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USE OF PIXE TO MEASURE SERUM COPPER, ZINC, SELENIUM, AND BROMINE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

Y. BEGUIN [1]*, V. BOURS [1]**, J-M. DELBROUCK [2], G. ROBAYE [2], I. ROELANDTS [2], G. FILLET [1] and G. WEBER [2]***

[1] *Departments of Hematology and* [2] *Experimental Nuclear Physics, University of Liège, Sart-Tilman B-15, 4000 Liège, Belgium*

The use of PIXE allowed for a simultaneous determination of serum copper (Cu), zinc (Zn), selenium (Se) and bromine (Br), in various groups of patients with hematologic malignancies. In 78 patients with acute nonlymphocytic leukemia, it was observed that (1) serum Se was significantly lower than in healthy controls and correlated inversely with the tumor burden; (2) serum bromine was normal at diagnosis but dropped dramatically after intensive chemotherapy, before recovering progressively over a period of months; and (3) pretreatment serum copper and zinc were significant prognostic factors of the chance to achieve a complete remission. In 50 patients with chronic lymphocytic leukemia, it was observed that (1) serum Cu and Cu/Zn ratio were useful indices of the disease activity, which were independent of a nonspecific acute phase reaction; and (2) Zn deficiency could contribute to immune dysfunction. In 119 patients with myeloproliferative disorders or myelodysplasic syndromes, serum Cu and Zn levels were mostly dependent on nonspecific factors, such as age and inflammation.

1. Introduction

We report our experience with the use of particle induced X-ray emission (PIXE) (1) to investigate the trace element status of patients with hematologic malignancies and to recognize potential needs for supplementation; (2) to evaluate the prognostic value of serum trace element (STE) levels; and (3) to identify factors responsible for alterations in STE levels before and after chemotherapy.

2. Patients and methods

Group I. We studied 78 patients with acute nonlymphocytic leukemia (ANLL), including 46 males and 32 females, whose age ranged from 11 to 85 yr (mean 50 yr). These patients received high-dose induction chemotherapy, including one or several of the following agents: cytarabine, daunorubicin, mitoxantrone, amsacrine, etoposide, vincristine, and melphalan. Complete remission (CR) was defined as a normocellular marrow with less than 5% blasts, and a peripheral blood with unsustained hemoglobin > 10 g/dl, platelets $> 100\,000/\mu$l, and neutrophils $> 1500/\mu$l. Partial remission, failure, and death were all classified as failures.

Group II. We also studied 50 patients with chronic lymphocytic leukemia (CLL). There were 31 men and 19 women, whose age ranged from 46 to 86 yr (mean 65 yr). The patients were classified into clinical stages 0 through 4 according to the criteria of the Rai classification system [1].

Group III. One hundred and nineteen patients, 65 males and 54 females, aged 12 to 81 yr (mean 59 yr), had a myeloproliferative disorder (MPD) or a myelodysplastic syndrome (MDS). There were 39 patients with polycythemia vera (PV), 27 with chronic myelogenous leukemia (CML), 13 with agnogeneic myeloid metaplasia (AMM). 15 with idiopathic thrombocythemia (IT), and 25 with a MDS.

PIXE measurements. STEs were measured par PIXE as described previously [2]. The copper-to-zinc ratio (Cu/Zn) was calculated from the actual Cu and Zn values. In patients with ANLL, serum samples were obtained before chemotherapy and thereafter twice weekly for four weeks, while in a few of these patients, samples were collected for several months after cessation of chemotherapy. Normal STE levels were determined in a group of 100 healthy subjects from the same local population as the patients. Results are expressed as Mean $\pm$ SEM. Student's t-tests, with pooled or separated variances as appropriate, were used to compare two groups. Paired Student's t-tests were used to compare pretreatment to post-treatment values. Univariate R correlation coefficients were computed on pairs of variables.

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3. Results

Group I. Pretreatment serum Se levels were lower in ANLL Patients than in controls (table 1). Se correlated negatively with the bone marrow blast + promyelocyte percentage ($R = -0.41$, $P < 0.01$), serum LDH ($R = -0.53$, $P < 0.001$), peripheral absolute blast cell count ($R = -0.62$, $P < 0.001$) and WBC count ($R = -0.58$, $P < 0.001$). Immediately after chemotherapy, serum Se levels increased significantly to a mean value of 0.108 ± 0.037 μg/ml on day 7 ($P < 0.01$ for comparison with pretreatment value). The difference between day 7 and day 0 (in μg/ml) correlated with the initial peripheral blast cell count ($R = 0.44$, $P < 0.01$). Mean serum Se levels on days 14, 21 and 28 remained essentially comparable to the levels observed in controls. Pretreatment serum Se levels were not predictive of response. However, day 7 Se levels were significantly higher in failures (0.131 ± 0.027 vs. 0.100 ± 0.037 μg/ml, $P < 0.005$). Thereafter Se levels remained stable in CRs while decreasing in failures.

Pretreatment serum Br levels in ANLL patients did not differ from those measured in normal subjects (table 1). Serum Br decreased progressively from 4.02 ± 2.11 μg/ml (range 2.0–10.6 μg/ml) before chemotherapy to 2.08 ± 0.48 μg/ml (range 1.1–3.6 μg/ml) on day 28 ($P = 0.0000$). Most of the decline occurred during the first week. Normal serum Br levels were gradually obtained after a few weeks or months in patients receiving no further chemotherapy.

Serum Zn levels were lower and serum Cu levels were higher in ANLL patients than in controls (table 1). Pretreatment serum Zn levels (0.96 ± 0.03 vs. 0.80 ± 0.05 μg/ml, $P < 0.01$) and serum Cu levels (1.46 ± 0.05 vs. 1.23 ± 0.09 μg/ml, $P < 0.02$) were higher in CRs than in failures.

Group II. Serum Br and Se levels in CLL patients did not differ from those measured in normal subjects (table 1). Cu was higher and Zn was lower in patients than in controls (table 1) in all stages of the disease. The erythrocyte sedimentation rate (ESR), fibrinogen, and alpha2-globulin, were within normal limits and were not modified from stage to stage. Cu increased with advancing clinical stage (1.30, 1.40, 1.50, 1.90 μg/ml, for stages 0, 1, 2, and 3–4, respectively; $P < 0.005$). Zn decreased with advancing clinical stage (NS) and the Cu/Zn ratio increased steadily from stage 0 to stages 3–4 ($P < 0.001$). Se was significantly lower in stages 3–4 than in stages 0–2 (0.079 ± 0.011 vs. 0.0108 ± 0.006 μg/ml, $P < 0.04$) and was inversely correlated with the lymphocyte count in patients with lymphocytes $> 20000/mm^3$ ($P < 0.05$). Cu was significantly associated with the lymphocyte count ($P < 0.01$), serum LDH ($P < 0.01$) and alpha2-globulin ($P < 0.01$), but not with ESR and fibrinogen.

Group III. Cu was slightly increased and Zn slightly decreased in patients with MPD or MDS as compared to controls, producing an elevation of Cu/Zn (table 1). Zn was significantly lower and Cu/Zn higher in patients with a MDS or AMM than in patients with CML, IT or PV. However, patients in the first two groups were significantly older. Cu correlated positively and Zn negatively with parameters of inflammation. Serum Zn correlated inversely with age and directly with albumin.

4. Discussion

Patients with ANLL were found to have decreased serum Se levels as compared to controls, suggesting that individuals with a low selenium status are at increased risk of developping ANLL or that ANLL is responsible for a decline of serum Se [3]. Pretreatment Se levels as well as the degree of increment after chemotherapy correlated inversely with several measurements of the tumor burden. In patients with resistant disease, Se tended to be lower before treatment, to increase more immediately after chemotherapy, and to fall gradually

Table 1
Serum trace elements (μg/ml, Mean ± SEM) in normal controls and in patients with ANLL (group I), CLL (group II), and MPD or MDS (group III). *P* values are given for comparisons with controls

	Controls	Group I	Group II	Group III
N	100	78	50	119
Cu	1.10 ±0.02	1.38 ±0.04 ($P < 0.001$)	1.50 ±0.06 ($P < 0.001$)	1.26 ±0.03 ($P < 0.001$)
Zn	1.10 ±0.02	0.91 ±0.03 ($P < 0.001$)	0.94 ±0.03 ($P < 0.001$)	0.96 ±0.02 ($P < 0.001$)
Cu/Zn	1.03 ±0.03	1.61 ±0.07 ($P < 0.001$)	1.68 ±0.09 ($P < 0.001$)	1.35 ±0.04 ($P < 0.001$)
Se	0.097±0.004	0.082±0.004 ($P < 0.01$)	0.107±0.005 (NS)	0.097±0.004 (NS)
Br	5.36 ±0.19	4.02 ±0.28 (NS)	5.43 ±0.51 (NS)	5.47 ±0.34 (NS)

later on. All these findings are in favor of an inverse relationship between serum Se levels and disease activity in ANLL. A similar relationship can be suspected in CLL, as serum Se correlated inversely with the lymphocyte count, and was significantly reduced in stages 3–4.

Bromine is not regarded as an essential element and the significance of its presence in human blood and tissues is not known [4]. We observed a marked decline of serum Br levels in all ANLL patients receiving induction chemotherapy. Pretreatment Br levels were only recovered after a few months in patients receiving no further chemotherapy. This phenomenon was also observed after chemotherapy for other hematologic malignancies and the magnitude of the decrease in serum Br levels appeared to be related to the intensity of the chemotherapy (data not shown). Interestingly, the bromine content of liver and kidneys was increased in mice treated with a chemotherapeutic agent not used in ANLL, cisplatin [5]. Further studies are warranted to determine the mechanism of serum Br decrease after chemotherapy and to identify possible sequestrating organs.

Pretreatment serum Zn and Cu levels were found to be significant prognostic factors of response to ANLL induction chemotherapy, both being higher in CRs than in failures. The use of these STE levels should be evaluated in the development of multivariate predictive models for response to chemotherapy.

In CLL, serum Cu was correlated with the lymphocyte count and serum LDH, and increased steadily from stage 0 to stage 4. The elevation of Cu in cancer patients has often been considered as part of a nonspecific acute phase reaction [6]. However, the ESR, fibrinogen and alpha2-globulin were normal in our patients, and Cu did not correlate with them. All these findings in patients with CLL support the value of serum Cu and Cu/Zn as indices of the disease activity, which appear to be independent of a nonspecific acute phase reaction.

Serum Zn levels were lower in CLL patients than in controls, and decreased in stages 3–4 as compared to stages 0–2. Zn deficiency has been shown to produce cell-mediated immunity dysfunction [7], particularly in patients with cancer [8]. Patients with CLL are also more susceptible to infections than normal subjects. This can be explained by the replacement of normal neutrophils and lymphocytes by tumor cells, but Zn deficiency could be an additional factor of immune dysfunction.

In patients with MPD and MDS, serum Cu levels were slighly elevated and weakly correlated with the blast count but not with the peripheral count of the proliferating cell line. Stronger correlations with ESR, fibrinogen, and alkaline phosphatase, suggested that nonspecific factors, such as inflammation and cholestasis, played a more important role in the elevation of serum Cu levels. We also observed a lower serum Zn level in our patients as compared to normal controls, particularly in those with AMM or a MDS. Serum Zn levels were mostly related to nonspecific factors such as albumin level, inflammation, and age.

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