mechanisms. Isolation of *Mycoplasma pneumoniae* from extrapulmonary sites provides evidence of a more invasive role in extrapulmonary manifestations, indicating that the organism may spread more frequently than thought. Demonstration of *Mycoplasma pneumoniae* outside the respiratory tract is hampered by the organism's fastidious nature, the invasive procedures required and the lack of thorough research.

Our case shows that some cases diagnosed as idiopathic pericarditis could have a mycoplasmal etiology, even in the absence of serological documentation. Large pericardial effusions, particularly in immunosuppressed patients or those with previous cardiac surgery, may be clues for suspicion of mycoplasmal pericarditis. Appropriate treatment with tetracycline or erythromycin might be effective in curing this severe complication of *Mycoplasma pneumoniae* infection.

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References

1. Pönka A: Carditis associated with *Mycoplasma pneumoniae* infection. Acta Medica Scandinavica 1979, 206: 77–86.
2. Naftalin JM, Wellisch G, Kahana Z, Diengott D: *Mycoplasma pneumoniae* septicemia. Journal of the American Medical Association 1974, 228: 565.
3. Kenney RT, Li JS, Clyde WA, Wall TC, O'Connor CM, Campbell PT, Van Trigt P, Corey GR: Mycoplasmal pericarditis: evidence of invasive disease. Clinical Infectious Diseases 1993, 17, Supplement 1: 58–62.
4. Collier AM: Attachment by mycoplasmas and its role in disease. Reviews of Infectious Diseases 1983, 5, Supplement 4: 685–692.
5. Pietsch K, Ehlers S, Jacobs E: Cytokine gene expression in the lungs of BALB/mice during primary and secondary intranasal infection with *Mycoplasma pneumoniae*. Microbiology 1994, 140: 2043–2048.
6. Bayer AS, Galpin JE, Theofilopoulos AN, Guze LB: Neurologic disease associated with *Mycoplasma pneumoniae* pneumonitis: demonstration of viable *Mycoplasma pneumoniae* in cerebrospinal fluid and blood by radioisotopic and immunofluorescent tissue culture techniques. Annals of Internal Medicine 1981, 94: 15–20.
7. Thomas M, Jones M, Ray S: *Mycoplasma pneumoniae* in tubo-ovarian abscess. Lancet 1975, ii: 774–775.
8. Cherry JD: Anemia and mucocutaneous lesions due to *Mycoplasma pneumoniae* infections. Clinical Infectious Diseases 1993, 17, Supplement 1: 47–51.
9. Davis CP, Cohran S, Lisse J, Buck G, DiNuzzo AR, Weber T, Reinarz JA: Isolation of *Mycoplasma pneumoniae* from synovial fluid samples in a patient with pneumonia and polyarthritis. Archives of Internal Medicine 1988, 148: 969–970.
10. Sillis M: The limitations of IgM assays in the serological diagnosis of *Mycoplasma pneumoniae* infections. Journal of Medical Microbiology 1990, 33: 253–258.
11. Marmion BP, Williamson J, Worswick DA, Kok T-W, Harris RJ: Experience with newer techniques for the laboratory detection of *Mycoplasma pneumoniae* infection: Adelaide, 1978–1992. Clinical Infectious Diseases 1993, 17, Supplement 1: 90–99.

Cryptococcal Meningitis Following Fludarabine Treatment for Chronic Lymphocytic Leukemia

Fludarabine (9-β-D-arabinofuranosyl-2-fluoradenine) is a purine antimetabolite that is highly active in the treatment of low-grade lymphoma, chronic lymphocytic leukemia and Waldenström's macroglobulinemia. The use of fludarabine in first- and second-line treatment has resulted in high response rates (1). Chronic administration of fludarabine causes lymphocytopenia, leading to decreased numbers of T-lymphocytes and, to a lesser extent, B-lymphocytes (2). It is hypothesized that fludarabine induces deficiency of the cell-mediated immunity, thereby predisposing to opportunistic infections (3). Opportunistic microorganisms, which are found characteristically in patients with impaired cellular immunity, may cause severe infections in fludarabine-treated patients (4, 5). We describe here a patient with chronic lymphocytic leukemia who was treated with fludarabine and who subsequently experienced a primary episode of cryptococcal meningitis.

A 47-year-old man who had developed chronic lymphocytic leukemia, Binet stage III, 10 years previously was treated from 1988 to 1993 with several cycles of chlorambucil and cyclophosphamide/prednisone. In 1993 he was treated with four cycles of fludarabine (25 mg/m^2 x 5, total of 975 mg) and prednisone. During the last course of fludarabine he experienced an unexplained episode of pulmonary infection with dyspnea and interstitial infiltrates in both lungs. No causative organisms were found in the bronchoalveolar lav-

age fluid; microscopic preparations and cultures for *Pneumocystis carinii* and cytomegalovirus were negative.

Four months after the last dose of fludarabine was administered, the patient was admitted to hospital for treatment with cyclophosphamide, adriamycine, vincristine and prednisone because of progression of the chronic lymphocytic leukemia to an intermediate-grade non-Hodgkin's lymphoma. One week after this treatment he reported back to the hospital with fever (40°C), nausea and vomiting. At this time no headache was reported. Anterior cervical and axillary and inguinal adenopathy was present. Neurological and physical examination was otherwise normal; there were no signs of meningism. Laboratory analysis revealed a leukocyte count of 6,400 cells/mm^3 (98.6 % lymphocytes). The erythrocyte sedimentation rate was 114 mm/h.

A chest radiograph showed a dense infiltrate in the left lower lobe, and empiric antibiotic therapy was started. Fever, however, remained high, and after seven days the patient developed a moderate headache. At this time antibiotic therapy was discontinued. After ten days *Cryptococcus neoformans* grew from blood cultures taken at admission. The serum cryptococcal antigen titer was 1:524,288. A lumbar puncture was performed, and encapsulated yeasts were seen in the India ink-stained specimen. The CSF cryptococcal antigen titer was 1:2,048. *Cryptococcus neoformans* was cultured from the CSF. Direct ophthalmoscopy showed bilateral papilloedema (unfortunately, the lumbar opening pressure was not measured). Treatment with amphotericin B (0.7 mg/kg) was started. The patient's temperature and headache did not improve, and *Cryptococcus neoformans* was still cultured from the CSF after two weeks of treatment with amphotericin B. Flucytosine (5 mg/day) was added to the treatment regimen, and in the following three weeks the patient's condition slowly improved. The cryptococcal antigen titers in serum and in CSF had decreased to 1:2,048 and 1:128, respectively. He was discharged from the hospital on fluconazole (400 mg/day) as maintenance therapy. In the months following his discharge, the patient received several courses of chemotherapy. Despite this, his condition deteriorated and he died later due to pulmonary insufficiency. At obduction *Aspergillus fumigatus* was cultured from both lungs. There were no indications of persistent cryptococcal infection.

We describe a patient with cryptococcal meningitis after treatment with fludarabine and prednisone. Fludarabine is a purine antimetabolite introduced in the early 1980s and used more extensively today in the treatment of chronic lymphocytic leukemia and non-Hodgkin's lymphoma. High response rates (> 50 %) are described after previous treatment with other regimens, and even higher response rates have been observed when fludarabine was used as initial treatment (overall response 79 %, with 75 % of the patients achieving complete remission) (4). The major complication during fludarabine therapy is infection, often with opportunistic microorganisms, especially when fludarabine is used in combination with prednisone (4, 5). This is probably due to the effects of fludarabine on the different subsets of lymphocytes. Lymphocytopenia develops within a few days after administration of fludarabine (2). While the reduction of B-lymphocytes is relatively small, with an average of 50 %, the T-lymphocyte counts decrease by about 90 % (6). Both CD4+ and CD8+ subpopulations decrease substantially, but CD4+ reduction is more substantial, resulting in a reversal of the CD4+:CD8+ ratio. This phenomenon is also seen in advanced stages of chronic lymphocytic leukemia and in HIV-infected patients (6). The reduction of CD4+ lymphocytes and the reversal of the CD4+:CD8+ ratio is thought to result in increased susceptibility to opportunistic infections. *Pneumocystis carinii*, *Listeria monocytogenes* and cytomegalovirus have been reported in patients treated with fludarabine with or without prednisone (3–5, 7). Sometimes the same features are seen in patients without underlying diseases. Cryptococcal infections are described in these patients, too, underscoring the importance of this phenomenon (8, 9).

As far as we know, ours is the first patient presented in the literature with cryptococcal meningitis after fludarabine therapy. Although cryptococcal meningitis was diagnosed only four months after the last dose of fludarabine, it is very likely that our patient acquired the infection as a consequence of this treatment. We have two reasons for this assumption. First, as stated previously, it is likely that the cellular immunosuppression caused by fludarabine continues to exist for months or even years after therapy is stopped (5). During this period cryptococcosis may have been clinically dormant and not specific, as occurs in AIDS patients (10). Second, during his last course of fludarabine, our patient had an episode of pneumonia for which no causative organism was found. It is possible that this episode was also caused by *Cryptococcus neoformans* (although

cryptococcal antigen titers in serum samples drawn at that time remained negative when tested 4 months later). We presume that under continued immunosuppression after fludarabine therapy, followed by further depression of the immunity caused by the cyclophosphamide, adriamycine, vincristine and prednisone regimen, the infection spread to the central nervous system.

Our patient's case history shows that opportunistic infections, including cryptococcal meningitis, should be considered in patients who have been treated with fludarabine several months previously, even when headache is not reported. Cryptococcosis can be easily diagnosed in these patients by determination of serum cryptococcal antigen titers.

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References

1. Keating MJ, Kantarijan H, Talpaz M, Redman J, Koller C, Barlogie B, Velasquez W, Plunkett W, Freireich EJ, McCredie KB: Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood 1989, 74: 19–25.
2. Boldt DH, Von Hoff DD, Kuh JG, Hersh M: Effects on human peripheral lymphocytes of in vivo administration of 9-β-D-arabinofuranosyl-2-fluoroadenine-5"-monophosphate (NSC 312887), a new purine antimetabolite. Cancer Research 1984, 44: 4661–4666.
3. Sanders C, Perez EA, Lawrence HJ: Opportunistic infections in patients with chronic lymphocytic leukemia following treatment with fludarabine. American Journal of Hematology 1992, 39: 314–315.
4. O'Brien S, Kantarjian H, Beran M, Smith T, Koller C, Estey E, Robertson LE, Lerner S, Keating M: Results of fludarabine and prednisone therapy in 264 patients with chronic lymphocytic leukemia with multivariate analysis-derived prognostic model for response to treatment. Blood 1993, 82: 1695–1700.
5. Wijermans PW, Gerrits WBJ, Haak HL: Severe immunodeficiency in patients treated with fludarabine monophosphate. European Journal of Haematology 1993, 50: 292–296.
6. Hautekeete ML, De Bock RF, Van Bockstaele DR, Colpin GC, Berneman ZN, Peetermans ME: Flow cytometric analysis of T-lymphocyte subpopulations in B-cell chronic lymphocytic leukemia: correlation with clinical stage. Blut 1987, 55: 447–452.
7. Anaissie E, Kontoyiannis DP, Kantarjian H, Elting L, Robertson LE, Keating M: Listeriosis in patients with chronic lymphocytic leukemia who were treated with fludarabine and prednisone. Annals of Internal Medicine 1992, 117: 466–469.
8. Ho DH, Cao Y, Zhu T, Farthing C, Wang N, Gu G, Schooley RT, Daar ES: Idiopathic CD4+ T-lymphocytopenia: immunodeficiency without evidence of HIV infection. New England Journal of Medicine 1993, 328: 380–385.
9. McNulty A, Kaldor JM, McDonald AM, Baumgart K, Cooper DA: Acquired immunodeficiency without evidence of HIV infection: national retrospective survey. British Medical Journal 1994, 308: 825–826.
10. Dismukes WE: Cryptococcal meningitis in patients with AIDS. Journal of Infectious Diseases 1988, 157: 624–628.

An Imported Fatal Case of Diphtheria in Italy

Confirmed diphtheria cases have been very rare in Italy for many years, and biochemical characterization or molecular biotyping of toxigenic *Corynebacterium diphtheriae* has not previously been described. We report the biological characteristics of a strain of *Corynebacterium diphtheriae* that was also biotyped by molecular methods to define its origin and to establish whether it was imported or indigenous.

In December 1993, a 38-year-old Peruvian woman, an illegal immigrant to Italy, died of diphtheria within one day of admission to a hospital in Rome. The woman had been febrile for six days before seeking treatment for acute respiratory distress. Autopsy and microbiological examination confirmed the initial suspicion of diphtheria at admission. A biopsy of the mucous membranes from the soft palate and pharyngeal wall, taken during tracheostomy (performed immediately after admission), was examined for the presence of *Corynebacterium diphtheriae*, which was found in association with *Streptococcus mutans*. The isolate of *Corynebacterium diphtheriae* was identified by microscopic morphology and biotyping (API Coryne, bioMérieux, France) which designated it as *Corynebacterium diphtheriae* var. *gravis*. Toxigenicity of *Corynebacterium diphtheriae* was determined using a DNA probe (T21) specific for the diphtheria toxin gene (1) and by the in vivo guinea pig subcutaneous virulence test (2). Southern blot hybridization of *Bam*HI-restricted *Corynebacterium diphtheriae* DNA