

## Hypocupremia and bone marrow failure

**Copper deficiency associated with neurological disorders is a well-documented condition. However, hypocupremia is less often recognized as a cause of cytopenias or bone marrow failure. We report an illustrative series of three new cases of bi-lineage cytopenia associated with copper deficiency. We have analyzed clinical features of current and historical cases to identify clues that could facilitate application of appropriate laboratory testing and heighten the level of clinical suspicion. By maintaining an appropriately high level of suspicion for potential copper deficiency and obtaining a serum copper level, bone marrow failure due to this condition can be correctly diagnosed and treated. We suggest that copper deficiency be included in the differential diagnosis of reversible causes of bone marrow failure syndromes including myelodysplastic syndrome.**

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### Introduction

In the evaluation of patients with pancytopenia, reversible causes of bone marrow failure such as nutritional deficiencies, viral infections, and medications need to be considered in the differential diagnosis. Copper deficiency can be easily treated but the diagnosis may be difficult due to comorbidities and low level of clinical suspicion.

Patients with copper deficiency most commonly present with neurological disorders, including myeloneuropathy, arthralgias and neuropathy.<sup>1-4</sup> A diagnosis of copper deficiency is usually established by measuring serum copper or ceruloplasmin levels; a serum copper level <10  $\mu$ mol/L or ceruloplasmin <180 mg/L is considered presumptive evidence of copper deficiency. The most common hematologic abnormalities associated with copper deficiency include anemia and neutropenia, while the platelet count usually remains normal.

The adult diet in Western countries contains 0.6 to 1.6 mg of copper/d. Water, shellfish, organ meats, nuts, and grains are all sources of dietary copper. Absorption occurs through the intestinal mucosa across the basement membrane into the blood stream via energy dependent mechanisms where the availability and saturation of copper may limit absorption. Of significant interest is the physiologic relationship between copper and zinc. High zinc levels result in increased metallothionein production in enterocytes. The high affinity of copper to metallothionein displaces zinc and thus leads to the accumulation of copper in the enterocytes. The enterocytes are ultimately shed into the gastrointestinal tract, leading to copper elimination.<sup>5</sup>

Copper plays a role as a cofactor for many enzymes, in electron-transporting proteins, and in antioxidant metabolism. The main copper-binding protein is ceruloplasmin which plays a role in copper transport and release and in protection of cells from oxidative damage. Both ferroxi-

**Table 1. Clinical characteristics of patients with cytopenias due to copper deficiency.**

Parameter	Case #1	Case #2	Case #3
Age/Sex	60/F	38/F	53/M
Malabsorption history?	Yes, pancreatitis	Yes, gastric bypass	No
Neuropathy?	Yes (severe)	Yes (mild)	Yes (mild)
Absolute neutrophil count $\times 10^9/L$	0.170	0.240	1.72
Hemoglobin (g/L)	85	72	100
MCV (fL)	95.7	94.1	99.3
Platelets $\times 10^9/L$	335	196	197
Bone marrow cellularity	Normocellular	Hypocellular	Not performed
Serum zinc N (8.4-22.9 $\mu$ mol/L)	14.9	12.4	33.5
Serum copper (13-24 $\mu$ mol/L)	<2	<2	<2

dase and ceruloplasmin may oxidize Fe<sup>2+</sup> to Fe<sup>3+</sup> facilitating the release of Fe from ferritin and its binding to transferrin.<sup>6</sup> Copper is required for a variety of enzymes, including amino oxidase, ferroxidase, cytochrome-C-oxidase and dopamine hydroxylase. Copper is a cofactor for cytochrome oxidase, methallothionein and superoxide dismutase (SOD) involved in antioxidative responses.<sup>6-8</sup>

The mechanism by which copper deficiency results in cytopenias is not well understood. In general, many copper-containing proteins are important for the proper function of the hematopoietic system. Copper deficiency decreases transferrin concentration in the plasma, and facilitates heme degradation secondary to an increase in heme oxygenase. Copper deficiency may result in a reduction in cytochrome-C-oxidase, leading to a slower rate of electron flow and ATP production, and ultimately diminished hemoglobin synthesis. Ceruloplasmin and copper-zinc SOD are decreased in copper deficiency, affecting the survival of erythrocytes.

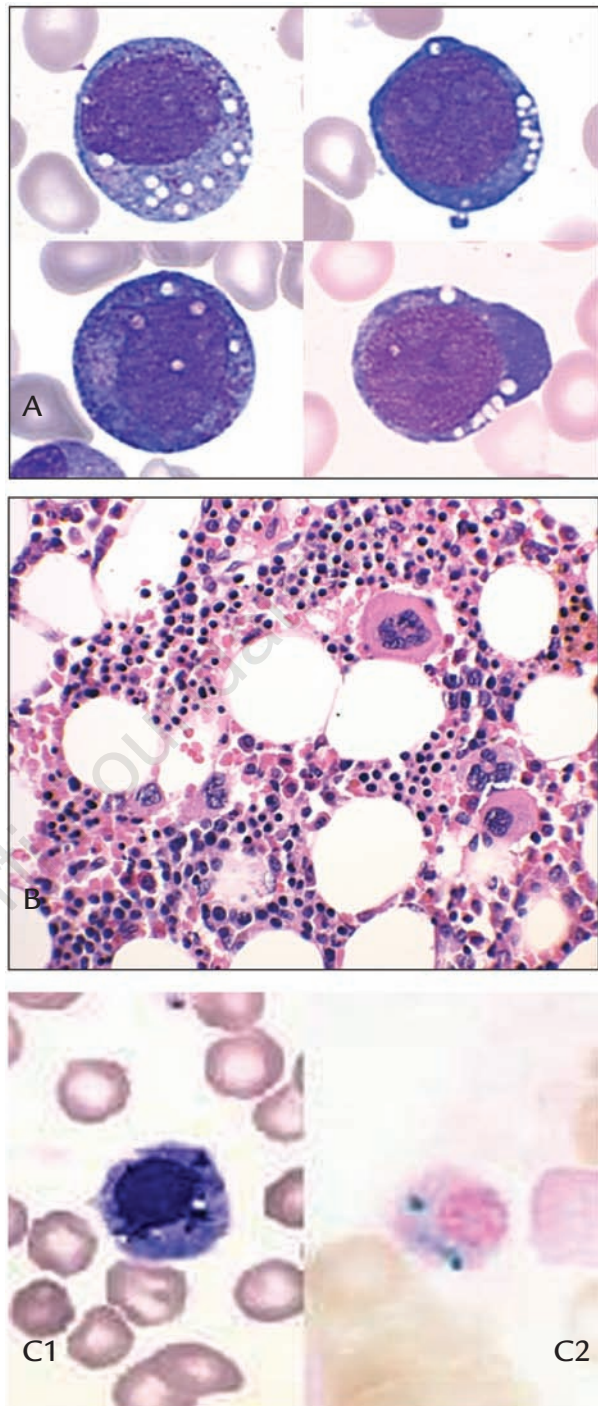
This report was inspired by the clinical observation of 3 very informative cases of copper deficiency associated with cytopenia (Table 1).

### Patients and literature

**Case 1.** A 60-year-old woman presented with an unexplained 80 lb weight loss over 4 months. She complained of discomfort and numbness in her fingertips and loss of sensation in her hands; she noted similar symptoms involving her lower extremities. She had gradually developed progressive gait abnormalities and eventually became wheelchair bound. Her past medical history included pernicious anemia, pancreatitis, and a cholecystectomy; there was no history of alcohol abuse. The patient had been treated for pernicious anemia without response. Physical exam showed mild weakness in both legs that was attributed to leg pain, reduced sensation to pain up to the anterior thighs bilaterally,

and impaired vibratory sensation up to her knees. Proprioception was impaired in the feet and the ankles. Her gait was wide-based. MRI of the brain and spine were normal. Laboratory studies showed a normocytic anemia with hemoglobin as low as 86 g/L, reticulocyte count of 1.6%, WBC:  $1.5 \times 10^9/L$  with absolute neutropenia ( $0.130 \times 10^9/L$ ), and a normal serum vitamin B12 level, normal methylmalonic acid level, negative HTLV-I serology, and negative heavy metal screen including arsenic, mercury and lead. Initial and repeat bone marrow biopsies were unremarkable with normal cellularity (Figure 1). Virtually no plasma cells were present in the aspirate smear. Based on the combination of the pancytopenia and the neurologic signs and symptoms copper deficiency was suspected. Serum copper was  $<2 \mu\text{mol/L}$  (normal: 13-24), ceruloplasmin level was 20 mg/L (normal: 210-450), and 24 hr urine for copper was 2  $\mu\text{g}/24 \text{ hrs}$  (normal 0-40). A diagnosis of copper deficiency was made, and the patient was treated with 2 mg oral copper twice daily; the dose was subsequently increased to 6 mg taken three times a day. She showed an excellent hematologic response approximately 6 months after starting treatment. Neurological symptoms stabilized with minimal improvement.

**Case 2.** A 38-year-old woman who had a gastric bypass operation four years earlier presented for evaluation of unsuspected anemia found when volunteering to become a blood donor. Upon further questioning, the patient admitted to symptoms of fatigue for more than a month, lightheadedness and dizziness. She had numbness in the distal part of her legs for a few months. She did not have any other significant past medical history or any other symptoms. Current medications included Mometasone Furoate, loratadine, zinc, calcium, prenatal vitamins, ester-CYP3A4, vitamin B12, vitamin B6, and ferrous sulfate. The physical examination was unremarkable. A blood cell count showed WBC:  $1.3 \times 10^9/L$  (normal: 4.0-11.0), Hgb: 72 g/L (normal: 120-160), MCV 94.1 fL (Normal: 80-100), and platelet count:  $196 \times 10^9/L$  (normal 150-400) (Table 1). There was severe neutropenia ( $0.240 \times 10^9/L$ ). Ferritin, vitamin B12, folic acid, and TSH were within normal limits. Additional laboratory studies showed serum copper:  $<2 \mu\text{mol/L}$  (normal: 13-24) and serum zinc:  $<12.4 \mu\text{mol/L}$  (normal: 8.4-22.9). Bone marrow aspirate and biopsy were slightly hypocellular for age (40%) with trilineage hematopoiesis, an erythroid predominance, and mild megakaryocytic hyperplasia. Bone marrow aspirate smears demonstrated multiple clear cytoplasmic vacuoles in proerythroblasts, also plasma cells containing hemosiderin pigment were present (Figure 1). A diagnosis of copper deficiency was established, and she was treated with oral copper 2 mg twice daily. After 3 months a blood cell count showed: WBC:  $7.55 \times 10^9/L$ , Hgb: 134 g/L, absolute neutrophil count:  $4.27 \times 10^9/L$  and serum copper: 17  $\mu\text{mol/L}$ . Her neurologic symptoms completely resolved.



**Figure 1.** Morphology of bone marrow in copper deficiency. (A) Bone marrow aspirate smears demonstrated multiple clear cytoplasmic vacuoles in promyelocytes (left panels) and proerythroblasts (right panels) from Case 1 (bottom panels) and Case 2 (top panels). Vacuolated cells were infrequent in both cases, and significant dysplastic features were not identified in either case. (Wright-Giemsa stain, original magnification 100x). (B) Bone marrow biopsy from Case 2 was slightly hypocellular for age (40%) with trilineage hematopoiesis, an erythroid predominance, and mild megakaryocytic hyperplasia. (H&E stain, original magnification 40x). (C) Hemosiderin (iron) containing plasma cells were noted in case 2. In the marrow aspirate approximately 25% of plasma cells contained cytoplasmic hemosiderin pigment (C1) that was highlighted in iron stain (C2). (H&E stain and prussian stains, original magnification 100x).

**Case #3.** A 53-year-old man presented with anemia, leukopenia and neurological symptoms (Table 1). His symptoms started five years prior to presentation with numbness in his feet; this was followed by progressive foot and hand weakness to the extent that he developed bilateral foot and wrist drop. His past medical history was significant for gastroesophageal reflux disease, frequent alcohol and tobacco use, and chronic normocytic anemia and leukopenia. Physical examination was negative except for bilateral weakness in the wrists and feet, and reduced pinprick sensation from toes to ankles and from fingers to wrists; vibratory sensation was absent at toes and ankles. Laboratory studies showed: WBC:  $2.32 \times 10^9/L$  (normal: 4.0-11), Hgb: 100 g/L (normal: 135-175), MCV: 99.3 fL (normal: 80-100), platelets:  $197 \times 10^9/L$  (normal: 150-400), and absolute neutrophil count:  $1.72 \times 10^9/L$ . Additional studies showed serum zinc:  $33.5 \mu\text{mol/L}$  (normal: 8.4-22.9), 24 hr zinc excretion: 4,940  $\mu\text{g}/24\text{hr}$  (normal: 150-1300), serum copper:  $<2 \mu\text{mol/L}$  (normal: 13-24), and serum ceruloplasmin: 40 mg/L (normal: 21-45). He was treated with oral copper (2 mg twice daily which was increased to 5 mg twice daily until the present time. Five months later his blood cell count and copper level normalized, but zinc level continued to be above the normal level.

**Summary of previous cases.** Systematic review of the literature from 1985 to 2005 was performed and identified 45 reported patients with severe copper deficiency in

addition to our three cases (Table 2). Of these, 47 (98%) had absolute neutropenia, 48 (100%) had anemia, and thrombocytopenia was seen in 5 (10%). The anemia was microcytic in only 2 (4%) and macrocytic in 17 (35%). The serum copper level was low in 47 (98%) patients. All patients were reported to be recovered either following copper supplementation or discontinuing zinc (if the copper deficiency was due to increased zinc ingestion). Twenty patients (41%) had documented neurological findings ranging from polyneuropathy, loss of sensation in the extremities, loss of vibratory sensation, sensory ataxia without involvement of cerebellum, gait abnormalities with wide-based gait, increased tone, weakness, exaggerated reflexes, loss of Babinski's reflex and neurocognitive defects. Importantly, hematologic abnormalities due to copper deficiency were not always associated with neurologic symptoms. Available bone marrow findings revealed cytoplasmic vacuolization in 27 patients (56%), refractory anemia with ringed sideroblasts (RARS) in 22/48 (41%), and 12/48 (25%) had a hypocellular bone marrow (Table 3).

### Discussion

The neurological symptoms of copper deficiency are well-defined. However, copper deficiency is only rarely recognized as a cause of pancytopenia. The causative relationship between decreased copper level and pancytopenia can be inferred from the excellent response to treatment with copper supplementation.

**Table 2. Review of reported patients with pancytopenia and copper deficiency (1985 to 2005).**

#	Reference	# of Cases	CNS	Hb	MCV	ANC	Platelets	Cytoplasmic vacuolization?	Ringed sideroblast?	↓ Cellularity	Response to copper?
1	23	1	No	↓	Low	↓	NL	N	N	N	Y
2	10	5	Yes	↓	NL	↓	NL	N/A	N/A	N/A	Y
3	20	1	Yes	↓	NL	↓	NL	N/A	N/A	N/A	Y
4	15	3	Yes	↓	NL	↓	NL	Y	Y	Y	Y
5	5	1	Yes	↓	High	↓	N/A	N	N	N	Zn stop
6	12	1	Yes	↓	NL	↓	High	N	Y	Y	Y
7	29	1	Yes	↓	High	↓	NL	N/A	N/A	N/A	Zn stop
8	27	1	Yes	↓	N/A	↓	Low	N	N	N	Y
9	25	1	Yes	↓	High	↓	NL	Y	Y	N	Zn stop
10	30	1	Yes	↓	NL	N/A	N/A	N/A	N/A	N/A	Y
11	22	1	Yes	↓	High	↓	NL	Y	Y	N	Y
12	26	1	Yes	↓	High	↓	NL	Y	Y	Y	Y
13	19	1	N/A	↓	N/A	↓	Low	Y	N	Y	Y
14	18	1	N/A	↓	N/A	↓	Low	Y	N	Y	Y
15	28	1	N/A	↓	Low	↓	NL	Y	Y	N	Y
16	11	6	N/A	↓	N/A	↓	NL	N/A	N/A	N/A	Y
17	9	10	No	↓	High	↓	NL	Y x6	Y x6	Y	Zn stop
18	17	1	No	↓	N/A	↓	Low	N/A	N/A	N/A	Y
19	24	1	No	↓	NL	↓	NL	Y	Y	N	Y
20	31	1	No	↓	NL	↓	NL	Y	Y	Y	Y
21	14	2	No	↓	N/A	↓	NL	Y	Y	Y	Zn stop
22	13	1	No	↓	High	↓	NL	Y	Y	N/A	Zn stop
23	21	1	No	↓	N/A	↓	NL	N/A	N/A	N/A	Y
24	16	1	Yes	↓	NL	↓	Low	Y	Y	N	Y
25	Our Case #1	1	Yes	↓	NL	↓	NL	Y	Y	N	Y
26	Our Case #2	1	Yes	↓	NL	↓	NL	Y	Y	Y	Y
27	Our Case #3	1	Yes	↓	High	↓	NL	N/A	N/A	N/A	Y

#Number of patients, CNS: Neurological manifestations, Y: yes, N: no, N/A- data not available, Zn Stop- discontinuation of Zinc supplementation, NL- Normal, ANC- absolute neutrophil count.

**Table 3. Review of MCV, ANC, platelet count and pathologic abnormalities in copper deficiency.**

Total n. of cases	No. of pts. with anemia	MCV	ANC	Platelet	Pathologic abnormalities in bone marrow
48	48 (100%)	NL 16/48 (33%)	Low 47/48 (98%)	Low 5/48 (10%)	Cytoplasmic Vacuolization 23/48 (48%)
		High 17/48 (35%)	N/A 1/48 (2%)	High 1/48 (2%)	Ringed sideroblasts 22/48 (41%)
		Low 2/48 (4%)		Normal 40/48 (83%)	Hypocellularity 12/48 (25%)
		Unknown 13/48 (27%)		Unknown 2/48 (4%)	

MCV, mean corpuscular volume; ANC, absolute neutrophil count; NL, normal; N/A, not available.

Our three well annotated cases of copper deficiency are very illustrative and serve to highlight the main features that may trigger targeted diagnostic tests to exclude copper deficiency as a cause of cytopenias. Historically, 48 cases of copper deficiency, including three from our institution, were associated with hematologic abnormalities. The largest single series of published cases included "10 patients.<sup>9</sup> All reported cases of copper deficiency showed anemia and absolute neutropenia. MCV values were variable, and ranged from low to high. Thrombocytopenia was absent in most cases, aside from patients receiving total parenteral nutrition. A rarely noted finding is the presence of prominent hemosiderin in plasma cells.<sup>26</sup> The majority of patients showed an improvement in their CBC or a complete hematologic response following either therapy with copper supplements or by eliminating excess dietary zinc or zinc supplements.<sup>10-12</sup>

Multiple reports have implicated low copper levels in the pathogenesis of selected cases of refractory anemia with ringed sideroblasts (RARS) responsive to copper; all cases had normal platelet counts.<sup>13-15</sup> Refractory sideroblastic anemia was reported in 22 patients, and often occurred in association with excessive zinc ingestion, a finding causally linked to decreased copper levels. Stopping zinc supplements or removing dietary sources of excess zinc was enough to achieve hematologic recovery in these patients. Less well characterized and more loosely associated were cytopenia and myeloneuropathy are signs and symptoms of copper deficiency were highlighted in several case reports.<sup>1,2,16-20</sup> The most common causes of copper deficiency are malabsorption and malnutrition, but as noted above, copper deficiency can also occur secondary to chronic use of large doses of zinc causing zinc overload syndrome.<sup>21,22</sup> Responsive to copper supplementation or zinc withdrawal in cases the diagnosis of RARS should be avoided, since the anemia is not refractory to treatment and true myelodysplastic syndrome is not present.

Chronic copper overdose, which is a very rare condition, may cause serious side effects including liver cirrhosis, episodes of hemolysis and kidney damage, and even death.

In conclusions our study demonstrates that copper and zinc levels should be checked in patients who pres-

ent with cytopenias, especially in cases lacking thrombocytopenia, or having vacuolated erythroid precursors in a marrow aspirate, or with increased RS. Copper deficiency usually presents with bicytopenia (neutropenia and anemia), but severe pancytopenia has been observed in some cases, especially those patients on TPN. Hematologic recovery can be achieved with oral copper supplements administered for a few weeks, but some patients may require supplementation using total parenteral infusions. In cases of hyperzincemia or zinc overdose, discontinuing the zinc supplementation can be sufficient for hematologic recovery.

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