Pancytopenia and Myelodysplastic Changes in Aceruloplasminemia: A Case with a Novel Pathogenic Variant in the Ceruloplasmin Gene

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Abstract:
A 72-year-old Japanese woman suffered from mild pancytopenia 3 years before her initial hospitalization. On admission, the levels of trace elements, particularly copper, and ceruloplasmin were significantly decreased in her blood serum. Abdominal lymphadenopathy and bone marrow dysplasia were detected. Hemosiderin deposition was observed in her lymph nodes and bone marrow, and magnetic resonance imaging suggested its deposition in various organs. A novel missense pathogenic variant (c.T1670G) was detected in the ceruloplasmin gene, resulting in an amino acid change (p.M557R). When copper deficiency is accompanied by cytopenia and dysplasia in a patient, it is worthwhile to consider a differential diagnosis of aceruloplasminemia.

Key words: aceruloplasminemia, pancytopenia, copper deficiency, Introduction

Case Report
A 72-year-old woman was diagnosed with mild pancytopenia (white blood cell count, 2,600/μL; hemoglobin level, 10.7 g/dL; platelet count, 105,000/μL) 3 years before her first hospitalization. Her bone marrow showed no evidence of myelodysplastic changes in three blood cell lineages and no chromosomal aberrations. There was also no apparent increase in the number of leukemic blasts on a flow cytometry analysis. Blood tests revealed microcytic hypochromic anemia and elevated ferritin levels. Among the trace minerals, the serum levels of copper were low (30 μg/dL) despite her consumption of copper supplements.

Three years later, she had become less active, so a full...
were also low (2 μg/dL [normal levels, 3-132 ng/mL]). Her urinary copper levels were low (Table). Her glucose tolerance and elevated soluble interleukin-2 receptor [normal levels, 3-132 ng/mL]). She also had impaired glycosuria concentration, 31.1%), and high ferritin levels (1,165 ng/mL). In the biopsy specimen. The low levels of copper and ceruloplasmin, the deposition of hemosiderin in abdominal lymph nodes and bone marrow (Figs. 2 and 4), the impaired glucose tolerance and elevated soluble interleukin-2 receptor levels (Table). Among the trace elements, the serum levels of copper, iron, and zinc were low (Table). Her urinary copper levels were also low (2 μg/dL [normal levels, ≤30 μg/dL]) and the serum ceruloplasmin levels were well below the limit of detection (<2 mg/dL [normal levels, 21-37 mg/dL]). Dysplastic myelocytes (with two nuclei) and giant platelets and neutrophils were observed in the bone marrow, but no leukemic blast cells were apparent (Fig. 1). There was also no increase in the immature blast cell fraction within the bone marrow smear, with a normal karyotype on G-banding. Whole-body computed tomography (CT) showed abdominal lymphadenopathy (3 cm diameter), and an abnormal fluorine-18-labeled deoxyglucose uptake was noted on positron emission tomography (Fig. 2A). A diagnosis of reactive lymphadenopathy was made based on the findings of a CT-guided abdominal lymph node biopsy. The structure of the lymph follicles was maintained, and the deposition of hemosiderin was observed (Fig. 2B and C). Susceptibility-weighted magnetic resonance imaging (MRI) of the brain detected a diffuse low signal of the brain surface, suggesting superficial siderosis (Fig. 3A). Abdominal MRI revealed hepatomegaly with a very low signal intensity in T1- and T2-weighted images (Fig. 3B and C), which also agreed with the hemosiderin deposition in the liver. The spleen showed similar findings. Para-aorta lymphadenopathies with a low signal intensity in T2-weighted images also agreed with the hemosiderin deposition. The Kayser-Fleischer corneal ring, which reflects copper deposition in the cornea, was not detected, and the urinary copper levels were low.

The patient had no history of gastrointestinal resection. Endoscopy of the upper and lower gastrointestinal tract revealed no abnormal findings and the deposition of amyloid in the biopsy specimen. The low levels of copper and ceruloplasmin, the deposition of hemosiderin in abdominal lymph nodes and bone marrow (Figs. 2 and 4), the impaired glucose tolerance, and neurological symptoms, such as mild cognitive impairment, were all highly suggestive of aceruloplasminemia. A genetic analysis detected a novel homozygous pathogenic variant in exon 9 of the ceruloplasmin gene (c.1670T>G, p.Met557Arg) (Fig. 5). An in silico analysis using the Polymorphism Phenotyping v2 (PolyPhen-2) (3) predicted that this pathogenic variant might be “possibly damaging.” Another analysis by Sorting Intolerant From Tolerant (SIFT) (4) resulted in a prediction of “damaging.” These findings led to a diagnosis of aceruloplasminemia in our patient.

The patient’s pancytopenia progressed gradually, and she suffered from neutropenic fever repeatedly, being resistant to antibiotic therapy. She ultimately died of infectious disease approximately one month later.

### Discussion

Aceruloplasminemia causes the accumulation of iron in the entire body, especially the brain, thyroid, heart, liver, and pancreas (1). CT and MRI are particularly useful means of evaluating iron deposition in organs (5, 6).

Copper deficiency is known to cause anemia and blood cell dysplasia, suggesting that the low levels of copper in this case might have contributed to pancytopenia. Wilson’s disease, aceruloplasminemia, malabsorption of the gastrointestinal tract, and Menkes disease are all considered differential diagnoses of low serum copper levels. In addition, be-
cause copper competes with zinc for absorption, the supplemental intake of zinc may lead to copper deficiency.

Since the first ceruloplasmin gene pathogenic variant was reported in 1995 (7), aceruloplasminemia causative mutations have been reported in more than 70 cases. Ceruloplasmin gene variants are thought to influence the age of disease onset and symptoms. Most ceruloplasmin gene variants are truncated mutations, leading to premature termination codons. The ferroxidase activity of ceruloplasmin depends on the presence of a copper cluster at the N-terminal-C-terminal interface, so copper plays an important role in the structural stability of human ceruloplasmin. Therefore, ceruloplasmin gene mutations are likely to influence the enzyme function by impairing the structural instability of the protein (1, 8).

SIFT predicts the influence of mutations on the protein function according to the conserved sequence of the homolog among other species (4). The Polymorphism Phenotyping v2 and Sorting Intolerant From Tolerant prediction software program analyzes samples using an algorithm, unlike SIFT, and predicts whether or not the mutation is damaging based on the predicted change in the highly advanced structure of the mutation protein (3). This mutation (M557R) was predicted to be “damaging” using SIFT and “possibly damaging” using the Polymorphism Phenotyping v2 and Sorting Intolerant From Tolerant prediction software program.

We consider the following three mechanisms to be the cause of pancytopenia in the present case: first, copper deficiency associated with myeloid cell maturation failure in the bone marrow may have reduced the number of neutrophils in peripheral blood, as reported previously (9, 10). Mice with a copper deficiency were observed to have leukopenia with an increased number of immature myeloid cells, suggesting that a lack of copper leads to a maturation defect of hematopoiesis (11). A maturation disorder of erythropoiesis in the bone marrow is also thought to occur by a similar mechanism. Several studies have reported pathological abnormalities such as myelodysplastic syndrome in association with copper deficiency (10, 12, 13), and the present case exhibited various signs of dysplasia, including myelocytes with two nuclei, giant platelets, giant neutrophils, and a pseudo-Pelger-Huet anomaly.

Vacuolation in the precursor cells of erythroid and myeloid lineages is characteristic of copper deficiency (13) and was also observed in our patient (Fig. 1). Furthermore, no specific karyotype abnormalities are linked with copper deficiency, in contrast to myelodysplastic syndrome (13). Second, it is possible that an iron metabolism disorder caused by aceruloplasminemia promoted anemia. When serum iron is used for the synthesis of hemoglobin, it is combined with Fe$^{3+}$ and transferrin. However, because Fe$^{2+}$ to Fe$^{3+}$ conversion is disrupted by ferroxidase function loss in aceruloplasminemia, it inhibits iron movement from cells to plasma. Therefore, microcytic anemia with increased ferritin levels is often observed, which is affected by increased iron storage. Third, iron overload and hemosiderin deposition in the bone marrow may have impaired hematopoiesis.
Iron overload is known to influence erythropoiesis. Several reports have described hematologic improvement after deferasirox use. Six studies of approximately 760 MDS patients receiving iron chelation therapy reported an increase in the hemoglobin level ranging from 6% to 44.5%. Of note, an increase occurred in not only the hemoglobin level but also the platelet count (from 13% to 61%) and neutrophil count (from 3% to 76%) (14). Hemosiderin deposition in bone marrow was also confirmed in our case, and an earlier diagnosis and intervention with iron chelation therapy might be effective.

There have been some case reports of myelosuppression associated with secondary aceruloplasminemia. One case report described congenital nephrotic syndrome due to a pathogenic variant NPHS1 gene (encoding nephrin). In that patient, ceruloplasmin loss to urine resulted in aceruloplas-
minemia and copper deficiency. Severe neutropenia was complicated, but it was resolved with copper supplementation (15). Another case report described a case complicated with myelodysplastic changes and aceruloplasminemia, similar to our case. However, the symptoms in that case might have been caused by zinc overload with denture cream and secondary aceruloplasminemia by copper deficiency. In that case, copper supplementation was effective (16).

We identified 42 reports with the full text or abstract available through a search of PubMed. Several case reports described microcytic anemia, as we already know. However, there were two case reports showing hematological data sug-

Figure 4. Histopathological features of the bone marrow biopsy from the iliac crest. The bone marrow cellularity was normocellular (A). Hematoxylin and Eosin staining revealed brown granules (B) that appeared blue by Fe staining (C), showing the deposition to be hemosiderin (magnified in the boxed area, arrow).

Figure 5. Sequencing of the ceruloplasmin gene. Direct sequencing of exon 9 of the ceruloplasmin gene revealed a missense mutation at the 1670th base causing a substitution of thymine to guanine (arrow) and an amino acid change from methionine to arginine.
gestive of myelodysplastic changes, as in our case. One previous report described a patient who had leukopenia, however, the exact cause of leukopenia in that case was unclear. In addition, it was unclear whether or not this case was associated with myelodysplastic changes since no hematological findings were available (i.e. no blood cell morphology findings or information on chromosomal abnormalities) (17).

The other report (18) said, "These bone marrow aspects were compatible, referring to the latest WHO classification and criteria for the myelodysplastic syndrome, to refractory cytopenia with multilineage dysplasia (RCMD)". We believe that myelodysplasia may have been overlooked in some cases.

Some cases have been examined on consultation with hematologists. One case report described a patient with an abnormal iron metabolism (bone marrow aspirate revealed mild dyserythropoiesis; iron staining showed abundant iron in RE cells and the absence of iron granules in erythroblasts) but did not mention dysplasia of other blood cell lineages (19). These reports suggest that myelodysplasia was overlooked in some but not all cases. The existence or nonexistence of myelodysplasia is one point of diversification for the phenotype of aceruloplasminemia.

The use of iron-chelating agents has been reported to control the progress of symptoms in cases of aceruloplasminemia that have been diagnosed early (1). However, the prognosis is poor when organ damage has already developed following iron deposition. According to a survey of 40 cases of cytopenia that have been diagnosed early (1). However, the prognosis is poor when organ damage has already developed following iron deposition. According to a survey of 40 cases of aceruloplasminemia. Case report. J Neurol Sci 339: 231-234, 2014.

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The authors state that they have no Conflict of Interest (COI).

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