titis documented the first HIV-1 infection in 1979 (probably the oldest documented in Italian IVDUs [5]). Subsequent studies revealed the presence of HTLV-I/II in the same population in the same year, with a prevalence that was comparable to that of HIV-1 (6%; unpublished data). The spread of HTLV-II therefore preceded or at least paralleled the initial spread of HIV-1 infection among IVDUs in the Milan area.

A second indication may be the detection of HTLV-II–infected subjects among blood donors who had no contact with drug users [6]. If confirmed by other studies, this would suggest that HTLV-II may have been transmitted in Italy not just by iv drug use and that it may even have been in circulation before the outbreak of HIV-1.

As far as the technical problems related to the detection of HTLV-II infection are concerned, various authors have reported a high frequency of false-negative anti–HTLV-I/II EIA results [7]. Using the polymerase chain reaction (PCR) and Western blot (WB), we recently detected HTLV-II infection in Italian blood donors who were seronegative at the screening test [6]. Furthermore, we found an antibody response to HTLV-II that is frequently incomplete in subjects with advanced HIV-1 infection (PN occurs during the advanced phases of HIV-1 disease) [8]. We also identified an EIA-negative but WB- and PCR-positive subject in our series of PN cases [1]. Soriano et al. [2] also report a high frequency of indeterminate WB patterns in HTLV-EIA–positive or borderline reactive subjects. However, it is possible that more extensive PCR investigations in HIV-1–positive IVDUs may reveal a number of HTLV-II infections not identified by EIA screenings.

These considerations and the discrepancies in the epidemiology of HTLV-II in Italy and Spain should be taken into account when considering our results [9] and those of Soriano et al. [2] concerning the prevalence of HTLV infection in HIV-1–positive persons with non-Hodgkin's lymphoma.

Finally, we disagree with Soriano et al. [2] only when they consider the prevalence of PN observed in Spanish HTLV-II/HIV-1–positive patients to be low. In the absence of specific information, we can only assume that their doubly infected patients may be in different stages of HIV-1 infection and that there is a good proportion of asymptomatic subjects with relatively high CD4 cell counts. If so, bearing in mind the data in the literature about the frequency of chronically manifested PN in HIV-1–infected patients [4], a prevalence of ~5% sounds to our ears more a confirmation of our results than a dissenting or discordant voice.

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References

1. Zehender G, De Maddalena C, Osio M, et al. High prevalence of human T cell lymphotropic virus infection in patients affected by human immunodeficiency virus type 1 associated predominantly sensory polyneuropathy. J Infect Dis **1995**;172:1595 8.
2. Soriano V, Gutiérrez M, Merino F, et al. Recent spread of human T lymphotropic virus type II among injecting drug users in Spain [letter]. J Infect Dis **1996**;174:891–2.
3. Zanetti AR, Zehender G, Tanzi E, et al. HTLV-II among Italian intravenous drug users and hemophiliacs. Eur J Epidemiol **1992**;8:702–7.
4. Bacellar H, Muñoz A, Miller EN, et al. Temporal trends in the incidence of HIV-1–related neurologic diseases: multicentric AIDS cohort study, 1985–1992. Neurology **1994**;44:1892–900.
5. Lazzarin A, Crocchiolo P, Galli M, et al. Milan as possible starting point of LAV/HTLV-III epidemic among Italian drug addicts. Boll Ist Sieroter Milan **1987**;66:9–13.
6. Zehender G, Girotto M, De Maddalena C, Francisco G, Moroni M, Galli M. HTLV infection in ELISA-negative blood donors. AIDS Res Hum Retroviruses **1996**;12:737–40.
7. Weiss SH. The evolving epidemiology of human T lymphotropic virus type II. J Infect Dis **1994**;169:1080–3.
8. De Maddalena C, Zehender G, Girotto M, et al. High frequency of false anti–HTLV-I/II negative results by EIA in HIV-1 positive patients and in blood donors in Italy [abstract 172]. J Acquir Immune Defic Syndr Hum Retrovirol **1995**;10:256.
9. Zehender G, Meroni L, Piconi S, et al. Frequent detection of antibodies directed against HTLV antigens in patients affected by AIDS-related non-Hodgkin lymphoma. AIDS Res Hum Retroviruses **1995**;11:823-7.

Detection of *Chlamydia pneumoniae* in Atheroma Specimens

To the Editor—Weiss et al. [1] recently reported detection of *Chlamydia pneumoniae* in only 1 of 58 coronary atherectomy specimens tested by culture and polymerase chain reaction (PCR). They also found a lower seroprevalence of anti–*C. pneumoniae* antibodies among the atherectomy patients than in a group of control patients. These results differ from those of numerous studies that indicated an association between *C. pneumoniae* and atherosclerotic cardiovascular disease [2–10]. This discrepancy may be due to a variety of factors that are deserving of additional comment.

Weiss et al. [1] divided the specimens, processed one portion for PCR testing, and inoculated the rest onto cell culture. By their admission, the divided specimens were quite small. Although a separate group of specimens was examined by electron microscopy and found to contain foam and smooth muscle cells, it is possible that some of the specimens tested by PCR did not contain adequate cellular material. Results of attempts to amplify the human β globin gene would have been helpful to determine whether DNA was extracted from the samples tested. One of the specimens was positive for *C. pneumoniae* by PCR; however, the organism was not isolated from any of the specimens tested. Although *C. pneumoniae* has been isolated from coronary [10] and carotid atheroma specimens (unpublished data), this method is not expected to be sensitive for detection of *C. pneumoniae*. Weiss et al. did not test their samples by immunocytochemistry (ICC), a method that can

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effectively detect the presence of *C. pneumoniae*–specific antigens in atheroma specimens. In a study by Campbell et al. [2], atherectomy samples were tested by PCR and ICC, and 45% (17/38) of the samples were ICC-positive; the organism was detected only by ICC in 21% (8/38) of the samples tested [2]. Failure to use this method further decreased the ability of Weiss et al. to detect the presence of *C. pneumoniae* in their specimens.

The authors attempted to assess the association between serologic evidence of past infection with *C. pneumoniae* and heart disease by comparing the prevalence of anti–*C. pneumoniae* antibodies among 65 atherectomy patients with that among 28 outpatients. The validity of this comparison is limited by the selection of a dissimilar control group that was not matched by age or sex to the case group. Using multivariate analysis, they found no difference in prevalence of anti–*C. pneumoniae* IgG titers ≤ 32 between the 2 groups, and a higher prevalence of titers ≥ 64 among the controls, which was of borderline statistical significance ($P = .05$). These results were interpreted as indicating that seroprevalence to *C. pneumoniae* did not correlate with the presence of heart disease. Not discussed, however, was the fact that the seroprevalence was extremely high among the controls: 93% (26/28) had IgG titers ≥ 32, and 86% (24/28) had titers ≥ 64. Therefore, even though the seroprevalence among the case-patients (72% had titers ≥ 32) was higher than that in many seroepidemiologic studies of *C. pneumoniae* and heart disease, it is not surprising that Weiss et al. [1] were unable to demonstrate a higher seroprevalence among the atherectomy patients than in their control group. The reason for the elevated seroprevalence among the control subjects is not clear but may have resulted from chance fluctuation due to the small sample size. This small sample size clearly limited their ability to detect a true difference in seroprevalence between the case and control groups. Even if the seroprevalence among the controls had been nearer to that expected for older adults (e.g., 75%), this study of 93 subjects would have had $<20\%$ power to detect an odds ratio of 2.0 (at $1 - \alpha = 95\%$), which is the approximate magnitude of risk reported in many of the previous seroepidemiologic studies.

Weiss et al. [1] cite differences in local epidemiology as one factor that may explain the differences between their results and those of other investigators and note, "it appears that if this organism can cause coronary heart disease, its role varies in different populations." We agree that the evidence supporting an association between *C. pneumoniae* and atherosclerosis does not prove a causal relationship. However, if differences in the populations studied is accepted as the explanation for the discrepancy of the results with those of other studies, then the group of Brooklyn subjects studied by Weiss et al. must be assumed to differ not only from populations in Seattle and South Africa, as they state, but also from the populations of much of the rest of the world. To date, a serologic association of *C. pneumoniae* and atherosclerotic disease or detection of the organism in atheroma has been reported in Finland [3], Sweden [4], the United Kingdom [5, 9], and Italy. In the United States, two nationwide studies [6, 7] and studies of patients in California [8], Kentucky [10], and Utah [11] have reported such associations. Of note, 6 authors of the report under discussion also contributed to the Kentucky study abstract [10]. In addition, results reported in several other recent abstracts have extended the regions to include the Netherlands, Germany, India, Japan, and Alaska. However, an abstract from investigators in Minnesota reported a failure to detect *C. pneumoniae* in 49 coronary artery autopsy specimens tested only by PCR.

Given the overall consistency of the results of studies from many different areas, it seems more likely that methodologic differences account for the results reported by Weiss et al. [1] rather than true differences between the populations studied. As they suggest, further studies are needed before it can be concluded that the presence of *C. pneumoniae* in atheroma or a serologic association with atherosclerotic cardiovascular disease varies by geographic location.

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References

1. Weiss SM, Roblin PM, Gaydos CA, et al. Failure to detect *Chlamydia pneumoniae* in coronary atheromas of patients undergoing atherectomy. J Infect Dis **1996**;173:957–62.
2. Campbell LA, O'Brien ER, Cappuccio AL, et al. Detection of *Chlamydia pneumoniae* (TWAR) in human coronary atherectomy tissues. J Infect Dis **1995**;172:585–8.
3. Saikku P, Leinonen M, Tenkanen L, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki heart study. Ann Intern Med **1992**;116:273–8.
4. Dahlén GH, Boman J, Birgander LS, Lindblom B. Lp(a) lipoprotein, IgG, IgA and IgM antibodies to *Chlamydia pneumoniae* and HLA class genotype in early coronary artery disease. Atherosclerosis **1995**;114:165–74.
5. Mendall MA, Carrington D, Strachan D, et al. *Chlamydia pneumoniae:* risk factors for seropositivity and association with coronary heart disease. J Infect **1995**;30:121–8.
6. Melnick SL, Shahar E, Folsom AR, et al. Past infection by *Chlamydia pneumoniae* strain TWAR and asymptomatic carotid atherosclerosis. Am J Med **1993**;95:499–504.
7. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15 to 35 years old). Proc Natl Acad Sci USA **1995**;92:6911–4.
8. Grayston JT, Kuo CC, Coulson AS, et al. *Chlamydia pneumoniae* (TWAR) in atherosclerosis of the carotid artery. Circulation **1995**:92;3397–400.
9. Ong G, Thomas BJ, Mansfield OA, Davidson BR, Taylor-Robinson D. Detection and widespread distribution of *C. pneumoniae* in the vascular system and its possible implications. J Clin Pathol **1996**;49:102–6.
10. Ramirez J, Ahkee C, Ganzel B, et al. Isolation of *Chlamydia pneumoniae* (Cp) from the coronary artery of a patient with coronary atherosclerosis [abstract B59]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, **1995**:36.
11. Muhlestein JB, Hammond EH, Carlquist JF, et al. Increased incidence of *Chlamydia* species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. J Am Coll Cardiol **1996**;27:1555–61.