The possible role of tumor antigen CA 15-3, CEA and ferritin in malignant and benign disease

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Abstract

Introduction: Serum CA15-3 has been one of the most reliable tumor markers used in monitoring of breast cancer patients. To increase its sensitivity, the combined measurement of other tumor markers (CEA and ferritin) with CA15-3 was investigated. The aim of this study was determination of CA 15-3, CEA and ferritin in female patients with breast cancer, lung cancer and mastitis

Methods: 300 patients with carcinoma, hospitalized at Department of Gynecologic Oncology and Department for Oncology at the University Clinics Center of Sarajevo and 200 healthy subjects were compared.

Results: In patients with breast cancer the mean value of tumor markers were CEA 155.61 ng/mL, CA 15-3 106.38 U/mL and ferritin 197.03 ng/mL. In patients with lung cancer CEA was 58.97 ng/ml, CA 15-3 40.62 U/mL and ferritin 544.16 ng/mL. Patients with mastitis had CEA 5.17 ng/mL, CA 15-3 112.67 U/mL and ferritin 174.92 ng/mL. The control group had values of tumor markers CEA 1.62 ng/mL, CA 15-3 11.72 U/mL and ferritin 85.35 ng/mL. We found good correlation between CA 15-3 and CEA correlation coefficient was r = 0.750. There was a low correlation between CA 15-3 and ferritin with correlation coefficient r = 0.274.

Conclusions: The CA 15-3 and CEA are useful markers in patients with confirmed diagnosis of breast and lung cancers. The ferritin concentration has not increased in patients with breast cancer but it increased in lung patients. The future study has to make investigations of tumor markers and ferritin in different stage of breast cancer.

Keywords: CA15-3, CEA and ferritin

Introduction

Although the measurement of tumor markers in breast cancer has been studied for nearly 20 years, their usefulness remains unclear. In patients with metastatic breast carcinoma, tumor markers appear to be useful during follow-up, but a wide range in rates of marker positivity has been reported: 50%–80% (1-3). Breast cancer is the most common malignancy in women. Successful treatment of breast cancer relies on a better understanding of the molecular mechanisms involved in breast cancer initiation and progression (4). The CA 15-3 concentrations increase was observed in various malignant tumors, but this is a useful marker for breast cancer metastasis and is determined in monitoring disease progression and success of therapy. It is not used as screening test or as a test for primary diagnosis because it has low diagnostic sensitivity (5). CA 15-3 alone, however, is not recommended as a marker for either diagnosis or detection of early recurrence of breast cancer according to the American Society of Clinical Oncology (ASCO) guidelines (6). Because of insufficient data, the ASCO also does not recommend the use of CA 15-3 alone as a marker for monitoring response. It should be noted that the elevation of CA 15-3 between 4 and 6 weeks after initiation of a new therapy, i.e. spurious early rise (surge), indicates poor prognosis. The proportion of patients exhibiting a surge in CA 15-3 level

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after chemotherapy initiation was reported to be 4.8%, and patients with surge may respond more poorly to chemotherapy than those without surge (7). CEA is also not recommended as a marker for diagnosis or routine surveillance after primary therapy, and a surge in CEA level may also occur between 4 and 6 weeks after initiation of primary treatment. The ASCO does not recommend CEA measurement in patients with positive CA 15-3; thus, the CEA level provides only supplementary information. However, if sufficient data regarding other factors are available, rising CEA level can be a useful indicator of treatment failure in patients without measurable disease (8). Efforts are directed to identify new markers of breast cancer, which might be used for early detection, staging and prognosis, and prediction of therapy response. Ferritin is a large macromolecule (450 kDa) which is synthesized in the liver, spleen, myocardium, placenta and other tissues and plays a major role in iron storage. It consists of 24 subunits which form protein shell (apoferritin) around an insoluble core of stored iron. There are two types of subunits, the basic L and acidic H type. Different isoferritins have different proportions of these two subunits (9). Ferritin is a sensitive indicator of iron deficiency, thus the main clinical application of serum ferritin measurement is in differential diagnosis of anaemia. Ferritin concentration may increase in case of iron overload (haemochromatosis or haemosiderosis), infection or inflammation, neurodegenerative disorders, malignancies and destruction of liver tissue (10). Ferritin is one of the proteins whose concentration may be altered due to breast cancer presence. Recent studies have suggested a crucial role of perturbations in ferritin levels and tightly associated with this, the deregulation of intracellular iron homeostasis; however, the underlying molecular mechanisms for the cancer-linked ferritin alterations remain largely unknown and often with conflicting conclusions (11). Therefore, this study was undertaken to define the role of ferritin in breast cancer. We have make determination of CA 15-3, CEA and ferritin at patents with breast or lung cancer and patients with diagnosis of mastitis. At our investigation we try to find a possible correlation between tumor marker CA 15-3 with tumor marker CEA and ferritin in patients with cancer.

Methods

Patients

The investigation included patients (n= 500) in period from February till October in 2011. It was retrospective study ant we included female patients with diagnosis breast cancer, lung cancer or with diagnosis of mastitis. All of 300 patients were hospitalized at Department of Gynecologic Oncology and Department for Oncology at the University Clinics Center of Sarajevo and 200 healthy subjects. The mean age of patients with cancer was 45.32-/+ 9.23, patents with mastitis 35.24 +/- 5.64 and mean age of control group was 43.45 -/+ 2.85. The patient samples of blood were collected in serum separation Vacutainer test tubes (Beckton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL. We used test tubes with gel. Serum samples were obtained by centrifugation at 3000 rpm using centrifuge (Sigma 4-10). After centrifuging, serum concentration of CEA, CA 15-3 and ferritin was determined. The investigation was done respecting ethical standards in the Helsinki Declaration.

Chemiluminescent microparticle immunoassay – CMIA

All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody. A label is a molecule that will react as a part of the assay, so a change in signal can be measured in the blood after added reagent solution. CMIA is noncompetitive sandwich assay technology to measure analytes. The amount of signal is directly proportional to the amount of analyte present in the sample. Architect ferritin, CEA and CA 15-3 assay is two-step immunoassay to determine the presence antigen in human serum using CMIA technology. In the first step, sample, assay diluent and anti-antibody-coated paramagnetic particles are combined. Ferritin, CEA or CA 15-3 present in the sample binds to the anti-coated micro particles. After incubation and wash, anti-acridinium-labeled conjugate is added in the second step. Following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The pre-trigger solution (hydrogen peroxide) performs the following functions:

- Creates an acidic environment to prevent

early release of energy (light emission).
- Helps to keep microparticles from clumping.
- Splits acridinium dye off the conjugate bound to the microparticle complex. This action prepares the acridinium dye for the next step.

The trigger solution (sodium hydroxide) dispenses to the reaction mixture. The acridinium undergoes an oxidative reaction when exposed to peroxide and an alkaline solution. This reaction causes the chemiluminescent reaction to occor. N-methylacridone forms and releases energy (light emission) as it returns to its ground state. The resulting chemiluminescent reaction is measured as relative light units (RLU). A direct relationship exists between the amount of ferritin, CEA or CA 15-3 in the sample and RLU detected by Architect System optics (8). The normal serum range of CA 15-3 between 0. 0 – 31.3 U/mL, CEA 0-5.00 ng/mL and ferritin 4.63 – 204.00 ng/mL.

Statistical analysis

The results were statistically analyzed using NCSS and statistical software SPSS version 12.0 software. Determined by the average value ( ), standard deviation (SD), Pearson correlation coefficient (r), equations of linear regression and Student t test with statistical significance level of $p < 0.05$.

Results

The CA 15-3 is cancer antigen that is used in the management of some patients with breast cancer. It is most effective at monitoring metastatic breast cancer, but has not had high success at detecting early stage breast cancers. Many studies are still conducted with the purpose of finding markers that could be used for early diagnosis and/or serve as possible reliable prognostic or predictive parameters, but with conflicting results. At present, no markers are available for an early diagnosis of breast cancer. The surveillance of patients with diagnosed breast cancer the most widely used serum markers are CA 15-3 and CEA which, in combination with other clinical parameters, could have clinical significance. The raised of serum ferritin concentrations in breast carcinoma patients might be attributed to stromal reaction rather than to tumor synthesis. In our study we have a female patients with diagnosis of breast and lung cancer. The patients with lung cancer have a primary cancer in breast. The

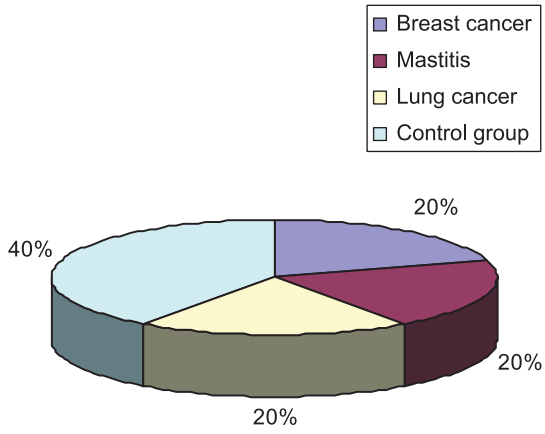

FIGURE 1. The patient diagnosis involved in the study

patients with cancer were hospitalized in Department of Gynecologic Oncology and Department for Oncology at the University Clinics Center of Sarajevo. The patients with mastitis were hospitalized in Department of Gynecologic at the University Clinics Center of Sarajevo. The percent of patients in our study is show in Figure 1. Abnormal CEA (>5 ng/mL) or CA 15.3 (>30 U/mL) serum concentrations were found in 15.4% and 27.2 % of the patients studied, respectively. In patients with breast cancer the mean value of tumor markers were CEA 155.61 ng/mL, CA 15-3 106.38 U/mL and ferritin 197.03 ng/mL. Our study have got results in patient with lung cancer CEA 58.97 ng/ml, CA 15-3 40.62 U/mL and ferritin 544.16 ng/mL. The results of our study have shown that the patients with mastitis have CEA 5.17 ng/mL, CA 15-3 112.67 U/mL and ferritin 174.92 ng/mL. The control groups have value of tumor markers CEA 1.62 ng/mL, CA 15-3 11.72 U/mL and ferritin 85.35 ng/mL. Serum levels for the three tumor markers in patients with metastatic diseases were significantly higher than those in patients without metastasis. This suggests that serum CA15-3 is the most reliable monitoring marker in patients with metastatic diseases. The CEA and ferritin were high in patients with lung cancer then CA 15-3 and it could be explain that CEA is more specific for lungs then CA 15-3. The raises serum ferritin in tumors might be due to tumor synthesis because the ferritin is reactant of acute phase. The mean value of tumor marker in our study is shown in Figure 2. We compared CA 15-3 and ferritin in patients with

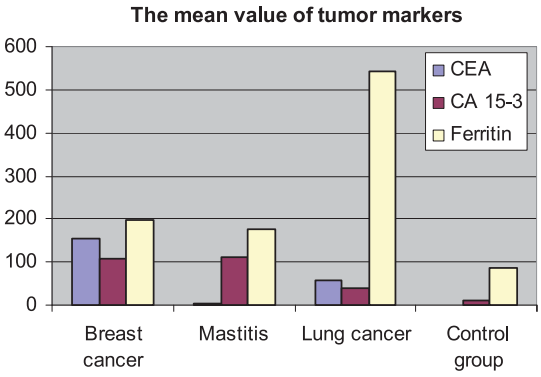

FIGURE 2. The mean concentration of CEA. CA 15-3 and ferritin in patients with tumor and benign disease

cancer in 200 blood samplers (r = 0.274), results is shown in Figure 3. Regression equation revealed a slope of 0.5597 and a y axis intercept of 264.64. The difference between the tumor marker CA 15-3 and ferritin was statistically significant for $p < 0.05$ according Student t-test. The low correlation (r = 0.274) was found between CA 15-3 and ferritin. The results of our comparison CA 15-3 and CEA are shown in Figure 4. Regression equation revealed a slope of 1.4334 and a y axis intercept of 118.39, correlation coefficient was r = 0.75. The difference between the tumor markers was statistically significant for $p < 0.05$ according Student t-test. The coefficient of correlation was r = 0.75, so we have good found correlation between CA 15-3 and CEA.

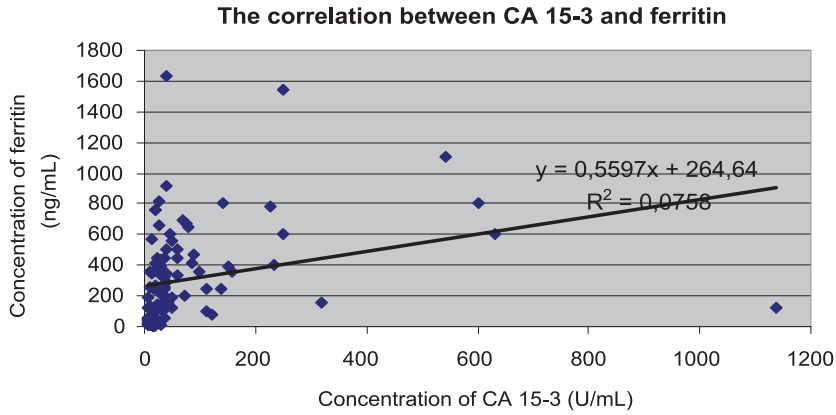

FIGURE 3. Comparison of CA 15-3 and ferritin in serum measured by Architect CMIA. The correlation coefficient r = 0.274.

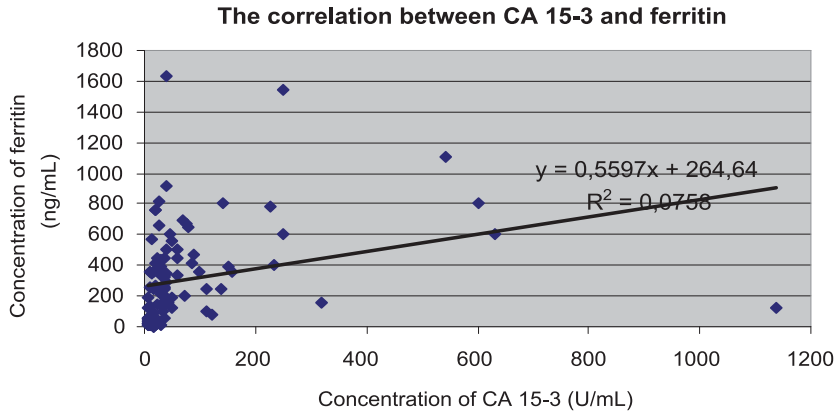

FIGURE 4. Comparison of CA 15-3 and CEA in serum measured by Architect CMIA. The correlation coefficient r = 0.75.

Discussion

The results of this study indicate that of the three tumor markers tested, serum CA15-3 is the most sensitive and specific in terms of the detection of breast cancer metastases. In our study the average concentration of CEA and CA 15-3 was higher in patients group with primary breast cancer, a results are shown in Figure 2. The CA 15-3 was higher in breast cancer but lower than CEA in lung cancer. The patients with mastitis diagnosis the have higher concentration of CA 15-3 but normal concentration of CEA. Serum CA15-3 has been one of the most reliable tumor markers used in monitoring breast cancer patients. It has been reported that the sensitivity and specificity of serum CA15-3 for detecting metastatic diseases are higher than those of CEA (12,13). In our study the concentration of CEA was mostly elevated in patients with breast and lung carcinoma. The recent study has reported that serum levels of CEA were high at patients with adenocarcinoma and squamous carcinoma (14). The other investigator has shown that CEA is significantly related to differential degree of lung cancers (15). The different staining patterns and positive rates and intensities of CEA may be helpful for the pathological classification of lung cancers. The CEA concentration was in reference rage in patients with mastitis and controls. In this study patients with cancer particularly with lung cancer have higher concentration of ferritin. This might be due to the potential additional regulators involved in ferritin synthesis. Iron is the main, but not the only regulator of ferritin expression. Hypoxia, often present in neoplastic tissue, is also one of the factors that promote ferritin increase independently of the iron status (16, 17). The ferritin concentrations may be a prognostic indicator in some patients with lungs and breast cancer. In this study the highest levels of ferritin were obtained in the group of patients with advanced disease, which was in agreement with previous reports (18). The other study groups have found that ferritin has been previously associated with breast cancer. Still, there is no consistent conclusion regarding its role or relevance in breast cancer (19). The possible limitation of our study is that we do not have information about ferritin level in patients before cancer or mastitis. Linear regression analysis of the values for the tumor markers revealed that serum CA 15-3 values were not correlated with serum ferritin values ($r = 0.274$) but it was good correlation with serum CEA values ($r = 0.750$). The correlation results are shown in Figure 3 and 4. In other investigation groups it was found that good correlation between CA 15-3 and CEA with coefficient of correlation $r = 0.96$ (20).

Conclusion

Serum concentrations of CEA, CA 15-3, are related to tumor extent, with significantly higher values seen in patients with breast cancer. The CA 15-3 is only specific in patients with metastasis because we have got high concentration in patients with mastitis. The serum value of CEA was higher in patients with lung cancer. Further prospective studies, of a large number of subjects premenopausal and postmenopausal women, are required to confirm such a statement and to validate the usefulness of ferritin determination in the serum of lung or breast cancer patients.

Conflict of interest

Authors declare no conflict of interest.

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