Consultations in Molecular Diagnostics

T Cell Receptor γ -Chain Gene Polymerase Chain Reaction to Diagnose Central Nervous System Involvement by Cutaneous T Cell Lymphoma

Robert Taylor,* Jo-Anne Vergilio,[†]
Michael Shapiro,[‡] David Raizen,* Jennifer Hunt,[†]
Cindy McGrath,[†] Alain Rook,[‡] and Adam Bagg[†]

From the Departments of Neurology, Dermatology,[‡] and
Pathology and Laboratory Medicine,[†] Hospital of the University of
Pennsylvania, Philadelphia, Pennsylvania*

The authors describe a patient who was suspected of having cutaneous T cell lymphoma involvement of the brain despite repeatedly negative cerebrospinal fluid (CSF) cytology, inconclusive flow cytometry, and no discrete lesion for brain biopsy. The diagnosis was made by polymerase chain reaction (PCR) analysis that showed a monoclonal T cell receptor γ -chain gene rearrangement in the CSF, identically sized to that present in a skin biopsy specimen. Thus, PCR could be used early and routinely to diagnose central nervous system spread of T cell lymphomas, because of its potentially superior sensitivity and specificity to CSF cytology. (*J Mol Diagn* 2002, 4:118–120)

Cutaneous T cell lymphoma (CTCL) is a low-grade, non-Hodgkin's lymphoma characterized by a malignant clone of helper T lymphocytes that homes to the skin to produce pruritic and scaly patches and plaques, tumors, and erythroderma. In advanced stages, malignant cells with hyperconvoluted nuclei (ie, Sézary cells) circulate in the peripheral blood, invade lymph nodes, and, more infrequently, spread to visceral organs such as the lungs, bone, gastrointestinal tract, and central nervous system (CNS). Autopsies have shown CNS spread to the meninges and almost any part of the brain parenchyma.¹ However, symptomatic CNS involvement is uncommon, as demonstrated by a review that found only 40 total reported cases and 28 reported cases in the English literature.² The most frequent neurological symptoms include mental status changes, motor and gait disturbances, and cranial nerve deficits.² Symptoms arise as early as 4 months and as late as 30 years from the time of initial diagnosis, with mean time to onset described as 3.8

years to 7.5 years.^{2,3} Pre-mortem diagnosis is usually made by cerebrospinal fluid (CSF) cytology, although cytology is often negative and the diagnosis is made at autopsy.^{2,4} Brain biopsy is an alternative diagnostic method.^{3,5}

We describe a patient who developed gait abnormalities, mental status changes, and brain magnetic resonance (MR) imaging abnormalities 6 years after the initial diagnosis of CTCL. Cytology was repeatedly negative, flow cytometry was inconclusive, and a brain biopsy was not performed because there was no discrete lesion for biopsy. Polymerase chain reaction (PCR) analysis of the T cell receptor (TCR) γ -chain gene of the CSF showed a monoclonal rearrangement, indicative of a monoclonal T cell population that was identical to that evident in a skin biopsy with known tumor involvement. CNS-active chemotherapeutic agents were subsequently initiated.

Case Report

A 70-year-old woman developed multiple erythematous and scaly patches and plaques, which were diagnosed as CTCL by skin biopsies in 1994. The disease was controlled with a combination of psoralen and ultraviolet light A, topical corticosteroids and nitrogen mustard, and intramuscular methotrexate for six years. In April 2000, she had recrudescence of cutaneous disease with tumors of the scalp and orbit.

In August 2000, she developed neurological symptoms of disorientation, poor concentration, a slow magnetic gait, and postural instability leading to multiple falls. These neurological symptoms worsened over the ensuing months and by November 2000, she was oriented only to name, was attentive only to simple tasks, and was bed-bound. An initial brain MR imaging scan in August was unrevealing, but subsequent MR scans over the next 2 months showed increased signal intensity on T2-

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Address reprint requests to Robert A. Taylor, Department of Neurology, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104. E-mail: rat@mail.med.upenn.edu.

Figure 1. CSF specimen demonstrating an admixture of neutrophils, monocytes, and non-atypical lymphocytes (Wright magnification, $\times 100$).

weighted and fluid-attenuated inversion recovery images in the periventricular white matter and right centrum semiovale, extending into the right cerebral peduncle, pons, and both basal ganglia. There was minimal enhancement outlining the basal ganglia after gadolinium administration.

Initial lumbar puncture revealed 12 white blood cells per μl that were 63% polymorphonuclear neutrophils, 17% morphologically normal lymphocytes, 19% monocytes, and 1% eosinophils with a protein level of 62 mg/dl. Bacterial and fungal cultures were negative. Four additional lumbar punctures showed a similar cellular profile (Figure 1). The cytopreparations were Wright (Fisher Healthcare, Houston, TX), Diff-Quik (EM Science, Gibbstown, NJ), and Papanicolaou-stained (EM Science) preparations. The first was initially reviewed by a hematopathologist (A.B.), and the latter two by a cytopathologist (C.M.), who independently came to the same conclusion, namely the absence of atypical cells. Following the identification of monoclonality at the molecular genetic level, both parties subsequently reviewed all preparations in conference and the negative cytology was reconfirmed. CSF flow cytometry on three specimens failed to identify a significantly expanded, immunophenotypically aberrant T cell population. On one occasion, 8% of the cells, accounting for less than 1 cell per μl were $\text{CD}2^+$, $\text{CD}4^+$, and $\text{CD}7^-$. Serum B12 and angiotensin converting enzyme (ACE) levels were normal, and a rapid plasma reagin (RPR) and treponema pallidum antibody (TPA) were negative. Human immunodeficiency virus serology was unreactive. PCR amplification assays of the CSF for varicella-zoster virus, JC virus, and cytomegalovirus were negative.

Despite the unrevealing cytological examinations and flow cytometry studies, the clinical suspicion for spread of CTCL to the brain remained high. A brain biopsy was deferred due to the high morbidity associated with the

procedure and the likelihood that it would miss the small regions of enhancement observed on MRI. Consequently, TCR γ -chain gene rearrangement studies to identify a clonal population of T cells were performed on cerebrospinal fluid specimens.

Two separate skin biopsy specimens from the patient, that were histologically and immunophenotypically diagnostic of CTCL, were used as positive controls, and a

Figure 2. Results of PCR of the V γ 9–11 region of the T cell receptor γ -chain gene, using three different upstream primers, one each to V γ 9, V γ 10, and V γ 11. The PCR products are analyzed using capillary electrophoresis. The specimens obtained from the patient included a skin biopsy (**A**), two samples of CSF (**B** and **C**) obtained approximately 1 week apart, and a sample of peripheral blood (**D**). The same monoclonal rearrangement is present in the skin and the CSF, demonstrated by peaks of identical size (at 175 bp and 194 bp). The finding of two different sized peaks indicates biallelic TCR γ -chain gene rearrangement. The peripheral blood shows polyclonality, demonstrated by many peaks of varying size. The x axis reflects the sizes of the products in bp. The y axis reflects fluorescent intensity in relative units.

peripheral blood sample from the patient was used as a negative control. The negative control of the patient's blood sample was additionally important to eliminate the possibility of a false positive CSF result due to contamination by circulating Sézary cells from a bloody tap. DNA was extracted from the CSF, a paraffin-embedded skin biopsy and peripheral blood samples by standard techniques using a commercially-available kit, and following the manufacturer's instructions (Qiagen, Valencia, CA). The DNA was then quantified spectrophotometrically. All samples were similarly handled however, the CSF specimens (5 to 10 ml) were first centrifuged to concentrate the cellular contents and then washed with phosphate-buffered saline before DNA extraction. PCR amplification was performed using two sets of fluorescently labeled primers that are complementary to the V and J regions of the TCR γ gene, as previously described.⁶

One set of primers used to perform PCR amplification contained a single upstream primer, a consensus to V γ 1–8, while the second set contained three upstream primers, one each to V γ 9, V γ 10, and V γ 11. The PCR amplification products were resolved using an automated high-resolution capillary electrophoresis system (ABI 310; Perkin Elmer Biosystems, Foster City, CA) with the following expected product sizes: V γ 1–8 primer (200 to 250 bp; midpoint, 225 bp) and V γ 9–11 primers (150 to 200 bp; midpoint, 175 bp). No consistent and reproducible monoclonal peak was discerned with the V γ 1–8 primer in either the CSF or in the skin. Using the V γ 9–11 primer set however, the analysis of both the CSF and skin biopsy specimen revealed the same two dominant amplification products of ~175 and ~194 bp (Figure 2). These PCR products represent a biallelic, monoclonal TCR γ -chain gene rearrangement, indicative of a clonal T cell population. By contrast, the same analysis on peripheral blood DNA displayed a polyclonal pattern, without the aforementioned dominant amplification products, indicating that there were no detectable circulating clonal T cells.

The detection of the same clonal population of T cells in the skin and the CSF indicates that the tumor was also present in the CSF. We inferred that the parenchymal CNS lesions represented tumor as well.

The patient was treated with high doses of intravenous steroids and methotrexate which resulted in an improvement in her mental status and a return of her ability to ambulate. The methotrexate was switched to Ara-C 4 months later, because of progressive renal failure. The patient died 1 month later (5 months from the time of initial diagnosis) at another hospital, where an autopsy was not performed, precluding the exact determination of the cause of death.

Discussion

The prognosis of CTCL involvement of the CNS is poor with a mean survival from the time of CNS disease diagnosis of about 3.9 months.² Earlier diagnosis and treatment can potentially improve outcome. The TCR γ -chain gene rearrangement PCR assay has already been shown to be a highly sensitive technique for detecting monoclonal T cell populations in skin biopsies of patients with CTCL, with the ability to detect a dominant clone constituting 0.01% to 0.1% of total specimen cells.⁷ The added sensitivity of this PCR-based assay provides the potential for earlier detection of clonal T cell populations in the CSF. A single positive PCR result, in isolation, does not imply the presence of monoclonality, with pseudoclonality⁸ (due to low numbers of lymphocytes) and canonical rearrangements⁹ (of the TCR γ -chain gene) being potential sources of confounding results. However, the ability of capillary electrophoresis to identify an accurate and precisely sized "fingerprint" of the T cell clone provided a tumor-specific marker (identical to that obtained at a remote, involved site), which allowed for the diagnosis of CNS involvement, in the absence of "conventional" pathological findings. In support of this was the patient's initial response to appropriate systemic therapy, and subsequent clinical course. To our knowledge, this is the first such reported case highlighting this finding.

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