Use of the Serum Copper/Zinc Ratio in the Differential Diagnosis of Ovarian Malignancy

Abraham Lightman, Joseph M. Brandes, Nlr Binur, Arle Drugan, and Oren Zinder

We measured copper and zinc in serum (n = 82) and tumor tissue (n = 41) from women with a palpable pelvic mass, admitted for suspected ovarian tumor. In serum, copper was increased and zinc decreased in the group (n = 40) subsequently proven to have a malignant ovarian tumor. The mean copper/zinc ratio in this group was 2.30 (SD 0.41), significantly (p < 0.001) higher than for the benign group (n = 42), 1.43 (SD 0.22). In tumor tissue the concentrations of copper and zinc followed the same pattern, the copper/zinc ratio being significantly (p < 0.001) higher in the malignant (0.158, SD 0.065) tumors than in the benign tissue (0.093, SD 0.04).

We conclude that the Cu/Zn ratio in serum reliably (specifically) indicates the presence of advanced ovarian malignant tumors. The decreased concentrations of zinc and the increased concentrations of copper in serum do not seem to result from a shift of zinc into or release of copper out of the malignant tumor tissue.

Additional Keyphrases: cancer • trace elements • atomic absorption spectrophotometry • cutoff value

The biological role of copper and zinc, as well as fluctuations in their concentrations in various physiological and pathological conditions, has been intensively investigated in recent years. In a comprehensive review, Prasad (1) described the clinical, biochemical, and pharmacological role of zinc. Concentrations of zinc in serum decrease in several pathological situations, e.g., some forms of malignancy (2,3) and liver disorders (4) and in myocardial infarction, ulcers, nephrotic syndrome, Down's syndrome, and multiple myeloma (5). Special interest has been focused on the role of zinc in nutrition and malabsorption (6), zinc having been shown to be essential for growth and development (7). Andrews (8) demonstrated a marked decrease in zinc concentrations in plasma from patients with carcinoma of the bronchus, but pointed out that zinc was also low in some other types of malignant solid tumors.

Andrews also demonstrated (7) an increase in the concentrations of copper in serum parallel to the decrease in zinc. Abnormally high concentrations of copper have been observed in serum from patients with various malignancies, especially Hodgkin's disease (8) but also non-Hodgkin's lymphomas (9) and acute leukemia (10). Inutake and Araki (11) demonstrated a significantly higher copper/zinc ratio in serum of patients with gastrointestinal cancer than in normal controls.

These changes in the concentrations of trace metals in serum prompted us to investigate whether there are also such changes in gynecological—specifically, ovarian—malignant tumors, and whether this reciprocal relationship between zinc and copper concentrations in serum can be used for diagnostic purposes.

Materials and Methods

Reagents. All reagents were of analytical grade. Only doubly distilled water was used in preparing solutions, for use as blanks, and for rinsing the equipment. Trichloroacetic acid (TCA) was from Fluka. All equipment was thoroughly cleaned with dilute (0.1 mol/L) HNO3 and rinsed well.

Standards. Copper and zinc standards (1 g/L) were prepared by dissolving 1 g of the pure metal ("Ventron", Alfa Products, Beverly, MA) in 40 mL of an equivalent solution of hydrochloric acid and water, then diluting to 1 L with water. They were stored in a tightly stoppered volumetric flask. On the day of determination, we diluted the stock solution with water to give the following concentrations of working standards: 0.5, 0.75, 1.00, 1.50, 1.75, 2.00, and 3.00 mg/L. Just before use in an assay, we diluted both the copper and the zinc working standards with an equal volume of 250 g/L TCA solution and mixed well.

Serum preparation. At the time of admission we obtained pre-prandial specimens of venous blood from 82 women hospitalized for suspected ovarian tumor (palpable pelvic mass). The blood was drawn into specially cleaned plastic disposable test tubes and stoppered. Serum was separated by centrifugation (1000 × g, 10 min) and duplicate samples were mixed with an equal volume of 250 g/L TCA solution. After letting the tubes stand on ice for 30 min to ensure complete precipitation of protein, we centrifuged them (1500 × g, 15 min) and removed the supernatants and stored them at -4°C until determination.

Tissue processing. Duplicate tissue samples (about 200 mg wet weight each) were taken from different areas of the tumor, weighed, and placed in a Teflon-lined stainless-steel decomposition vessel (Unisal Co. Ltd., Haifa, Israel). We added 2.5 mL of concentrated (18 mol/L) nitric acid, placed the closed vessel in an oven at 120°C for 2 h, then allowed the vessels to cool at room temperature for 30 min. The acid solution containing the decomposed tissues was transferred to a drying vessel (e.g., a small Erlenmeyer flask), from which the liquid was removed by boiling on a hot plate in a fume hood. We dissolved the residue in 2.5 mL of 0.1 mol/L HNO3, then kept this solution at -20°C until assay. Before assay for copper and zinc, we added 2.5 mL of 250 g/L TCA solution, then followed the precipitation steps as for serum.

Trace-metal determinations. We determined copper and zinc with a Varian 1200 atomic absorption spectrophotometer set at 324.7 and 213.8 nm, respectively, with a 2-mm slit width and an air–acetylene flame. Within-batch and day-to-day coefficients of variation were consistently <10%.

Samples were aspirated directly from the supernate remaining after the TCA protein-precipitation step. Concentrations were determined by comparison with the standard curves obtained with the TCA-diluted standards.

Tumor identification. Diagnosis of the suspected tumor was based on histological determinations (biopsies were
taken during laparotomy). Staging of the malignancy was based upon clinical and surgical findings. Of the 82 women examined, 40 were found to have carcinoma of the ovary; the rest had benign tumors (ovarian cysts, myoma uteri).

**Results**

We found a highly significant difference ($p < 0.001$) between the copper/zinc ratio for the benign group and that for the malignant group (Table 1). This difference is the result of both higher values for copper and lower values for zinc in serum from the malignant group as compared with normal persons or with those patients with benign tumors. As in our former study (17), most of the malignant cases were in stage III or IV, the stages in which most cases of malignant ovarian tumors are first seen by the physician; only a few of our cases were in other stages (Table 1). In all cases of proven malignancy at these advanced stages, the Cu/Zn ratio was significantly higher than the mean for the benign group. A positive correlation between the Cu/Zn ratio and the stage of malignancy was observed, as we have previously shown (17).

To investigate whether the changes in concentrations of copper and zinc in serum and their concentration in ovarian tissues are conversely related in the presence and absence of malignant disease, we determined the concentrations of these metals in biopsies of the excised tissues. Table 2 summarises our findings for samples of tissues from 23 benign and 18 malignant tumors. Although the two groups did not differ significantly with respect to copper concentrations, the zinc concentration was significantly lower ($p < 0.001$) in the malignant tumor tissue (Figure 1)—and the copper/zinc ratio was higher for the malignant tumor tissue.

We plotted the ratio of serum copper to serum zinc (Figure 2) in all 82 cases. We set as the cutoff value for normal the mean for Cu/Zn in the benign group (1.43 ± 0.22) plus 2 SD; i.e., Cu/Zn ratios exceeding 1.87 were considered to indicate malignancy. Ratios between 1.65 (mean ± 1 SD over the mean benign group ratio) and 1.87 were considered indeterminate, to be interpreted with caution, and ratios of <1.65 were considered nonmalignant. Use of such cutoff values resulted in a very small percentage of false-positive (1/42) and false-negative (2/40) results. The two false-negative cases were stage I ovarian malignant tumor.

**Discussion**

The last decade has seen a major effort to identify tumor markers by simple, reliable laboratory methods. New markers recently discovered for various tumors, through use of sophisticated instrumentation and recent advances in molecular biology, are being assessed for specificity (12, 13). Determinations of these markers are as yet expensive and time consuming, and clinicians must still use a combination of more suitable markers. One of the better described markers has been copper in serum, which increases in cases of lymphoma, especially in Hodgkin's lymphoma (8). The use of copper concentrations in serum for differentiating malignant solid tissue from normal or benign tissue has not been as well documented; the magnitude of alteration seems to be quite dependent on the tumor site (14, 15).

Concentrations of zinc in serum have also been suggested as a marker for malignancy in solid tumors, a decrease being indicative of malignancy. This marker has been useful in the differential diagnosis of breast cancer, cervical cancer, osteosarcoma, lung cancer, and intestinal malignant tumors (2, 7, 14, 16). In some of those studies the copper/zinc

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**Table 1. Ovarian Tumor Stage and Concentrations of Copper and Zinc in Serum**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cu, mg/L</td>
<td>16.0 ± 1.7</td>
<td>19.0 ± 0.6</td>
</tr>
<tr>
<td>Zn, mg/L</td>
<td>7.8 ± 1.5</td>
<td>8.1 ± 1.4</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>2.05 ± 0.51</td>
<td>2.34 ± 0.22</td>
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Results are ± SD for 36 of the 40 cases of proven malignancy. In Stage III vs benign $p < 0.001$ (Student's $t$ test).

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**Table 2. Concentrations of Copper and Zinc in Serum and Tumor Tissue of Patients with Benign and Malignant Ovarian Tumors**

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc in serum</td>
<td>n = 42</td>
<td>n = 40</td>
</tr>
<tr>
<td>Cu, mg/L</td>
<td>13.3 ± 1.7</td>
<td>17.3 ± 2.0*</td>
</tr>
<tr>
<td>Zn, mg/L</td>
<td>8.4 ± 1.4</td>
<td>7.7 ± 1.0*</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>1.43 ± 0.22</td>
<td>2.30 ± 0.41*</td>
</tr>
<tr>
<td>Conc in tumor</td>
<td>n = 23</td>
<td>n = 18</td>
</tr>
<tr>
<td>Cu μg/g wet wt.</td>
<td>1.95 ± 0.64</td>
<td>2.17 ± 0.64*</td>
</tr>
<tr>
<td>Zn μg/g wet wt.</td>
<td>21.9 ± 4.1</td>
<td>14.7 ± 3.5*</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>0.093 ± 0.039</td>
<td>0.158 ± 0.065*</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD. *Significantly different from values in benign group at $p < 0.001$ (Student's $t$ test). **Difference not significant ($p = 0.05$).
The serum copper/zinc ratio must be interpreted cautiously in cases of suspected malignancy that are accompanied by acute and chronic infections, chronic liver diseases, pregnancy, or other conditions known to affect copper and zinc in serum. Although we excluded from our study patients with these clinical conditions, there were still a few false-positive (one out of 42) and false-negative (two out of 40) results.

Use of the Cu/Zn ratio for staging an ovarian tumor and for diagnosis of very early stage malignancy is as yet difficult to advocate with assurance, because of the relatively small sample of patients we have studied so far and because most ovarian malignancies are first seen by the physician at a fairly advanced stage, very few patients presenting with in situ or stage I tumors. From preliminary studies we believe that the Cu/Zn ratio might have greater applicability in the followup and management of patients after surgical removal of the malignant tumor.

References