

# Evaluation of a Serum Assay of s-Adenosylmethionine in the Diagnosis of *Pneumocystis carinii* Pneumonia

*Andy Miller*

## A. Study Purpose and Rationale

The purpose of this study is to establish the serum SAM assay as a sensitive and specific test in the early diagnosis of PCP pneumonia.

*Pneumocystis carinii* pneumonia (PCP) is a common cause of admission, one of the most common AIDS-defining illnesses, and the most common cause of death, among AIDS patients not taking regimens of highly-active antiretroviral therapy (HAART).<sup>1</sup>

Although the history, physical exam, laboratory values, and chest X-ray often lead to correct presumptive diagnosis of PCP during the initial evaluation of the patient, there is no single accurate test for which results are available early in treatment. The standard method of diagnosing PCP is cytological staining of sputum, bronchoalveolar lavage (BAL), or transbronchial biopsy samples; the sensitivity of diagnosis by sputum is much lower than with other clinical samples (in the range of 55-78% compared to BAL, despite various technical advances to improve yield).<sup>2,3,4</sup>

Bronchoscopy with subsequent immunofluorescent and/or histochemical staining of BAL or biopsy tissue is the gold standard for the diagnosis of PCP. Unfortunately, bronchoscopy has known risks and is costly and uncomfortable. Sputum samples must often be sent to establish the absence of *Mycobacterium tuberculosis* before the bronchoscopy is performed, and there is a non-negligible laboratory turnaround time, which means definitive diagnoses of PCP often take up to a week. A sensitive and specific test for PCP, performed early in the workup of the AIDS patients with pulmonary symptoms, would be helpful in making treatment decisions and in deciding whether to obtain bronchoscopies on specific patients. In particular, a high sensitivity would allow clinicians to "rule out" PCP early with a negative test, avoiding the uses, risks, and diagnostic confusions which accompany the use of steroids for severe PCP.

Other methods for early non-bronchoscopic diagnosis of PCP exist but have not gained clinical acceptance for a variety of reasons.

- Sputum: see above
- PCR: Several PCR-based assays for the diagnosis of PCP have been developed although none are yet FDA-approved for clinical use (reviewed in Murdoch 2003).<sup>5</sup> Although sensitivity of these tests is generally high, bronchoscopy-negative, symptom-free controls have often been identified as PCR-positive, especially with nested-PCR assays. Most probably these false-positives arise from a failure of PCR to distinguish colonization from infection. Quantitative PCR may prove a solution to this problem but has yet to be rigorously evaluated. Finally, turnaround time of PCR, although theoretically quite short, in practice may approach the time needed to perform microscopic evaluations of BAL or sputum samples.
- High-resolution CT scanning (HRCT): This technique has been shown to be of value in the diagnosis of PCP among AIDS patients; in one small study of 30 patients the sensitivity of HRCT (compared to bronchoscopy) was 100%, with a specificity of 83%.<sup>6</sup>

Experiments aiming to grow *P. carinii* in culture showed markedly improved growth of the organism in the presence of S-adenosylmethionine.<sup>7</sup> This observation, with results showing that *P. carinii* was in fact a SAM auxotroph (and the only known organism which cannot synthesize SAM), led to a recent study observing SAM levels in human patients with and without PCP.<sup>8</sup> (A variety of other studies have evaluated SAM's questionable role in dementia, liver disease, and other disease states, but

are not relevant to this trial). This study compared serum SAM levels in 15 AIDS patients with PCP (only half of which were proven by histological diagnosis of clinical specimens, including induced sputum; the rest by clinical assessment), 12 healthy controls, and 33 patients with a variety of other pulmonary infections. All patients with PCP had SAM levels below 8 nmol/L (14/15 undetectable); all of the other patients and controls had SAM levels 47-189 nmol/L.

The limitations of the above study include:

- failure to diagnose PCP using a single gold standard (bronchoscopy).
- unclear numbers of PCP-negative AIDS patients with CD4 counts below 200 in the control group (CD4 counts in the PCP group were all likely below 200).

In summary, the SAM assay appears to be a potentially useful test in the early diagnosis of PCP pneumonia, most likely as an adjunct test, but its accuracy in defined clinical populations remains unclear. If SAM levels correlate strongly with bronchoscopy results, the use of steroids in SAM-*detectable* (PCP-negative) patients and the use of bronchoscopy in SAM-undetectable (PCP-positive) patients could be decreased, leading to significant improvement in the clinical care of AIDS patients with pulmonary symptoms. In addition, the prospect of serial SAM levels as a predictor of clinical outcome and response to therapy is intriguing, but not addressed in this study.

## B. Study Design and Statistical Analysis

This study will be conducted in 2 phases: **Phase I** in which serum SAM levels will be measured in AIDS patients with pulmonary symptoms undergoing bronchoscopy, and **Phase II** in which all AIDS patients with pulmonary symptoms will be prospectively evaluated with the SAM assay and with bronchoscopy/BAL.

Subjects in both phases of the study will satisfy the following criteria:

- The diagnosis of AIDS on or prior to admission (<200 CD4 cells/mm<sup>3</sup>).
- Clinical symptoms of PCP infection (fever, cough, dyspnoea, elevated serum LDH, hypoxia) leading to empiric diagnosis of, and presumptive treatment for, PCP.
- Patients may not be taking SAM-containing nutritional supplements or medications at the time of presentation.

The study requires a total of 53 subjects in Phase I, and 33 subjects in Phase II. This allows for an 80% likelihood of detecting a difference ( $\beta = 0.80$ ) in sensitivity between a theoretically desirable sensitivity of a diagnostic test (set at 95% in Phase I and 85% in Phase II) and the presumed sensitivity of the SAM assay (99.5%), when  $\alpha = 0.05$ .

Sensitivity, specificity, and predictive values of the serum SAM assay will be computed in comparison to the gold-standard results of bronchoscopy with BAL or biopsy.

## C. Study Procedure

**Serum SAM assay:** This assay, performed on a sample of blood obtained by venipuncture, consists of precipitation of serum proteins with 10% perchloric acid and high-pressure liquid chromatography as described in (7). A colorimetric HPLC assay for plasma SAM levels is available as an alternative.<sup>9</sup> It is expected that the turnaround time of the assay, once the assay is set up in ICCR laboratory, will not be more than a day, although results will not be available to the clinical team or research subject. The SAM assay will be performed on blood samples obtained within 24 hours of the institution of PCP therapy, and/or prior to steroid therapy.

**Bronchoscopy:** This procedure involves the insertion of a flexible fiber optic bronchoscope into the airways and lungs with the intent of visualizing the airways and obtaining clinical samples. Clinical samples can be obtained by lavage of the airways with a small volume of isotonic saline and subsequent

analysis of the aspirated liquid, or by transbronchial biopsy (which is not typically done when evaluating for PCP). The procedure usually requires conscious sedation and local anesthesia to prevent gagging and coughing; patients typically experience some amount of discomfort. The risks include bleeding, aspiration of gastric contents and subsequent infection or pneumonitis, and pneumothorax.

Schedule: In Phases I and II, blood samples for SAM will be obtained as previously stated (within 24 hours of therapy, but before steroids are administered). In Phase II, in patients whose primary clinicians have decided not to pursue bronchoscopy, consent for bronchoscopy will be requested at that time.

The likely duration of the study is approximately 1-2 years, depending on the number of appropriate subjects and the rate of recruitment into the study.

#### **D. Study Drugs**

No drugs are being studied.

#### **E. Medical Device**

No devices are being studied.

#### **F. Study Questionnaires**

No questionnaires are currently planned for this study.

#### **G. Study Subjects**

Phase I: AIDS patients with pulmonary symptoms admitted to CPMC whose primary medical doctor(s) refer the patient for bronchoscopy.

Phase II: AIDS patients with pulmonary symptoms, regardless of the need for bronchoscopy (patients whose primary medical team does not intend to obtain a bronchoscopy will be asked to voluntarily undergo the procedure with compensation).

#### **H. Recruitment of Subjects**

Potential subjects for Phase I will be identified after admission to the CPMC Medical Service by housestaff and faculty. Although most patients are likely to be admitted to the AIDS/TB unit, appropriate patients assigned to all other medical teams will be approached once identified.

In accordance with CPMC policy, the patient will not be included in the study unless that patient's primary physician is in agreement and discusses participation before study investigators approach the patient.

#### **I. Confidentiality of Study Data**

Study data will be coded and stored in a secure location in accordance with CPMC IRB regulations.

#### **J. Potential Conflict of Interest**

None known

#### **K. Location of the Study**

This study will take place in clinical areas (inpatient wards and procedure rooms) at CPMC.

#### **L. Potential Risks**

There are no foreseen risks of the SAM assay. The risks of bronchoscopy, which are listed above, will be discussed with each patient by the primary medical team, the pulmonary specialist performing the procedure, and/or (for subjects whose primary team did not intend to undertake bronchoscopy) the investigators.

#### **M. Potential Benefits**

Study subjects: There are no likely benefits of the SAM assay to the subjects in this study. There is the possibility that a bronchoscopy will benefit certain subjects through diagnosis of unsuspected pulmonary disease or confirmation of suspected PCP.

Social benefits: There is a possibility that this assay would decrease health care costs and improve diagnostic accuracy in the care of AIDS patients with pulmonary disease.

#### **N. Alternative Therapies**

This is not a therapeutic trial, and alternative therapies for PCP will not be discussed with subjects by study investigators. Alternate diagnostic tests, as listed above, are not in clinical practice and will not routinely be offered to study subjects.

#### **O. Compensation to Subjects**

No compensation will be offered to patients in Phase I of the study (who would undergo bronchoscopy as part of the clinical workup).

For those subjects in Phase II whose primary doctors do not intend to bronchoscope, monetary compensation for the bronchoscopy will be offered (preliminarily \$300).

#### **P. Costs to Subjects**

No additional costs are foreseen for subjects in this study.

#### **Q. Minors as Research Subjects**

No minors will participate in this study.

#### **R. Radiation or Radioactive Substances**

This study will not expose any subject to radiation or radioactive substances.

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