CASE REPORT

Quantitative polymerase chain reaction test for molecular diagnosis of intraocular relapse of acute lymphoblastic leukemia: a case report

Jennifer CH Hung,[1] *MBBS (UK), MRCSEd (Ophth), FCOphth (HK), FHKAM (Ophthalmology); Ian YH Wong,*[2] *MBBS (HK), FRCS, FHKAM (Ophthalmology)*
[1]*Department of Ophthalmology, United Christian Hospital, Hong Kong*
[2]*Department of Ophthalmology, Hong Kong Sanatorium Hospital, Hong Kong*

Correspondence and reprint requests:
Dr Jennifer CH Hung, United Christian Hospital, 130 Hip Wo Street, Kwun Tong, Hong Kong
Email: hch395@uch.org.hk

Abstract

Leukemia relapse rarely presents with ophthalmic manifestations. We report on a 62-year-old woman with relapse of Philadelphia chromosome–positive acute lymphoblastic leukemia who presented with ocular infiltration as the sole presentation. The diagnostic difficulties are also discussed.

Key words: Leukemia, lymphoid; Molecular diagnostic techniques; Polymerase chain reaction

Introduction

Patients with leukemia often have ocular manifestations secondary to direct leukemic infiltration or indirect effects of hematological abnormalities, central nervous system involvement, infections, or therapy.[1] However, leukemia relapse presenting with ocular involvement is uncommon. We report a case of adult Philadelphia chromosome–positive acute lymphoblastic leukemia relapse with orbital infiltration as the sole presentation. The diagnostic difficulties are also discussed.

Case presentation

In April 2019, a 62-year-old woman was referred to our ophthalmology department with a 2-day history of sudden vision loss, swelling, and redness of the right eye. She had been diagnosed with Philadelphia chromosome–positive acute lymphoblastic leukemia 1 year earlier. After the first course of chemotherapy, she relapsed but responded to further chemotherapy and haplo-allogeneic bone marrow transplant. She was receiving blinatumomab immunotherapy, along with low-dose steroids, prophylactic antivirals, antibiotics, and antifungals. During the first year of treatment, she had fungal lung infection and made a full recovery.

She had no ocular history of laser, surgery, or trauma except for right eye retinal barrier laser over 30 years earlier. On examination, visual acuity was light perception in the right eye and 20/20 in the left eye. The right eye had relative afferent pupillary defect. Intraocular pressure was 20 mmHg. Extraocular movements were restricted in all directions, with marked proptosis. Biomicroscopy examination showed severe chemosis and conjunctival injection. The anterior chamber was quiet with no cells, with an immature cataract. Fundoscopy revealed optic disc swelling with whitish infiltrate at the macula, along with

retinal hemorrhages in all four quadrants. The vitreous was clear with no cells. Ocular coherence tomography of the macula and disc showed marked thickening of the macula and disc with significant subretinal fluid (**Figure 1**). Anterior segment examination and dilated fundoscopy of the left eye was unremarkable. Orbital infiltration secondary to leukemia relapse was suspected, with proptosis and compression of the optic nerve resulting in a central retinal vein occlusion pattern and orbital inflammation. One differential diagnosis was orbital infection secondary to the immunocompromised state. However, the patient had no history of fever, and her vitals were stable, and her peripheral blood count did not show any leukocytosis suggestive of sepsis. In addition, she had no history of diabetes mellitus, hypertension, or hypercoagulable states that might have contributed to the central retinal vein occlusion. Another differential diagnosis was idiopathic orbital inflammatory syndrome. It was important to rule out leukemia relapse.

Peripheral blood smear did not show any blasts and was negative for bcr-abl p190 quantitative polymerase chain reaction (qPCR). Owing to existing thrombocytopenia, the patient did not undergo lumbar puncture or bone marrow aspirate. Magnetic resonance imaging of the brain and orbit showed proptosis of the right eye with increased heterogenous enhancement at the intraconal space (**Figure 2**). Sclerochoroidal complex, extraocular muscles, and optic nerve were also thickened with contrast enhancement along the nerve. The eyelid and preseptal fat also enhanced abnormally. These findings suggested orbital inflammation with possible infiltration of the optic nerve and orbit.

Owing to the high index of suspicion for leukemia relapse, the patient was treated with a tapering course of intravenous dexamethasone. She also received topical levofloxacin, timolol 0.5% and intensive lubricants.

To obtain a molecular diagnosis and rule out infection, orbital tissue biopsy was proposed, but it has a high risk of hemorrhage owing to the patient's thrombocytopenia. The patient refused invasive procedure but agreed to undergo an aqueous tap for cytological examination and microbiological cultures, although aqueous was likely to have a lower yield of positive results because of the lack of cells in the anterior chamber. Flow cytometry result was negative for leukemic cells, but qPCR result was positive for bcr-abl p190, which provided a molecular diagnosis of localized leukemia relapse (**Figure 3**).

Within 2 days of starting intravenous steroids, orbital inflammation improved markedly, with eventual resolution of chemosis and disc swelling, resulting in a pale and atrophic disc. The patient underwent orbital radiotherapy (15 Gy) over the next 3 weeks. Unfortunately, her vision did not recover, with no light perception. The patient continued with immunotherapy, but the disease continued to progress. She eventually received palliative care overseas and died 4 months later.

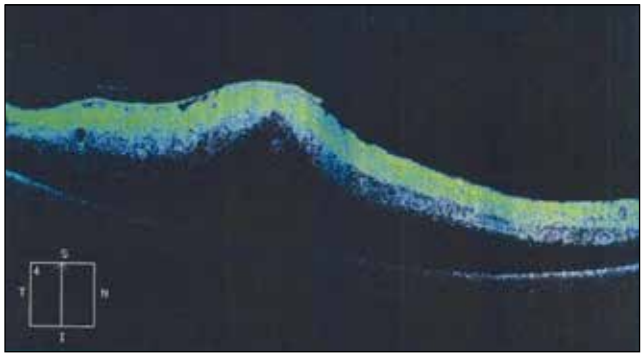

Figure 1. Ocular coherence tomography of the right eye showing marked thickening of the macula and disc with significant subretinal fluid.

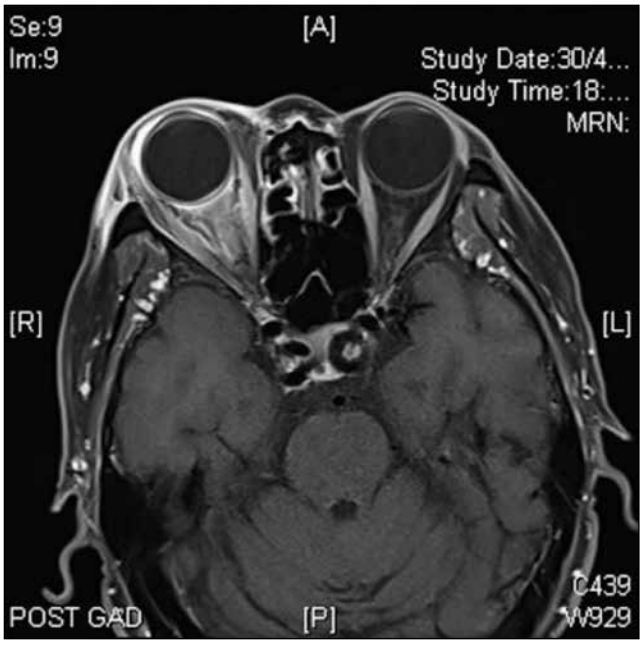

Figure 2. T1-weighted magnetic resonance imaging of the brain and orbit showing proptosis of the right eye with heterogenous enhancement in the intraconal space and thickened optic nerve. Sclerochoroidal complex and extraocular muscles are also thickened. The eyelid and preseptal fat enhance abnormally.

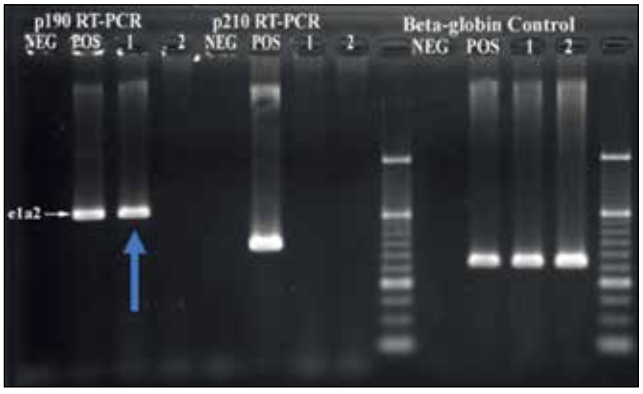

Figure 3. Quantitative polymerase chain reaction result from an aqueous sample is positive for bcr-abl p190 genetic abnormality (arrow).

Discussion

To the best of our knowledge, this is the first case in which aqueous aspirate flow cytometry was negative for leukemic cells but positive for molecular PCR diagnostics. This case highlights the diagnostic difficulties and the importance of molecular diagnostics in addition to flow cytometry, especially when tissue biopsy is not feasible.

Leukemia may affect the eye in various ways (**Table**). Leukemia manifested as direct infiltration of the eye is uncommon. Indirect manifestations secondary to hematological abnormalities (such as hyperviscosity syndromes and thrombocytopenia), chemotherapy, radiation, bone marrow transplantation, and infection are more common.[1] Epidemiological studies have reported that around 40% of patients with newly diagnosed leukemia exhibit ocular signs, although they may not necessarily cause visual symptoms.[2,3] Leukemia relapse with sole involvement of the orbit is rare and is likely to occur in acute lymphoblastic leukemia. The central nervous system (CNS) may act as a sanctuary for leukemic cells, because the blood-brain barrier prevents systemic chemotherapy from entering the CNS.[4] Malignant cells may infiltrate the optic nerve and orbit and cause relapse. Orbital involvement may present as an orbital abscess secondary to infection of necrotic infiltrated orbital tissues.[5] In a review of 2780 children with acute lymphoblastic leukemia followed up over 10 years, only 20 had intraocular relapse, accounting for 2% of all acute lymphoblastic leukemia relapses.[6] For adults, isolated cases of eye involvement during relapse have been reported. In a review of 458 adults with remission from acute myeloid leukemia, the 5-year cumulative incidence of meningeal relapse was only 0.3% in those treated with modern chemotherapy protocols,[7] compared with 5% in adult patients with acute lymphoblastic leukemia in remission.[8] Optic nerve infiltration may be categorized as disease relapse involving the CNS; therefore, the proportion of patients with intraocular involvement in relapse is even smaller. CNS relapse of leukemia is associated with a poor prognosis. In a UK study in 1992, the 5-year survival rate was 21.4% in pediatric patients with ocular involvement of acute leukemia, compared with 45.7% in those without ocular involvement.[9]

Treatment for leukemic infiltration of the eye should target the underlying systemic malignancy. Newly diagnosed patients should undergo a treatment protocol of induction, consolidation, and maintenance, usually involving intensive systemic chemotherapy. Intrathecal chemotherapy and radiotherapy may be required for CNS involvement. Allogeneic bone marrow transplants may be required for leukemias with aggressive clinical course and unfavorable cytogenetics. Relapse protocols depend on the affected site. Relapse at the orbit or optic nerve is usually managed as CNS relapse. Patients usually require orbital or cranial irradiation as well as systemic and intrathecal chemotherapy.[5] However, no formal guidelines exist for the treatment of orbital relapse.

Diagnosing leukemia relapse in immunosuppressed patients is difficult, as they are susceptible to opportunistic infections that may mask or mimic relapse. Ocular leukemic infiltration may masquerade as uveitis and infection, and therefore a high index of suspicion and a cytological or molecular diagnosis is important to initiate prompt and appropriate treatment. In our patient, the severe proptosis, chemosis, vision loss, and optic nerve swelling indicated possible orbital infection or inflammation in addition to leukemic infiltration.

Ideally, vitreous, retinal, or optic nerve biopsy should be performed for immunohistochemistry and direct visualization of leukemic cells. However, biopsy of such tissue samples may not be safe, as in our case, optic nerve biopsy may have resulted in irreversible visual loss, and the bleeding risk was high owing to thrombocytopenia. Lumbar puncture should

Table. Leukemic involvement in the eye (adapted from Sharma T, Grewal J, Gupta S, Murray PI. Ophthalmic manifestations of acute leukaemias: the ophthalmologist's role. Eye 2004;18:663-72.)

Eye part	Manifestation
Lids	Ectropion, edema, mechanical ptosis
Conjunctiva	Chemosis, conjunctival mass, corkscrew vessels, conjunctivitis
Cornea	Keratitis-limbal infiltration or secondary to immunosuppression or graft-versus-host disease, sterile ring ulcers, pannus, melt syndrome, dry eyes, epithelial changes secondary to chemotherapy
Orbit	Exophthalmos, orbital/preseptal cellulitis, endophthalmitis, dacryocystitis
Iris, angle, anterior chamber, and lens	Glaucoma, uveitis, hyphema, pseudohypopyon (yellow/grey), heterochromia, cataract secondary to treatment
Retina	Hemorrhage at all levels, perivascular infiltrates, retinitis, vitreous hemorrhage, microaneurysms, cotton wool spots, peripheral neovascularization, retinal detachments, drusen, vascular occlusion, retinitis secondary to opportunistic infections
Choroid	Thickened with associated serous retinal detachment
Optic nerve and central nervous system	Nausea, vomiting, lethargy, seizures, diplopia, asymptomatic papilloedema, blurred vision as a result of compromised optic nerve function
Miscellaneous	Anterior segment ischemia, lacrimal gland infiltration, opportunistic infections
Extramedullary relapse in eye	Uveitis, proptosis due to retro-orbital mass, retinal detachment, disc swelling

be considered to confirm CNS relapse involving the optic nerve, but it was contraindicated in our patient owing to thrombocytopenia.

An aqueous tap is relatively safe and easy to obtain, with low infection risk. Vitreous taps may have lower yield because vitreous may plug the needle during aspiration, preventing large amounts of vitreous fluid from being aspirated. Aqueous aspirate can be sent for microbiological examination and cultures, cytological examination, flow cytometry, and/or molecular diagnostics such as gPCR. The feasibility and logistics of performing qPCR and flow cytometry on aqueous samples or vitreous biopsies should be discussed with laboratories on a case-by-case basis, as not all laboratories have the capability of performing and validating these tests on such samples.

Flow cytometry uses a laser probe to detect, count, and profile cells within the fluid sample. Cell surface markers are fluorescently labelled to enable detection by the laser. Flow cytometry is useful to aid in the diagnosis of intraocular lymphoma.[10] Several case reports described using flow cytometry and qPCR to diagnose intraocular infiltration of leukemia.[11,12]

The success of cytological examination and flow cytometry depends on obtaining sufficient leukemic cells for detection. Some recommend >10 mL of cerebrospinal fluid, which is not feasible for aqueous aspirate assuming that affected aqueous humor has similar cell counts to cerebrospinal fluid.[13] Turbid aqueous, such as those taken from hypopyons, may act like highly proteinaceous effusions with large amounts of cell debris and reactive cells that may obscure leukemic cells.[10] All these samples should be processed in a timely fashion, as cells may deteriorate rapidly for adequate identification.

In our patient, flow cytometry of the aqueous aspirate was negative for leukemic cells. This could be due to a low lymphocyte cell count within the sample, as CD45+ lymphocytes accounted for 10.5% of nucleated cells, and B cells accounted for only 7% of these lymphocytes.

qPCR is a molecular diagnostic test widely used in the diagnosis of leukemia.[4] It amplifies and detects specific target DNA sequences or gene rearrangements in a sample. Our patient had bcr-abl p190 abnormal RNA sequence based on genetic phenotyping of her Philadelphia chromosome–positive acute lymphoblastic leukemia when she was initially diagnosed. We could extract RNA from the aqueous sample and detect an abnormal bcr-abl p190 sequence through qPCR to confirm the diagnosis.

The positive result from qPCR may be due to a small number of infiltrative tumor cells causing subclinical uveitis in the anterior chamber or the presence of tumor cells within the chemotic conjunctiva that contaminated the aqueous sample (but the latter cannot be proven without orbital tissue biopsy). qPCR is a useful and sensitive test to confirm leukemia relapse, owing to its ability to amplify DNA and RNA sequences. This test should be promptly processed to prevent RNA-ase degrading the sample. To confirm suspected intraocular involvement of leukemia, qPCR and flow cytometry of intraocular specimens should be performed promptly. Close cooperation with the hematologist and histopathologist may speed up the process. To improve the diagnostic yield, larger samples taken from vitreous, retinal, or optic nerve biopsy are preferred, in addition to lumbar puncture. However, patients with systemic condition may not be suitable for invasive procedures, especially retinal biopsy, which requires post-operative posturing for intraocular gas or oil tamponade. Instead, aqueous or vitreous taps should be considered, although they have a lower yield and may not be supported by all laboratories. qPCR is preferable to flow cytometry, as it requires a smaller sample cell count to detect disease, but qPCR is not widely available. If qPCR is not available, then cytology may be the first step to identify disease relapse. Lumbar puncture should be performed, if possible, to support a histopathological diagnosis of intraocular leukemic involvement.

Conclusion

A high index of suspicion is needed to diagnose leukemia relapse in patients presenting with ocular symptoms only. Infection may mimic leukemic infiltration. Aqueous aspirate is a useful and safe procedure to obtain samples for cytological and molecular diagnosis. qPCR may confirm the diagnosis even if flow cytometry result is negative. Urgent orbital or cranial radiotherapy should be performed to reduce the risk of irreversible visual loss. Full peripheral blood and marrow examination should be performed to identify other systemic relapse.

Ethics approval

This study was approved by the Medical Group Research Committee of the Hong Kong Sanatorium Hospital (reference: RC-2020-07), and the patient was treated in accordance with the Declaration of Helsinki. Written informed consent for publication was not obtained from the patient before she died. Her next of kin was unable to be contacted for consent.

Conflicts of interest

All authors have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Author contributions

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

References

1. *Talcott KE, Garg RJ, Garg SJ. Ophthalmic manifestations of leukemia. Curr Opin Ophthalmol 2016;27:545-51.* Crossref
2. *Koshy J, John MJ, Thomas S, Kaur G, Batra N, Xavier WJ. Ophthalmic manifestations of acute and chronic leukemias presenting to a tertiary care center in India. Indian J Ophthalmol 2015;63:659-64.* Crossref
3. *Reddy SC, Jackson N, Menon BS. Ocular involvement in leukemia: a study of 288 cases. Ophthalmologica 2003;217:441-5.* Crossref
4. *Larson RA. Managing CNS disease in adults with acute lymphoblastic leukemia. Leuk Lymphoma 2018;59:3-13.* Crossref
5. *Sharma T, Grewal J, Gupta S, Murray PI. Ophthalmic manifestations of acute leukaemias: the ophthalmologist's role. Eye (Lond) 2004;18:663-72.* Crossref
6. *Somervaille TC, Hann IM, Harrison G, et al. Intraocular relapse of childhood acute lymphoblastic leukaemia. Br J Haematol 2003;121:280-8.* Crossref
7. *Martínez-Cuadrón D, Montesinos P, Pérez-Sirvent M, et al. Central nervous system involvement at first relapse in patients with acute myeloid leukemia. Haematologica 2011;96:1375-9.* Crossref
8. *Larson RA. Managing CNS disease in adults with acute lymphoblastic leukemia. Leuk Lymphoma 2018;59:3-13.* Crossref
9. *Ohkoshi K, Tsiaras WG. Prognostic importance of ophthalmic manifestations in childhood leukaemia. Br J Ophthalmol 1992;76:651-5.* Crossref
10. *Davis JL, Miller DM, Ruiz P. Diagnostic testing of vitrectomy specimens. Am J Ophthalmol 2005;140:822-9.* Crossref
11. *Cerdà-Ibáñez M, Bayo-Calduch P, Manfreda-Domínguez L, Duch-Samper A. Acute vision loss as the only sign of leukemia relapse. Retin Cases Brief Rep 2018;12:10-1.* Crossref
12. *Hiraoka M, Ohguro H, Ikeda H, Furuya D, Takahashi S. Intraocular infiltration of Philadelphia chromosome-positive acute lymphoblastic leukemia diagnosed by polymerase chain reaction from the aqueous humor: a case report. Medicine (Baltimore) 2020;99:e18872.* Crossref
13. *Leach M, Drummond M, Doig A. Limitations. In: Practical Flow Cytometry in Haematology Diagnosis. John Wiley & Sons: 2013:20-30.*