POSSIBLE SIGNIFICANCE OF ANTIBODIES TO *LEGIONELLA PNEUMOPHILA* IN HIV-1 INFECTED PATIENTS WITH ACUTE RESPIRATORY ILLNESS+

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Summary. – Eighty-six serum samples from 52 HIV-1 infected patients with complicating pneumonia illness were assayed for the presence of antibodies to *Legionella pneumophila.* In 25 of these patients *Pneumocystic carinii* has been previously diagnosed. Among all patients investigated only 4 had antibodies to *L. pneumophila* of significant titer of 128. In one patient *L. pneumophila* was demonstrated coexisting with *P. carinii.* Despite of a small proportion of patients with a significant titer of antibodies against *L. pneumophila,* the presence of this microorganism should be carefully investigated in AIDS patients with complicating pneumonia especially when the aetiological diagnosis is not defined; the reason is to improve the therapeutic treatment.

Key words: AIDS; HIV; *Legionella pneumophila*; serology; indirect immunofluorescence

Lower respiratory tract infections are the most common finding in human immunodeficiency virus-1 (HIV-1) infected patients with acquired immunodeficiency syndrome (AIDS) (Murray *et al.*, 1984; Stover *et al.*, 1985). The spectrum of pulmonary diseases in these patients is usually caused by *P. carinii* alone or combined with cytomegalovirus, and other less frequently isolated opportunistic pathogens (Polsky *et al.*, 1986; Suster *et al.*, 1986). Opportunistic infections are responsible for both more than 90 % of death and most of the morbidity in patients infected with HIV-1 (Antoniskis *et al.*, 1990). Several of those opportunistic pathogens take advantage of abnormalities in T-cell and macrophage functions as described in the first reports on AIDS (Clifford Lane *et al.*, 1983; Durack *et al.*, 1981; Siegal *et al.*, 1981; Masur *et al.*, 1981; Gottlieb *et al.*, 1981). The facultative intracellular pathogen *L. pneumophila* plays a role in pulmonary infections observed in the immunologically compromised host with a defect in cell-mediated immunity (Marrie *et al.*, 1988; McDade *et al.*, 1977; Saravolatz *et al.*, 1979; Schlanger *et al.*, 1984). Indeed *Legionella* appears with increasing frequency either alone or together with *P. carinii* as responsible for pneumonia in patients with AIDS (Allam and Kamholz, 1989; Bangsborg *et al.*, 1990; Gottlieb, 1983; Jensen *et al.*, 1990; Khardori *et al.*, 1987; Guenot *et al.*, 1984; Storch *et al.*, 1980; Witt *et al.*, 1987). On this basis the aim of this study was to assess the frequency of *L. pneumophila* infection in HIV-1 antibody-positive individuals with pulmonary complications by the evaluation of the serological response against *L. pneumophila.*

L. pneumophila serology was performed by indirect fluorescence antibody (IFA) test. Immunoglobulin G, A, M (IgG, IgA, IgM) class antibody concentrations in sera of HIV-1 infected patients with pneumonia illness were determined using a polyvalent mixture of *L. pneumophila* serogroups 1 to 6 as the antigen substrate (SciMedx corp., Denville, NJ, USA).

All patients had been previously confirmed as HIV-1-antibody positive by both ELISA and Western blot analysis. A total of 86 serum samples were obtained from 52 AIDS patients with pulmonary complications. According to the CDC criteria of classification (CDC, 1986), 40 patients had clinical AIDS, six had AIDS-related complex (ARC), five were symptom-free, and one had persistent generalized lymphadenopathy (Table 1). The infec-

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Table 1. Clinical stage of HIV-1 infection in 52 patients grouped according to CDC criteria

	Group	No. of patients
I	Acute infection	0
II	Asymptomatic infection	5
III	Persistent generalized lymphadenopathy	1
IVa	ARC	6
IVb	AIDS	40

tion with *P. carinii* had been demonstrated by morphological identification of the organism in the bronchial alveolar lavage in 25 patients, whereas 27 patients were without aetiological diagnosis of pulmonary complications. In order to evaluate the probable seroconversion, sera were studied either at the onset or during the convalescent phase of pulmonary manifestation. The initial serological screening was carried out with sera dilutions of 1:64 and 1:128. Only the reactive sera were then taken to higher dilutions.

Of the fifty-two HIV-1 infected patients ten (19.2 %) exhibited antibodies to *L. pneumophila* serogroups 1 to 6. There were six sera (11.5 %) with titer of 64, four sera (7.7 %) with titer of 128, whereas no antibodies were detected in sera diluted 1:256 (Table 2).

Table 2. Serological response to L. pneumophila in HIV-1 patients with acute pulmonary illness

Antibody titer	No. of patients	Group	%
64	2	II	3.8
64	4	IVb	7.7
128	4	IVb	7.7
256	0	–	–

Besides the IFA test also a direct staining, namely the Gimenez method has been successfully used (Harrison and Taylor, 1988). However, the IFA test is considered as extremely rapid and most frequent confirmatory tool in diagnosis of *Legionella* infection (Harrison and Taylor, 1988). Similarly to other authors (Storch *et al.*, 1980; Harrison and Taylor, 1988), we have considered as positive sera with titer of 128 or higher. This value is usually accepted as an evidence of a presumptive case (Harrison and Taylor, 1988).

Our results show a low incidence of antibodies to *L. pneumophila* in HIV-1 infected patients with pulmonary complications. This finding is in agreement with other studies (Murray *et al.*, 1984; Schlanger *et al.*, 1984; Khardori *et al.*, 1987; Allam and Kamholz, 1989; Witt *et al.*, 1987). The susceptibility to *L. pneumophila*, which is a facultative intracellular bacterium readily replicating in human monocytes/macrophages (Horwitz and Silverstein, 1980) correlates with the host's cellular immune defence system (Eisenstein and Friedman, 1985). In addition it should be noted that patients with AIDS have a weaker immune response and produce lower antibody titers to foreign agents (Yuan-Hu Wang *et al.*, 1992). On this basis one could expect a higher frequency of *Legionella* infection in those patients with a decreased response. The matter could be at least partially explained by late disfunction of macrophages during HIV-1 infection (Yuan-Hu Wang *et al.*, 1992).

In one of our HIV-1-infected patients *L. pneumophila* was coexisting with *P. carinii* previously diagnosed. This association has been reported also by others (Storch *et al.*, 1980; Bangsborg *et al.*, 1990). In this regard one might speculate that *L. pneumophila* plays and important role in association with other opportunistic pathogens (i.e. dual infection). Apart from this pathophysiological point of view which remains to be clearly defined, the presence of *Legionella* should be taken into consideration especially when the aetiology of pulmonary complications in AIDS patients is not well defined and the appropriate therapeutic treatment cannot be properly carried out.

References

Allam, A. A., and Kamholz, S. L. (1989): *Legionella* pneumonia and AIDS. *Chest* **95**, 707–708.

Antoniskis, D., Larsen, R. A., and Sattler, F. R. (1990): Current approaches to management of opportunistic infections. *Curr. Op. inf. Dis.* **3**, 100–107.

Bangsborg, J. M., Jensen, B. N., Friis-Moller, A., and Bruun, B. (1990): Legionellosis in patients with HIV infection. *Infection* **18**, 342–346.

Centers for Disease Control (1986): Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. *Ann. intern. Med.* **105**, 234–237.

Clifford Lane, H., Masur, H., Edgar, L. C., Whalen, G., Rook, A. H., and Fauci, A. S. (1983): Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *New Eng. J. Med.* **309**, 453–458.

Durack, D. T., and Phil, D. (1981): Opportunistic infections and Kaposi's sarcoma in homosexual men. *New Engl. J. Med.* **305**, 1465–1467.

Edelstein, P. H. (1987): Laboratory diagnosis of infections caused by legionellae. *Eur. J. clin. Microbiol.* **6**, 4–10.

Eisenstein, T. K., and Friedman, H. (1985): In S. M. Kadz (Ed.): *Legionellosis*, Vol. II, CPR Press, Boca Raton, p. 119.

Gottlieb, M. S. (1983): The acquired immunodeficiency syndrome. *Ann. intern. Med.* **99**, 208–220.

Gottlieb, M. S., Schroff, R., Schanker, H. M., Weisman, J. D., Fan, P. T., Wolf, R. A., and Saxon, A. (1981): *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *New Engl. J. Med.* **305**, 1425–1431.

Guenot, O., Dournon, E., Coste, F., Tremolieres, F., Martin, E., and Dromer, F. (1984): Legionellose au cours d'un sydrome d'immunodeficience acquise. *La Presse Medicale* **13**, 2150–2151.

Harrison, T. G., and Taylor, A. G. (1988): In T. G. Harrison and A. G. Taylor (Eds): *A laboratory Manual for Legionella*, Wiley-Interscience Publ., Chichester, UK, pp. 103–135.

Horwitz, M. A., and Silverstein, S. C. (1980): Legionnaires' disease bacterium *Legionella pneumophila* multiplies intracellularly in human monocytes. *J. clin. Invest.* **66**, 441–450.

Jensen, B. N., Gerstoft, J., Højlyng, N., Backer, V., Paaske, M., Gomme, G., and Skinhoj, P. (1990): Pulmonary pathogens in HIV-infected patients. *Scand. J. inf. Dis.* **22**, 413–420.

Khardori, N., Haron, E., and Rolston, K. (1987): *Legionella micdadei* pneumonia in the acquired immunodeficiency syndrome. *Amer. J. Med.* **83**, 599–600.

Marrie, T. Z., and Martin, R. S. (1988): Persistent *Legionella pneumophila* bacteremia in an immunocompromised host. *J. Infection* **16**, 203.

Masur, H., Michelis, M. A., Greene, J. B., Onorato, I., Vande Stouwe, R. A., Holzman, R. S., Wormser, G., Brettman, L., Lange, M., Murray, H. W., and Cunningham-Rundles, S. (1981): An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *New Engl. J. Med.* **305**, 1431–1438.

Mathys, W., Junge, E., Sobek-Pfeiffer, C., Bosenberg, B., and von Eiff, M. (1988): The importance of different laboratory methods in *Legionella* diagnosis in medical care. *Zbl. Bakt. Hyg. A.* **270**, 122–130.

McDade, J. E., Shepard, C. C., Fraser, D. W., Tsai, D. W., Redus, M. A., and Dowdle, W. R. (1977): Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory diseases. *New Engl. J. Med.* **297**, 1197–1203.

Murray, J. F., Felton, C. P., Garay, S. M., Gottlieb, M. S., Hopewell, P. C., Stover, D. E., and Teirstein, A. S. (1984): Pulmonary complications of the acquired immunodeficiency syndrome. *New Engl. J. Med.* **310**, 1682–1688.

Parry, M. F., Stampleman, L., Hutchinson, J. H., Folta, D., Steinberg, M. G., and Krasnogor, L. J. (1985): Waterborne *Legionella bozemanii* and nosocomial pneumonia in immunosuppressed patients. *Ann. intern. Med.* **103**, 205–210.

Polsky, B., Gold, J. W. M., Whimbey, E., Dryjanski, J., Brown, A. E., Schiffman, G., and Armstrong, D. (1986): Bacterial pneumonia in patients with the acquired immunodeficiency syndrome. *Ann. intern. Med.* **104**, 38–41.

Saravolatz, L. D., Burch, K. H., Fisher, E., Madhavan, T., Kiani, D., Neblett, T., and Quinn, E. L. (1979): The compromised host and Legionnaires' disease. *Ann. intern. Med.* **90**, 533–537.

Schlanger, G., Lutwick, L. I., Kurzman, M., Hoch, B., and Chandler, F. W. (1984): Sinusitis caused by *Legionella pneumophila* in a patient with the acquired immunodeficiency syndrome. *Amer. J. Med.* **77**, 957–960.

Siegal, F. P., Lopez, C., Hammer, G. S., Brown, A. E., Kornfeld, S. J., Gold, J., Hassett, J., Hirschman, S. Z. Cunningham-Rundles, C., Adelsberg, B. R., Parham, D. M., Siegal, M., Cunningham-Rundles, S., and Armstrong, D. (1981): Severe acquired immunodeficiency in male homosexuals, manifestated by chronic perianal ulcerative herpes simplex lesions. *New Engl. J. Med.* **305**, 1439–1444.

Storch, G., Hayes, P. S., Meyers, J. D., Sulzer, A., and Baine, W. B. (1980): Legionnaires' disease bacterium. Prevalence of antibody reacting with the organism in patients suspected of having infection with *Pneumocystis carinii*. *Amer. Rev. resp. Dis.* **121**, 483–486.

Stover, D. E., White, D. A., Romano, P. A., Gellene, R. A., and Robeson, W. A. (1985): Spectrum of pulmonary diseases associated with the acquired immunodeficiency syndrome. *Amer. J. Med.* **78**, 429–437.

Suster, B., Akerman, M., Orenstein, M., and Wax, M. R. (1986): Pulmonary manifestations of AIDS. Review of 106 episodes. *Radiology* **161**, 87–93.

Witt, D. J., Craven, D. E., and McCabe, W. R. (1987): Bacterial infections in adult patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Amer. J. Med.* **82**, 900-906.

Yuan-Hu Wang, R., Wai-Kuo Shih, J., Grandinetti, T., Pierce, P. F., Hayes, M. M., Wear, D. J., Alter, H. J., and Lo, S.-C. (1992): High frequency of antibodies to *Mycoplasma penetrans* in HIV infected patients. *Lancet* **340**, 1312–1316.