

Combined Diagnostic Approach to AIDS-Related Primary CNS Lymphoma Using EBV-DNA Polymerase Chain Reaction in CSF, Thallium-201 Single-Photon Emission Computed Tomography, and Toxoplasma gondii Serologies. Is a Brain Biopsy Always Necessary?

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A. Study Purpose and Rationale

Focal brain lesions (FBL) in HIV-infected patients remain a diagnostic dilemma. Approximately 30-40% of all patients with AIDS show central nervous system (CNS) disease [1,2]. This number increases to 75% or more in autopsy studies. Approximately 10% of AIDS patients with CNS disease have one or more mass lesions [2]. The differential diagnosis of these mass lesions is broad and can include primary CNS lymphoma (PCNSL), Toxoplasmosis, progressive multifocal leucoencephalopathy (PML), HIV encephalopathy, cytomegalovirus (CMV)-encephalitis, herpes simplex virus (HSV)-encephalitis, tuberculoma, cryptococcoma, vasculitis, and abscess [3]. Toxoplasmosis and PCNSL account for over 50% of FBL [2], with toxoplasmosis being the most common cause.

Lesions caused by toxoplasmosis and PCNSL are often difficult to distinguish from each other by radiographic appearance on CT or MRI [4,7,8,9,10,11]. Toxoplasmosis typically causes ≥ 2 ring-enhancing lesions, often with associated edema, and lesions commonly occur in frontal, basal ganglia, and parietal regions [9]. PCNSL classically causes a single or multiple hypodense lesions that enhance homogeneously; however, central necrosis can cause a ring-enhancing appearance. Lesions are often located in the cerebrum, basal ganglia, cerebellum, and, occasionally, the brain stem, and are typically periventricular. Subependymal spread is pathognomonic for PCNSL. However, there is considerable overlap between the appearance of these two entities. For example, 21% of toxoplasmosis lesions are single lesions on MRI, and PCNSL causes multiple lesions in 50% of cases.

Brain biopsy is the gold standard for the diagnosis of FBL. Serious complications due to brain biopsy have been reduced with the use of stereotactic biopsy procedures [1, 10]. Complications from stereotactic brain biopsy include hemorrhage, neurological impairments, which may be transient or permanent, and, rarely, infection at the biopsy site. In one study, perioperative morbidity after stereotactic biopsy was 12%, while perioperative mortality was 2% [6]. Hemorrhage was the most common complication and, interestingly, all hemorrhages occurred in patients found to have PCNSL [3]. The diagnostic yield of stereotactic biopsy is high, often $>90\%$ [1, 2, 4, 6, 12].

Many diagnostic algorithms have been developed in order to identify HIV-infected patients, presenting with neurological signs or symptoms, who would most likely benefit from a brain biopsy [1, 6, 12]. Figure 1 and Figure 2 [12] show the most commonly used algorithms.

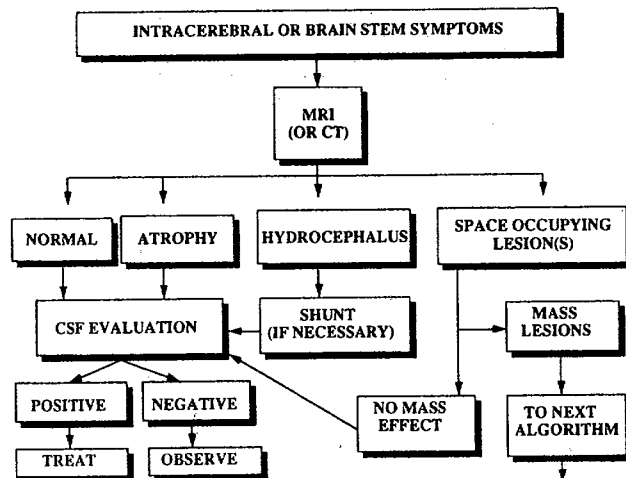


FIG. 1. Algorithm for the evaluation of patients with HIV infection and neurological signs of symptoms.

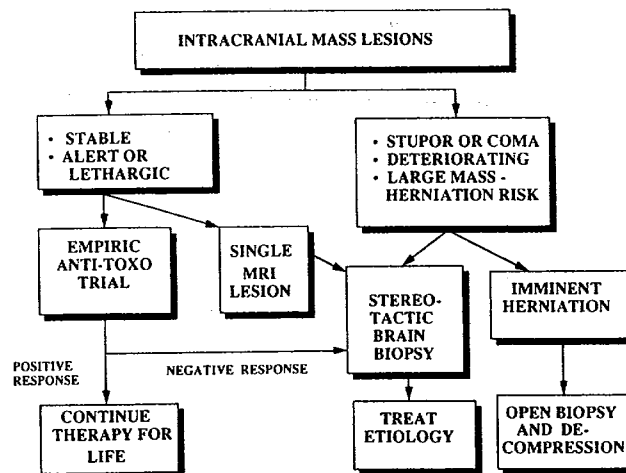


FIG. 2. Algorithm for the evaluation of patients with HIV infection and intracranial lesions.

Typically, patients are treated with anti-*Toxoplasma* therapy for 1-2 weeks. Those patients who do not show clinical or radiologic improvement while on anti-*Toxoplasma* therapy then undergo brain biopsy. It is generally believed that the earlier the diagnosis of PCNSL is made and the sooner treatment is started, the better the outcome [4,5], therefore, other methods of diagnosis have been examined [3,4,5,6,7,10]. The most promising of these include the detection of EBV-DNA in CSF samples by polymerase chain reaction (PCR) and Thallium-201 single-photon emission computed tomography (SPECT) scan.

PCNSL is almost always a high grade B-cell lymphoma [3,4,5,6]. It is not surprising, therefore, that the EBV genome can be detected in lymphoma cells in nearly 100% of cases. Cingolani *et al.* [3] demonstrated the high sensitivity and specificity of EBV-DNA detection in the CSF by PCR for PCNSL. They showed a sensitivity of 80% and specificity of 100% for PCNSL in a population of HIV-infected patients with FBL who underwent LP and brain biopsy.

Thallium-201 SPECT scan has been shown to be beneficial in distinguishing lymphoma from other brain lesions [4, 10]. Thallium-201, a radionuclide, is taken up by active transport into tumor cells. Normal increased thallium-201 uptake also occurs at the skull base, orbits, nasopharynx, and scalp. Infections and nonneoplastic brain conditions are typically negative to thallium-201 uptake. False

negatives can also be obtained in areas of tumor necrosis, and lesions smaller than 6-8 mm are undetectable by SPECT scan. False positives have been identified in 2 cases: a *Candida* brain abscess and a bacterial brain abscess. False positives are also more common when SPECT scan results are analyzed visually (see table below).

Table 5. The findings of single-photon emission CT thallium-201 studies of patients with AIDS.

Reference	No. of subjects	Method of interpretation	Sensitivity, %	Specificity, %
[119]	32	Uptake ratios	55	100
[133]	13	Subjective	100	86
[134]	37	Uptake ratios	100	100
[135]	18	Uptake ratios	100	90
[136]	37	Uptake ratios	75	96
[137]	49	Subjective	96	76
[138]	36	Uptake ratios	83	96
[139]	21	Subjective	100	80
[140]	31	Uptake ratios	92	89
[141]	38	Uptake ratios	86	83
Total	312		92 ^a	89 ^a

^a Weighted mean.

[ref 4]

To reduce errors in scan interpretation, lesion activity can be quantified by calculating a lesion to background (L/B) ratio (see table). The background is typically the activity of the scalp or the activity of the homologous area in the contralateral brain [14]. SPECT scan images can also be fused, or correlated, with CT or MRI images to confirm lesion size and location.

Antinori *et al.* examined the value of combining EBV-DNA detection and thallium-201 SPECT scan for the diagnosis of PCNSL in a population of HIV-infected patients with FBL [5]. In their study, SPECT scan had a sensitivity of 92.3 % and a specificity of 88.9 % for PCNSL, with L/B ratios ranging from 1.90-2.00 having the highest sensitivity (92%) and specificity (89%). EBV-DNA detection by PCR had a sensitivity of 84.6% and a specificity of 100%. Combining these modalities gave a sensitivity of 76.9% and a specificity of 100% for PCNSL. Antinori *et al.*, however, did not consider the benefit of *Toxoplasma gondii* serology results in further distinguishing the etiology of FBL.

Toxoplasmosis is caused by an intracellular parasite, *Toxoplasma gondii* [4,9]. The prevalence of toxoplasmosis varies from region to region. In the United States, for example, the seroprevalence varies from 3-30%, while in France, the seroprevalence varies from 73-90%. HIV-infected patients develop toxoplasmic encephalitis primarily by reactivation of latent infection. Because of the high prevalence of infection with *Toxoplasma*, a positive serology alone cannot confirm the diagnosis of toxoplasmosis; however, <3-6% of patients with toxoplasmosis have negative serologies, therefore a negative result makes the diagnosis of toxoplasmosis very unlikely [4].

The purpose of this study is to evaluate the diagnostic specificity of a combined approach with EBV-DNA detection in the CSF, thallium-201 SPECT scan, and *Toxoplasma* serology for the diagnosis of PCNSL. The goal is to identify a subgroup of HIV-infected patients with FBLs who are likely to have PCNSL, according to these relatively noninvasive diagnostic procedures, and therefore, do not need to undergo brain biopsy.

B. Study Design and Statistical Analysis

This will be a multicenter, prospective study on consecutively enrolled HIV-infected patients with at least one focal brain lesion by head CT or brain MRI. All patients will undergo serology testing

for *Toxoplasma gondii*, lumbar puncture for EBV-DNA detection, thallium-201 SPECT scan, and brain biopsy (as described below). The sensitivity and specificity will be determined for the following combined result: EBV-DNA detected in CSF, thallium-201 SPECT scan consistent with lymphoma, and negative *Toxoplasma* serology.

Histology (either from brain biopsy or autopsy specimen) is considered the gold standard for the diagnosis of PCNSL and has a specificity of 100%. This study will have an 80% power ($p=0.05$) to detect a difference of 2%, requiring approximately 225 subjects with PCNSL. In other words, this study will be able to detect the true specificity of this combined result if it is less than 98%. Using the conservative estimate that 6% of FBL in HIV-infected patients are due to PCNSL [6], the estimated size of the study population needed is 3750 subjects, which will be recruited from medical institutions in 6 major cities.

C. Study Procedure and Data Collection

All patients in this study will undergo initial head CT with intravenous contrast (unless known contrast allergy or baseline serum creatinine ≥ 2) or brain MRI with and without gadolinium. For each patient, demographic and epidemiologic features, including CD4 count, Karnofsky performance status [13], history of AIDS-defining event, concurrent anti-*Toxoplasma* prophylaxis, and interval since onset of neurologic symptoms, will be recorded. Serum samples will be collected from all patients and tested for *Toxoplasma* IgG. A lumbar puncture will be performed after assessing herniation risk, including neurological signs, papilledema, or mass effect on CT or MRI, and obtaining written informed consent. All CSF samples will be tested for cryptococcal antigen; evaluated by PCR for EBV, JC virus, CMV, HSV, and *Mycobacterium tuberculosis*; and cultured for bacteria, mycobacteria, and fungi. EBV-DNA detection will be performed by a nested PCR technique, as described by Antinori *et al.* [1].

All patients will undergo a thallium-201 SPECT scan 30-minutes following IV infusion of standard thallium-201 dose, according to the standard guidelines of the Nuclear Medicine department. Scans will be analyzed visually by an experienced nuclear medicine specialist who is blinded to the patient's serology, CSF, and histology results. An uptake ratio will then be calculated by dividing the activity of the lesion by the activity of the homologous contralateral area (L/B ratio). If more than one suspicious lesion is identified, an L/B ratio for all lesions will be determined and the highest value will be selected, as per Antinori *et al.*[1].

Ideally, all patients will undergo brain biopsy; however, it is standard practice to start an anti-*Toxoplasma* regimen (pyrimethamine/sulfadiazine, or pyrimethamine/clindamycin if unable to tolerate sulfadiazine) prior to biopsy. Patients will, therefore, be started on anti-*Toxoplasma* therapy. Indications for biopsy will include the following: 1.) no clinical or radiologic improvement following 10 days of anti-*Toxoplasma* treatment, 2.) thallium-201 SPECT scan results not consistent with toxoplasmosis and EBV-DNA detected in CSF, and 3.) clinical deterioration with herniation risk. This last group will require craniotomy for decompression and an open biopsy. Patients will be excluded if they are unable to have brain biopsy and refuse autopsy. Histologic analysis of specimens from brain biopsy or autopsy will be performed by an experienced pathologist who is blinded to the results of the EBV-DNA PCR, SPECT scan, and *Toxoplasma* serology.

The diagnostic criteria that will be used in this study include the following: TE: histology or radiographic lesions with mass effect or enhancement, plus (+) Toxo serology and response to anti-Toxo treatment; PML: histology or compatible MRI, plus (+) JC in CSF; CMV or HSV encephalitis: histology or compatible clinical and radiological findings, plus detection of viral DNA in CSF; HIV encephalopathy: histology; cryptococcus: histology and positive culture; TB: histology and positive culture; PCNSL: histology (biopsy or autopsy) or exclusion of other diagnosis with negative *Toxoplasma* serology, no response to anti-*Toxoplasma* treatment, and thallium-201 SPECT scan consistent with lymphoma.

D. Study Drugs

No drug is being studied.

E. Medical Device

No medical devices are being studied.

F. Study Questionnaires

No questionnaires will be used. A data abstraction instrument will be developed to collect demographic, epidemiologic, clinical, and diagnostic data.

G. Study Subjects

All HIV-infected patients, who are 18 years of age or older, with one or more FBL, presenting to any participating institution will be eligible for enrollment in this study. Patients who refuse to undergo LP, SPECT scan, or either brain biopsy or autopsy will be excluded from the study. Patients who have increased bleeding risk due to coagulopathy or thrombocytopenia will have the defect reversed by appropriate therapy prior to either LP, biopsy, or both. Patients who are unable to undergo LP or brain biopsy due to herniation risk or rapidly deteriorating clinical state will be excluded from the study if they also refuse autopsy.

H. Recruitment of Subjects

Since all HIV-infected patients presenting with FBL will be considered eligible, unless they meet one of the above exclusion criteria, subjects will be identified by a daily survey of new admissions recorded in the medical admitting resident (MAR) log book, or equivalent log at other institutions. At Columbia Presbyterian Medical Center, all patients admitted to the medical services, including AIDS/TB, General Medicine, and Hospitalist services, must be entered into the MAR notebook with admitting diagnosis and pertinent laboratory and radiologic findings. To identify patients with FBL who have been admitted to Neurology or Neurosurgical services prior to the diagnosis of HIV-infection, weekly communication with the On-Call Neurology and Neurosurgery residents will occur. Flyers will be placed in General Medicine, AIDS/TB, Neurology, and Neurosurgical floors to remind teams of the on-going clinical study. Informed consent will be obtained prior to entry into the study, including consent for SPECT scan, LP, and brain biopsy, if not already obtained.

I. Confidentiality of Study Data

All study subjects will be given a unique study identification number, which will be used for test processing. A list of the patient identification numbers will be kept in a separate location. All data will be kept in the investigator's locker.

J. Potential Conflicts of Interest

None identified.

K. Location of Study

Multicenter trial involving 5 major medical institutions in each of 6 major U.S. cities.

L. Potential Risks

Lumbar puncture is generally believed to be a minimally invasive procedure. The most common complication is headache [14]. Less common complications include bleeding, infection, and nerve damage.

As described above, brain biopsy complications include perioperative hemorrhage, neurologic impairments, and, much less common, infection at the biopsy site. Perioperative morbidity after stereotactic biopsy is approximately 12%, while peroperative mortality is 2% [6].

Radiation exposure from CT scan and thallium-201 SPECT scan is discussed below. Allergic reactions to IV contrast are not common and range from mild to anaphylactic. Radiology suites are prepared to treat such reactions.

M. Potential Benefits

Identification of a subgroup of patients with PCNSL who can be identified by minimally invasive procedures and begin therapy relatively early.

N. Alternative Therapies

No experimental therapies are being studied.

O. Compensation

Subjects will not be compensated for their participation in this study.

P. Cost to Subjects

Subjects will not incur any additional costs as a result of participating in this study.

Q. Minors as Research Subjects

The study does not involve the participation of minors.

R. Radiation or Radioactive Substances

Head CT: A computed tomography (CT) scan is a common radiologic procedure that is performed daily. Although it does involve exposure to radiation in the form of x-rays, it is considered an intermediate exposure when compared with all radiological procedures. The typical radiation dose is equivalent to the amount of natural background radiation received over a year. The abdomen and pelvis will be covered with a lead apron to avoid unnecessary exposure. Women who are pregnant or possible pregnant will be excluded. Nursing mothers will be instructed to wait 24 hours after contrast injection before resuming breastfeeding. [15]

Thallium-201 SPECT scan: Thallium has been used to evaluate myocardial perfusion since the 1970s. It is a safe radionuclide with no adverse effects reported. The amount of radioactivity from a standard dose is equivalent to 1-2 years of natural background radiation. Women who are pregnant or nursing will be excluded from the procedure. [16]

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