

Contribution of the seric cytokeartin fragment 21.1 (cyf 21.1) as a tumor marker in a population of algerien women with breast cancer undergoing chemotherapy

Apport du fragment de cytokératine sérique 21.1(cyfra21.1) comme marqueur tumoral chez une population de femme algéroise atteinte de cancer du sein sous chimiothérapie

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ABSTRACT

In Algeria, breast cancer accounts for 37.8% of new cancer cases in women. It is an epithelial cell tumor in most cases. CA15.3 (carbohydrate antigen) is the marker of choice for therapeutic monitoring, although ASCO (the American Society of Clinical Oncology) does not recommend its routine use, preferring CYFRA21.1 (cytokeratin19 fragment) to assess response to treatment. The aim of this study was to estimate the degree of correlation between these two biological markers as a function of tumour histological type and the presence or absence of metastases. In a prospective descriptive study, sera from 110 breast cancer patients (mean age 49 ± 7.23 years) were measured using the electrochemiluminescence (ECL) technique to assess CYFRA 21.1 and CA 15.3 levels. Comparison of CA15.3 and CYFRA21.1 levels between patients and controls (79 controls) showed a highly significant association ($p < 0.0001$) between these two markers and breast cancer. The significant correlation between CA15.3 and CYFRA21.1 ($r = 0.245$; $P < 0.0001$) suggests that reliance on CYF 21.1 for monitoring breast cancer patients undergoing chemotherapy is possible. The results show a good correlation between the two markers, especially for the two histological types luminal a and luminal b+HER2 ($r = 0.602$; $p < 0.0001$, $r = 0.505$; $p < 0.0001$ respectively). This study also assessed the correlation between CA15.3 and CYFRA21.1 levels in patients with and without metastases. A significant correlation was also found between CA15.3 and CYFRA 21.1 in the presence of metastases ($r = 0.472$, $p < 0.0001$). A multi-marker approach could provide a more comprehensive assessment of tumor burden and response to treatment.

Keywords: breast cancer, CA15.3(carbohydrate antigen), CYFRA21.1(cytokeratin19 fragment), therapeutic monitoring.

RÉSUMÉ

En Algérie, le cancer du sein représente 37,8 % des nouveaux cas chez les femmes. C'est une tumeur à cellules épithéliales. CA15.3 (antigène glucide) est le marqueur de choix pour le suivi thérapeutique, bien que l'ASCO (American Society of Clinical Oncology) ne recommande pas son usage routinier, préférant CYFRA21,1 (fragment de cytokératine19) pour évaluer la réponse au traitement. L'objectif de cette étude était d'estimer le degré de corrélation entre ces deux marqueurs biologiques en fonction du type histologique de tumeur et de la présence ou absence de métastases. Dans une étude descriptive prospective, les sérums de 110 patientes atteintes de cancer du sein (âge moyen $49 \pm 7,23$ ans) ont été mesurés à l'aide de la technique d'électrochimiluminescence (ECL) pour évaluer les niveaux CYFRA 21,1 et CA 15,3. La comparaison des niveaux de CA15,3 et CYFRA21,1 entre les patientes et les témoins (79 témoins) a montré une association très significative ($p < 0,0001$) entre ces deux marqueurs et le cancer du sein. La corrélation significative entre CA15,3 et CYFRA21,1 ($r = 0,245$; $P < 0,0001$) suggère qu'il est possible de s'appuyer sur le CYF 21.1 pour surveiller les patientes atteintes de cancer du sein en chimiothérapie. Les résultats montrent une bonne corrélation entre les deux marqueurs, notamment pour les deux types histologiques luminal a et luminal b+HER2 ($r = 0,602$; $p < 0,0001$, $r = 0,505$; $p < 0,0001$ respectivement). Cette étude a également évalué la corrélation entre les niveaux de CA15,3 et CYFRA21,1 chez les patients avec et sans métastases. Une corrélation significative a également été trouvée entre CA15,3 et CYFRA 21,1 en présence de métastases ($r = 0,472$, $p < 0,0001$). Une approche multi-marqueurs pourrait permettre une évaluation plus complète de la charge tumorale et de la réponse au traitement.

Mots clés : Cancer du sein, CA15.3 (antigène des glucides), CYFRA21.1 (fragment de cytokératine19) , Surveillance thérapeutique

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INTRODUCTION

Breast cancer is a real public health problem. It accounts for 37.8% of all new cases of cancer in women, and ranks first in the wilaya of Algiers (1). Most breast cancers are epithelial tumours (carcinomas) that develop from the cell membranes of ducts or lobules ; cancers developed from stromal cells (e.g. angiosarcoma, primary sarcoma, phyllodes tumour) are less frequent. Breast cancer spreads locally, initially disseminating to regional lymph nodes via the bloodstream. Metastases can affect any organ in the body, such as the liver, lungs, bones, brain and skin (2), in which case prognostic and predictive factors guide the clinician in establishing an appropriate treatment regimen based on a set of criteria such as tumor size, lymph node status, grade of differentiation, presence or absence of metastases and tumour markers, making it increasingly a controllable disease in the majority of cases.

Medical biology plays an important role in breast cancer, with the use of tumor or tissue markers to optimize therapeutic decision-making or improve patient follow-up. CA15-3 (Carbohydrate Antigen 15-3) has been used as a serum marker for therapeutic follow-up and for detecting recurrence in breast cancer patients. A high-molecular-weight glycoprotein (300 to 400 kDa) belonging to the mucin family, it is composed of two subunits: the carboxy-terminal subunit, located in the intracellular environment, is involved in cancer formation and cell proliferation; the amino subunit, with a highly glycosylated extracellular domain, interacts with adhesion proteins and inhibits immune cell access to tumors. CA15.3 is encoded by the MUC-1 gene, located on chromosome 1 (1q21-24), and is one of the epitopes of the MUC-1 gene expression product, encoding the PEM glycoprotein (Polymorphic Epithelia Mucin: Mucins are the main component of mucus and are most often synthesized by epithelial cells specialized in storage and secretion functions, of which there are several variants. MUC-1 gene products are implicated in carcinogenesis, notably in epithelial tumors(3) . It can be detected in blood and other biological fluids.(4)

But in 2001, the American Society of Clinical Oncology (ASCO) declared that neither CA 15.3 nor CEA are recommended for routine use in screening, diagnosis and monitoring after primary treatment, or for monitoring response to treatment, or after advanced cancer. In these cases, the Society suggests the cytokeratin fragment 21.1 (CYFRA 21.1) assay(5) . CYFRA21.1 (a soluble structural protein fragment of cytokeratin 19: CK19) is a cytokeratin of the intermediate filament family and the main component of the cell cytoskeleton, as well as a major component of simple epithelia, and its expression persists in the corresponding epithelial tumors. Its molecular weight is relative to 40 KDa. In immunohistochemistry, this fragment is expressed in the cytoplasm of epithelial tumor cells, including lung cancers, certain ovarian and breast cancers, pancreatic and cervical cancers and, more specifically, squamous cell carcinomas .(6)

The objective of this study is to determine the contribution of CYFRA 21.1 as a tumor marker in breast cancer, and whether there is a correlation between the two biological markers: the carbohydrate antigen CA15.3 and the

CYFRA21.1 marker.

The question is: Is there a correlation between the levels of these two markers in breast cancer patients undergoing chemotherapy, depending on the histological type of tumour and the presence or absence of metastases?

METHODS

Sample collection

This was a prospective descriptive study with retrospective recruitment, carried out at ROUIBA hospital central biology laboratory biochemistry unit. This study included Algerian women suffering from breast cancer and followed up at the oncology department of ROUIBA hospital. Inclusion criteria were: age over 18, biopsy-confirmed diagnosis of breast cancer, undergoing chemotherapy, and female. Exclusion criteria were the presence of a second cancer other than breast cancer, including cheek cancer, liver cancer and ovarian cancer.

A control group of healthy women was also included. Inclusion criteria for the control group were: age over 18, absence of cancer and chronic inflammatory diseases. Controls were recruited from volunteers at the Rouiba hospital in the blood transfusion center.

Our study was carried out between November 2023 and March 2024. Epidemiological and immunohistological characteristics of the tumor were analyzed according to the following criteria: age (years) (mean $\pm$ standard deviation), family situation, breastfeeding, history of contraception, family history, circumstances of discovery, performance score of Eastern Cooperative Oncology Group (ECOG) (0: normal activity without restrictions, 1: restricted activity for physical activities, 2: ambulant and able to take care of himself, but bedridden for close to 50%. 3: spends more than 50% of time in bed or in a chair. 4: totally confined to bed or chair), histological type and presence of metastases. The characteristics were reported on an information sheet.

Methods

Blood samples for CA15.3 and CYFRA21.1 were taken from venous blood in tubes containing the anticoagulant lithium heparinate. The fasting is not recommended. blood was centrifuged for 5 minutes at 400rpm to obtain plasma, which was analyzed immediately or frozen for later analysis. Blood samples were analyzed using the electrochemical luminescence technique on the COBAS e411. Normal values for CA15.3 and CYFRA21.1 were <34.5U/mL and <2.37ng/mL respectively.

Informed written consent was taken from each participant for the study. The study got approval from the ethical committee of the university of algiers 1.

The data are collected in Excel and the results are processed using Excelstat 24 software. The Kolmogorov-Smirnov test is used to assess the normality of quantitative variables. Variables that show a normal distribution will be described as the mean $\pm$ standard deviation (SD) and variables that do not follow the normal distribution will be described as the median with the 1st quartile; group means will be compared using the T-Student test and the Wilcoxon

test to compare medians. Correlations are assessed using Pearson's or Spearman's correlation coefficients. Statistical significance is set at $p < 0.05$.

RESULTS

Characteristics of the study population

The epidemiological and immunohistological characteristics of the tumor in the study population are reported in the following table (Table 1).

Table 1. Epidemiological and immunohistological characteristics of the tumor in the study population.

Information n=110	Results
Age (years) (Mean±standard deviation)	49.0 ± 7.2
Family situation	Married (86.3%)
Breastfeeding	Yes (62.7%)
History of contraception	Yes (62.9%)
Family history of cancers	Present (58.2%)
Circumstances of discovery	Individual screening (34.6%)/ Organized screening (36.7%)/ Clinical event (16.3%)/Don't know (12.2%)
Performance score	0 (59.4%)/1 (24.7%)/2(12.8%)/3 (2.9%)/4 (0%)
Histological type	Luminal a (14.5%) /Luminal b (60%)/ HER2+ (19.1%)/ Luminalb+HER2(11.8%)/TN (6.3%)
Metastases	Present (59.1%)

HER2: human epidermal growth factor receptor 2, TN: triple negative. Performance score: 0: normal activity without restrictions. 1:restricted activity for physical activities. 2: ambulant and able to take care of himself, but bedridden for close to 50%. 3: spends more than 50% of time in bed or in a chair. 4: totally confined to bed or chair.

The age distribution of our population is shown in figure 1.

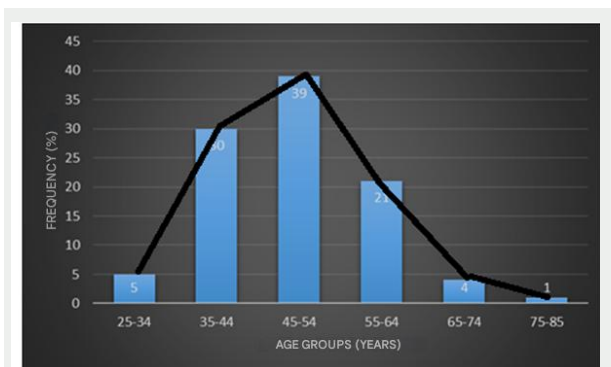


Figure 1. Histogram showing patient distribution by age group.

The association of CA 15.3 and CYFRA 21.1 with breast cancer

Comparison of CA15.3 and CYFRA 21.1 concentrations in patients versus healthy controls to assess the relationship between breast cancer and these two markers yielded the results summarized in Table 2.

A p value < 0.0001 was obtained for both markers, indicating a strong association between breast cancer and these two markers.

Table 2. Comparison of CA15.3 and CYFRA21.1 in patients and controls

The marker	Patients n=110	controls n=79	p-value
CA15.3 (U/mL)	81.7 ±101.0(5.2-1882)	26.3±14.7(1,8-169.6)	<0.0001
CYFRA21.1 (ng/mL)	11.4±14.8(0.8-387)	3.2±1.8(0.3-23.0)	<0.0001

The results are represented as mean±standard deviation (minimum-maximum),CA15.3: Carbohydrate antigen 15.3, CYFRA21.1: cytokeratin fragment 21.1

Degree of correlation between CA15.3 and CYFRA 21.1 with breast cancer according to histological type

Correlation results between these two markers according to histological type are shown in Table 3.

Table 3. Correlation results between CA15.3 and CYFRA21.1 according to histological type.

	CA15.3 (U/mL)	CYFRA21.1 (ng/mL)	correlation coefficient" r"	P-value
All histological types (n=110)	21.6±14.1 (5.2-1882)	2.6 ±1.8 (0.832-387)	0.245	<0.0001
Luminal a (n=16)	21.0±19.7 (9.8-39.5)	2.9 ± 2.3 (1.2-11.9)	0.602	<0.0001
Luminal b (n=66)	24.5±16.6 (5.2-300)	2.5± 1.9 (0.83-74.6)	0.211	<0.0001
HER2+ (n=12)	19,1±15.2 (7.5-1327)	3.0±2.7 (1.1-187.5)	0.246	<0.0001
TN (n=6)	300±45.4 (15.3-1882)	2.3± 2.1 (1.6-387)	0.486	0.017
Luminal b+HER2 (n=10)	20.1±18.3 (7.9-246.7)	2.8±1.7 (1.1- 60.7)	0.505	<0.0001

The results are represented as median±1st quartile (minimum-maximum),HER2: human epidermal growth factor receptor 2, TN: triple negative, CA15.3: Carbohydrate antigen 15-3, CYFRA21.1: fragment of cytokeratin 21.1.

Degree of correlation between CA15.3 and CYFRA 21.1 according to the presence or absence of metastasis

Correlation results between CA15.3 and CYFRA 21.1 according to the presence or absence of metastasis are shown in Table 4.

Table 4. Correlation results between CA15.3 and CYFRA21.1 according to the presence or absence of metastasis.

Presence or absence of metastasis	CA15.3 (U/mL)	CYFRA21.1 (ng/mL)	Spearman coefficient r	p-value
Patients with metastasis (n=65)	24.5±19.1 (6.6-1882)	4.2±3.6 (2-387)	0.472	<0.001
Patients without metastasis (n=45)	20.7±14.5 (5.2-897.5)	1.7±1.2 (0.8-2.2)	0.029	<0.001

The results are represented as median±1st quartile (minimum-maximum), CA15.3: Carbohydrate antigen 15-3, CYFRA21.1: fragment of cytokeratin 21.1

DISCUSSION

The results of this study examine CYFRA21.1 and CA15.3 levels in Algerian women with breast cancer as a function of tumour histology and the presence or absence of metastases. The preliminary results of our study indicate a

significant but weak correlation between the two markers CA 15.3 and CYFRA 21.1 ($r=0.245$; $p<0.001$). However, a more detailed analysis reveals a strong significant correlation between these two markers for the two histological types luminal a and luminal b+HER2 ($r=0.602$ and 0.505 respectively). The presence of metastases also appears to influence the correlation between the two markers.

CA 15.3 is linked to breast cancer, and more specifically to the presence of MUC1. Nevertheless, high serum levels of CYFRA 21.1 have been observed in various malignancies, in particular in lung cancer(7) but also in breast cancer, as it has been clearly demonstrated that breast cancer cells express cytokeratin-19 fragments.(5)

Our results reveal a significant correlation between CA15.3 and CYFRA21.1 levels in general (although $r=0.245$ but $p<0.001$), and if we focus on histological types, we note that there is a strong correlation between these two markers for the two histological types: luminal a and the luminal b+HER2 association (0.602 and 0.505 respectively). This correlation can be explained by the biochemical nature of each marker and the distinct release mechanisms: CA 15.3 is a glycoprotein excreted by the cell surface, whereas CYFRA 21.1 is a polypeptide, which is most likely released after cell death(8) and therefore the mechanism of decrease under chemotherapy is also different. In the case of CA 15.3, chemotherapy destroys cancer cells and consequently reduces tumour mass, which in turn reduces CA 15.3 production. On the other hand, chemotherapy blocks the formation of new blood vessels in the tumor, which reduces the supply of oxygen and nutrients needed by cancer cells to form CA 15.3. In the case of CYFRA 21.1, chemotherapy reduces inflammation, thereby reducing CYFRA 21.1 levels (inflammation increases CYFRA 21.1 levels). Thus, the production and release of CYFRA 21.1 varies from one individual to another, depending on factors such as age, kidney and liver function, and history of inflammatory disease.

In breast cancer patients, CA 15.3 can be used as a primary marker to monitor disease progression and treatment efficacy. However, in cases where metastasis is suspected, the incorporation of additional markers such as CYFRA 21.1 is necessary(9). The significant correlation ($r=0.47$ and $p<0.001$) between CA15.3 and CYFRA21.1 in our metastatic patients suggests a relationship between the two markers, so CYFRA 21.1 may be useful for disease monitoring in cases of metastasis. Rodriguez et al (2002) examined serum titers of CYFRA 21.1, CEA and CA 15.3 in their 40 patients with metastatic breast cancer and found that CYFRA 21.1 was a sensitive tumor marker of breast cancer compared to CEA or CA 15.3(9). Measurement of CYFRA 21.1 may be useful for detecting disease relapse and assessing surgical and chemotherapeutic efficacy in the presence of metastatic disease(10). According to the study by Brothrick et al. CYFRA 21.1 is a fragment of cytokeratin 19, a protein specifically expressed by epithelial cells, and one of the major components of the cytoskeleton(6). Malignant transformation is characterized by changes in the cytoskeletal structure of the cells involved. Cytokeratin (CK) protein and messenger RNA (mRNA) expression were examined in malignant and benign breast tissues. Flow

cytometry results demonstrated a significant correlation between specific protein expression of cytokeratins 8, 18 and 19 and axillary lymph node metastases ($P = 0.01$). Only cytokeratin 19 showed a significant correlation with increased tumour size ($P = 0.006$). The results indicate that cytokeratin expression in breast cancer may be associated with tumor progression(11). However, Giovanella et al (2002) reported that CYFRA 21.1 was less accurate in assessing primary and recurrent breast cancer than CA 15.3(12). Clinicians could use this information to refine their strategies for monitoring cancer patients.

Although our study provides important information on the relationship between CA 15.3 and CYFRA 21.1, it has certain limitations: The number of patients in this study was too small. It is essential to define the patients' metastasis stage in order to clarify the differences in correlation observed in the study population. Unfortunately, this information was not provided in the patient files.

Further prospective studies are desirable, taking into account the following points:

- More patients are needed before the CYFRA 21.1 marker can be recommended for routine clinical use.
- Exploring the other underlying biological mechanisms of release and production that lead to the differential expression of these markers could provide a deeper understanding of their response to chemotherapy.
- The combined use of CA 15.3 and CYFRA 21.1 in conjunction with other emerging markers such as BCA225(13), c-erbB-2(14) and tissue polypeptide antigen(15), CA 549(16) could also improve therapeutic follow-up strategies for patients.

CONCLUSION

This study allowed us to understand the influence of the two histological types of breast cancer: luminal a; luminal b+HER2 and the presence of metastasis on the two tumor markers CYFRA21.1 and CA15.3 levels, and to deduce the relative usefulness of these two markers in monitoring the disease.

Although, further investigations are required to confirm this observation. A multi-marker approach could improve more accurate and comprehensive assessment of tumor burden and response to treatment.

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