LETTERS TO THE EDITOR

"Opportunist" mycobacterial infections

We were greatly impressed by the Joint Tuberculosis Committee guidelines on the management of opportunist mycobacterial infections.¹ We do, however, wonder why the word "opportunist" has been used to describe the mycobacteria, other than the *M tuberculosis* complex, that cause human disease. All mycobacteria causing disease, even the *M tuberculosis* complex, are opportunists. Thus, the latter are often spoken of as causing opportunist disease in HIV positive persons. Since the causative role of these other mycobacteria in human disease was established in the middle of the 20th century, a wide range of collective nouns has been applied to them—atypical, anonymous, MOTT (mycobacteria other than tuberculosis), non-tuberculous, and tuberculoïd—as well as opportunist.

The distinguishing feature of almost all mycobacteria other than members of the *M tuberculosis* complex is that they live freely in the environment. For this reason the expression "environmental mycobacteria" has been in widespread use in recent years. May we suggest that, for uniformity, this expression should be universally adopted.

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1 Subcommittee of the Joint Tuberculosis Committee of the British Thoracic Society. Management of opportunist mycobacterial infections: Joint Tuberculosis Committee guidelines 1999. *Thorax* 2000;55:210-8.

AUTHORS' REPLY The term "opportunist mycobacteria" was suggested by Marks.¹ He argued that *M tuberculosis*, *M bovis*, and *M leprae* were obligate pathogens which, if they did get into the environment, could not survive for any significant length of time. The other mycobacteria that cause disease in humans are, as Drs Davies and Grange say, free living environmental organisms and we are all continually exposed to them. However, comparatively few people become infected. Those who do usually have some pre-existing condition which predisposes them to infection—for example, chronic bronchitis and emphysema, bronchiectasis, previous tuberculosis, or some form of immunosuppression. The mycobacteria that are free living in the environment thus need an opportunity to cause disease—hence "opportunist mycobacteria". It should also be pointed out that not all environmental bacteria cause disease. The nomenclature was discussed both by the Working Party and the full Joint Tuberculosis Committee; in both it was

agreed by substantial majorities. This decision is also supported by the current and former directors of the Mycobacterium Reference Unit for England and Wales. We had hoped that the nomenclature argument about this group of mycobacteria could have been laid to rest once and for all after these decisions.

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1 Marks J. Nomenclature of the mycobacteria. *Am Rev Respir Dis* 1964;90:278.

Antibiotic prescribing in the community

Macfarlane *et al* present a comprehensive and thorough review of the multiplicity of factors affecting therapeutic decision making by general practitioners for patients presenting with acute lower respiratory tract symptoms.¹ They do, however, pass very briefly over the evidence base for the use of antibiotics in this common and important clinical situation, citing only one original study, one review, and one meta-analysis to justify the statement that "antibiotics have little impact on the duration of symptoms of acute bronchitis". For such an important and fundamental cause of morbidity in primary care there is an extraordinary dearth of studies to inform evidence-based decision making; the published studies are small, variable in quality, and use various antibiotics, dosage regimes, and outcome measures. In the quoted meta-analysis by Fahey *et al* of randomised controlled trials comparing antibiotics with placebo, only nine studies investigating a total of 700 randomised patients were found for analysis. Only six of these studies were suitable for the analysis of some of the key outcomes. The authors' conclusion that antibiotic treatment has no effect on the resolution of acute cough was subsequently criticised.²⁻⁵

Although the clinical improvements analysed in the antibiotic treated group failed to reach statistical significance, quite narrowly for some outcomes, the results did favour antibiotics for an effect on both resolution of cough and clinical improvement at re-examination, suggesting a trend favouring the use of antibiotics over placebo. The wide confidence limits and the small numbers point to the need for further data. The Cochrane meta-analysis of the same data⁶ reached very different conclusions, commenting that "the review confirmed the impression of clinicians that antibiotics have some beneficial effects in acute bronchitis". The benefits are probably small and confined to certain patient subgroups, but the quantification of benefit and the definition of the characteristics of responder groups need further studies to delineate.

All responsible clinicians must be in favour of appropriate use of antimicrobial drugs and efforts to "raise the trigger line" for the use of such agents are laudable. The assertion that the majority of British GPs and their European colleagues are ignoring a good evidence base when they prescribe antibiotics in this situation would, however, appear to be premature. Clarification of which patients with acute lower respiratory symptoms will benefit and by how much can only assist us in

targeting and restricting the use of antimicrobials. Increasingly well informed patients and GPs attempting to practice evidence-based medicine need such information to make rational decisions on appropriate management options. There is a need for well designed prospective placebo controlled, randomised trials performed in real world primary care settings with adequate power to provide definitive answers.

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- 1 Macfarlane J, Holmes WF, Macfarlane R. Issues at the interface between primary and secondary care in the management of common respiratory disease: do hospital physicians have a role in reducing antibiotic prescribing in the community? *Thorax* 2000;55:153-8.
- 2 Fahey T, Stocks N, Thomas T. Quantitative systematic review of randomized controlled trials comparing antibiotic with placebo for acute cough in adults *BMJ* 1998;316:906-10.
- 3 Cates CJ. Data do not justify study's conclusions. *BMJ* 1998;317:1014.
- 4 Shakespeare TP, Bourke RC. Too few subjects were studied for useful conclusions to be drawn. *BMJ* 1998;317:1014.
- 5 Harris J. Quality scores showed poor agreement. *BMJ* 1998;317:1014.
- 6 Becker L, Glazier R, McIsaac W, *et al*. Antibiotics for acute bronchitis. In: Douglas R, Bridges-Webb C, Glasziou P, *et al*, eds. *Acute respiratory infections*. Cochrane Database of Systematic Reviews.

AUTHORS' REPLY We are grateful to Dr Thomas for interest in our review¹ and pleased that he found it comprehensive and thorough. In his letter he debates the evidence base for the use of antibiotics for acute bronchitis or lower respiratory tract illness. There are problems with studies in this area relating to size of the studies, differing definitions of acute bronchitis, and identification of easily measurable and clinically important end points. There does seem to be a consistent message from the different studies that, overall, there is not much clinical benefit from antibiotics for acute bronchitis. This does not mean that all patients with acute bronchitis will not benefit from antibiotic use and the view that antibiotics are never indicated is unhelpful and impractical. However, we suspect that the proportion who need antibiotics is nearer to the 25% of patients in our studies in whom the GP stated that antibiotics were definitely clinically indicated than the 75% of patients consulting with acute bronchitis who are actually given antibiotics. We agree with Dr Thomas that the challenge is identifying that small group of patients in whom antibiotics are clinically indicated and it is here that further research is indicated, along with clearly described illness definitions and clinically relevant end points.

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Genetic susceptibility to COPD

We read with interest the report by Yim *et al* of a failure to observe an association between polymorphisms of the microsomal epoxide hydrolase (mEPHX) gene and chronic obstructive pulmonary disease (COPD).¹ This contrasts with the findings of earlier studies.²

There is debate in the literature on the place of association studies in the investigation of late onset complex disorders.³ Failure to replicate an initial report of a positive association is common⁴⁻⁶ and it is important that the reasons for this are established.

The authors correctly state that their failure to replicate the results of earlier studies may be a reflection of the marked racial differences in the frequency of the mEPHX gene within their population. However, their study also lacks power. Given, for example, their reported frequency of 75% for the wild type homozygous exon 4, a sample size of 80 subjects would only be able to detect a difference of 22% (e.g. 75% versus 53%) between the case and control groups (two tailed p value = 0.05, power = 0.8).

Phenotypic heterogeneity is a problem in the genetic dissection of complex traits and hampers comparisons between studies. The authors are rigorous in their spirometric criteria used to define cases. However, their COPD group also includes never smokers and those with minimal pack-year histories. It is not restricted to adult onset disease, potentially containing chronic asthmatics. This phenotypically heterogeneous group could reduce the likelihood of demonstrating an association. The calculation of phenotypic "scores" is one solution to the clinical diversity which the label "COPD" describes.⁷

Finally, the importance of age and sex matching cannot be understated, not only because controls may develop disease, but also to minimise the effects that will occur to the gene pool as the population ages. We would therefore urge caution before abandoning a role for this candidate gene in this population. Further studies in extended populations with rigorously matched controls are clearly needed.

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- 2 Smith CAD, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630-3.
- 3 Editorial. Freely associating. *Nature Genetics* 1999;22:1-2.
- 4 Blacker D, Wilcox M, Laird NM, *et al*. Alpha-2-macroglobulin is genetically associated with Alzheimer's disease. *Nature Genetics* 1998;19:357-60.
- 5 Dow DJ, Lindsay N, Cairns NJ, *et al*. Alpha-2-macroglobulin and Alzheimer's dementia risk in the UK. *Nature Genetics* 1999;22:16-17.
- 6 Rogaeva EA, Premkumar S, Grubber J, *et al*. An alpha-2-macroglobulin polymorphism insertion-deletion in Alzheimer's disease. *Nature Genetics* 1999;22:19-22.
- 7 Panhuysen CI, Bleeker ER, Koeter G, *et al*. Characterisation of obstructive airway disease in family members of probands with asthma. *Am J Respir Crit Care Med* 1998;157:1734-42.

AUTHORS' REPLY We thank Dr Ruse and colleagues for their interest and comment on our study.¹ They mentioned three points: (1) sample size, (2) the possibility of including asthmatic patients in the COPD groups, and (3) failure of age and sex matching between the disease and control groups.

We agree with them that our sample size was not large enough to detect small differences between the two groups (COPD 83, control 76). The strict criteria used in our study to select patients with disease or healthy smokers made our sample size smaller.

They suggested the possibility that we may have included asthmatic patients in the COPD groups because of the minimal smoking history in some patients. It is well known that there are risk factors for developing COPD other than smoking history such as environmental tobacco smoking (passive smoking), ambient air pollution, and occupation. It is therefore possible for non-smokers to develop COPD. Although we vigorously excluded patients with minimal asthmatic features in order to select a phenotypically homogeneous group, it is true that some patients with chronic asthma cannot be differentiated from patients with COPD by any method.

Gene frequencies do not vary according to sex in the general population and the lack of sex matching in our study may not influence the result. When we excluded six women from the COPD group the result was the same. Although we adjusted for the effect of age by stratification, it is clear that an age matched control group would have been better. The first and only study which suggested the role of genotypes of microsomal epoxide hydrolase (mEPHX) in the pathogenesis of COPD also lacked age and sex matching because the control group was anonymous.²

We agree with Dr Ruse and colleagues that further large scale rigorously matched case control studies are needed to clarify the role of this candidate gene in the pathogenesis of COPD.

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- 2 Smith CAD, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630-3.

Jae-Joon Yim *et al*¹ reported that genetic polymorphisms in microsomal epoxide hydrolase (mEPHX), glutathione-S transferase (GST) M1, and GST T1 genes are not associated with the development of chronic obstructive pulmonary disease (COPD) in Koreans. However, we strongly propose the possibility that the frequency for the mutant type of mEPHX exon 3 polymorphism (codon 113) was overestimated in their study as we have recently found a haplotype with a novel polymorphism (codon 119, accession #AB035519) within the antisense primer they used, and thus half of the individuals heterozygous at codon 113 could be misclassified as homozygous mutant using the same protocol in Japanese subjects.² In fact, the allele frequency for mEPHX exon 3 polymorphism reported by Yim *et al*¹ was not in Hardy-Weinberg equilibrium, suggesting the existence of some problems.

We have investigated the association between mEPHX gene and susceptibility to COPD in Japanese subjects and identified a novel single nucleotide polymorphism at codon 119 (AAG to AAA) 20 bp downstream of the codon 113 polymorphism (estimated allele frequency 0.29). This is a silent substitution and is unlikely to have any biological significance by itself. However, the variant type of this polymorphism (AAA) showed strong linkage disequilibrium with the wild type at codon 113. Since the novel polymorphism at codon 119 existed within the antisense primer used for codon 113 polymorphism, in individuals with 113:wild and 119:variant in one allele and 113:variant and 119:wild in another, the latter allele with the higher homology to the antisense primer was preferentially amplified as if it was an homologous variant for codon 113. In the Japanese population about half of the wild allele for codon 113 showed variant at codon 119 and almost all the variant alleles for codon 113 showed wild at codon 119.² As a consequence, about half the individuals heterozygous at codon 113 were misclassified as homozygous variants and the allele frequency was not in Hardy-Weinberg's equilibrium using the primer set used by Yim *et al*¹ and Smith and Harrison.³ The miscalculated allele frequency was the same as that reported by Yim *et al*.¹ The true genotype at mEPHX codon 113 could be determined by direct sequencing of the PCR products amplified with an antisense primer designed outside the original one. Since previous reports^{3,4} in Caucasians using the same protocol reported quite a low frequency of the homozygous mutant at codon 113, and the allele frequency was in Hardy-Weinberg's equilibrium, the novel polymorphism at codon 119 is unlikely to exist in Caucasians. Thus, the novel polymorphism at codon 119 or the haplotype at codons 113 and 119 are probably specific for Asians and we strongly suggest that Yim *et al* should carry out direct sequence analyses for codon 113 using an antisense primer outside the codon 119 to determine the true genotype and to re-

1 Yim JJ, Park GY, Lee CT, *et al*. Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. *Thorax* 2000;55:121-5.

evaluate the relationship with COPD in the Asian population.

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- 2 Yoshikawa M, Hiyama K, Ishioka S, *et al.* Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese. *Int J Mol Med* 2000;5:49-53.
- 3 Smith CAD, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630-3.
- 4 Benhamou S, Reinikainen M, Bouchardy C, *et al.* Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res* 1998;58:5291-3.

AUTHORS' REPLY We appreciate the comment by Dr Yoshikawa and colleagues on the possibility of overestimating the frequency of homozygous mutant genotype of microsomal epoxide hydrolase (mEPHX) exon 3 and agree that further explanation is needed for the fact that in our study¹ the allele frequencies of mEPHX in exon 4 are in Hardy-Weinberg equilibrium but those of exon 3 are not. The suggestion by Yoshikawa *et al.*² that patients with a heterozygous genotype of mEPHX exon 3 can be misclassified as a homozygous mutant due to polymorphism at codon 119 may be a good explanation for this observation, and we plan to sequence the PCR product of exon 3 amplified with an antisense primer outside the original one we used. We expect this to reveal the prevalence of a single nucleotide polymorphism at codon 119 of exon 3.

There is one fact which is overlooked by Dr Yoshikawa and colleagues. In our opinion there is no reason to assume that the prevalence of a single nucleotide mutation at codon 119 of mEPHX is different between patients with COPD and healthy smokers and, if the prevalence of mutation at codon 119 of mEPHX exon 3 is similar in the two groups, the real distributions of genotypes of mEPHX exon 3 are also similar.

As mentioned above, the sequencing of the PCR product of exon 3 amplified with an antisense primer outside the original one will clarify this confusion and further research on the functional significance of a single nucleotide polymorphism at codon 119 of mEPHX exon 3 will provide us with a more complete understanding of this polymorphism.

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- 2 Yoshikawa M, Hiyama K, Kishioka S, *et al.* Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese. *Int J Mol Med* 2000;5:49-53.

Fluticasone in asthma

The paper by Meijer *et al.*¹ compares the effects of inhaled fluticasone propionate (2 mg and 0.5 mg daily) and oral steroids (prednisolone 30 mg daily) in patients with mild to moderate asthma. Many patients with severe asthma are dependent upon corticosteroids, although inhaled steroids can effectively replace oral prednisolone.²

The biological effects of oral versus inhaled steroids can be compared in terms of lung function, airway responsiveness, and blood eosinophil number and activity, but suppression of the hypothalamic-pituitary-adrenal (HPA) axis is very difficult to assess. Prednisolone is so chemically similar to cortisol that most analytical methods (particularly radioimmunoassay, as used by Meijer *et al.*) cannot distinguish between the two steroids. Although the authors attempted to overcome this problem, the correction of the measured level of cortisol for a potential cross reaction with prednisolone is not valid. If there is any possibility of cross reaction from other steroids, cortisol assays are only specific if performed using high performance liquid chromatography or mass spectrometry.³ It is therefore incorrect for the authors to conclude that cortisol levels after 30 mg oral

prednisolone were comparable to those after inhaled fluticasone in a dose of 2 mg/day. The paper did not describe the precise dosages and timings for fluticasone or prednisolone administration and these could have large effects on 08.00 hour serum cortisol concentrations. The increased systemic effect of prednisolone compared with fluticasone is strongly supported by the significantly higher serum ECP level and blood eosinophil count.

Many papers on the function of the HPA axis in the context of safety of oral and inhaled steroids fail to take account of the normal function of the axis, the way in which the axis is perturbed by exogenous steroids, and the best methods for testing the axis, particularly when patients are also taking oral corticosteroids. Care should be taken at the outset to select analytical methods appropriate to the design of such studies to avoid misinterpretation.

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- 1 Meijer RI, Kerstens HAM, Arends LR, *et al.* Effects of inhaled fluticasone and oral prednisolone on clinical and inflammatory parameters in patients with asthma. *Thorax* 1999;54:894-9.
- 2 Noonan M, Chervinsky P, Busse WW, *et al.* Fluticasone propionate reduces oral prednisone use while it improves asthma control and quality of life. *Am J Respir Crit Care Med* 1995;152:1467-73.
- 3 Chrousos GP, Harris AG. Hypothalamic-pituitary-adrenal axis suppression and inhaled corticosteroid therapy. *Neuroimmunomodulation* 1998;5:277-87.

AUTHORS' REPLY We thank Dr Honour for his valuable comments. We are currently in the process of re-analysing our samples by an HPLC method as suggested by him, but we have had problems finding a laboratory able to perform the HPLC measurements with the required quality. We would like to respond as soon as the data are available.

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