community were not different from cluster sizes of the drug-resistant isolates, suggesting that the drug-resistant isolates are transmitted as easily as the drug-susceptible isolates. In addition, the well-documented spread of the multidrug-resistant New York strain W is further evidence for transmissibility (as a virulence measure) of multidrug-resistant tuberculosis (7). Our data from South Africa and the New York study demonstrate transmissibility of highly resistant strains and these strains have the ability to cause disease within a defined time interval. This may be interpreted as a strong indicator of strain virulence, where virulence is defined as the ability to be transmitted and cause disease. Thus, we believe that resistance to high concentrations of isoniazid does not (in general) imply lower strain virulence in the human as a function of impaired catalase activity. Virulence in these strains may be compensated for by the upregulation of additional virulence factors. Transmission of these resistant strains may create a large reservoir of individuals infected by resistant strains, who in the near future could develop multidrug-resistant disease. Therefore, an increased understanding of the molecular mechanisms associated with mycobacterial virulence in the human and efficient procedures for the quick diagnosis of drug resistance in *Mycobacterium tuberculosis* are important for prevention of transmission of these highly pathogenic strains.

Acknowledgement

This work was supported by the Glaxo Wellcome Action TB Initiative.

T.C. Victor[1]*, R. Warren[1], N. Beyers[2], P.D. van Helden[1]

[1] Department of Medical Biochemistry and [2]Department of Pediatrics and Child Health, University of Stellenbosch/Medical Research Council Centre for Molecular and Cellular Biology, PO Box 19063, University of Stellenbosch, Tygerberg, 7505 South Africa.

References

1. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC: A comparison of the virulence in guinea pigs of South India and British tubercle bacilli. Tubercle 1960, 41: 1–22.
2. Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry CE, Stover CK: Compensatory *ahpC* gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. Science 1996, 272: 1641–1643.
3. Ordway DJ, Sonnenberg MG, Donahue SA, Belisle JT, Orme IM: Drug-resistant strains of *Mycobacterium tuberculosis* exhibit a range of virulence for mice. Infection and Immunity 1995, 63: 741–743.
4. Ausina V, Riutort N, Viñado B, Manterola JM, Ruiz Manzano J, Rodrigo C, Matas L, Giménez M, Tor J, Roca J: Prospective study of drug-resistant tuberculosis in a Spanish urban population including patients at risk for HIV infection. European Journal of Clinical Microbiology & Infectious Diseases 1995, 14: 105–110.
5. Warren R, Hauman J, Beyers N, Richardson M, Schaaf HS, Donald P, Van Helden PD: Unexpectedly high strain diversity of *Mycobacterium tuberculosis* in a high incidence community. South African Medical Journal 1996, 86: 45–49.
6. Warren R, Richardson M, Sampson S, Hauman JH, Beyers N, Donald PR, Van Helden PD: Genotyping of *Mycobacterium tuberculosis* with additional markers enhances accuracy in epidemiological studies. Journal of Clinical Microbiology 1996, 34: 2219–2224.
7. Bifani PJ, Plikaytis BB, Kapur V, Stockbauer K, Pan X, Lutfey ML, Moghazeh SL, Eisner W, Daniel TM, Kaplan MH, Crawford JT, Musser JM, Kreisworth BN: Origin and interstate spread of a New York city multidrug-resistant *Mycobacterium tuberculosis* clone family. Journal of the American Medical Association 1996, 275: 452–457.

Detection of *Mycobacterium avium-intracellulare* in the Blood of HIV-Infected Patients by a Commercial Polymerase Chain Reaction Kit

In North America and Europe, disseminated infections with mycobacteria other than tuberculosis occur frequently in the late stages of AIDS (1). In a prospective study of patients with positive mycobacterial blood cultures in Switzerland, *Mycobacterium genavense* was responsible for 12.8% of the infections (2), and *Mycobacterium avium* accounted for 82%.

Until recently, the only method available to diagnose these infections was culture in liquid medium (Bactec; Becton Dickinson, USA). Such cultures require a long incubation time (up to 14 weeks) to become positive, which delays diagnosis and treatment. Amplification methods such as the polymerase chain reaction (PCR) applied directly to blood specimens may accelerate diagnosis. The few studies that have attempted to detect mycobacteria directly in blood have used nested PCR methods to increase sensitivity (3–5). However, PCR performed on blood presents particular methodologic problems because of the neces-

Table 1: Detection of *Mycobacterium avium, Mycobacterium intracellulare*, and *Mycobacterium tuberculosis* in blood from HIV-infected patients by the Amplicor PCR test and culture.*

	Culture positive			Culture negative	PCR	
	M. avium (n = 32)	*M. intracellulare* (n = 1[a])	*M. tuberculosis* (n = 6)	*M. avium, M. intracellulare* and *M. tuberculosis* (n = 162[b])	Sensitivity (%)	Specificity (%)
PCR positive	14	0	1	0	42[c]	100[c]
PCR negative	18	1	5	162		

*The positive culture specimens were hybridized with the *M.avium*, *M. intracellulare*, and *M. tuberculosis* probes according to their identification by biochemical tests. The negative culture specimens were final hybridized with the *M. avium* probe.
[a] Also positive for *M. avium*.
[b] Four blood cultures positive for *M. genavense*, 1 positive for *M. haemophilum*; 157 blood cultures negative.
[c] Sensitivity and specificity calculated for *M. avium/M. intracellulare* Amplicor test (values of the *M. tuberculosis*-positive cultures excluded).

sity to eliminate inhibitors of the PCR, such as the heme compound, during preparation of the DNA sample (6).

We report here the results of a retrospective study in which 43 of 200 Ficoll gradients from the blood of HIV-infected patients were positive for mycobacteria by culture in Bactec liquid medium. Samples were analyzed by a commercial PCR kit (Amplicor MAI; Roche Molecular Systems, Switzerland) that uses a simple PCR method to target the first variable region of the 16S rRNA gene. When used previously for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens, this PCR method had an overall sensitivity of approximately 70% and a specificity exceeding 98% (7, 8).

Blood samples were collected from patients in our hospital during a two-year period. Five ml of whole blood was cultured in Bactec 13A medium, and 5 ml was used to isolate the polynuclear and mononuclear blood cells in Ficoll/Hypaque solution. These Ficoll samples were frozen and then analyzed in batches using the Amplicor MAI PCR assay according to the standard protocol provided by the manufacturer. Briefly, the samples were prepared by washing the sample pellet three times with mycobacteria blood wash solution, lysing mycobacteria with mycobacteria blood lysis reagent and incubating at 60 °C for 45 min, and adding a neutralization reagent to provide the magnesium necessary for amplification. The amplification target is a 582 bp of the mycobacterial 16S rRNA gene, and the amplicons are detected using a probe specific for *Mycobacterium avium* or *Mycobacterium intracellulare*.

Of the 157 negative blood cultures, 43 were obtained from patients harboring mycobacteria in other specimens such as sputum, bronchoalveolar lavage fluid, or biopsies; 17 negative blood cultures were contaminated by bacteria other than mycobacteria. Of the 43 positive blood cultures, 31 were positive for *Mycobacterium avium*, one was mixed (*Mycobacterium avium* and *Mycobacterium intracellulare*), four were positive for *Mycobacterium genavense*, six were positive for *Mycobacterium tuberculosis*, and one was positive for *Mycobacterium haemophilum*. The six cultures positive for *Mycobacterium tuberculosis* were amplified by the Amplicor test, and the PCR products were hybridized with the *Mycobacterium tuberculosis* complex probe available in a commercial respiratory kit (Amplicor *M. tuberculosis* complex; Roche Diagnostic System, Switzerland).

The results of PCR compared with the results of Bactec culture, which is considered the gold standard, are presented in Table 1. The sensitivity of the Amplicor *Mycobacterium avium/Mycobacterium intracellulare* test on blood specimens was low (42%), whereas the specificity was excellent (100%). Another technique to extract DNA from blood (QIAamp blood kit; Qiagen, Switzerland) was also performed, but no additional mycobacteria-positive sample was detected by PCR. The weak sensitivity was not due to PCR inhibition, since an internal control was successfully coamplified in each sample tested. The only blood sample positive for *Mycobacterium avium* and *Mycobacterium intracellulare* was tested by hybridization with the *Mycobacterium avium* and the *Mycobacterium intracellulare* probes. These two hybridization results were negative. The amplification products of the six blood cultures positive for *Mycobacterium tuberculosis* were hybridized with the *Mycobacterium tuberculosis* complex probe supplied by the manufacturer (9). Only one blood culture was positive by PCR.

The PCR results for *Mycobacterium avium* did not correlate with the time to positivity in the Bactec

system. The mean Bactec detection time was 16 days and 14 days for positive and negative PCR results, respectively ($p > 0.2$). The time to positivity in Bactec is correlated with the inoculum. Therefore, the lack of correlation we observed suggests that a low concentration of mycobacteria in the samples is not the only explanation for PCR negativity. This result differs from results with the Amplicor kit for respiratory specimens, in which sensitivity is higher for samples that are positive by microscopy. It also differs from an internal study conducted by Roche Diagnostic Systems (V. Tevere, Benchmark, Journal of Amplicor PCR Diagnostics 1996, 3:6), which compared Amplicor MAI to quantitative culture to determine the detection limit of the test. Depending on the pathogen load, a range of sensitivities was observed: 36.4% when < 1 cfu/ml was tested; 66.7% with 1–100 cfu/ml; and 100% with > 100 cfu/ml.

In our study no correlation was seen between the PCR results and the clinical characteristics of the patients (CD4+ cell counts, levels of p24 antigen and β2 microglobulin, presence of antimycobacterial treatment). For the same patient during the same period of hospitalization, some blood specimens were clearly positive by PCR, while others were devoid of positive signals. Our results are in accordance with those of Schneider et al. (36th ICAAC, 1996, Abstract no. 1165), who found the sensitivity of the Amplicor test to be 50% and the specificity 98%.

In our opinion the MAI Amplicor test is not sensitive enough to be used prospectively to detect *Mycobacterium avium* or *Mycobacterium intracellulare* directly in the blood samples of all HIV-infected patients. The development of a nested PCR, along with improved methods for DNA preparation, may be essential to detect mycobacterial DNA in more culture-positive blood samples.

B. Ninet*, R. Auckenthaler, P. Rohner, O. Delaspre, B. Hirschel

Division of Infectious Diseases, Hôpital Cantonal Universitaire, CH-1211 Geneva, Switzerland.

References

1. Chan ISF, Neaton JD, Saravolatz LD, Crane LR, Osterberger J: Frequencies of opportunistic diseases prior to death among HIV-infected persons. AIDS 1995, 9: 1145–1151.
2. Pechere M, Opravil M, Wald A, Chave JP, Bessesen M, Sievers A, Hein R, Von Overbeck JP, Clark RA, Tortoli E: Clinical and epidemiologic features of infection with *Mycobacterium genavense*. Swiss HIV cohort study. Archives of Internal Medicine 1995, 155: 400–404.
3. Schluger NW, Condos R, Lewis S, Rom WN: Amplification of DNA *Mycobacterium tuberculosis* from peripheral blood of patients with pulmonary tuberculosis. Lancet 1994, 344: 232–233.
4. Emler S, Böttger EC, Broers B, Cassis I, Perrin L, Hirschel B: Growth-deficient mycobacteria in patients with AIDS: diagnosis by analysis of DNA amplified from blood or tissue. Clinical Infectious Diseases 1995, 20: 772–775.
5. Iralu JV, Shritharan VK, Pieciak WS, Wirth DF, Maguire JH, Barker RH Jr: Diagnosis of *Mycobacterium avium* bacteremia by polymerase chain reaction. Journal of Clinical Microbiology 1993, 31: 1811–1814.
6. Akane A, Matsubara K, Nakamura H, Takahashi S, Kimura K: Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. Journal of Forensic Science 1994, 39: 362–372.
7. Chin DP, Yajko DM, Keith Hadley W, Sander CA, Nassos PS, Madej JJ, Hopewell PC: Clinical utility of a commercial test based on the polymerase chain reaction for detecting *Mycobacterium tuberculosis* in respiratory specimens. American Journal of Respiratory and Critical Care Medicine 1995, 151: 1872–1877.
8. D'Amato RF, Wallman AA, Hochstein LH, Colaninno PM, Scardamaglia M, Ardila E, Ghouri M, Kim K, Patel RC, Miller A: Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. Journal of Clinical Microbiology 1995, 33: 1832–1834.
9. Stauffer F, Mutschlechner R, Hasenberger P, Stadlbauer S, Schinko H: Detection of *Mycobacterium tuberculosis* complex in clinical specimens by a commercial polymerase chain reaction kit. European Journal of Clinical Microbiology & Infectious Diseases 1995, 14: 1046–1051.

Edwardsiella tarda Septicemia with Cellulitis in a Patient with AIDS

A 39-year-old woman was admitted to hospital in August 1994 with fever, rigors and confusion. She was known to be HIV-positive since 1990, and at the time of admission had a CD4+ cell count of $6/mm^3$. She had a history of pulmonary tuberculosis in 1990, cerebral toxoplasmosis in 1991, cryptococcal meningitis in 1993, sclerosing cholangitis since 1994 and chronic hepatitis B (HBsAg positive, HBV DNA negative). A venous access device was implanted in September 1993 for administration of prolonged i.v. amphotericin B treatment, but removed in March 1994 due to cen-