INT J TUBERC LUNG DIS 12(11):1340–1343

SHORT COMMUNICATION

Laboratory features for presumptive diagnosis of disseminated tuberculosis in HIV-infected patients

R. P. dos Santos, K. L. Scheid, L. Z. Goldani

Section of Infectious Diseases, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

SUMMARY

Rapid diagnosis is crucial for adequate treatment of disseminated mycobacteriosis. We conducted a retrospective cohort study to identify clinical and laboratorial features of disseminated mycobacteriosis in human immunodeficiency virus (HIV) infected patients that could help to differentiate tuberculosis (TB) from non-tuberculous mycobacteria (NTM) disease. All patients diagnosed from 1996 to 2006 were reviewed. TB was diagnosed in 65 patients and NTM in 31. Patients with TB had higher median levels of aspartate aminotransferase (AST) (69.0 vs. 45.0, $P = 0.02$) and lactate dehydrogenase (LDH) (725.0 vs. 569.0, $P = 0.03$). AST and LDH may be valuable tools in differentiating disseminated TB from NTM in HIV-infected patients.

KEY WORDS: mycobacteria; AIDS; tuberculosis; non-tuberculous mycobacteria

MYCOBACTERIAL DISEASE is an important cause of morbidity and mortality in patients with the acquired immune-deficiency syndrome (AIDS).[1] In immunosuppressed human immunodeficiency virus (HIV) infected persons, tuberculosis (TB) and non-tuberculous mycobacteriosis (NTM) often present in a disseminated form that is difficult to differentiate on clinical presentation. Rapid laboratory diagnosis is crucial for adequate treatment; however, diagnosis depends on the growth of a slow growing microorganism, and complex methods such as DNA tests may not be feasible for developing countries.

Considering that mycobacterial infections are the most common cause of fevers of unknown origin in patients with AIDS in low-resource countries,[2] specific laboratory features of TB and NTM disease might be important clues in the early diagnosis of HIV-infected patients with disseminated mycobacteriosis. The goal of our study was to identify relevant laboratory markers in HIV-infected patients with disseminated TB and NTM disease.

MATERIALS AND METHODS

We performed a retrospective cohort study at Hospital de Clínicas de Porto Alegre, a 735-bed tertiary care hospital in southern Brazil. From 1996 to 2006, patients with AIDS and disseminated mycobacterial disease were reviewed. The ethics committee of the hospital approved the study.

Disseminated mycobacterial disease was diagnosed by identification of *Mycobacterium tuberculosis* or NTM in blood, bone marrow or liver biopsy cultures.

For isolation of *Mycobacterium sp*., a radiometric system (BACTEC 460 TB, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) and two non-radiometric systems (BACTEC 9240, Becton Dickinson, and BacT/Alert 240, BioMérieux, Marcy-l'Etoile, France) were used. For species identification, the *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test was used.[3,4] In cases where the NAP test could not be performed, species identification was performed by visualisation of the aspect of the colony, and presence or absence of cord formation.[5]

Laboratory analyses included complete blood count, blood electrolytes, aminotransferase, bilirubins, alkaline phosphatase, lactate dehydrogenase (LDH), creatinine, albumin and serology for hepatitis C and B. The data were collected within 7 days of the hospitalisation of the patient. The CD4 lymphocyte count and HIV viral loads were collected within a 6-month period before hospitalisation.

A descriptive analysis for the variables was performed. The χ^2 test or Fisher's exact test was used for univariate analysis. Associations were considered statistically significant when $P < 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Correspondence to: Rodrigo Pires dos Santos, Infectious Disease Unit/Infection Control Department, Hospital das Clínicas de Porto Alegre, Room 2225, Ramiro Barcelos 2350, 90035-003 Porto Alegre, Brazil. Tel: (+55) 51 2101 8643. Fax: (+55) 51 2101 8000. e-mail: rpsrodrigo@gmail.com

Article submitted 5 February 2008. Final version accepted 17 June 2008.

Table 1 Demographic characteristics of HIV-infected patients with disseminated mycobacterial disease

	All patients* ($N = 96$) *n* (%)	*Mycobacterium tuberculosis* ($n = 65$) *n* (%)	Non-tuberculous mycobacteria ($n = 31$) *n* (%)	*P* value
Median age, years	32.0	32.0	34.0	0.43
Men	71 (74.0)	49 (75.4)	22 (71.0)	0.83
Race†				0.05
White	63 (66.3)	39 (60.9)	24 (77.4)	
Black	23 (24.2)	20 (31.3)	3 (9.7)	
Other	9 (9.5)	5 (7.8)	4 (12.9)	
HIV exposure				
Sexual	46 (57.5)	28 (52.8)	18 (56.7)	0.34
Intravenous drug use	34 (42.5)	25 (47.2)	9 (33.3)	
Hepatitis C serology	26 (35.6)	18 (37.5)	8 (32.0)	0.83
Hepatitis B serology	6 (8.8)	4 (8.5)	2 (9.5)	0.74

*Patients diagnosed with TB and non-tuberculous mycobacterial infection.
† *P* value related to a comparison of Black and White race groups.
HIV = human immunodeficiency virus.

RESULTS

One hundred and seven HIV-infected patients with disseminated mycobacterial disease were identified from 1996 to 2006. Eleven patients were excluded from the comparative analysis: six (5.6%) due to incomplete data registry, three (2.8%) because of simultaneous diagnosis of TB and NTM disease and two (1.9%) because speciation was not performed for technical reasons. Of the 96 remaining patients, 65 (67.7%) had disseminated TB and 31 (32.3%) had NTM disease.

The baseline characteristics of the patients are shown in Table 1.

There was no statistical significant difference when comparing the clinical presentations of HIV-infected patients with disseminated TB and those with NTM. The most common clinical manifestations were weight loss (92.7%), fever (89.6%), respiratory symptoms (75.0%) and gastrointestinal manifestations (68.8%).

Laboratory data of patients are shown in Table 2. Patients with disseminated NTM disease were more immunosuppressed than those with TB (median CD4 lymphocyte count 13 vs. 42 cells/mm^3, $P = 0.002$).

HIV-infected patients with disseminated TB had higher median levels of aspartate aminotransferase (AST) (69.0 vs. 45.0 U/l, $P = 0.02$) and LDH (725.0 vs. 569.0 U/l, $P = 0.03$), and lower median albumin levels (2.5 vs. 2.8 g/dl, $P = 0.05$).

If we consider a cut-off point of 45 international units (IUs)/l for AST, the sensitivity and specificity for the diagnosis of TB is respectively 77% and 48%. For LDH, a cut-off point of 850 IU/l resulted in a sensitivity of 35% and specificity of 84% in the diagnosis of TB. If these cut-off points are taken together (AST $>$ 45 U/l and LDH $>$ 850 U/l), the sensitivity for the diagnosis of TB is 59% and specificity 80%; the OR for TB diagnosis was 3.64 (95%CI 1.3–10.6, $P = 0.01$).

When we stratified the patients for the lowest CD4 lymphocyte count strata (CD4 $\leqslant$ 50 cells/mm^3), higher

Table 2 Laboratory results of HIV-infected patients and disseminated mycobacterial infection

	All patients*		*Mycobacterium tuberculosis*		Non-tuberculous mycobacteria		
	Median (range)	*n*	Median (range)	*n*	Median (range)	*n*	*P* value
Alkaline phosphatase, U/l	603.0 (75–8224)	89	579.5 (75–4.991)	58	778.0 (189–8.224)	29	0.24
ALT, U/l	38.0 (7–429)	91	41.0 (9–429)	60	34.0 (7–229)	29	0.37
LDH, U/l	608.0 (111–2329)	89	725.0 (172–2.329)	57	569.0 (111–1.537)	30	0.03
AST, U/l	64.0 (12–1575)	95	69.0 (12–1575)	62	45.0 (18–724)	31	0.02
Bilirubin, mg/dl	1.0 (0.3–8.1)	94	1.0 (0.3–8.1)	61	0.9 (0.3–5.8)	31	0.80
Haematocrit, %	26.0 (8–46)	98	26.0 (8–43)	65	25.0 (17–46)	31	0.88
Haemoglobin, g/dl	8.5 (4.6–14.8)	98	8.7 (4.6–13.4)	65	7.8 (5.3–14.8)	31	0.47
Leucocytes, cells/µl	4205.0 (250–16400)	98	4420.0 (490–12650)	65	3800.0 (250–16400)	31	0.14
Creatinine, mg/dl	0.8 (0.1–3.2)	96	0.8 (0.2–2.9)	64	0.7 (0.1–3.2)	30	0.42
Albumin, g/dl	2.6 (1.4–4.0)	81	2.50 (1.4–4.0)	53	2.80 (1.8–4.0)	26	0.05
Median CD4, cells/mm^3	28.0	53	42.0	34	13.0	19	0.002
Median viral load, log/ml	5.2	11	4.7	5	5.4	6	0.83

* Patients diagnosed with TB and non-tuberculous mycobacterial infection.
HIV = human immunodeficiency virus; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase.

AST and LDH serum levels were still observed more often in HIV-infected patients with disseminated TB than in patients with NTM disease. The median albumin levels were no longer statistically different.

DISCUSSION

Previous studies have investigated the use of laboratory parameters as a tool to differentiate TB from NTM infection in HIV patients. In the study by Hsieh et al., a prospective cohort of 37 patients with disseminated mycobacterial disease, elevated levels of serum alkaline phosphatase, gamma-glutamil transpeptidase and leucopaenia favoured the diagnosis of *M. avium* complex (MAC).[6] Rolla et al. reported that higher levels of LDH were associated with the diagnosis of disseminated MAC infection in their case-control study; however, this study did not compare disseminated MAC disease and TB.[7]

In our study, HIV-infected patients with disseminated TB infection had higher levels of AST and LDH compared with patients with disseminated NTM, with a specificity of 84% for LDH and sensitivity of 74% for AST levels. Higher levels of AST and LDH, which are present in many organs (lung, kidney, heart, pancreas, muscle), may be a consequence of widespread tissue involvement in patients with disseminated TB, in contrast to MAC, where bacteraemia diagnosis may precede tissue spread.[8]

Tissue necrosis is also a characteristic feature of TB granulomas and may explain the higher values of LDH in this disease. In one study, necrosis was observed in most tissue samples of patients with disseminated TB.[9] The higher levels of LDH in TB disseminated disease may reflect the greater burden of the disease and granuloma formation, and a greater propensity of the liver, spleen and bone marrow to release the enzyme.[10]

Our results emphasise the importance of specific laboratory features such as AST and LDH levels as valuable tools to help differentiate TB and NTM disseminated infection in HIV-infected patients, considering the variable clinical presentation of both diseases. Recognition of these features can lead to a tentative diagnosis so that appropriate treatment can be instituted before the results of mycobacterial culture become available.

Acknowledgement

The authors would like to thank the Fundo de Incentivo a Pesquisa (FIPE), Hospital de Clínicas de Porto Alegre, Brazil, for partial support of this study.

References

1 Armstrong W S, Katz J T, Kazanjian P H. Human immunodeficiency virus-associated fever of unknown origin: a study of 70 patients in the United States and review. Clin Infect Dis 1999; 28: 341–345.
2 Lambertucci J R, Rayes A A, Nunes F, Landazuri-Palacios J E, Nobre V. Fever of undetermined origin in patients with the acquired immunodeficiency syndrome in Brazil: report on 55 cases. Rev Inst Med Trop Sao Paulo 1999; 41: 27–32.
3 Crump J A, Tanner D C, Mirrett S, McKnight M, Reller L B. Controlled comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB and ISOLATOR 10 systems for detection of mycobacteremia. J Clin Microbiol 2003; 41: 1987–1990.
4 Morgan M A, Doerr K A, Hempel H O, Goodman N L, Roberts G D. Evaluation of the *p*-nitro-α-acetylamnino-β-hydroxypropiophenone differential test for identification of *Mycobacterium tuberculosis* complex. J Clin Microbiol 1985; 21: 634–635.
5 Koksalan O K, Aydin M D, Eraslan S, Bekiroglu N. Reliability of cord formation in BACTEC 12B/13A media for presumptive identification of *Mycobacterium tuberculosis* complex in laboratories with a high prevalence of *Mycobacterium tuberculosis*. Eur J Clin Microbiol Infect Dis 2002; 21: 314–317.
6 Hsieh S M, Hung C C, Chen M Y, Hsueh P R, Chang S C, Luh K T. Clinical features and outcome in disseminated mycobacterial diseases in AIDS patients in Taiwan. AIDS 1998; 12: 1301–1307.
7 Rolla V, Jacomet C, Salause B, et al. Clinical and laboratory findings of disseminated *Mycobacterium avium* complex infection (DMAC) in a pair matched case-control study. Rev Inst Med Trop Sao Paulo 1999; 41: 273–277.
8 Torriani F J, McCutchan J A, Bozzette S A, Grafe F R, Havlir D V. Autopsy findings in AIDS patients with *Mycobacterium avium* complex bacteremia. J Infect Dis 1994; 170: 1601–1605.
9 Hill A R, Premkumar S, Brustein S, et al. Disseminated tuberculosis in the acquired immunodeficiency syndrome era. Am Rev Respir Dis 1991; 144: 1164–1170.
10 Kagawa F T, Kirsch C M, Yenokida G G, Levine M L. Serum lactate dehydrogenase activity in patients with AIDS and *Pneumocystis carinii* pneumonia. Chest 1998; 94: 1031–1033.

RÉSUMÉ

Un diagnostic rapide est essentiel pour le traitement adéquat des mycobactérioses disséminées. Nous avons mené une étude rétrospective de cohorte pour identifier les caractéristiques cliniques et de laboratoire des mycobactérioses disséminées chez les patients séropositifs pour le virus de l'immunodéficience humaine (VIH) afin d'aider à la différentiation entre la tuberculose (TB) et la maladie due aux mycobactéries non tuberculeuses (NTM). On a revu tous les patients diagnostiqués entre 1996 et 2006. On a diagnostiqué la TB chez 65 patients et une NTM chez 31 patients. Chez les patients TB, les niveaux médians d'aspartate aminotransférase (AST) et de déshydrogènase lactique (LDH) étaient plus élevés (respectivement 69,0 vs. 45,0 ; $P = 0{,}02$ et 725,0 vs. 569,0 ; $P = 0{,}03$). L'AST et la LDH pourraient constituer un outil valable pour aider à différencier une TB disséminée et une NTM chez les patients infectés par le VIH.

RESUMEN

En el tratamiento adecuado de la enfermedad microbacteriana diseminada es primordial el diagnóstico precoz. Se llevó a cabo un estudio retrospectivo de cohortes con el fin de determinar las características clínicas y de laboratorio que diferencian la tuberculosis (TB) diseminada de la enfermedad por micobacterias atípicas (NTM), en pacientes con infección por el virus de la inmunodeficiencia humana (VIH). Se estudiaron todos los pacientes con diagnóstico establecido de enfermedad micobacteriana diseminada entre 1996 y 2006. Hubo 65 pacientes con diagnóstico de TB y 31 pacientes con enfermedad por NTM. Los pacientes con TB presentaron una concentración mediana más alta de aspartato-aminotransferasa (AST) (69,0 vs. 45,0 ; $P = 0,02$) y de lactato deshidrogenasa (LDH) (725,0 vs. 569,0 ; $P = 0,03$). La determinación de AST y de LDH podría constituir una herramienta valiosa en la diferenciación entre TB y enfermedad por NTM en pacientes infectados por el VIH.