEW PREDICTIVE MARKER FOR CHEMOTHERAPY EFFICIENCY IN SUPPRESSION OF BREAST CANCER

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Abstract

Breast cancer is a prevalent disease that has a detrimental effect on women's health and is one of the top causes of mortality due to cancer on a global scale. Recent research has demonstrated that the pattern recognition receptor known as C-type lectin domain family 4 member E (CLEC4E) may recognize related proteins produced during necrotic cell death, cholesterol crystals, and b-glucosylceramide released by injured cells. CLEC4E is expressed by phagocyte cells. In the current investigation, the concentration of CLEC4E was measured in the sera of thirty-two female patients with malignant breast tumors before they had chemotherapy; thirty-two female patients with benign breast tumors (pathological control group); and thirty-two healthy females served as the control group. The most elevated level of CLEC4E was found in the sample of a cancer patient who was in the third stage of the disease and had a history of breast cancer in their family. During the course of chemotherapy, CLEC4E levels steadily went down, which is an indication that the body responded favorably to the treatment and that fewer cancer cells were present. CLEC4E levels were found to be significantly lower in the group of breast cancer patients who had received a planned amount of chemotherapy. On the other hand, CLEC4E levels were found to be marginally lower in the group of healthy controls, which suggests that CLEC4E may be a possible biomarker for the diagnosis and prognosis of breast cancer. According to the findings of this study, CLEC4E has the potential to be used as a hopeful diagnostic marker between malignant and benign breast tumors, even after the malignant tumor has been surgically removed. In addition, CLEC4E has the potential to be an effective tool for monitoring patients' responses to treatment throughout the various phases of chemotherapy. It may be useful to study the possibility of this parameter in diagnosing tumor infection and determining the stage of the cancerous tumor, which may contribute to the designation of CLEC4E as a new marker for this kind of cancer, and evaluating the levels of CLEC4E in patients with breast tumor when diagnosing and before receiving surgical treatment may be useful for this purpose.

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1. Introduction

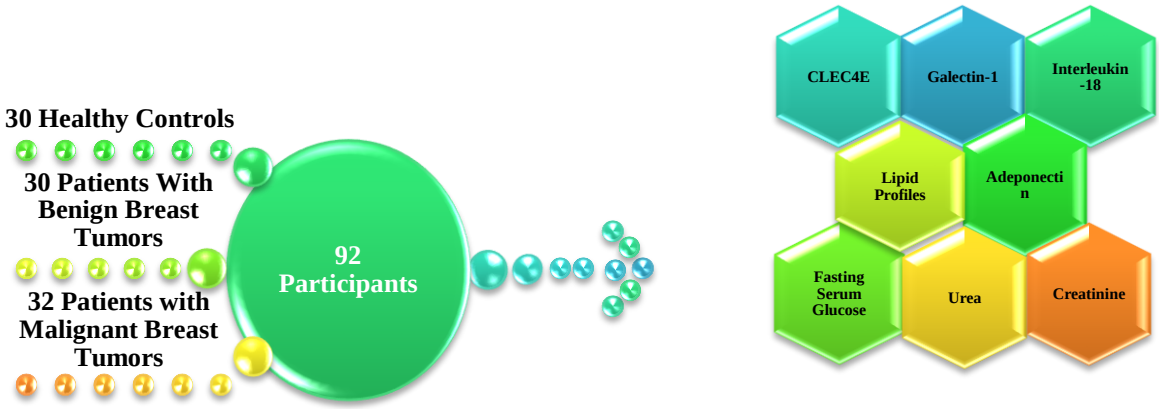
In medical literature that date back more than 5,000 years, in texts from ancient manuscripts, cases of breast cancer have been documented. These cases emerge with a frequency that is likely higher than that of any other type of cancer. The Edwin Smith and Ebers Papyrus, which dates back to between 3000 and 2500 BC, contains the earliest written evidence that is suggestive of breast cancer. While cauterization was utilized to treat certain breast diseases at the time, it is unclear as to whether or not these included breast cancers [1]. Surveillance was the primary method of treatment for breast cancer for the majority of the time that passed before the birth of Christ. During this time period, women who were found guilty of breast cancer were punished by having their breasts amputated, and breast surgery was not considered a viable option for definitive therapy [2]. Breast cancer is a genetically and clinically complex illness with a wide variety of subtypes. If treatment is not received, the cancerous cells will eventually travel to other parts of the body if they continue to proliferate abnormally in the breast. It is nearly often the female gender that is affected by breast cancer [3]. Breast cancer in women is currently the most common form of the disease to be detected, surpassing lung cancer as the leading cause of cancer deaths worldwide. In 2021, it is expected that there were 226 1419 new instances of the disease detected in females all over the world [4. In the United States, a diagnosis of breast cancer is made in a greater number of women than any other type of cancer, with the exception of skin cancer. The condition is responsible for one out of every three new cases of cancer diagnosed in women each year. The number of women in the United States who were diagnosed with invasive breast cancer in 2020 is projected to be 287850, while the number of women who were diagnosed with non-invasive breast cancer is projected to be 51400. Since the middle of the 2000s, the number of women being diagnosed with invasive breast cancer has climbed by around half of one percent per year [5]. Cancer of the breast in women ranks as the fifth biggest cause of mortality across the globe. According to estimates, breast cancer claimed the lives of 684996 women over the world in 2021. It is anticipated that there will be 43250 deaths of women in the United States caused by breast cancer in the year 2021 [6]. The most recent figure places breast cancer at the top of the list for the number of newly diagnosed cases that are registered annually around the globe: 2088849. It is the second most common cancer in women after lung cancer, when it constitutes 23% of all cancer cases in women, and it presents the first in global mortality (18.6% of cancer deaths, 626679 cases) [7]. Breast cancer is a common disease that has a negative effect on women's health, and it is one of the leading causes of death related to cancer. Breast cancer is the second most common cancer in women after lung cancer, when it constitutes 23% of all cancer cases in women. Syria has the largest documented number of breast cancer patients of any Arab country, making it the region's leader in this statistic. It is rated 11th in the world with 14.17 deaths, behind countries such as Lebanon, which has 12.19 deaths, Somalia, which has 12.18 deaths, and Iraq, which has 11.70 deaths but is placed 37th globally. This indicates that Iraq is in the first quarter and ranks 45th out of the total countries in the globe. According to the information provided by the World Health Organization (abbreviated as "WHO"), breast cancer was the most prevalent form of cancer in women in Iraq in 2018, accounting for 5141 (20.3%) of all cancer cases. Lung cancer was the second most prevalent form of cancer [8].

C-type lectin domain family 4 member E (CLEC4E) is a member of the human C-Type lectins family. It comprises 219 amino acids, a mass of 25.1 kilodaltons, water-soluble and complementary proteins in the cell membrane, and a sequential amino acid

sequence with approximately 15% carbohydrate content [9]. C-type lectin domain family 4 member E (CLEC4E) is one of the human C-Type lectins. Calcium ion (Ca2+) is one of the basic requirements in the process of carbohydrate binding and in the efficiency of metabolism [10]. [Note: Ca2+ is also an abbreviation for calcium].CLEC4E was initially characterized as a receptor for the glycolipid (cord factor) that is found in the mycobacterial cell wall. Recent research has shown that in addition to its function in viral disorders, CLEC4E is able to identify related protein, which is released during the necrotic process of cell death, cholesterol crystals, and b-glucosylceramide, which is released by cells that have been injured. Therefore, cell damage might be detected by CLEC4E, which would then set off an immunological response [11].CLEC4E is a C-type lectin pattern recognition receptor that is produced by phagocytic cells such as monocytes and macrophages; it binds cholesterol crystals and other cellular debris and makes it easier for these substances to be incorporated into the cell and then broken down further inside the cell [12].

Population of the Study

Within the scope of the present investigation were 92 female participants who were recruited from the beginning of October 2021 all the way through the month of July 2022. The individuals who took part in the study were divided into three distinct categories according to their health status (patients, healthy women, and healthy women patients) as well as the kind of tumor that the patients in the study were afflicted with. The first group consisted of 32 female patients who were diagnosed with malignant breast tumors prior to getting chemotherapy; their ages varied from 32 to 67 years. The second group, which served as the pathological control group, consisted of 30 female patients who were diagnosed with benign breast tumors; their ages ranged from 31 to 68 years. The third group consisted of thirty different women, ranging in age from 32 to 62, all of whom were considered to be part of a healthy control group. Figure 1 contains an in-depth breakdown of the data gathered from each of the investigated groups.



(A) (B)

Figure 1: (A) Distribution of Participants in the Different Study Groups (B) The Evaluated Parameters

Tumor cases were collected based on the questionnaire that was prepared according to the opinion of specialized doctors. The questionnaire included complete information on the following topics: age, place of residence, occupation, period of

onset of symptoms, medical history, stage of malignancy, location of the breast (as shown in Figure 2), and treatments that were utilized by patients. Before beginning chemotherapy, patients who had cancerous tumors were observed at the National Hospital for Oncology and Hematology, where samples from those individuals were obtained, and they were also monitored while they were getting chemotherapy. Patients suffering from malignant tumors had surgical treatment anywhere from three to five weeks before receiving their first dose of chemotherapy. Thirty of the patients who were diagnosed with cancer were married and had at least one child each.

(A) (B)

Figure **2**: Stage (A) and Location (B) of Malignant Tumor

In the meantime, instances of benign breast tumors were collected from a number of hospitals and centers in the Najaf Governorate. These facilities include the Breast Cancer Early Detection Unit in Al-Sadder Medical City, the Private Al-Ameer Hospital, the Private Al-Ghadeer-2 Hospital, the Private Al-Najaf Hospital, and the Private Al-Batoul Hospital. Patients who were found to have benign breast tumors were married and had between two and seven children. Housewives, postgraduate students, and staff at the centers and hospitals where infected samples were taken all provided healthy samples for the study. These healthy samples were collected from the area surrounding the study population. In rare instances, a cesarean section was performed during delivery only because a surgical intervention was performed previous to the injury.

Exclusion Criteria of the Study

Due to the nature of the current investigation, the following cases have to be excluded: All participants (patients with breast tumors or healthy controls) who had suffered from chronic diseases as a result of taking part in the current study, including hepatic, renal, cardiovascular, and metabolic diseases as well as diabetes, hypertension, and morbid obesity. Patients who were later diagnosed with breast cancer did not previously have breast cancer. Smoker ladies. Patients whose disease symptoms coincided with taking oral or intravenous contraceptives or who took oral contraceptives for three years in a row before to the beginning of symptoms were considered to have a higher risk of developing the condition. cases that had surgical intervention within the previous 5 years.

Samples Collection

After the participants in the study had gone without food for at least 8 hours, venous blood samples measuring 5 milliliters were drawn from each of them using gel tubes. The participants included both patients and healthy people. After using a centrifuge to separate the serum from the research samples for a period of five minutes at a speed of 5000 xg. Eppendorf tubes were used to keep the serum samples at a temperature of -20 degrees Celsius until they were needed.

Assessment of CLEC4E in the Sera of Patients and Control Groups

The Sandwich Enzyme-Linked Immune Sorbent Assay (Sandwich-ELISA) method was used to determine the level of CLEC4E present in the participants' blood serums during the course of the research.

Statistical Analysis of the Data

The results of the current study were analyzed using the statistical analysis system (SAS) version 25 software program from the statistical package for the social sciences (SPSS) as well as the statistical package excel. In order to demonstrate the variables, we used mean and standard deviation, minimum and maximum values, frequencies, percentages, and cumulative percentages. Charts in the form of pies and bars were used to present the graphics. In order to illustrate the degree of sensitivity exhibited by the analyzed parameters, a rock curve was plotted. The following were included in the inferential data analysis: For the purpose of investigating the potential differences between the biochemicals that were tested, a one-way analysis of variance (ANOVA) was carried out. It was determined using the independent student's t-test whether or not there was a significant difference in the levels of the tested parameters before and after chemotherapy treatment. The Pearson's correlation was used to analyze the data in this study in order to discover the relationship between the various biochemical indicators. If the p-value is lower than 0.05, the probability of deflection is judged to have statistically significant when compared to the controls. According to the statistical methods used in biomedicine, the percentages of sensitivity and specificity were computed.

2. Results and Discussion

In the current investigation, malignant breast tumors were recognized for patients who were through early diagnostic stages (independent of the phases of cancerous injury), and none of these instances had yet been subjected to chemotherapy or radiotherapy. The concentration of CLEC4E was measured in the sera samples taken from patients with breast tumors as well as from healthy people. When compared to the benign tumor group (p=0.000) and the healthy control group (p=0.000), the levels of CLEC4E were significantly higher in the group with malignant tumors before therapy. On the other hand, when the two control groups (those with benign breast tumors and healthy individuals) were examined together, researchers did not find any statistically significant differences in the concentration of CLEC4E. This can be shown in Table 1.

Table 1: Levels of CLEC4E in the Samples of Study Individuals

Parameters	**Subjects (N) Mean ± S.D. Minimum-Maximum**			**p-value**
	Malignant Tumors 32	**Benign Tumors 30**	**Healthy Controls 30**	
CLEC4E(ng/mL)	**4936.63±753.085 3016-5250**	**2890.13±1928.726 115-5250**	**2252.67±1395.036 993-1368**	**0.000 For M vs B 0.000 For M vs C**

				0.229 For B vs C

M: Malignant Tumor Patients, B: Benign Tumor Patients, C: Healthy Controls. Significant at $p<0.05$

The highest level of CLEC4E (5250 ng/mL) was found in the sample of a malignant patient who was in the third stage of the disease. This patient had a family history of breast cancer in her relative aunt, and she had undergone full mastectomy of her right breast. When the levels of CLEC4E were measured during the chemotherapy phase of treatment, a steady decline in the levels of this parameter was seen that was proportional to the advancement of the phase of treatment that the breast cancer patients were receiving (Figure 3).

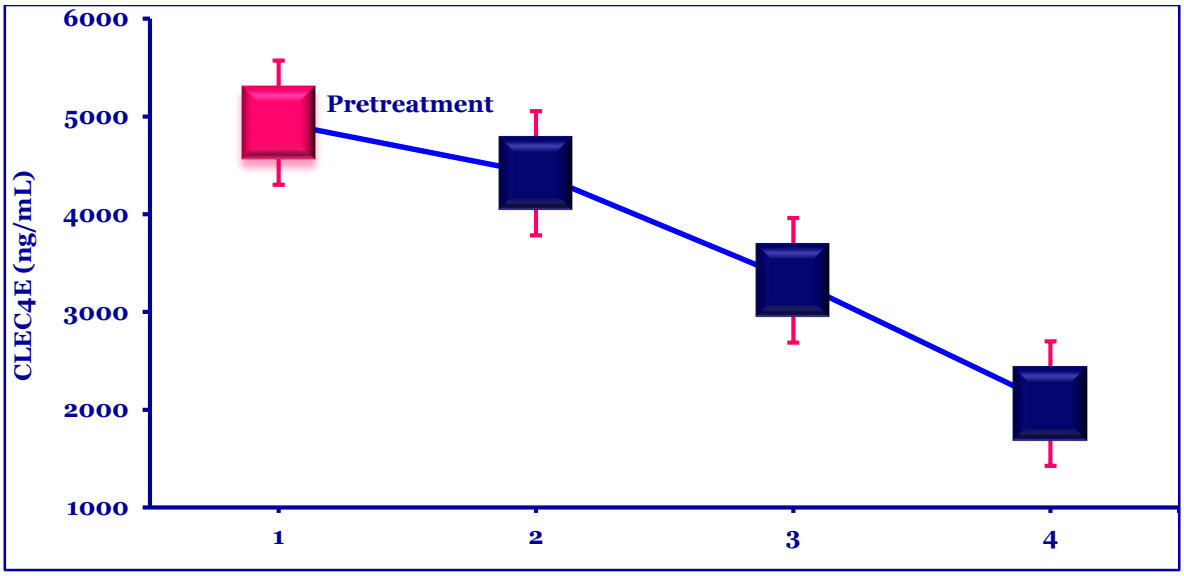


Figure 3: Follow-up of CLEC4E Levels during Consecutive Chemotherapy

The steady decline in CLEC4E levels that occurs during the course of chemotherapy may be an indication of the body's response to the treatment; additionally, it may be a reference to the reduction in the number of cancer cells, which may create a shortfall in the production of CLEC4E. Both of these possibilities are possible explanations for the phenomenon.

In the current investigation, we are going to compare the levels of CLEC4E that were found in breast cancer patients who had received a scheduled dose of follow-up with the levels that were found in the control group. The levels of CLEC4E in the group of cancer patients were significantly lower than those in the group of healthy controls (the levels of the CLEC4E post last chemotherapy dosage were represented approximately 40% of CLEC4E pretreatment), as shown in Figure4. In addition, the levels of CLEC4E in the cancer patients group were lower, but not significantly so, than those in the group of healthy controls.

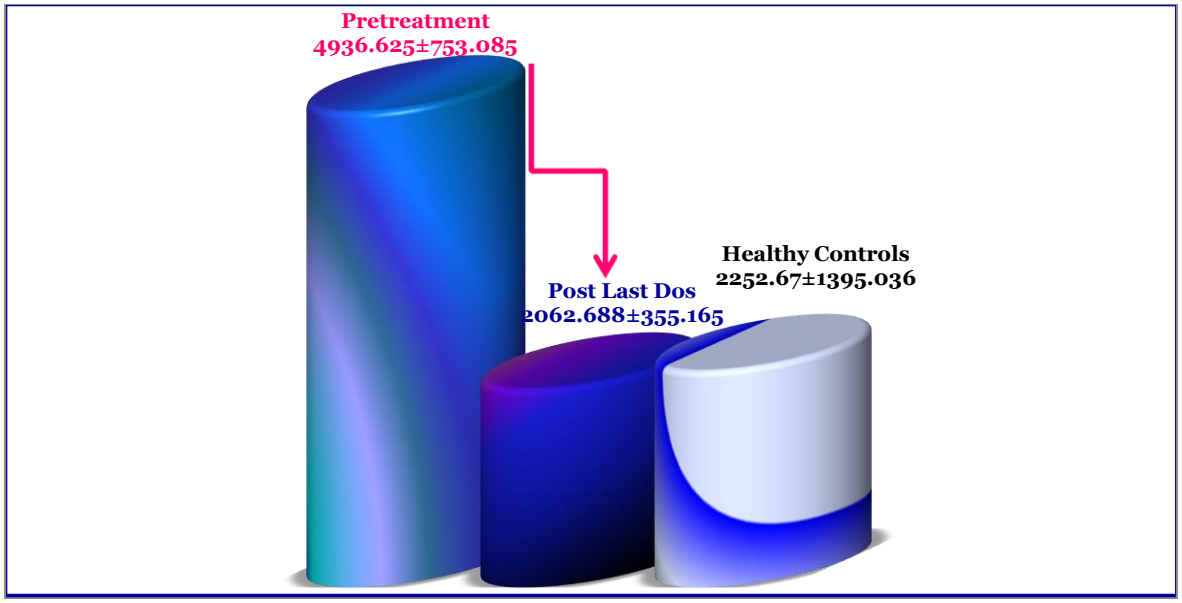


Figure 4: Comparison Levels of CLEC4E in Cancerous Patients at Pre and Post Chemotherapy Treatment

According to the findings of this investigation, the levels of lectin found in the sera of patients suffering from malignant breast tumors were much higher than those found in healthy individuals. On the other hand, the levels of serum lectin in patients who had benign breast tumors stayed within the lectin levels of normal individuals, and the serum of patients who had breast disorders that were not caused by tumors did not exhibit any significant alterations when compared with the serum of healthy individuals. In addition, patients who had benign breast cancers exhibited outcomes that were roughly equivalent to those of healthy individuals.

The ROC-AUC curve was examined in order to provide an illustration of the diagnostic capacity of CLEC4E for breast cancer. When all of the malignant cases displayed CLEC4E levels that were higher than the highest CLEC4E that was observed in the healthy controls, the sensitivity of CLEC4E was determined to be one hundred percent. When 24 of the benign tumor cases were registered with lower CLEC4E levels than the cutoff threshold, the specificity of CLEC4E was calculated to be greater than 80%.

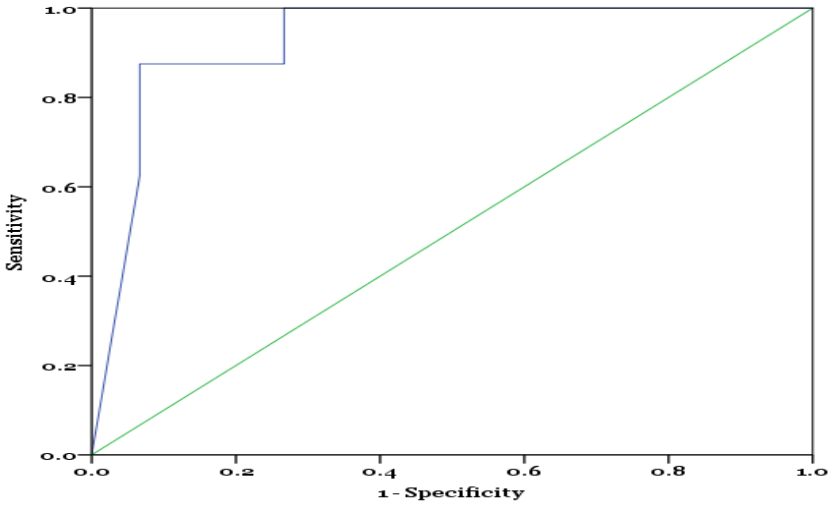


Figure 5: ROC-AUC Curve of CLEC4E

Table 2: Full Information about ROC-AUC test

Area	S. E. [a]	Asymptotic Sig.[b]	Asymptotic 99% Confidence Interval	
			Lower Bound	Upper Bound
0.929	0.051	0.000	0.798	1.000

An increase in CLEC4E levels in malignant tumor specimens may have numerous possible explanations, including the following hypotheses: According to the hypothesis that CLEC4E may play an important role in the immune response of the body by attaching to foreign invaders and (pathogens), which causes inflammation and causes levels of this protein to rise, the increase in the levels of this protein may be due to an exacerbation of the inflammatory condition caused by the progression of the cancerous injury and decreased defensibility [12]. This hypothesis is based on the fact that the cancerous injury causes decreased defensibility.The fact that a significant percentage of patients had a family history of cancer led the researchers conducting this study to make the assumption that a number of factors, including other genetic and environmental factors, are involved in the development of CLEC4E rise and vulnerability to malignant infection.The results demonstrate that an increase in the number of mutation cells is associated with an increase in the concentration of CLEC4E, which in turn is associated with an increase in the number of mutant cells. Additionally, an increase in the concentration of CLEC4E is associated with an increase in the number of mutated cells. As a result of the distinctions between benign and malignant tumors, researchers discovered that patients with benign breast tumors had serum CLEC4E levels that remained within the normal range for healthy individuals. There is a growing body of evidence suggesting that lectins play an active role in the identification of malignant cells. After reviewing the relevant prior research, the authors of the present study hypothesize that theirs is the first study to assess the CLEC4E level in breast cancer patients; nonetheless, this factor has been estimated in a number of diseases.CLEC4E was estimated in a previous non-tumoral study, Kawther's study, which was done on the type 2 diabetics during dialysis. This study revealed a significant difference when comparing renal failure patients undergoing hemodialysis and healthy individuals, where the recorded results showed an increase in the levels of CLEC4E in the sera of the patients group in comparison to the controls group [13].The outcomes of the CLEC4E measurement performed in the current work were in agreement with the findings of Human's study, which focused on the pediatric patients suffering from acute lymphocytic leukemia (ALL) and undergoing chemotherapy. These patients were younger than 12 years old. The levels of CLEC4E in ALL patients were found to be much higher than those seen in healthy children, according to the findings of this study. On the other hand, the levels of CLEC4E were shown to significantly drop in 80% of patients who had received at least two chemotherapy treatments. In contrast to the findings of Hadeer's study, which was conducted on GIT disorders and found a statistically significant reduction in the concentration of CLEC4E in malignant GIT tumor samples in compared to benign GIT tumors, non-tumoral GIT diseases, and healthy individuals, respectively, the results of the current investigation showed an increase in the level of CLEC4E in benign GIT tumor samples. When benign GIT tumors and healthy individuals were examined together, a highly significant variation was seen; however, when non-tumoral GIT were compared with both of the benign GIT tumors and healthy

individuals groups, no such results were recorded [14].

3. Conclusions

Based on results that are unambiguous after a malignant breast tumor has been surgically removed, the CLEC4E protein may be used as a hopeful diagnostic marker to differentiate between a malignant breast tumor and a benign breast tumor. In addition, based on the results that were achieved, CLEC4E has the potential to be an effective tool that may be used to monitor patients' responses to treatment throughout the various phases of chemotherapy.

Recommendations

Evaluating the levels of CLEC4E in patients with breast tumors at the time of diagnosis and before surgical treatment in order to study the possibility of this parameter in diagnosing tumor infection and determining the stage of the cancerous tumor, which may contribute to the designation of CLEC4E as a new marker for this kind of cancer. This evaluation is being done in order to determine whether or not CLEC4E should be designated as a new marker for breast cancer.

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