Research letters

S-adenosylmethionine concentrations in diagnosis of *Pneumocystis carinii* pneumonia

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***Pneumocystis carinii* is unable to synthesise S-adenosylmethionine and thus scavenges this intermediate. We aimed to test whether measurement of concentrations of this metabolic intermediate in plasma could provide a new method for rapid diagnosis of *Pneumocystis carinii* pneumonia (PCP). We measured S-adenosylmethionine plasma concentrations in 12 healthy controls, 16 patients with confirmed or suspected PCP, and 36 patients with other infections. Median concentration in healthy controls was 106 nmol/L (range 86–128), but the protein was undetectable in eight patients with histologically proven and seven with suspected PCP, and was 8 nmol/L in another confirmed case (p<0·0001). In 36 patients with other infections, S-adenosylmethionine concentrations were much the same as in controls: 18 had bacterial pneumonia, two tuberculosis, five cryptococcal meningitis, three had other infections, and eight had asymptomatic HIV-1 infection. After treatment for PCP, S-adenosylmethionine concentrations rose rapidly in all but one patient who died of the disease. Measurement of plasma S-adenosylmethionine concentrations could prove useful for diagnosis of PCP and assessment of patients' response to treatment.**

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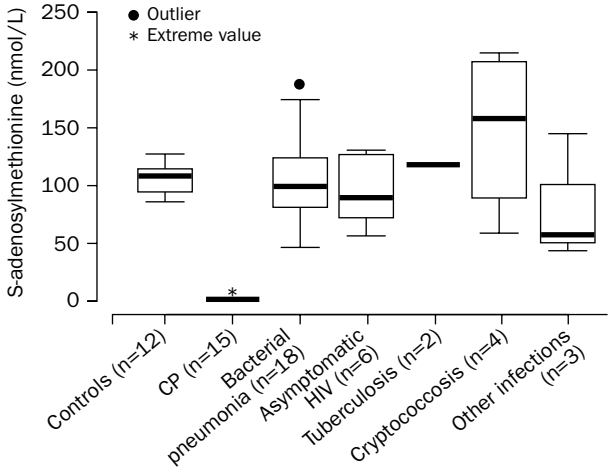
Although frequency of *Pneumocystis carinii* pneumonia (PCP) has fallen in the past decade, it continues to be one of the most common AIDS-defining illnesses.[1] PCP is usually diagnosed by histological identification of the organism or by clinical assessment.[2]

S-adenosylmethionine is a critical biochemical intermediate in many cellular functions.[3] Organisms, apart from *P carinii*, can synthesise S-adenosylmethionine from ATP and methionine using S-adenosylmethionine synthetase. *P carinii* needs exogenous S-adenosylmethionine to maintain an axenic culture,[4] and depletes the protein from the plasma of experimentally infected animals.[5] We aimed to test whether measurement of plasma S-adenosylmethionine concentrations could be used to diagnose PCP and assess patients' response to treatment.

We enrolled patients with asymptomatic HIV-1 infection who attended the virology clinics, or were admitted to Bellevue or Manhattan Veterans Administration hospitals for febrile illnesses. Healthy controls were recruited from laboratory and medical staff in the hospitals. The study protocol was approved by the Institutional Review Board at each hospital and we obtained written informed consent from all participants before samples were taken.

PCP was suspected in patients with dry cough, fever, or progressive dyspnoea and one or more of the following signs: chest radiograph suggestive of PCP, a positive gallium scan, hypoxia with arterial saturation of less than 85%, abnormal diffusing capacity, or raised serum lactic dehydrogenase concentrations. Diagnosis was confirmed by histological identification of *P carinii* in induced sputum, bronchoalveolar lavage, or transbronchial biopsy. Criteria for a diagnosis of probable bacterial pneumonia were clinical or radiographic evidence of response to treatment for bacterial pneumonia and evidence of a new infiltrate on chest radiograph, and one or more of fever, cough, pleuritic chest pain, or new onset of purulent sputum production. Bacterial pneumonia diagnosis was confirmed by isolation of a bacterial pathogen from blood culture, bronchoalveolar lavage, or transbronchial biopsy. We diagnosed tuberculosis if *Mycobacterium tuberculosis* were present in sputum culture. Cryptococcosis was diagnosed by a positive culture or identification of capsular polysaccharide in blood or cerebrospinal fluid and aspergillosis by identification of the organism in sputum and a chest radiograph suggestive of the illness.



Plasma S-adenosylmethionine concentrations
Bar in centre of box=median. Box=quartiles enclosing 50% of data. Upper whisker=highest data point not an outlier (ie, ⩾75th percentile+1·5×IQR). Lower whisker=lowest data point not an outlier (ie, ⩽25th percentile –1·5×IQR). Data points away from the median by 1·5–3 times the IQR are termed outliers, and those away from the median by >3 times the IQR are extreme values.

We took 5 mL of blood from all participants at study entry, which for patients was within 2 days of admission, apart from a patient who developed PCP 28 days after admission. When possible, we also took blood from patients with bacterial pneumonia or PCP on days 3, 5, 7, and 14 after enrolment. The sample processing and detection method has been described.[5]

Between Sept 1, 2000, and April 30, 2001, we enrolled 12 healthy controls (five men) and 48 patients (36 men). Of 15 patients with PCP, seven had histologically confirmed disease, five improved and left hospital without bronchoscopy, two refused bronchoscopy, and one died before bronchoscopy could be done. Of 18 patients with bacterial pneumonia, five had the diagnosis confirmed by associated pneumococcal

	S-adenosylmethionine (nmol/L)				
	Day 1	Day 3	Day 5	Day 7	Day 14
PCP					
1*	8		30	40	106
2*	<0·5	<0·5	<0·5	48	99
3*	<0·5	28	50	48	ND
4*	<0·5	<0·5	49	ND	ND
5*	<0·5	67	ND	117	ND
6*	<0·5	<0·5	ND	ND	ND
7*	<0·5	<0·5	ND	ND	ND
8*	<0·5	ND	ND	ND	ND
9	<0·5	0·9	ND	ND	ND
10	<0·5	<0·5	ND	ND	ND
11	<0·5	<0·5	ND	ND	ND
12	<0·5	ND	ND	ND	ND
13	<0·5	ND	ND	ND	ND
14	<0·5	ND	ND	ND	ND
15	<0·5	ND	ND	ND	ND
Bacterial pneumonia					
1*	189	348	67	87	ND
2*	175	116	ND	100	ND
3	167	164	147	ND	ND
4	124	146	145	126	101
5	124	146	106	101	ND
6*	106	70	183	ND	ND
7	106	ND	ND	ND	ND
8	101	200	285	ND	ND
9	101	ND	ND	ND	ND
10	98	121	ND	ND	ND
11*	87	156	ND	ND	ND
12	82	98	ND	ND	ND
13	82	ND	ND	ND	ND
14	77	ND	ND	ND	ND
15	76	162	350	178	ND
16*	73	45	ND	ND	ND
17	59	ND	ND	ND	ND
18	47	ND	126	235	ND

ND=not done. Within groups, patients are ranked by baseline S-adenosylmethionine concentration. Concentrations <0·5 nmol/L were below the lower limit of detectablity of the assay. *Diagnosis confirmed by histological analysis; other patients had probable infection, clinically assessed.

Plasma S-adenosylmethionine concentrations after treatment for pneumocystis or bacterial pneumonia

patients and cryptococcosis in four. Four of the six asymptomatic outpatients with HIV-1 infection were on antiretrovirals. Of other patients originally admitted for fever, three were classed as having other conditions: one viral meningitis, one pulmonary aspergillosis, and one congestive heart failure complicated by bronchitis.

The figure shows baseline plasma S-adenosylmethionine concentrations. Of seven patients with histologically confirmed PCP and eight with probable PCP, 14 had undetectable concentrations of plasma S-adenosylmethionine and one had a concentration of only 8 nmol/L ($p<0·0001$, Mann-Whitney U test). Concentrations in other groups did not differ significantly from those in healthy controls.

Plasma S-adenosylmethionine concentrations were measured by high performance liquid chromatography, and were done more than once for 13 of 18 patients with bacterial pneumonia and ten of 15 with PCP (table). Patients with bacterial pneumonia showed no reduction in S-adenosylmethionine concentration over time, although increases were noted in some patients. In patients with PCP, initially low concentrations rose with effective treatment. Of the three patients with PCP who were assessed during the second week of treatment, each had plasma S-adenosylmethionine within the normal range. Of the original 15 PCP patients, two died of respiratory failure: one on day 3 with an undetectable plasma S-adenosylmethionine and no explanation other than PCP, and the other on day 5 with a rising concentration (49 nmol/L), but with coexistent pulmonary aspergillosis, and severe wasting.

We considered the possibility that medication used to treat HIV could have an effect on plasma S-adenosylmethionine concentrations, so we included a group of asymptomatic patients with varying CD4 counts and viral loads, some of who were taking antiretrovirals. Overall, S-adenosylmethionine concentrations in asymptomatic individuals with HIV-1 infection were similar to those in healthy controls, thus there was no association between S-adenosylmethionine concentrations and antiretrovirals (figure).

The consistency of the pattern of association between PCP and S-adenosylmethionine concentration, and the return of the protein concentration to normal values with effective treatment suggests that severe depletion of S-adenosylmethionine is characteristic of PCP. Reduced plasma S-adenosylmethionine concentration was a sensitive and specific test for PCP in our patients, with high positive and negative predictive value. The return of plasma S-adenosylmethionine concentrations towards normal values could be useful in monitoring the course of treatment for PCP.

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2 Barry SM, Johnson MA. *Pneumocystis carinii* pneumonia: a review of current issues in diagnosis and management. *HIV Med* 2001; **2:** 123–32.

3 Chiang PK, Gordon RK, Tal J. S-adenosylmethionine and methylation. *FASEB J* 1996; **10:** 471–80.

4 Merali S, Frevert U, Williams JH, Chin K, Bryan R, Clarkson AB. Continuous axenic cultivation of *Pneumocystis carinii*. *Proc Natl Acad Sci USA* 1999; **96:** 2402–07.

5 Merali S, Vargas D, Franklin M, Clarkson AB. S-adenosylmethionine and *Pneumocystis carinii*. *J Biol Chem* 2000; **275:** 14958–63.

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Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease

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Kawasaki disease is an acute vasculitis of possible infectious cause, which in particular affects the coronary arteries. Young children rely mostly on their innate immune system for protection against invading microorganisms, of which mannose-binding lectin is an important component. We aimed to investigate the possible role of the gene for this molecule (*MBL*) in white Dutch patients with Kawasaki disease. In 90 patients, frequency of mutations in the *MBL* gene was higher than in healthy children. In children younger than 1 year, those with mutations were at higher risk of development of coronary artery lesions than were those without (odds ratio 15·7, 95% CI 1·4–176·5, p=0·026). Our findings suggest that the innate immune system contributes differently to pathophysiology of Kawasaki disease at various ages.

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Kawasaki disease is an acute inflammatory disorder characterised by persistent fever, lymphadenopathy,