Use of the Polymerase Chain Reaction to Detect B- and T-Cell Gene Rearrangements in Vitreous Specimens From Patients With Intraocular Lymphoma

Valerie A. White, MD, FRCPC; Randy D. Gascoyne, MD, FRCPC; Katherine E. Paton, MD, FRCSC

Objective: To determine whether the polymerase chain reaction for B- and T-cell gene rearrangements could be applied to vitreous specimens to aid in the diagnosis of intraocular lymphoma.

Methods: Vitreous washing specimens from 4 patients were received in balanced saline solution and centrifuged, and a portion of the pellet was used to make routine cytospins. The remainder was used to make a crude extract of DNA that was amplified for immunoglobulin heavy chain and T-cell receptor γ gene rearrangements and the 14;18 translocation by polymerase chain reaction.

Results: One patient had 2 specimens 2 years apart. In each, there was an identical band corresponding to the minor cluster region breakpoint of the bcl-2 oncogene, indicating the presence of a 14;18 translocation. One patient showed an immunoglobulin heavy chain gene rearrangement indicating a B-cell lymphoma. Two patients showed rearrangements of the T-cell receptor γ gene, indicating the presence of a T-cell lymphoma.

Conclusions and Clinical Relevance: Vitreous washing specimens can be used successfully to detect B- and T-cell gene rearrangements by polymerase chain reaction. This may be useful to confirm the diagnosis of intraocular large cell lymphoma in cases suggestive of the diagnosis. Prompt handling of the specimens is necessary to prevent degradation of the DNA.


The diagnosis of large-cell lymphoma involving the vitreous in the absence of central nervous system (CNS) disease can be difficult. Typically, the clinical picture is characterized by initial misdiagnosis and treatment for inflammatory uveitis, delaying definitive diagnosis. Diagnosis requires an invasive procedure, and often sufficient material is received to only perform routine cytological examination. Procedures such as immunohistochemical analysis, which might aid in diagnosis, cannot often be used. The major diagnostic difficulties with vitreous washings are in the distinction of inflammatory lymphoid infiltrates from intraocular lymphoma or, when only a few atypical cells are present, being confident of the diagnosis of lymphoma. The polymerase chain reaction (PCR) method for the detection of immunoglobulin heavy chain (IgH) gene rearrangements, T-cell receptor gene rearrangements, and the bcl-2 translocation has been used for several years to confirm the diagnosis of lymphoma at other sites. We sought to determine whether PCR could be applied to vitreous samples in a similar manner for diagnosis.

REPORT OF CASES

CASE 1

A 30-year-old woman was examined in September 1992 because of a red, irritated right eye, blurred vision, and 2 seizures that had occurred over the past 12 to 18 months. A computed tomographic (CT) scan showed a left parietal abnormality, and a magnetic resonance image showed multiple periventricular enhancing lesions that were initially diagnosed as multiple sclerosis. Visual acuity was 20/200 OD and 20/20 OS. After an 18-month course of corticosteroid treatment for uveitis, vitrectomy was performed in the right eye in December 1992, and intraocular large-cell lymphoma was diagnosed. The patient underwent whole-brain and bilateral orbital irradiation of 3500 Gy in 20 fractions and also received systemic che-
MATERIALS AND METHODS

CYTOLOGICAL METHODS

Vitreous specimens were received in balanced saline solution (Balanced Salt Solution Plus; Alcon Canada Inc, Mississauga, Ontario) in the vitrectomy cassette within 2 hours of surgery. They were centrifuged in a benchtop centrifuge (Silencer model 103NAD; Silecencer, Japan) at 1500 rpm for 10 minutes. The supernatant was removed and the cell pellet resuspended in 1 to 2 mL of Hanks buffered saline solution. Aliquots (0.5 mL) of the cell pellet were spun onto glass slides in a cytocentrifuge (Shandon Southern Instruments Inc, Sewickley, Pa) at 500 rpm for 5 minutes. The slides were postfixed for at least 2 minutes in Clark fixative (12% glacial acetic acid in 70% alcohol) and stained with a rapid hematoxylin–eosin stain, or air dried and stained with Giemsa stain. If lymphoma was identified on the initial slides, the remainder of the specimen was stored overnight at 4°C and DNA was extracted the next day. If there was no suggestion of lymphoma when it was strongly suspected, the remainder of the specimen was used to prepare more slides.

MOLECULAR METHODS

The remainder of each cell pellet was used to make a crude extract of DNA by digesting overnight at 55°C with proteinase K (60 µg/mL). After digestion, the samples were heated to 95°C for 10 minutes to denature the proteinase K, centrifuged in a microfuge for 30 seconds to clear the supernatant, and diluted 1:4 before PCR. The DNA was amplified for IGH and T-cell receptor gene rearrangements as previously described. With all amplifications, known positive controls and samples containing no patient DNA were run. All patients’ specimens were amplified for the ß-globin gene (310 base pairs) as a test for DNA integrity. As negative controls, 14 vitreous specimens from 14 patients whose cytological studies showed mixed inflammation, consisting of lymphocytes, histiocytes, plasma cells, and/or occasional neutrophils, were also examined by PCR for evidence of gene rearrangement.

no further investigation or treatment was carried out, and CNS involvement did not recur. After cataract extraction, the patient’s visual acuity was 20/20 bilaterally.

CASE 2

An 83-year-old woman was seen in August 1995, 3 months after phacoemulsification surgery because of inflammation that developed in her right eye. This had been treated with prednisone, 40 mg daily, but had failed to clear. Before vitrectomy her visual acuity had been counting fingers OD and 20/50 OS. The family reported that the patient had seemed tired and weak for approximately 21 months, but that this had worsened along with a decreased appetite, weight loss of 9 kg, and some confusion in the last 2 months. A CT scan of the brain showed hypodense lesions in the central white matter, thought to be lacunar infarcts. A cerebrospinal fluid specimen did not show lymphoma cells, but the protein level was increased. A CT scan of the orbits showed no optic nerve thickening. A vitrectomy performed in September 1995 disclosed large-cell lymphoma. After vitrectomy, the patient’s visual acuity was 20/200 OD and 20/60 OS. Fundus examination showed an atrophic choroid with no evidence of lymphomatous deposits. She subsequently received 2000 cGy of radiation in 5 fractions over 5 days to the right anterior orbit and eye. The patient was alive 2½ years after diagnosis, but her general status was unchanged.

CASE 3

A 73-year-old woman was seen in July 1996 because of a 1-month history of increasing floaters in both eyes and a feeling of looking through fog. Chest radiograph was unremarkable, but the result of skin testing for purified protein derivative, Candida, and Trichophyton was anergic. There was no notable previous illness, and review of systems was negative, with no weight loss. Visual acuity was 20/25 OD and 20/30 OS. There were mild lenticonular changes in both eyes, and intraocular pressure was 18 mm Hg in both eyes. Fundus examination showed moderate vitreous cells in both eyes that appeared creased and of varying shapes. There were small macular drusen and pigmentary changes.

A pars plana vitrectomy was performed in October 1996 and intraocular large-cell lymphoma was diagnosed. There was no evidence of systemic lymphoma; CT scans of the brain, orbits, abdomen, and pelvis were normal, and bone marrow and cerebrospinal fluid were negative for lymphoma. She received 3500 cGy of radiation in 20 fractions over 4 weeks to the brain, orbits, base of the skull, and brain stem to C2, and intravenous chemotherapy consisting of methotrexate, folinic acid, dexamethasone, doxorubicin, vincristine, and procarbazine.

During the ensuing months, the patient developed a cataract in the left eye, and cataract extraction and intraocular lens placement were performed in September 1997.

CASE 4

A 50-year-old man was examined in October 1997 with left vitreitis. Although lymphoma was considered, it was
excluded because of lack of other signs and his young age. In January 1998, he had a CT scan that disclosed a mass in the corpus callosum suggestive of a lymphoma. At this time he was slightly confused. Visual acuity was 20/100 OS and 20/20 OD. A diagnostic vitrectomy was carried out and a diagnosis of lymphoma made. He began undergoing treatment.

RESULTS

CYTOLOGICAL FINDINGS

The slides from the first vitrectomy on the right eye in patient 1 showed necrotic cells and karyorrhectic debris. Numerous large lymphocytes with high nuclear to cytoplasmic ratio, irregular nuclear membranes, and prominent nucleoli were present singly and in groups within the necrotic debris. Occasional histiocytes and lymphocytes were also present. The second vitrectomy, performed 2 years later, showed the left eye to be less cellular, but with otherwise similar findings (Figure 1).

The specimen from patient 2 was moderately cellular, with large numbers of necrotic cells. Only occasional large malignant lymphocytes were present, scattered throughout the necrotic debris (Figure 2).

The specimen from patient 3 showed 8 to 10 large malignant lymphocytes on 2 slides. These were admixed with other inflammatory cells, including histiocytes, small lymphocytes, and plasma cells.

The specimen from patient 4 was paucicellular and showed only occasional large atypical, degenerated cells in a background of macrophages, lymphocytes, plasma cells, and neutrophils.

MOLECULAR FINDINGS

Both specimens from patient 1 showed identical rearrangements of the bcl-2 oncogene at the minor cluster region, indicating the presence of a follicle center cell-derived B-cell lymphoma (Figure 3). No IgH gene rearrangement was present by PCR.

In cases 2 and 3, the specimens showed rearrangements of the T-cell receptor γ gene, indicating the pres-
COMMENT

These 4 cases demonstrate that the PCR can be used successfully with vitreous specimens to confirm the diagnosis of large-cell lymphoma. In the first 2 cases, the cytological diagnosis was not in doubt, as large malignant lymphocytes were present. However, in the third and fourth cases, only a few atypical cells were present, admixed with inflammatory cells, making the diagnosis more difficult. To our knowledge, this is the first report in which the diagnosis of intraocular lymphoma together with lineage assignment has been confirmed by molecular techniques on vitreous fluid alone, rather than on biopsy material from another site, in patients with ocular disease (MEDLINE, 1966-1998).

We did not do a cell count on the vitreous as received for a number of reasons: it was admixed with a variable amount of vitrectomy fluid; the count would have included inflammatory as well as malignant cells; and we did not feel that we could spare any specimen for this extra test. For this last reason, it was impossible to do rigorous testing of different vitrectomy solutions to determine how these would affect viability of the cells, and we could not test how long a specimen would survive before degradation of the DNA; this would also depend on how viable the cells were when removed from the patient. We did not think that testing these variables would add to the information obtained, as we were most interested in making a definitive diagnosis by cytological examination and PCR, and we handled the specimens as soon as was reasonably possible. Sensitivities were not established directly on the vitrectomy specimens themselves for similar reasons. We have established sensitivities for our methods, as previously reported.1,5

It should be emphasized that, while a positive result for clonality testing in the presence of malignant or atypical lymphocytes confirms the presence of lymphoma, a negative result does not necessarily rule it out. A specimen may be falsely negative on PCR testing because of degradation of or insufficient DNA, or lack of amplification by the primers used. If a patient is suspected of having lymphoma, but this cannot be proved by either cytological examination or PCR, it may be necessary to repeat the procedure.

Recently, Katai et al6 reported on 2 patients with previous B-cell lymphomas of the orbit and chest wall who developed vitreitis after initial treatment. Although vitrectomies did not disclose malignant cells, IgH gene rearrangement was detected in each case, and the authors diagnosed ocular involvement by malignant lymphoma. Rhodes et al7 recently described their experience with the use of PCR to aid in the diagnosis of lymphomatous meningitis. Of 20 specimens that were negative for or suggestive of lymphoma, PCR for IgH gene rearrangement was clonal in 10. Two of 4 specimens that were positive on cytological examination were also clonal. The specimens were not tested for rearrangements of the T-cell receptor genes or the bcl-2 oncogene. Rhodes et al postulated that useful DNA may be present in karyorrhectic nuclei as well as free in the cerebrospinal fluid, making it important to ensure that specimens are handled promptly to prevent further degradation of DNA.

Intraocular lymphoma may be present with primary CNS lymphoma, and multiple procedures are often required before a definitive diagnosis can be established. Whitcup et al8 studied 12 patients with intraocular lymphoma and found that 3 had normal findings on vitrectomy initially: 1 case was diagnosed on the second vitrectomy, 1 on the third, and 1 on enucleation of a blind eye. Five of 11 patients eventually had findings positive for lymphoma on lumbar puncture, but 3 only on the second attempt. Peterson et al8 studied 24 patients with intraocular lymphoma, 23 of whom had CNS lymphoma at some point in their course. Vitrectomy was positive for lymphoma in 10 of 15 patients, but only on the second attempt in 2. Their 7 nondiagnostic vitrectomies were all in patients who had received previous corticosteroid therapy, a problem also mentioned by Whitcup et al. Freeman et al9 published an earlier series of 32 cases of intraocular lymphoma, of which only 18 were diagnosed...
on vitrectomy. Four were diagnosed by enucleation and 10 at autopsy. The use of molecular techniques should be useful in eliminating false-negative vitreous specimens, such as those described above, thus allowing prompt intervention.

Primary T-cell lymphoma of the vitreous is rare. Brown et al10 presented 2 cases, reviewed the literature, and found that 12 (21%) of 57 cases of intraocular lymphoma in which cell marker studies had been performed were of T-cell lineage. Two well-documented cases have been described. Novak and Katzin11 presented a case formed were of T-cell lineage. Two well-documented cases of primary CNS lymphoma in which cell marker studies had been performed, insufficient cells were obtained for immunophenotypic analysis. The diagnosis of T-cell lymphoma was made on material obtained from a brain biopsy specimen that showed that 100% of cells were CD3 positive by flow cytometry and that multiple rearranged bands were detected by the T-cell receptor β-chain gene probe with the use of Southern analysis. Goldey et al12 reported a case of a patient with unilateral anterior uveitis in which all of the aspirated cells were large granular lymphocytes that were CD3 positive. This patient was subsequently found to have a high-grade T-cell lymphoma involving the small bowel and retroperitoneal lymph nodes, which was confirmed by Southern analysis. Intraocular lymphoma is often associated with primary CNS lymphoma. In a review, Ferracini et al13 stated that 27 cases of primary T-cell lymphoma of the CNS were reported in the literature from 1983 to 1992.

Jellinger and Paulus14 studied 5 cases of primary CNS lymphoma by immunohistochemistry and Southern analysis and found no bcl-1 or bcl-2 rearrangements; therefore, our case 1 may be unique in this regard. Bcl-2 gene rearrangements can be found at diagnosis in systemic diffuse large-cell lymphoma with frequencies ranging from 14% to 40%.15-17

As the incidence of primary CNS lymphoma and therefore of intraocular lymphoma appears to be increasing, PCR gene rearrangement studies on vitreous specimens for the determination of both lineage and clonality offer a useful adjunct for diagnosis in difficult cases.18

Accepted for publication January 8, 1999.

Cases 1 and 2 were presented at the International Society of Ophthalmic Pathology biennial meeting, Chicago, Ill, October 25, 1996.

Corresponding author: Valerie A. White, MD, FRCPC, Department of Pathology, Vancouver General Hospital, 910 W 10th Ave, Vancouver, British Columbia, Canada V5Z 1L8 (e-mail: vwhite@vanhosp.bc.ca).

REFERENCES