Ocular manifestation in myeloid/NK cell precursor acute leukemia: a case report. Diagnosed by flow cytometry and PCR from aqueous humor

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Abstract
Background: Myeloid/NK cell precursor acute leukemia (MNKL) is a rare type of leukemia, and ocular complications have not previously been reported. We now report a patient with MNKL who developed intraocular infiltrates during follow-up.

Methods and Results: A 13-year-old boy diagnosed with MNKL developed left eye pain 3 months after starting treatment. Examination of the left eye revealed a visual acuity of counting fingers at 20 cm, ciliary hyperemia, small corneal keratic precipitates, hypopyon, grade 4 vitreous opacities, and an obscured fundus. The differential diagnosis was between an opportunistic infection associated with immunodeficiency and an intraocular leukemic cell infiltrate. Therefore, a sample of aqueous humor was aspirated. Multiplex PCR/broad-range PCR of the aqueous humor was below detection limits for viruses, bacteria, and fungi. Flow cytometry (FCM) detected NK-related CD56-positive cells, thus leading to a diagnosis of ocular infiltrates due to MNKL. With treatment of the ocular infiltrates by systemic chemotherapy including intrathecal methotrexate (MTX), there was clearing of the vitreous opacities; and optic disc swelling, retinal hemorrhages, exudates, and protuberant lesions were now seen. With the addition of local radiation therapy to the eye, there was a dramatic treatment response, with regression of the optic disc findings and retinal lesions, and an improved visual acuity of 1.5.

Conclusion: We encountered the first case of MNKL in which ocular infiltrates developed during follow-up. Multiplex PCR and FCM of the aqueous humor were useful in rapidly distinguishing leukemic cell infiltrates from an opportunistic infection. This case highlights the usefulness of intrathecal MTX and local radiotherapy in treating ocular infiltrates in patients with MNKL.

Abbreviations: Ara-C = arabinoctydine, CD = cluster of differentiation, CMV = cytomegalovirus, CT = computed tomography, EBV = Epstein–Barr virus, FCM = flow cytometry, HDC = hydrocortisone, HHV = human herpes virus, HSV = herpes simplex virus, HTLV = human T-cell leukemia virus, L-asp = L-asparaginase, MIT = mitoxantrone hydrochloride, MNKL = myeloid/NK cell precursor acute leukemia, MRI = magnetic resonance imaging, MTX = methotrexate, NK = natural killer, VP-16 = etoposide, VZV = varicella zoster virus.

Keywords: flow cytometry, myeloid/NK cell precursor acute leukemia, ocular infiltration, ocular manifestation, PCR

1. Introduction

Myeloid/NK cell precursor acute leukemia (MNKL) is a rare type of leukemia characterized by extramedullary lesions mainly involving the lymph nodes and mediastinum. Usually, complete blood counts are mildly abnormal, with few leukemic cells in the peripheral blood. MNKL is a tumor at a stage similar to pluripotent stem cells originating as natural killer (NK) and myeloid cell precursors. Despite negative peroxidase staining on light microscopy, MNKL is characterized by myeloid antigen-positive, CD7-positive, and CD36-positive cells. No definitive treatment has been established, and because MNKL is usually refractory to chemotherapy, the prognosis is poor, with a median survival time of less than 2 years.

We now report the first known patient with MNKL who developed an ocular lesion and in whom PCR and flow cytometry (FCM) of the aqueous humor enabled a rapid diagnosis of an ocular leukemic cell infiltrate.

2. Case presentation

A 13-year-old boy noticed cervical lymph node swelling in October 2012 and was referred the following month to the Tokyo Medical and Dental University Hospital for further evaluation. Lymph node biopsy and bone marrow examination yielded a diagnosis of MNKL. In December 2012, he started to receive acute myeloid leukemia (AML)-oriented chemotherapy (cytarabine [Ara-C], anthracyclines, etoposide [VP-16], and triple intrathecal therapy [TIT] with methotrexate [MTX], Ara-C, and hydrocortisone [HDC]) combined with L-asparaginase (L-asp), which yielded complete remission after 2 courses of induction.
During the first course of consolidation therapy (high-dose Ara-C, mitoxantrone (MIT), L-asn, TIT) in February 2013, the patient developed pain and decreased vision in the left eye.

The initial eye examination showed a corrected visual acuity of 1.2 in the right eye and counting fingers at 20 cm in the left eye. Intraocular pressure was normal: right eye 17 mm Hg, left eye 13 mm Hg. Slit lamp examination of the right eye showed no abnormalities. However, in the left eye, there were 2+ ciliary injection, 1+ corneal mutton-fat keratic precipitates, 2+ anterior chamber cells, 3+ flare, and severe hypopyon (Fig. 1A). The right eye fundus was normal, but in the left eye, the fundus was obscured because of grade 4+ vitreous opacities (Fig. 1B).

First, an opportunistic infection was considered in the differential diagnosis, so aqueous humor was aspirated, and a multiplex PCR/broad-range PCR method that we developed was performed. All results were negative, including herpes simplex virus (HSV)-1,2 (−), varicella zoster virus (VZV) (−), cytomegalovirus (CMV) (−), Epstein–Barr virus (EBV) (−), human herpes virus (HHV)-6–8 (−), human T-cell leukemia virus (HTLV)-1 (−), bacteria (−), fungi (−), toxoplasma (−), and toxascaris (−). Thus, no genes for pathogens causing opportunistic infections were detected. Next, an ocular leukemic cell infiltrate was considered in the differential diagnosis, so FCM was performed. FCM detected CD56-positive NK cells in the aqueous humor (Fig. 2). Cranial and orbital computed tomography (CT)/magnetic resonance imaging (MRI) showed no abnormalities of the orbit or the optic nerve (Fig. 3). Based on this comprehensive evaluation, ocular infiltrates associated with MNKL were diagnosed.

Systemic chemotherapy including intrathecal MTX and anterior-oblique 2-port irradiation of the left orbit was planned. In March 2013, systemic chemotherapy with intrathecal MTX was started, the corrected visual acuity of the left eye improved to 0.6, and the vitreous opacities cleared. Examination of the fundus, which could now be visualized, showed retinal hemorrhages, exudates, protuberant lesions in the retinal periphery, and optic disc swelling (Fig. 4A). After starting radiation therapy, the protuberant lesions in the retinal periphery and the retinal exudates decreased in size.

After 4 months of radiation therapy (total radiation dose: 1.5 Gy × 17), the left eye visual acuity was 1.5, the optic disc swelling improved, the protuberant lesions in the retina disappeared, and only a few retinal exudates remained (Fig. 4B). The patient continues to be in remission for 2 years after completing the therapy.

3. Discussion

Previously reported ocular manifestations in leukemia include direct invasion by leukemic cells, retinopathy caused by hematopoietic injury, optic neuropathy secondary to CNS leukemia, and opportunistic infections.

In this first reported case of ocular manifestations in MNKL, the aspiration of aqueous humor enabled a definitive diagnosis in a short time. Patients with leukemia are prone to developing opportunistic infections because of their decreased immune

![Figure 1.](image1.png) Slit lamp examination at initial evaluation. There was ciliary injection and a hypopyon in the left eye (white arrow). (B) Funduscopy at initial evaluation. The fundus was obscured due to grade 4 vitreous opacities.

![Figure 2.](image2.png) Flow cytometry of the aqueous humor. CD56-positive natural killer (NK) cells were detected in the aqueous humor. (A) Gating of infiltrating cells in the aqueous humor. (B) Confirmation of CD56-positive cell infiltrates (upper left panel).

![Figure 3.](image3.png) Cranial computed tomography (CT) and magnetic resonance imaging (MRI) findings. No infiltrates of the orbit or optic nerve were present.

![Figure 4.](image4.png) (A) Fundus after systemic chemotherapy with intrathecal methotrexate (MTX). Corrected visual acuity was 0.6. Examination of the fundus, which could now be visualized, showed retinal hemorrhages, exudates, protuberant lesions in the retinal periphery (white arrow), and swelling of the optic disc. (B) After radiation therapy (1.5 Gy × 17). Corrected visual acuity was 1.0. At 4 months after starting treatment, the optic disc swelling improved, the protuberant lesions in the retina disappeared, and only a few retinal exudates remained (white arrow).
competence, so without therapy directed at pathogens, infectious endophthalmitis can progress and lead to a permanent loss of vision. Therefore, initial evaluation with multiplex PCR/broad-range PCR is useful in ruling out viral, bacterial, and fungal infections.\textsuperscript{[7–10]} Further testing with FCM of the aqueous humor detected NK cell–derived CD56, a major marker expressed on leukocytes in MNKL patients. This result led to a diagnosis of ocular infiltrates. Although CD34 expression was lost in ocular-infiltrated leukemic cells, leukemic cells occasionally alter their surface antigen expression pattern based on environmental conditions or differentiation stage.

Thus, PCR and FCM of the aqueous humor were able to distinguish leukemic infiltrates from an infection and were useful in helping to rapidly select appropriate treatment. This approach can be very effective in leukemic patients with ocular manifestations.

Treatment of ocular leukemic cell infiltrates includes chemotherapy for the primary disease, radiation therapy, and other treatment options such as vitrectomy.\textsuperscript{[4]} However, treatment of ocular infiltrates due to MNKL has not been previously reported, so no definitive treatment has been established. In our patient, treatment with systemic chemotherapy including intrathecal MTX and local radiation therapy to the eye effectively resolved the infiltrates. This combination may be useful for treatment of MNKL-related ocular infiltrates.

It is possible that the ocular infiltrates developed due to the treatment itself rather than MNKL. Thus, the chemotherapy may be a potential mechanism for the development of ocular infiltrates. It has been confirmed that the breakdown of the blood–ocular barrier can be induced after treatment with systemic drugs such as cidofovir, rifabutin, bisphosphonates, interferon, and TNFα inhibitors.\textsuperscript{[11]} The drug-induced autoimmunity and toxicity mainly result in anterior segment uveitis.

As for the clinical features in this case, previously reported inductive drugs were not used and infiltrates were seen not only in the anterior segment but also in the intermediate and posterior segments. Hypopyon, vitreous opacity, retinal disorders including exudates, hemorrhage, and protuberant lesions seen in this case were similar to leukemic manifestations rather than drug-induced manifestations.\textsuperscript{[4–12]}

The causative relationship between ocular infiltration and MNKL could be explained by the CD56-positive infiltrating cells detected from aqueous humor by FCM. CD56 expression is associated with extramedullary leukemic infiltration.\textsuperscript{[13–15]} CD56 is a neural cell adhesion molecule isoform that plays an important role in migration through cell-to-cell adhesion via homophilic adhesion.\textsuperscript{[16,17]} In addition to the expression of CD56 on NK cells, CD56 is also expressed on the surface of neurons and glia.\textsuperscript{[18]} Therefore, it is possible that CD56 expression in leukemic cells could allow infiltration through the adhesion of leukemic cells to intraocular components that also express CD56, such as neurons, Müller cells, astroglia, and microglia in the retina.\textsuperscript{[16,17]} Thus, ocular involvement in MNKL may result from the recruiting of leukemic cells to the eye via homophilic adhesion of CD56.

Taken together with PCR and FCM data, we diagnosed this patient as having ocular infiltrates due to MNKL. However, the recent findings make it difficult to perfectly describe the mechanism based on our current understanding. Further investigations need to be performed to identify the mechanism of ocular infiltration in patients with leukemia.

4. Conclusion

To the best of our knowledge, no previous cases of ocular infiltrates in patients with MNKL have been reported. For differential diagnosis of the ocular lesion in our patient, multiplex PCR of the aqueous humor was performed to rule out an infection, and then FCM was performed, which rapidly led to a definitive diagnosis of leukemic cell infiltrates. The combination of PCR and FCM of the aqueous humor may be an important test in leukemia patients with ocular lesions. Our case study suggests that a combination of intrathecal MTX and radiation therapy is an effective treatment for ocular infiltrates in MNKL.

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