New strains of bacteria and exacerbations of COPD [letter]

Bresser, P.; van Alphen, L.; Lutter, R.

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Correspondence

New Strains of Bacteria and Exacerbations of COPD

To the Editor: I find the choice of denominators by Sethi et al. (Aug. 15 issue)[1] curious, in that they compare patients with new strains during exacerbations with stable patients without new strains. This appears to be comparing apples and oranges, particularly given the wide range of base-line characteristics in the study population. For example, the forced expiratory volume in one second (FEV_1) at base line ranged from 0.47 liter to 4.07 liters, and the FEV_1 as a percentage of the predicted value ranged from 15 percent to 99 percent. Furthermore, over the 56-month study period, the number of patient visits to the clinic ranged from 2 to 65. Therefore, it seems feasible that a healthier population of patients with more stable chronic obstructive pulmonary disease (COPD) was serving as the control group. A more appropriate comparison would have been between patients who had exacerbations with new strains and those who had exacerbations without new strains.

The safest conclusions to be drawn from the study are that new strains occasionally appear in the sputum of patients with COPD both during stable periods (181 instances in the study) and during exacerbations (89 instances), and that new strains may be causally related to exacerbations in a minority of cases. In this carefully conducted longitudinal study that used advanced molecular techniques, it is unfortunate that no attempts were made to identify, characterize, and elucidate the emerging role of viruses in acute exacerbations of chronic bronchitis.[2]

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1. Sethi S, Evans N, Grant BJB, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 2002; 347:465-71.
2. Seemungal T, Harper-Owen R, Bhowmik A, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001; 164:1618-23.

To the Editor: Sethi et al. diagnosed exacerbations of COPD more commonly when certain new bacterial strains appeared in the sputum than when they were absent, suggesting that they caused the attacks. The new strains were unassociated with exacerbations of COPD in most cases (66 percent), so an alternative explanation is that they are not pathogens but, rather, that they more readily colonize inflamed bronchi during exacerbations (precipitated by other causes) than they do the airways of stable patients. The evidence offered by Sethi et al. supporting a strain-specific immune response in exacerbations rests on old, contradictory serologic investigations[1] and an unsatisfactory study involving only two patients and no controls.[2]

The accompanying editorial by Anthonisen[3] misinterprets the article's confusing, incomplete tables, thereby exaggerating the frequency of bacterial "pathogens" during exacerbations of COPD. The percentage of samples without these organisms was 62 percent, not 18 percent — a proportion that is consistent with other studies, in which 50 to 70 percent of cultures were negative, whether they were of expectorated or bronchoscopic specimens.[1]

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1. Hirschmann JV. Do bacteria cause exacerbations of COPD? Chest 2000;118:193-203.
2. Yi K, Sethi S, Murphy TF. Human immune response to nontypeable Haemophilus influenzae in chronic bronchitis. J Infect Dis 1997;176:1247-52.
3. Anthonisen NR. Bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 2002;347:526-7.

To the Editor: In their prospective study with 81 patients with chronic bronchitis, 91 percent of whom had moderate-to-severe airway obstruction, Sethi and colleagues showed

an association between exacerbation of the disease and acquisition of new strains of *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pneumoniae.* Recently, we completed a prospective, longitudinal study in which strains of *H. influenzae* were cultured and analyzed phenotypically and genotypically (with analyses of major outer-membrane proteins[1] and random amplified polymorphic DNA[2]) from 105 of 142 sputum samples (74 percent) collected at four-week intervals for six months from 19 patients with mild-to-moderate chronic obstructive bronchitis and chronic *H. influenzae* infections. We observed persistence, simultaneous persistence, and reinfection with related and unrelated strains. Forty-four strains with distinct patterns of major outer-membrane proteins were cultured; 22 (50 percent) were newly acquired strains, whereas only 3 of these were associated with an exacerbation. Thus, 86 percent of newly acquired strains were not associated with an exacerbation, whereas 67 percent of exacerbations occurred when no new strain was acquired.

Our findings appear to refute the theory that newly acquired strains of *H. influenzae* have a causative role in many acute exacerbations of chronic bronchitis, as suggested by Sethi et al. — and, indeed, by us in an earlier study involving patients with chronic bronchitis who were not chronically infected.[3] Possibly, the contribution of newly acquired strains to the inflammatory process is obscured by an already marked inflammation of the airway, especially in chronically infected patients with chronic bronchitis,[4] and therefore they do not lead to an exacerbation of the disease.

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1. van Alphen L, Riemens T, Poolman J, Zanen HC. Characteristics of major outer membrane proteins of Haemophilus influenzae. J Bacteriol 1983;155:878-85.
2. van Belkum A, Duim B, Regelink A, Möller L, Quint W, van Alphen L. Genomic DNA fingerprinting of clinical Haemophilus influenzae isolates by polymerase chain reaction (PCR) amplification: comparison with major outer-membrane protein and restriction fragment length analysis. J Med Microbiol 1994;41:63-8.
3. Groeneveld K, van Alphen L, Eijk PP, Visschers G, Jansen HM, Zanen HC. Endogenous and exogenous reinfections by Haemophilus influenzae in patients with chronic obstructive pulmonary disease: the effect of antibiotic treatment on persistence. J Infect Dis 1990;161:512-7.
4. Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R. Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic Haemophilus influenzae airway infection: comparison with noninfected patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:947-52.

The authors reply:

To the Editor: Dr. Kureishi has expressed concern that the patients with new strains during exacerbations are different from patients without new strains. The longitudinal design of our study allowed us to sample the same patients repeatedly. The unit of analysis was not the patient but the clinic visit. Therefore, patients with exacerbations associated with new strains are often the same patients as those who do not have new strains at other visits. Specifically, 88 percent of the patients who had exacerbations associated with new strains also had exacerbations without new strains. Therefore, the severity of the underlying COPD was not a confounding variable in our analysis.

Dr. Hirschmann implies that since new strains were not always associated with exacerbations, alternative explanations need to be considered. Because children and adults continuously acquire and clear strains of *H. influenzae* and other bacterial pathogens from the respiratory tract, to expect that every acquisition of a new strain will result in an exacerbation is too simplistic. A complex host–pathogen interaction most likely determines the outcome of the acquisition of a new strain by a patient; the determinants include the virulence of the pathogen, the host inflammatory response, preexisting immunity, the perception of symptoms, and others.

We agree with Dr. Hirschmann that definitive conclusions regarding strain-specific immune responses to bacterial pathogens in COPD cannot be drawn on the basis of available published data. Serologic investigations that use laboratory strains, rather than homologous isolates, are not capable of showing strain-specific immune responses, which accounts for the contradictory results of older serologic studies. However, a growing number of studies that use the homologous infecting isolates have demonstrated strain-specific immune responses in animal models, adults with COPD, and children with otitis media.[1-5] We are currently analyzing the results of immunoassays using homologous isolates from the patients from our longitudinal study.

The interesting observations of Bresser et al. appear to reflect a subgroup of patients with COPD who have chronic *H. influenzae* infection. The frequency of positive sputum cultures for *H. influenzae* in their patients was 74 percent, whereas the frequency of positive cultures for *H. influenzae* in our samples from unselected patients with COPD was 21 percent. Indeed, we have observed a similar subgroup of patients with high levels of colonization and infection within our study population. These observations emphasize the heterogeneity of patients with COPD with regard to the role of bacteria in the course and pathogenesis of disease.

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2. Duim B, Vogel L, Puijk W, et al. Fine mapping of outer membrane protein P2 antigenic sites which vary during persistent infection by *Haemophilus influenzae.* Infect Immun 1996;64:4673-9.
3. Yi K, Sethi S, Murphy TF. Human immune response to nontypeable *Haemophilus influenzae* in chronic bronchitis. J Infect Dis 1997;176:1247-52.
4. Yi K, Murphy TF. Importance of an immunodominant surface-exposed loop on outer membrane protein P2 of nontypeable *Haemophilus influenzae.* Infect Immun 1997;65:150-5.

5. Faden H, Bernstein J, Brodsky L, et al. Otitis media in children. I. The systemic immune response to nontypable *Haemophilus influenzae*. J Infect Dis 1989;160:999-1004.

The editorialist replies:

To the Editor: Dr. Hirschmann is correct; I did some bad math in analyzing Table 3 of the paper of Sethi et al. In more than 60 percent of sputum samples obtained during exacerbations, no bacterial pathogens were isolated. This fact does not change the findings of Sethi et al. that the risk of an exacerbation was increased when pathogens were isolated and that this risk was further increased when a new strain of bacteria was detected. The correction tends to emphasize the point made in the editorial that bacterial infection is very likely not the only cause of exacerbations.

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Immunosuppression and BKV Nephropathy

To the Editor: The study by Hirsch et al. (Aug. 15 issue)[1] provides important new data in the emerging field of nephropathy associated with the polyomavirus type BK (BKV). One point, however, is not supported by their data and may be misleading. The authors repeatedly state that BKV nephropathy is associated with the use of tacrolimus or mycophenolate mofetil. They also made this assumption in an earlier report,[2] and in the current study, they limited their patients to those receiving tacrolimus or mycophenolate mofetil. However, all the patients receiving mycophenolate mofetil were also receiving cyclosporine, making the determination of a causative agent in this group impossible.[1] The single prospective trial that compared the incidence of BKV viremia and viruria in patients randomly assigned to receive tacrolimus or cyclosporine demonstrated no significant difference between the two agents as well as no significant association between mycophenolate mofetil and BKV infection.[3] Other authors have reported retrospective cases of BKV nephropathy in patients receiving cyclosporine as well as tacrolimus, even reporting stabilization or improvement of renal function in patients whose therapy was changed from cyclosporine to tacrolimus.[4,5] Therefore, we believe it is more plausible that patients whose immunosuppression is maintained at a higher total level, rather than with a specific agent, have an increased incidence of BKV nephropathy.

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1. Hirsch HH, Knowles W, Dickenmann M, et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 2002;347:488-96.
2. Binet I, Nickeleit V, Hirsch HH, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. Transplantation 1999;67:918-22.
3. Lopez-Rocafort L, Wang C, Miller B, et al. A prospective evaluation of BK virus infection in renal transplant patients. Am J Transplant 2002;2: S260. abstract.
4. Barri YM, Ahmad I, Ketel BL, et al. Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. Clin Transplant 2001;15:240-6.
5. Howell DN, Smith SR, Butterly DW, et al. Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. Transplantation 1999;68:1279-88.

The authors reply:

To the Editor: BKV nephropathy may arise in the autologous kidneys of patients with inherited, acquired, or drug-mediated immune dysfunction,[1,2] but most cases have occurred in the renal allografts of patients treated with tacrolimus or mycophenolate mofetil since 1995, after widespread clinical use of these drugs.[3] The initial use of both drugs in high-dose rescue protocols suggested that intense immunosuppression might be a risk factor for BKV nephropathy.[4] Their different modes of action also suggest that immunosuppression is the common denominator. However, drug-specific mechanisms promoting BKV nephropathy have not been investigated. Intense immunosuppression is difficult to define and might occur at different drug levels in different patients. In our prospective study, the use of tacrolimus and azathioprine or of cyclosporine and mycophenolate mofetil was not sufficient for the development of BKV nephropathy without rejection and corresponding antirejection treatment: 68 of our 78 patients (87 percent) remained free of BKV viremia and nephropathy, and 55 of the 78 (71 percent) were free of decoy-cell shedding. Data from the literature indicate that neither drug is necessary, since BKV nephropathy has been described in patients treated with cyclosporine and azathioprine, albeit in only a few cases.[3] In fact, reexamination of allograft-biopsy specimens from patients with decoy-cell shedding between 1985 and 1995 revealed no additional cases at our center.[4]

We propose that BKV nephropathy has a multifactorial pathogenesis, with complementing key elements being viral (fitness, genotypes, or serotypes), cellular (injury and regeneration elicited by rejection, inflammation, drug toxicity, or coinfection), and immunologic (immunosuppression, primary or secondary infection with a new subtype, or inherited or acquired immunodeficiency) in nature. Clearly, further epidemiologic study as well as laboratory investigation is needed.

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1. de Silva LM, Bale P, de Courcy J, Brown D, Knowles W. Renal failure due to BK virus infection in an immunodeficient child. J Med Virol 1995; 45:192-6.
2. Haririan A, Ramos ER, Drachenberg CB, Weir MR, Klassen DK. Polyomavirus nephropathy in native kidneys of a solitary pancreas transplant recipient. Transplantation 2002;73:1350-3.

3. Hirsch HH. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. Am J Transplant 2002;2:25-30.
4. Binet I, Nickeleit V, Hirsch HH, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. Transplantation 1999;67:918-22.

Voriconazole versus Amphotericin B for Invasive Aspergillosis

To the Editor: Herbrecht et al. (Aug. 8 issue)[1] show the superiority of voriconazole over amphotericin B for primary therapy of invasive aspergillosis. However, we are concerned about the striking difference in the duration of treatment: a median of 10 days with amphotericin B, as compared with 77 days with voriconazole. How can one be so sure that "the superiority of voriconazole . . . was not the result of excessive interruptions" of amphotericin B? First, the use of one of the lipid formulations of amphotericin B, which are tolerated better by the kidneys than amphotericin B deoxycholate,[2] would probably have reduced the number of discontinuations in the control group. Second, an alternative antifungal agent was given to 107 patients in the amphotericin B group and to 52 patients in the voriconazole group. Because severe adverse events accounted for 45 discontinuations in the amphotericin B group and for 26 discontinuations in the voriconazole group, we wonder whether the other interruptions were explained by a poor response to initial therapy (which may be problematic in an open-label study). Therapy was switched from amphotericin B to itraconazole, the efficacy of which remains debatable in this setting, in 38 patients and from voriconazole to itraconazole in only 17 patients. This difference may partly account for the worse results in the amphotericin B group. The excess mortality in the amphotericin B group was evident after about 10 days, which coincides with the median duration of amphotericin B treatment.

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1. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002;347:408-15.
2. Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest 2002;121:1988-99.

To the Editor: In the study by Herbrecht et al., treatment of invasive aspergillosis was planned to take place over a 12-week period, so amphotericin B in a liposomal formulation would have been the more appropriate drug to answer the question of whether amphotericin B or voriconazole would be the optimal method of treatment for this infection. Herbrecht et al. show that use of amphotericin B is not the optimal approach, because it is not safe to administer this drug as required for an extended period. The crucial question of whether initial treatment with liposomal amphotericin B is as efficacious as treatment with voriconazole remains unanswered but is still of utmost clinical importance.

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The authors reply:

To the Editor: We agree with Blot and colleagues that the reasons for early discontinuation are important to the analysis. Thus, we have carefully reviewed the reasons for discontinuation in all the patients who received at least one dose of study drug (185 patients in the amphotericin B group and 194 patients in the voriconazole group) to avoid any misinterpretation of the difference in the duration of the initial, randomly assigned therapy. Progression of the infection (as assessed by the data-review committee, which was blinded to the study-drug assignment) and toxic effects accounted for discontinuation in 72 patients (38.9 percent) and 80 patients (43.2 percent), respectively, in the amphotericin B group and in 54 patients (27.8 percent) and 14 patients (7.2 percent), respectively, in the voriconazole group. Other reasons (in 21 patients in the amphotericin B group [11.4 percent] and in 20 patients in the voriconazole group [10.3 percent]) included progression of underlying cancer, withdrawal of consent, and the investigator's decision. Hence, there were no excess unjustified discontinuations in the amphotericin B group. Restriction of the analysis to the modified intention-to-treat population does not change the conclusion.

As the study results suggest, conventional amphotericin B is no longer considered a sustainable treatment for invasive aspergillosis. However, when the study was designed, intravenous amphotericin B and oral itraconazole were the only drugs approved for primary therapy of invasive aspergillosis. None of the lipid formulations were licensed in any country for primary therapy. Thus, no lipid formulation was chosen for use in a reference group in our study.

Whether a lipid formulation would have improved the results in the amphotericin B group, as suggested by Karthaus, is a speculation not supported by any data from the literature. We accept the superiority of lipid amphotericin B over conventional amphotericin B in terms of tolerance. Unfortunately, there is no evidence from any large randomized study that the lipid formulation has improved efficacy in invasive aspergillosis. A recent study showed that amphotericin B colloidal dispersion and conventional amphotericin B have similar efficacy, with 18.0 percent and 22.7 percent rates of complete or partial response, respectively.[1] In that study, the rate of response to amphotericin B was identical to the rate of response in our study at the end of amphotericin B therapy (21.8 percent), although the median duration of treatment was slightly longer (14.5 days, as compared with 10 days in our study). This identical response rate confirms the poor efficacy of amphotericin B for the treatment of invasive aspergillosis.

The benefit in terms of survival for patients treated with voriconazole became evident after only two weeks of therapy. These data suggest that the first days of therapy are

critical for the outcome of invasive aspergillosis and also indicate the insufficient efficacy of amphotericin B given as initial treatment.

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1. Bowden R, Chandrasekar P, White MH, et al. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. Clin Infect Dis 2002;35:359-66.

Paternal Inheritance of Mitochondrial DNA

To the Editor: Schwartz and Vissing (Aug. 22 issue)[1] describe a patient in whom paternal inheritance of mitochondrial DNA (mtDNA) was confirmed. However, the notion of paternal inheritance of mtDNA has been raised before — in one instance, in the *Journal*.[2] In that 1983 report, 30 families were studied for transmission of mitochondrial cytopathy. Maternal inheritance occurred in the majority of cases, but three cases showed evidence of paternal transmission. Although the authors recognized this possibility, it may have been that the dominant view at that time[3] prompted them to provide some alternative explanations as well, and therefore, they did not fully recognize the potential for paternal inheritance. Time and again, we are reminded that what we teach our students today may not necessarily be valid later. This lesson should also be coupled with an admonition to question vigorously data that appear to lie "outside of the box."

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2. Egger J, Wilson J. Mitochondrial inheritance in a mitochondrially mediated disease. N Engl J Med 1983;309:142-6.
3. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci U S A 1980;77:6715-9.

To the Editor: Schwartz and Vissing note that written consent was not required by the institutional review board because the genetic studies were considered to be part of clinical care. One wonders why this was so. For the patient, identification of a mitochondrial base-pair deletion could not be considered to be part of clinical care, since reparative gene therapy for such a deletion is not feasible. For the parents, the sister, and the paternal uncle, mitochondrial haplotype analysis could not be considered to be clinical care, since none had symptoms of skeletal or cardiac myopathy, and even if they had, they would not have benefited from such testing. The benefit here was the generation of new knowledge, and as such, it would have to be weighed by each subject against the potential loss of privacy from having the mitochondrial genome sequenced.[1] Such a deliberation would require an informed-consent process that explicitly acknowledged the lack of direct benefit and that clearly described the risk of a loss of confidentiality. At our medical center, such a study would have required approval by a convened institutional review board and written informed consent.

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1. Fuller BP, Kahn MJ, Barr PA, et al. Privacy in genetics research. Science 1999;285:1359-61.

The authors reply:

To the Editor: Dr. Gustafson states that the possibility of paternal inheritance of mtDNA has been raised before. It is true that it has been speculated on multiple times,[1,2] but it has not previously been proved to occur in humans. We believe that the occurrence of a pathogenic mtDNA mutation is necessary for the accumulation of paternal mtDNA. Only further investigation of sporadic cases in persons harboring mtDNA mutations can determine the frequency of this phenomenon.

Dr. Heckerling's concern that approval by an institutional review board and written consent were not obtained in our study was also raised during the review process for our article. Before acceptance, the paper was submitted to the central institutional review board in Copenhagen; this board approved the protocol and did not request any written consent procedure. Obviously, consent procedures differ among countries.

We disagree that "identification of a mitochondrial base-pair deletion could not be considered to be part of clinical care." Patients with mitochondrial myopathy are often given a misdiagnosis of being "terribly out of shape" or of having poor effort and motivation. Extensive pulmonary and cardiac investigations are often performed, particularly when muscle is the only affected organ, as in our patient. Our patient had had multiple investigations performed since childhood. When a specific diagnosis is made, not only are patients spared further, unnecessary testing, but often the diagnosis provides answers to many of their questions and helps them to explain their condition to other people.

In our experience, patients with muscle diseases generally want molecular characterization of their conditions. In addition, the detection of mutations in patients with mitochondrial myopathy has prognostic implications, since the outcome varies according to the load of mutation in different tissues. This information was clinically relevant not only for our patient, but also for his sister, who wanted to

know whether she harbored the mutation before she became pregnant. In our opinion, the failure to provide genetic testing for a capable person who requests such testing and who understands the pros and cons is nihilistic.

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2. Eyre-Walker A, Smith NH, Smith JM. How clonal are human mitochondria? Proc R Soc Lond B Biol Sci 1999;266:477-83.

Nurses' and Social Workers' Experience with Patients Who Requested Assistance with Suicide

To the Editor: Ganzini et al. (Aug. 22 issue)[1] surveyed nurses and social workers regarding their perceptions of patients' motivations for requesting lethal doses of medication. Apparently, the authors believe that their data are not subject to the bias of physicians who prescribe lethal doses, who may be "subject to an inherent conflict of interest and . . . may have failed to recognize depression or explore existential and social issues sufficiently." It could be argued, however, that nurses and social workers are subject to the same bias. Certainly, nurses who provide such lethal doses would like to think that their practice is consistent with statutory requirements — namely, that their patients' requests are not overly motivated by such factors as depression and uncontrolled pain. Nurses thus have some of the same inherent conflicts of interest as physicians do. Moreover, even nurses who have not provided lethal doses of medication have an interest in thinking that patients in their practice setting receive care that is consistent with the law. These considerations may thus bias nurses' responses to the study questionnaire.

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1. Ganzini L, Harvath TA, Jackson A, Goy ER, Miller LL, Delorit MA. Experiences of Oregon nurses and social workers with hospice patients who requested assistance with suicide. N Engl J Med 2002;347:582-8.

To the Editor: Ganzini et al. present results suggesting that hospice staff members believe that depression was among the least important reasons why patients requested physician-assisted suicide. Staff members also reported that patients requesting physician-assisted suicide had a level of depression that was similar to that of other hospice patients. These results contradict evidence from direct, standardized assessments showing that terminally ill patients who request physician-assisted suicide have higher levels of depression.[1]

There are several serious methodologic problems in this study. The results are based on retrospective reports by staff members on patients who requested physician-assisted suicide as compared with other hospice patients who did not; they covered a four-year period. This long period covered by the retrospective reports and the task of comparing the reports with global impressions of other hospice patients are subject to many biases. Clinicians often inadequately identify depression,[2] and recall of depression was based on unstandardized assessments. Psychological research suggests that such retrospective questions are answered by construction, not recall, based on the subjective viewpoints of participants.[3] Confirmation bias[4] is also likely to have led staff members to recall patients' characteristics according to their personal beliefs. We recommend a comparison between the ratings by staff members and the self-reported attitudes about physician-assisted suicide in order to partially address this issue of personal bias in recall.

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2. Block SD. Assessing and managing depression in the terminally ill patient. Ann Intern Med 2000;132:209-18.
3. Ross M. Relation of implicit theories to the construction of personal histories. Psychol Rev 1989;96:341-57.
4. Nickerson RS. Confirmation bias: a ubiquitous phenomenon in many guises. Rev Gen Psychol 1998;2:175-220.

The authors reply:

To the Editor: We agree that "nurses who provide such lethal doses" might be subject to the same conflicts of interest as physicians. Nurses, however, have no role under the Oregon Death with Dignity Act in providing lethal medications. Most hospices in Oregon either discourage or prohibit employees from being present when the lethal medication is ingested. Other studies support the belief that nurses, when offered anonymity as was offered in our study, are willing to provide information regarding illegal experiences with euthanasia.[1]

There are currently no studies using standardized assessments that demonstrate a relation between depression and requests for assisted suicide. The studies referenced by Haley et al. report that between 10 percent and 56 percent of terminally ill patients have a general interest in physician-assisted death and that depression is associated with a general interest, but whether these patients made actual requests is unknown.[2] In Oregon, 1 in 1000 deaths is from assisted suicide, including 4 in 1000 deaths of patients with cancer.[3] These figures suggest that only 1 in 100 patients expressing a general interest in assisted suicide dies by it. Among those interested in assisted suicide, there are no data to suggest that depression is a risk factor for receiving a lethal prescription. It is possible that very ill and depressed patients may be at a disadvantage in marshaling the focus and determination needed to obtain these prescriptions.[4]

Nurses' reports about the reasons for these requests were unrelated to their support of or opposition to the Oregon Death with Dignity Act, except that those who opposed the law rated dying at home as a less important reason than those who supported it ($P=0.002$). Nurses' ratings of the importance of depression for patients requesting a lethal prescription were not associated with nurses' support of or opposition to the law ($P=0.24$).

Clinicians who lack expertise in mental health do overlook depression. We reported that hospice social workers, who have expertise in evaluating mood disorders in dying patients, rated depression as the least important reason for the request for assisted suicide. Otherwise, as we noted in the article, we agree that the degree to which the nurses' responses accurately represent the patients' views is unknown, and studies of persons in Oregon who request assistance with suicide are needed to validate the importance of all these reasons.

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1. Asch DA. The role of critical care nurses in euthanasia and assisted suicide. N Engl J Med 1996;334:1374-9.
2. Emanuel EJ. Euthanasia and physician-assisted suicide: a review of the empirical data from the United States. Arch Intern Med 2002;162:142-52.
3. Sullivan AD, Hedberg K, Hopkins D. Legalized physician-assisted suicide in Oregon, 1998–2001. N Engl J Med 2001;344:605-7.
4. Ganzini L, Dobscha SK, Heintz RT, Press N. Oregon physicians' perceptions of patients who request assisted suicide and their families. J Palliative Med (in press).

Polymyalgia Rheumatica and Giant-Cell Arteritis

To the Editor: The prognosis for patients with polymyalgia rheumatica and temporal arteritis may not be as benign as intimated by Salvarani et al. (July 25 issue).[1] Despite the rapid initial response to corticosteroids, treatment is usually required for at least two years in the majority of patients. Studies in the United States suggest a shorter duration of therapy, in contrast to the European experience; this difference may be a manifestation of variations in demographic characteristics, the selection of patients, or the study design. In a study in Sweden, only 24 percent of patients with polymyalgia rheumatica, 16 percent of patients with polymyalgia rheumatica and temporal arteritis, and 5 percent of patients with temporal arteritis were able to discontinue prednisolone after two years of treatment.[2] In another study, less than 50 percent of patients with polymyalgia rheumatica had discontinued treatment after a mean of 23 months, and among those with both polymyalgia rheumatica and temporal arteritis, only 28 percent had stopped treatment after a mean of 31 months.[3] We have previously reported that only 24 percent of patients with polymyalgia rheumatica were able to discontinue corticosteroids successfully after two years.[4]

The results of a preliminary study suggest that severe polymyalgia rheumatica may be identified on the basis of a combination of the erythrocyte sedimentation rate and the interleukin-6 level.[5] If this finding is confirmed, prognostication may improve, leading to clearer guidelines on the length of treatment required. We currently tell patients that treatment is usually required for at least two years and may be required for a longer period. Furthermore, a small proportion of patients require treatment indefinitely, and such patients have a greatly increased incidence of corticosteroid complications.

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1. Salvarani C, Cantini F, Boiardi L, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. N Engl J Med 2002;347:261-71.
2. Myklebust G, Gran JT. Prednisolone maintenance dose in relation to starting dose in the treatment of polymyalgia rheumatica and temporal arteritis: a prospective two-year study in 273 patients. Scand J Rheumatol 2001;30:260-7.
3. Narváez J, Nolla-Solé JM, Clavaguera MT, Valverde-García J, Roig-Escofet D. Longterm therapy in polymyalgia rheumatica: effect of coexistent temporal arteritis. J Rheumatol 1999;26:1945-52.
4. Kyle V, Hazleman BL. The clinical and laboratory course of polymyalgia rheumatica/giant cell arteritis after the first two months of treatment. Ann Rheum Dis 1993;52:847-50.
5. Hunder GG. Polymyalgia rheumatica new treatment (new steroid sparing agents). Presented at the Second Annual European League Against Rheumatism Congress, Prague, Czech Republic, June 13–16, 2001. abstract.

To the Editor: Salvarani et al. report that giant-cell arteritis causes visual loss from "ischemic optic neuritis." No such disease exists. They have conflated optic neuritis and ischemic optic neuropathy. The former is an inflammatory disease often associated with multiple sclerosis; the latter is an ischemic disease that can occur in patients with giant-cell arteritis.[1] The authors also recommend that, when possible, a temporal-artery biopsy be performed before treatment is initiated. In patients with visual symptoms, corticosteroids should be administered immediately, not withheld until a biopsy has been performed. There is no evidence that a few days of corticosteroid treatment will mask the histopathological features of giant-cell arteritis. However, this delay is plenty of time for a patient to go blind.

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1. Miller NR, Newman NJ, eds. The essentials: Walsh & Hoyt's clinical neuro-ophthalmology. 5th ed. Baltimore: Williams & Wilkins, 1999:303-68.

To the Editor: Salvarani et al. suggest the use of calcium and vitamin D supplementation to prevent glucocorticoid-

induced osteoporosis. In its 1996 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis,[1] the American College of Rheumatology suggests the use of bisphosphonates only in patients with reduced bone mineral density.

Salvarani et al. did not consider that the American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis updated those recommendations in 2001,[2] suggesting that for patients who are beginning therapy with glucocorticoids (a prednisone equivalent of 5 mg per day), with plans for three months or more of treatment, bisphosphonates should always be prescribed, irrespective of the bone mineral density. As the authors say, in these patients serious corticosteroid-related complications are very frequent; thus, we think it is important to stress their correct prevention.

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1. American College of Rheumatology Task Force on Osteoporosis Guidelines. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Rheum 1996;39:1791-801.
2. American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis: 2001 update. Arthritis Rheum 2001;44:1496-503.

To the Editor: Salvarani et al. do not mention positron-emission tomography (PET) with the use of ^{18}F-fluorodeoxyglucose (FDG) as an imaging tool. My colleagues and I and other groups have shown that this scintigraphic technique can be used to visualize thoracic large-vessel inflammation in polymyalgia rheumatica and giant-cell arteritis.[1,2] In patients with mainly systemic symptoms (e.g., fever, weight loss, and malaise), its sensitivity reaches 75 percent (Fig. 1), with a specificity exceeding 95 percent.[1] Our results also demonstrate that in the majority of cases, giant-cell arteritis involves not only the temporal arteries but also the aorta and its proximal branches. One examination can demonstrate vasculitic involvement in the whole body. Therefore, I suggest that FDG PET scintigraphy be performed, in addition to arteriography, computed tomography, and magnetic resonance angiography, as Salvarani et al. suggest, whenever extracranial giant-cell arteritis is suspected. FDG PET scintigraphy is of great value for the diagnosis of giant-cell arteritis and of polymyalgia rheumatica when typical clinical signs are absent, when systemic symptoms predominate, or when temporal-artery biopsies are negative.

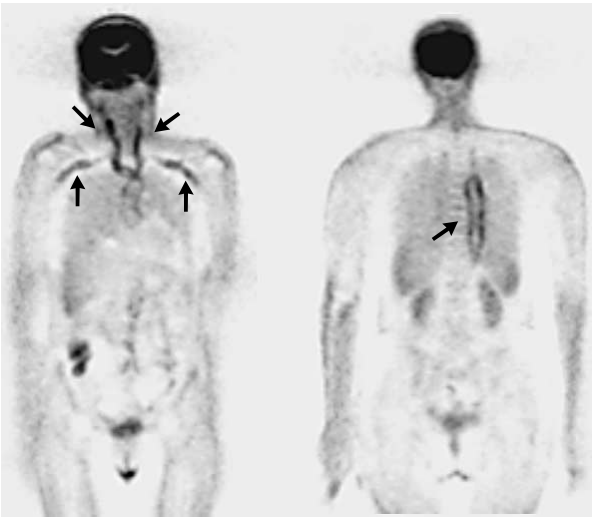

Figure 1. PET Scan Showing Increased Uptake of ^{18}F-Fluorodeoxyglucose in the Subclavian and Carotid Arteries and Thoracic Aorta (Arrows) in a 59-Year-Old Woman with Weight Loss and Fatigue Due to Giant-Cell Arteritis.

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1. Blockmans D, Stroobants S, Maes A, Mortelmans L. Positron emission tomography in giant cell arteritis and polymyalgia rheumatica: evidence for inflammation of the aortic arch. Am J Med 2000;108:246-9.
2. Turlakow A, Yeung HW, Pui J, et al. Fludeoxyglucose positron emission tomography in the diagnosis of giant cell arteritis. Arch Intern Med 2001; 161:1003-7.

The authors reply:

To the Editor: We did not mean to imply that polymyalgia rheumatica and giant-cell arteritis are benign conditions. Some cases of giant-cell arteritis are fatal, and with both syndromes, the majority of patients have important adverse effects of treatment. However, overall, we have found no significant reduction in life span. Drs. Ostor and Hazleman raise an important question about reported prognostic differences in northern European and U.S. studies of polymyalgia rheumatica and giant-cell arteritis. The reports suggest the presence of two subgroups of patients. One subgroup presents with mild, self-limiting disease requiring short-term treatment; the other subgroup has persistent disease requiring long-term treatment.[1,2] The conflicting data on the duration of corticosteroid therapy in polymyalgia rheumatica and giant-cell arteritis may be related to differences in the study setting. Studies in the United Kingdom and Scandinavian countries, which report a longer duration of corticosteroid therapy, enrolled patients with more severe disease who were recruited at secondary or tertiary referral centers. Our studies were population-based and included all diagnosed cases, without selection bias.

We are also convinced that some cases of polymyalgia rheumatica and giant-cell arteritis are overtreated. Corticosteroid therapy is sometimes continued late in the course of the disease for indeterminate musculoskeletal symptoms and normal or slightly elevated values for the erythrocyte sedimentation rate, C-reactive protein, or both. Such atypical symptoms are more likely due to noninflammatory causes such as corticosteroid withdrawal than to active polymyalgia

rheumatica. Aside from the erythrocyte sedimentation rate and C-reactive protein level, interleukin-6 appears to be a promising marker but is not routinely used in clinical practice.

We thank Dr. Horton for his clarification. We agree that ischemic optic neuropathy is the more correct term for the visual lesion in giant-cell arteritis. We agree that in the presence of visual symptoms, corticosteroids should be administered immediately rather than withheld until a biopsy has been performed. Temporal-artery biopsy specimens may show arteritis after more than two weeks of corticosteroid therapy.[3]

In the American College of Rheumatology's recommendations for the prevention and treatment of corticosteroid-induced osteoporosis, men and postmenopausal women who are beginning corticosteroid therapy, with a planned treatment duration of at least three months, are differentiated from those already receiving long-term corticosteroid therapy.[4] Bisphosphonate therapy is recommended only in the first group, whereas in the second group, it is recommended when the T score for bone mineral density at either the lumbar spine or the hip is below normal. Apart from these recommendations, in our clinical practice, we initiate bisphosphonate therapy only in patients with a reduced T score for bone mineral density. However, we always measure bone mineral density when initiating corticosteroid therapy for polymyalgia rheumatica and giant-cell arteritis, and we repeat the measurements annually in patients receiving long-term treatment.

We discussed FDG PET in our review. Although promising, it is an experimental technique for the diagnosis of giant-cell arteritis and is not routinely used in clinical practice.

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1. Salvarani C, Macchioni PL, Tartoni PL, et al. Polymyalgia rheumatica and giant-cell arteritis: a 5-year epidemiologic and clinical study in Reggio Emilia, Italy. Clin Exp Rheumatol 1987;5:205-15.
2. Ayoub WT, Franklin CM, Torretti D. Polymyalgia rheumatica: duration of therapy and long-term outcome. Am J Med 1985;79:309-15.
3. Achkar AA, Lie JT, Hunder GG, O'Fallon WM, Gabriel SE. How does previous corticosteroid treatment affect the biopsy findings in giant cell (temporal) arteritis? Ann Intern Med 1994;120:987-92.
4. American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis: 2001 update. Arthritis Rheum 2001;44:1496-503.

Self-Cardioversion of Paroxysmal Lone Atrial Fibrillation with Exercise

To the Editor: Endurance athletes may be at increased risk for lone atrial fibrillation.[1-5] We describe a middle-aged physician athlete with paroxysmal lone atrial fibrillation in whom cardioversion consistently occurs with vigorous exercise.

At 45 years of age, the patient had atrial fibrillation at an average ventricular rate of approximately 55 beats per minute. The results of physician examination, echocardiography, tests of thyroid function, and measurements of electrolytes were normal. After 24 hours of observation, external electrical cardioversion was attempted at progressive energy levels up to 400 J, without success. The patient was discharged home with instructions to take aspirin. The day after discharge, after being in atrial fibrillation for 48 hours, the patient resumed his schedule of normal exercise with a cross-country ski machine. Despite dyspnea with exertion, he achieved a maximal ventricular rate of approximately 170 beats per minute for 20 minutes, at which point he converted to sinus rhythm.

The patient had recurrences of atrial fibrillation at a slow ventricular rate during the following year, all of which were successfully converted to sinus rhythm with exercise. Noninvasive evaluation by an electrophysiologist, including multiple-event recording, resulted in a final diagnosis of paroxysmal atrial fibrillation, most likely a focal atrial fibrillation variant. Its features were not suggestive of vagally mediated atrial fibrillation.

Approximately 30 episodes of atrial fibrillation occurred during the following eight years. The patient successfully terminated all known episodes of atrial fibrillation through exercise, with the use of either a cross-country ski machine or an elliptical trainer. The interval between the onset of atrial fibrillation and the initiation of exercise ranged from approximately 1 hour to 48 hours. The total duration of exercise necessary for cardioversion ranged from approximately 20 minutes to 240 minutes. A ventricular rate of more than 160 beats per minute was achieved before successful cardioversion. Episodes of paroxysmal atrial fibrillation terminated with exercise were recorded with a multiple-event recorder.

Paroxysmal lone atrial fibrillation appears to be more common in endurance athletes than in the general population,[1-5] with a reported incidence of approximately 5.3 percent in a selected population of athletes.[5] Methods of treatment of atrial fibrillation include ablation, electrical cardioversion, drug-assisted cardioversion, heart-rate control, and anticoagulation. Vigorous exercise may be a noninvasive method of managing atrial fibrillation in athletes.

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1. Hood S, Northcote RJ. Cardiac assessment of veteran endurance athletes: a 12-year follow up study. Br J Sports Med 1999;33:239-43.

2. Furlanello F, Bertoldi A, Dallago M, et al. Atrial fibrillation in elite athletes. J Cardiovasc Electrophysiol 1998;9:Suppl:S63-S68.
3. Sivertssen E, Benestad AM, Rykke E, Brekke M, Smith G. Er det farlig å drive toppidrett? En 25 års etterundersøkelse av 24 elitelangrennsløpere. Tidsskr Nor Laegeforen 1994;114:3300-4.
4. Link MS, Homoud MK, Wang PJ, Estes NA III. Cardiac arrhythmias in the athlete. Cardiol Rev 2001;9:21-30.
5. Karjalainen J, Kujala U, Kaprio J, Sarna S, Viitasalo M. Lone atrial fibrillation in vigorously exercising middle aged men: case-control study. BMJ 1998;316:1784-5.

Neonatal Hair Analysis as a Biomarker for in Utero Alcohol Exposure

To the Editor: Alcohol is the most prevalent human teratogen. Fetal alcohol spectrum disorder is estimated to affect up to 1 percent of infants born in North America. The ascertainment of gestational exposure to alcohol is paramount for the diagnosis of the disorder. It is widely recognized that maternal reporting results in underestimates of the prevalence and amount of exposure to alcohol.

Until recently, there have been no objective biologic markers for the detection of long-term in utero exposure to alcohol. Studies in adults have documented that circulating ethanol is transesterified with fatty acids to fatty acid ethyl esters.[1] On the basis of this finding, fatty acid ethyl esters (ethyl linoleate, ethyl laurate, and ethyl stearate) have been identified in meconium.[2] In infants who were not exposed to alcohol in utero, the concentrations of these compounds in meconium are very low, whereas in infants with in utero alcohol exposure, the concentrations are high, sometimes up to 50 times as high as the values in controls.[2] Since meconium is available only during the first two or three days after birth, we have measured fatty acid ethyl esters in neonatal hair, which is available for up to two to three months after birth.[3]

Four fatty acid ethyl esters — ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate — have been identified in the hair of adults who drink socially or heavily.[4] We analyzed hair samples from a woman who admitted that she had drunk alcohol socially and used cocaine throughout pregnancy and from her newborn girl for fatty acid ethyl esters, cocaine, benzoylecgonine, and cocaethylene. The hair samples were analyzed for fatty acid ethyl esters with the use of a recently described method.[4] Briefly, samples of approximately 10 mg were extracted overnight with a mixture of *n*-heptane and dimethyl sulfoxide. The extract was then evaporated, and the residue, after resuspension in phosphate buffer, was reextracted with the use of headspace solid-phase microextraction. The final extract was analyzed by gas chromatography–mass spectrometry.

The hair samples from both the mother and the newborn were highly positive for cocaine, with the use of a method described previously,[5] with concentrations of 150 ng per milligram of hair from the mother and 12.9 ng per milligram of hair from the infant. The samples from both the mother and the infant were negative for cocaethylene but were positive for fatty acid ethyl esters, at 2.6 pmol per milligram and 0.4 pmol per milligram, respectively. The limit of detection for this gas chromatographic–mass spectrometric method is 0.25 pmol per milligram of hair when 5 mg of hair is analyzed; the highest yields of fatty acid ethyl ester extraction are obtained with a hair sample of 5 mg.[4] In our analyses, we used 5.1 and 6.7 mg of hair from the mother and her infant, respectively.

On the basis of the literature, the fatty acid ethyl ester concentration in the mother's hair corroborates her report of social drinking during pregnancy.[4] To the best of our knowledge, this is the first report of use of neonatal hair for the analysis of fatty acid ethyl esters. Our ability to measure fatty acid ethyl esters in the hair of a neonate whose mother was a self-reported social drinker suggests that exposure to larger amounts of alcohol will also be detectable. Large population-based studies are needed to establish a correlation between maternal intake of alcohol and fatty acid ethyl ester concentrations in neonatal hair.

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1. Laposata M. Fatty acid ethyl esters: ethanol metabolites which mediate ethanol-induced organ damage and serve as markers of ethanol intake. Prog Lipid Res 1998;37:307-16.
2. Klein J, Karaskov T, Koren G. Fatty acid ethyl esters: a novel biologic marker for heavy *in utero* ethanol exposure: a case report. Ther Drug Monit 1999;21:644-6.
3. Klein J, Koren G. Testing for drugs of abuse in the pediatric population. In: Mieczkowski T, ed. Drug testing technology: assessment of field applications. Boca Raton, Fla.: CRC Press, 1999:161-77.
4. Auwärter V, Sporkert F, Hartwig S, Pragst F, Vater H, Diefenbacher A. Fatty acid ethyl esters in hair as markers of alcohol consumption: segmental hair analysis of alcoholics, social drinkers, and teetotalers. Clin Chem 2001; 47:2114-23.
5. Klein J, Karaskov T, Koren G. Clinical applications of hair testing for drugs of abuse — the Canadian experience. Forensic Sci Int 2000;107:281-8.