Case Study

Three Cases of Aggressive Natural Killer Cell Leukemia with a Lethal Hemorrhagic Complication

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Aggressive natural killer cell leukemia (ANKL) is a rare malignant disease of NK cells that has a median survival of less than 2 months and a strong association with the Epstein-Barr virus. Herein, we report three Japanese cases of the disease. A 21-year male patient, a 31-year female patient, and a 76-year female patient presented with high fever, lymphadenopathy, hepatosplenomegaly, and severe liver damage. All three cases had granular lymphocytes in both peripheral blood and bone marrow. The phenotype of these cells was CD2+CD3-CD56+HLA-DR+. All cases had a high copy number of serum Epstein-Barr virus DNA in the peripheral blood and were diagnosed with ANKL. Case 1 and Case 2 were treated with chemotherapy, but suffered from gross intestinal bleeding or massive bleeding in the cerebellum, resulting in death. Although not treated with chemotherapy, Case 3 also suffered gross bleeding from an atypical duodenal ulcer and died from hemorrhagic shock 15 days after admission. There have been no previous reports of such acute lethal hemorrhagic complications with ANKL. The present cases suggest that patients with ANKL need a sufficient supply of coagulation factors, and that chemotherapy for this disease should be carefully designed with promising agents. [J Clin Exp Hematopathol 52(2) : 101-106, 2012]

Keywords: aggressive natural killer cell leukemia, lethal hemorrhagic complication, chemotherapy, intestinal bleeding, cerebellar hemorrhage

INTRODUCTION

Aggressive natural killer cell leukemia (ANKL) is a rare malignancy of NK cells that is refractory to several therapies, including intensive combined chemotherapy, and has a median survival of less than 2 months. Patients with the disease generally have hepatomegaly, splenomegaly, lymphadenopathy, and bone marrow involvement of large granular lymphocytes. ANKL may be closely associated with the Epstein-Barr (EB) virus because patients with ANKL generally have a high copy number of EB virus DNA in the peripheral blood. ANKL cells have the CD3-CD56+ phenotype. We present three patients with ANKL who died from lethal hemorrhagic complications.

CASE REPORTS

Case 1

A 21-year-old Japanese male patient suffered from general fatigue, high fever (> 38°C), and chills. The fever did not improve after treatments with antibiotics, and he presented with splenomegaly and elevated aminotransferases. Therefore, he was admitted to hospital for liver damage. Hepatitis B and C were both negative, and there was no history of drug administration. After 4 months of hospitalization, he still had a fever of 38°C that did not improve after administration of several antibiotics. He was referred to another hospital and a liver biopsy was performed. A pathological analysis indicated non-specific hepatitis suggestive of autoimmune hepatitis. Oral prednisolone (30 mg) was started, and the fever and hepatic failure were transiently improved. However, after tapering of the steroid, he suffered from high fever and hepatic failure again. Finally, he was referred to our hospital and subjected to several examinations. He had high fever (38.5°C), general fatigue, sore throat, and appetite loss, and his performance status was 1. Blood tests showed elevated levels of aspartate aminotransferase (290 U/L), alanine aminotransferase (273 U/L), lactate dehydrogenase (1,145 U/L), and alkaline phosphatase (3,188 U/L) (Table 1). An increased number of large granular lymphocytes was recognized in May–Giemsa staining of peripheral
blood (42%; Fig. 1a) and bone marrow (data not shown) smears, and the phenotype of the large granular cells was CD2−CD3−CD56+HLA-DR+. He was found to have a high copy number of serum EB virus DNA (1.8 × 10⁶ copies/μg DNA). Southern blot analysis of DNA from bone marrow cells revealed a monoclonal increase in EB virus-infected cells. Computed tomography (CT) revealed severe hepatosplenomegaly. He was finally diagnosed with ANKL.

He was treated with CHOP plus etoposide (Fig. 2a), and soon his fever improved and his lactate dehydrogenase decreased to within the normal range. However, his temperature returned to 40°C after 8 days of chemotherapy. On the following day, he felt abdominal distension and pain followed by massive hematemesis. An emergency CT scan revealed obstructive ileus caused by bleeding in the intestinal mucosa (Fig. 3a). Six days later, he experienced left visual loss and was diagnosed with bilateral subretinal hemorrhages, including a left submacular hemorrhage (Fig. 3b). After another 8 days, he suffered massive intestinal bleeding again and received angiography of the intestinal arteries, followed by coil treatment of the responsible arteries. Despite these treatments, he died from hemorrhagic shock.

**Case 2**

In January 2009, a 31-year-old Japanese female patient presented with a sore throat, painful lymphadenopathy, and
She subsequently suffered from upper abdominal pain, nausea, vomiting, and discomfort from abdominal distension. She attended an outpatient clinic, and blood tests revealed hyperbilirubinemia (total bilirubin, 11.1 mg/dL). She was admitted to our hospital because of suspected fulminant hepatitis. She had high fever (39°C), erythema of the face and body, lymphadenopathy of surface lymph nodes, and hepatosplenomegaly, and her performance status was 3. She had elevated total bilirubin (12.8 mg/dL), aspartate aminotransferase (559 U/L), alanine aminotransferase (225 U/L), lactate dehydrogenase (4,186 U/L), and alkaline phosphatase (1,885 U/L), and was negative for hepatitis B and C infections (Table 1). Peripheral blood and bone marrow smears revealed increased numbers of immature large granular lymphocytes with a basophilic cytosol (Fig. 1b). The phenotype of the large granular lymphocytes was CD2⁺CD3⁻CD56⁺HLA-DR⁺. She had a very high copy number of serum EB virus DNA (1.7 × 10⁶ copies/μg DNA) and a Southern blot analysis of DNA from bone marrow cells revealed a monoclonal increase in EB virus-infected cells. A CT scan revealed lymphadenopathy of the surface lymph nodes and hepatosplenomegaly. Taking these findings together, she was finally diagnosed with ANKL. Although she had no sign of a bleeding tendency, she was administered fresh frozen plasma, antithrombin, nafamostat mesilate, and danaparoid.

She was treated with 100 mg of etoposide (day 1 only) and 250 mg of methylprednisolone (days 1-3) (Fig. 2b). Her lactate dehydrogenase gradually decreased. After 4 days of treatment, she suddenly developed a severe headache and fell into a coma. A CT scan revealed a massive cerebellar hemorrhage (Fig. 4), and she finally died from brain death caused by the cerebellar hemorrhage.
Case 3

In April 2010, a 76-year-old Japanese female patient presented with high fever (38°C) and liver damage, and was admitted to hospital. A liver biopsy revealed non-specific acute liver disease. Hepatitis B and C were both negative, and there was no history of relevant drug administration. Antinuclear antibodies were negative, and administration of 30 mg of prednisolone did not improve the liver damage. Blood tests revealed increased granular lymphocytes (21%) and a high copy number of serum EB virus DNA (4.9 × 10^5 copies/μg DNA). She was referred to our hospital and diagnosed with ANKL. She had high fever (39°C), and elevated total bilirubin (2.5 mg/dL), aspartate aminotransferase (135 U/L), alanine aminotransferase (117 U/L), and lactate dehydrogenase (360 U/L) (Table 1), and her performance status was 3. Peripheral blood and bone marrow smears revealed increased numbers of immature large granular lymphocytes (Fig. 1c) and the phenotype of these cells was CD2^-CD3^-CD56^+HLA-DR-. She also presented with a cytomegalovirus infection that suggested an immunosuppressed status. Since she had a cytomegalovirus infection and a history of cerebral infarction, we decided not to treat her with intensive chemotherapy. On day 7 after admission, she suffered from hematemesis and an endoscopic examination revealed bleeding from a duodenal ulcer (Fig. 5). The mucosa of the edge of the...
ulcer was irregular, which may have been suggestive of invasion by malignant NK cells. An endoscopic biopsy was not performed because of the risk of massive bleeding. Instead, endoscopic clipping was performed and she was treated with proton pump inhibitors. Six days later, she suffered from bleeding from the duodenal ulcer again and died from bleeding shock despite re-treatment with endoscopic clipping (Fig. 2c).

DISCUSSION

Herein, we have reported three patients with ANKL who had lethal hemorrhagic complications. All three patients had high fever, hepatic failure with hyperbilirubinemia, elevation of alanine aminotransferase and aspartate aminotransferase, and increased numbers of NK large granular lymphocytes in both peripheral blood and bone marrow smears. The first two patients received chemotherapy, but soon thereafter suffered from lethal hemorrhagic complications in the intestine or brain, while the third patient without chemotherapy suffered from lethal duodenal ulcer bleeding. From 2001 to 2010, we have only diagnosed these three patients with ANKL among a total of 850 adult patients with hematological malignancies in our hospital, and all three patients suffered from lethal hemorrhagic complications. There are no other case reports of ANKL patients with lethal hemorrhagic complications. It is not apparent whether this type of ANKL is dependent on Japanese ethnicity or whether we are only seeing a subtype of ANKL. Since the incidence of ANKL is very low, it is possible that lethal hemorrhagic complications may not be recognized as a particular feature of ANKL by physicians.

Case 1 was first administered 30 mg of prednisolone for autoimmune hepatitis, and subsequently treated with a standard dose of CHOP plus etoposide for ANKL. Case 2 only received etoposide and methylprednisolone because the patient had severe hepatic failure and we believed that she could not be treated with intensive chemotherapy. Irrespective of the treatment intensities, both patients suffered from lethal hemorrhagic complications. We were unable to elucidate the mechanisms of these bleeding complications because the families of the patients would not give consent for autopsies. It is possible that the severe hepatic failure resulted in an increased risk of severe bleeding owing to low concentrations of serum coagulation factors. However, since these patients suffered from severe bleeding, there may have been other mechanisms inducing their bleeding rather than only low concentrations of coagulation factors caused by hepatic failure. In previous studies, pathological analyses revealed that the leukemia cells in ANKL patients are destructive and permeative, and induce necrosis, apoptosis, angiogenesis, and angiodestruction.4,5,14 Therefore, it is reasonable to consider that leukemia cells infiltrated into the vessels and tissues, and that the antigenic agents induced apoptosis of these infiltrating leukemia cells, leading to subsequent rupture of the vessels or destruction of the tissues that induced lethal bleeding. To prevent such lethal bleeding, it is very important to investigate the mechanisms of these incidents involving lethal bleeding. Since we were not able to elucidate the mechanisms involved, we need to perform chemotherapy carefully in ANKL patients with severe hepatic failure.

Although ANKL patients can be treated with several chemotherapy regimens, most of them do not achieve complete remission. Only some patients who received allogeneic stem cell transplantation achieved long-term complete remission.11,12 Therefore, we need to perform allogeneic hematopoietic stem cell transplantation to cure such patients because they are generally relatively young (median age: 42 years).12 To perform allogeneic bone marrow transplantation, initial treatments that achieve complete remission are critical for rescuing the patients. Overall, 3 of 13 patients who received intensive chemotherapy including anthracycline achieved complete remission in previous reports.11-12 It has also been reported that L-asparaginase-containing regimens achieved complete remission of ANKL, especially before allogeneic bone marrow transplantation,14-22 suggesting that we may need to include L-asparaginase in our chemotherapy regimens, even though L-asparaginase is hard to administer for patients with hepatic failure. We treated Case 1 with CHOP plus etoposide. However, this chemotherapy regimen might not have been appropriate, and instead, L-asparaginase could have been utilized for Case 1 because his performance status was relatively good compared with the other cases. Alternatively, ANKL patients may need to be treated with long-term administration of corticosteroid to improve the hepatic failure, which may prevent lethal hemorrhagic complications by normalizing the serum concentrations of coagulation factors. Case 2 may be such a patient because she had severe hepatic failure and suffered a cerebellar hemorrhage after only 4 days of chemotherapy. In contrast, although Case 1 received long-term steroid treatment, he suffered from intestinal bleeding after 9 days of chemotherapy. ANKL patients may need to be treated with corticosteroid for long periods, possibly before and after chemotherapy, to prevent hepatic failure. Case 3 could not be treated with chemotherapy and may have needed to be treated with corticosteroid for a longer period. In addition, patients with ANKL should be supplied with sufficient amounts of coagulation factors, including fresh frozen plasma and antithrombin.

In conclusion, both careful initial treatment, including available promising agents like L-asparaginase, and prophylaxis of bleeding by supplementation with coagulant factors are necessary for the treatment of ANKL patients.

REFERENCES

1 Chan J, Jaffe E, Ralfkiaer E, Ko Y: Aggressive NK-cell


4 Chan JK, Sin VC, Wong KF, Ng CS, Tsang WY, et al.:


7 Kwong YL, Wong KF, Chan LC, Liang RH, Chan JK, et al.:


 Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia 18:763-770, 2004


 Sustained CR of refractory aggressive natural killer cell leukemia following unrelated cord blood transplantation. Bone Marrow Transplant 45:1760-1762, 2010

 Aggressive NK-cell leukemia with sustained relapse-free survival after allogeneic peripheral blood stem cell transplantation. Rinsho Ketsueki 51:258-263, 2010