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Serum ceruloplasmin as a diagnostic marker of cancer

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Abstract

The pursuit of the ideal tumor marker has generated many tests for use in the diagnosis and management of cancer, several of which are now widely available. The objective of this study was to evaluate the diagnostic utility as a cancer marker of plasmatic levels of ceruloplasmin. Ceruloplasmin is a glucoprotein that transports serum copper. A case-control design was used. Serum values were evaluated in 144 patients and 103 normal controls by receiver operating characteristic (ROC) curve analysis to define the optimal cut-off levels for the serum values of ceruloplasmin in the diagnosis of cancer. The ROC analysis showed that ceruloplasmin is considerably sensitive in men (80%) at the specificity level of 80.3% and in women the sensitivity (Se) was (63.2%) and the specificity (Sp) was (63.3%). According to this study, it would seem optimal to use the cut-off level of 358 mg/l in men and 383 mg/l in women. In conclusion, serum ceruloplasmin was significantly elevated in advanced stages of solid malignant tumors, however, locally advanced or locoregionally spreading tumors did lead to significant increases ($P < 0.01$). Finally, the results of ROC curve analysis suggest that the ceruloplasmin is characteristic of good diagnostic markers. © 1997 Elsevier Science Ireland Ltd.

Keywords: Ceruloplasmin; Tumor marker; Cancer; Neoplasms

1. Introduction

Ceruloplasmin (blue plasma protein) is a glucoprotein that transports serum copper 90–95% [1], or 60% [2]. The molecular structure of this 132 kDa protein consists of a 1046 amino acid-long polypeptide chain and four linked oligosaccharides [1,3].

Ceruloplasmin is synthesized in the liver, from whence it transports copper to tissues that need this element for metalloenzyme functioning. The presence of specific membrane receptors for ceruloplasmin on tissues was first shown in the wall of the aorta by Stivens et al. [4]. Synthesis of the protein is regulated by HSF III/II (hepatocyte-stimulating factors) and by interleukin-6 (IL-6), both of which are potentiated by glucocorticoids [5]. Ceruloplasmin can also be synthesized by tumor cells [6,7].

In addition to its role in copper homeostasis and transport, ceruloplasmin shows ferro-oxidase, amino-oxidase and superoxide dismutase activity [1] and can mobilize copper and act as an acute phase reactant [8]. It also displays a weak direct immunomodulating action [9] or indirect modifying plasma copper [10].

Conforti et al. [11] reported a clear correlation between increases in ceruloplasmin and serum copper

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concentrations in normal rats and animals with experimental inflammation. Similar findings have also been reported in humans [12–14]. Factors that can increase serum concentrations of ceruloplasmin in humans include physical exercise, third trimester pregnancy, ovarian hyperfunction, arteriosclerosis, epilepsy, chronic inflammatory processes (e.g. alcoholic and biliary liver cirrhosis, active chronic hepatitis, rheumatoid arthritis, pyelonephritis and rheumatoid spondylitis) and malignant tumors (e.g. of the stomach, lung and bone and Hodgkin's disease) [15].

Transitory reversible increases have been reported in women taking oral contraceptives and persons with acute myocardial infarction [16].

Some years after the relation between elevated serum copper levels and some malignant tumors was first established [17–19], it was found that the increase in serum copper was traceable mainly by an increase in ceruloplasmin [20,21]. This raised the possibility of using the protein as a diagnostic marker for cancer with initial work centering on breast cancer [22,23].

Experimental work with Yoshida sarcoma and Zajdela ascites hepatoma [24] showed that five acute phase reactant proteins, including ceruloplasmin, underwent biphasic changes with small changes in the short initial phase and a large increase during the second phase that was proportional to increasing tumor mass up to the time of death. This work laid the scientific foundation for the clinical use of ceruloplasmin in monitoring tumoral progression, rather than as an aid to early diagnosis.

To test serum ceruloplasmin for the diagnosis of solid tumors, Linder et al. [21] measured its oxidase and antigenic activity and determined its relation with serum copper levels. Although all three approaches were similarly effective, the simplest and most economical method was to measure antigenic activity. High preoperative values correlated with a poor prognosis and elevated postoperative values correlated with residual tumor or recurrences. In a study of 145 patients with different types of solid tumor, Chakraborty et al. [25] concluded that ceruloplasmin is an excellent marker for monitoring tumoral involution after treatment. Arumanayagam et al. [26] found that ceruloplasmin was able to distinguish between large inoperable tumors of the cervix and early carcinomas and could thus be used as a prognostic marker. Protein malnutrition can influence the alterations in acute phase protein levels and both the cachexia itself and the alterations in acute phase proteins can contribute to the development of infection in patients with tumors [27].

In an earlier study of serum ceruloplasmin as a diagnostic marker for cancer, we found that the highest results obtained with a radial immunodiffusion method were less accurate and less reproducible than nephelometric values. The diagnostic utility of serum alpha-1-alpha-antiprotease (A1AP), measured by nephelometry, was also tested in cancer patients [29]. In the present study we used this method to measure serum ceruloplasmin and A1AP simultaneously, in order to compare the sensitivity and specificity of these two tumoral markers when used individually and jointly.

A diagnostic marker of cancer is a statistical predictor of cancerous disease. Such markers can potentially be used for screening, positive diagnosis, prognosis and monitoring of tumoral disease [30]. The statistical parameters that define the effectiveness of a tumoral marker are its sensitivity, specificity, positive and negative predictive value, positive and negative diagnostic weight, receiver operating characteristics (ROC curve) [30] and a cut-off value that effectively distinguishes patients (cases) from healthy individuals (controls).

2. Subjects and methods

A case-control design was used in which the observer was blind to whether a given serum was a case or a control sample. Cases were confirmed by histological examination and normal control subjects were healthy individuals with no significant antecedents of disease and no signs or symptoms of disease, who fulfilled statistical criteria of normality.

Patients were selected in a non-random manner as consecutive patients who had had a solid malignant tumor within the preceding year and were studied prior to treatment. In all cases the diagnosis was confirmed histologically; some patients were included in the study before histological confirmation was received and 38 cases were recurrences. A total of 144 patients (71 men and 73 women, mean age

51.05 years (range 49–53 years)) and 103 normal control subjects (47 men and 56 women, mean age 37 years (range 13–63 years)) participated. Table 1 shows the locations of the tumors in the patient group.

Serum samples were obtained by venipuncture on the patients and normal control and all serum samples were stored frozen at −20°C until analyzed.

As the main predictors we measured serum ceruloplasmin and A1AP by nephelometry with a Behring Nephelometer 100 analyzer (Behringwerke AG, Marburg, Germany) and specific antibodies (Behring Institute, Marburg, Germany). Calibration curves were prepared for each batch [31]. As dependent variables we recorded case/control status, anatomoclinical diagnosis of the tumor and histological diagnosis and tumor stage (TNM). Tumors were classified as group I (localized in the organ of origin), group II (regional extend disease) and group III (disseminated tumor or tumor spreading via the bloodstream or lymphatic system).

The results were analyzed statistically with conventional methods (Student's *t*-test and logistic regression) included in the SYSTAT statistical package [32]. Confidence limits for mean values were found with the method of Gardner and Altman [33] and cut-off values, sensitivity and specificity were calculated from normal distribution curves for serum ceruloplasmin in patients and healthy controls according to Strike [34]. the diagnostic weight was found with the method of Rembold and Watson [35] and ROC were obtained with the method of Weinstein and Fineberg [36]. Sensitivity and specificity of plasma ceruloplasmin and A1AP were calculated with the procedure of Roulston and Leonard [37].

3. Results

3.1. General findings

The mean serum ceruloplasmin concentration was 450 mg/l (95% CI 426–473 mg/l) in patients and 324 mg/l (95% CI 305–342 mg/l) in healthy control subjects. The difference was significant at $P < 0.001$.

The mean serum A1AP concentration was 409 mg/l (95% CI 378–439 mg/l) in patients and 257 mg/l (95% CI 239–274 mg/l) in control subjects. This difference was also significant at $P < 0.001$.

Table 1

Locations of the tumor in the group of patients with cancer

	n	Men	Women	Age range (years)
Control subjects	103	47	56	13–63
Cancer patients	144	71	73	49–53
Lung	24	24	0	
Breast	47	0	47	
Head and neck	26	26	0	
Gastrointestinal	14	7	7	

3.2. Sex differences

In men, the mean serum ceruloplasmin concentration was 296 mg/l (95% CI 270–310 mg/l) in control subjects and 460 mg/l (95% CI 430–480 mg/l) in patients ($P < 0.001$). In women, the mean serum ceruloplasmin concentration was 346 mg/l (95% CI 310–370 mg/l) in control subjects and 440 mg/l (95% CI 400–470 mg/l) in patients ($P < 0.001$). The difference between control men and control women was significant at $P < 0.05$.

The mean serum concentration of A1AP in men was 252 mg/l (95% CI 237–266 mg/l) in control subjects and 425 mg/l (95% CI 385–464 mg/l) in patients ($P < 0.001$). In women, the mean serum A1AP concentration was 261 mg/l (95% CI 231–290 mg/l) in control subjects and 394 mg/l (95% CI 347–440 mg/l) in patients ($P < 0.001$). The difference between control men and control women was not significant ($P > 0.05$).

3.3. Age differences

No correlation was found between serum ceruloplasmin and age in healthy subjects or patients of either sex ($P > 0.05$).

3.4. Tumor location

In 24 patients with lung cancer, the mean serum ceruloplasmin value was 469 mg/l (95% CI 415–522 mg/l). In 47 patients with breast cancer, the mean value was 407 mg/l (95% CI 358–457 mg/l). In 26 patients with larynx cancer, the mean value was 466 mg/l (95% CI 424–508 mg/l). In 14 patients with gastrointestinal cancer, the mean value was 546 mg/l

(95% CI 480–612 mg/l). Other locations were too infrequent to analyze separately. The four groups mentioned above differed significantly from same-sex healthy controls. However, significant differences in comparison with the whole patient group were found only for larynx ($P < 0.01$) and gastrointestinal tumors ($P < 0.01$).

3.5. Tumor volume

The mean serum ceruloplasmin concentration was 404 mg/l (95% CI 391–416 mg/l) in 27 patients with a group I tumor, 423 mg/l (95% CI 384–461 mg/l) in 39 patients with a group II tumor and 581 mg/l (95% CI 543–619 mg/l) in 40 patients with a group III tumor. The rest of the patients, 38 cases, had recurrences or tumors that could not be classified with certainty on tumor volume and were excluded from this analysis. There was no significant difference between values in patients with group I and group II tumors ($P > 0.05$), but the difference between group I and group III patients was significant at $P < 0.05$.

3.6. Determining the cut-off value

The cut-off value for serum ceruloplasmin was 358 mg/l in men and 383 mg/l in women. For A1AP the cut-off value was 306 mg/l in both men and women.

3.7. Diagnostic utility

Diagnostic utility indices were calculated from the normal distribution curves in healthy control subjects and patients with the cut-off value of each group. In men, the sensitivity (Se) of serum ceruloplasmin was 80%, the specificity (Sp) was 80.3%, the positive diagnostic weight was 4.1 and the negative diagnostic weight was −1.39. In women, Se was 63.2%, Sp was 63.3%, the positive diagnostic weight was 0.54 and the negative diagnostic weight was −0.54.

For A1AP, Se was 70.7% and Sp was 70.3% in both men and women. The positive diagnostic weight was 0.86 and the negative diagnostic weight was −0.87 in both sexes.

The overall comparative Se in men was 77 46% for serum ceruloplasmin and 68.05% for A1AP. The comparative Sp was 73.22% for ceruloplasmin and 83.3% for A1AP [37]. We did not calculate the comparative Se or Sp for women, as serum ceruloplasmin concentrations vary according to hormonal influences.

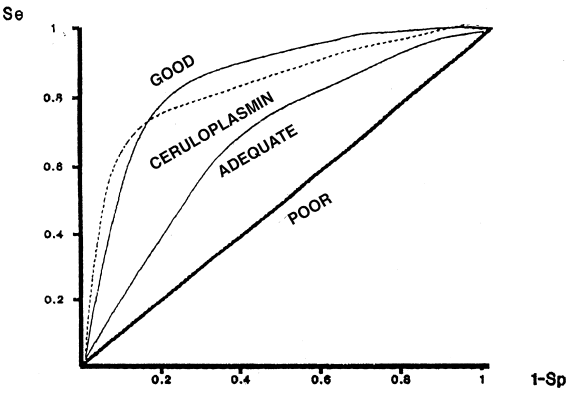

Fig. 1. Receiver operating characteristics (ROC) curves for serum ceruloplasmin.

3.8. Receiver operating characteristics curves (ROC)

Plots of the ROC curves showed that serum ceruloplasmin can be considered as an acceptable to good diagnostic marker according to the criteria of Weinstein and Fineberg [36] (Fig. 1).

3.9. Logistic regression

Linear regression with a constant showed an Se of 65% for both markers used jointly; with the same model very similar values were obtained for ceruloplasmin (63%) and A1AP (62%) separately. Joint Sp was calculated at 51% with similar values for ceruloplasmin (49%) and A1AP separately (48%). There were no significant differences between the joint use of both markers and the use of either one separately ($P > 0.05$).

4. Discussion

Serum ceruloplasmin was significantly elevated in metastasic disseminated solid malignant tumors; the difference was significant in comparison with healthy control subjects and patients with smaller tumors in the same location. Localized tumors were associated with non-significant increases in serum ceruloplasmin (group I). However, locally advanced or locoregionally spreading tumors (group II) did lead to significant

increases ($P < 0.01$). Although the increase in this tumoral marker was proportional to tumor volume (tumoral burden), the exact proportion of increase attributable to each of these factors remains to be determined.

The small increase in serum ceruloplasmin in patients with a localized tumor is compatible with experimental findings and with the subsequent increase in later stages of tumoral development [24]. This marker therefore cannot be used for the early detection of malignant tumors, although it is useful in monitoring later tumoral growth.

Although ceruloplasmin is an acute phase reactant, this does not detract from its diagnostic usefulness. Sensitivity, specificity and diagnostic weight are more important determinants of diagnostic usefulness than the mechanisms that increase serum ceruloplasmin levels and these three variables were found to be acceptably accurate markers in the present study. In addition, ceruloplasmin, like carcinoembryonic antigens, can be produced by tumoral cells [6,7,21].

In cancer patients, serum ceruloplasmin concentrations correlate significantly with A1AP, another acute phase reactant that is also used as a marker of cancer. In normal individuals there is no correlation between serum values of these two proteins. However, in patients with cancer the correlation is statistically significant. The mechanisms that increase circulating levels of both proteins in cancer may be similar or even the same, although they may be regulated independently in persons without cancer.

The highly significant difference in the mean serum values of ceruloplasmin between men and women has important implications for the use of this protein as a diagnostic marker. Like other authors [2,21,28], we found that normal values, Se, Sp and diagnostic weights differed between men and women, a finding apparently related with the fact that estrogen production is greater in women [15,21]. The lower diagnostic utility of ceruloplasmin in women appears to be traceable to the higher normal value in healthy women, which is not translated into a proportional increase in serum levels in women with cancer. We found no age-related differences in serum ceruloplasmin in healthy subjects or patients.

Several factors make serum ceruloplasmin a useful diagnostic marker of cancer. The values differ clearly between patients with neoplasms and healthy controls when men and women are compared separately. Its Se is relatively high (80.0% in men, 63.2% in women) and is better than the Se of A1AP. In addition, it shows relatively high Sp (80.4% in men, 63.3% in women) and lacks organ specificity (in contrast with many other tumor markers). Its positive diagnostic weight is good in men, although less favorable in women. Finally, its ROC curve is characteristic of good diagnostic markers [36].

The joint measurement of serum ceruloplasmin and A1AP in a given patient did not improve the diagnostic efficacy of ceruloplasmin determination alone. This finding is similar to the results of earlier studies in patients with larynx cancer, who were found to have higher serum ceruloplasmin values than healthy controls. Andrzejewska et al. [38] reported a correlation between serum ceruloplasmin and clinical stage of larynx cancer and Krecicki et al. [39] noted that this determination was useful in the prognosis and monitoring of these patients.

The nephelometric determination of serum ceruloplasmin is an automated technique that is as simple and economical as radial immunodiffusion. Although nephelometric values are significantly lower than those obtained with radial immunodiffusion, their diagnostic usefulness is greater.

It would be useful to know whether the increase in serum ceruloplasmin in patients with advanced tumors occurs early enough to be useful as a clinical indicator. Another potentially useful avenue for future studies may well be the search for evidence of a correlation between serum ceruloplasmin and Ca 15.3 [40], which would support the use of the former in monitoring patients with breast cancer and other solid carcinomas (lung, gastrointestinal, head and neck).

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