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non-obese groups. Such imbalance is unavoidable in this type of study, but we used multivariate analyses to correct for confounding factors.[1] Contrary to their statement, the imbalance between the two groups did not affect the results, since obesity was not an independent risk factor in our multivariate analyses. Slim and colleagues also question the statistical power of our analyses. We agree that the study would be too small to detect a difference of 20%. However, we calculated a statistical power of 80%, assuming a reduction of 40% in morbidity (15% in non-obese patients and 25% in obese patients). Slim and colleagues also raise the issue of whether surgeons' performance could be a confounding factor. We did not, however, assess this issue in our study. At our centre, a staff surgeon is present in every case.

R McCarthy and co-workers point out the limitations of a non-randomised and observational methodology. According to criteria of evidence-based medicine, our study ranks as a level 2, which presently provides the best evidence to identify outcome data in this population. A randomised study—ie, surgery in obese patients versus surgery in obese patients after weight loss—is hardly feasible taking into account that losing weight in obese patients is rarely successful. McCarthy and co-workers propose POSSUM as a comparative audit tool. The POSSUM scoring system has mainly been validated for hospital mortality rather than morbidity. Moreover, the POSSUM score includes intraoperative variables, such as intraoperative bleeding, which are typically biased by factors relating to the surgeon.[2] Another difficulty with the POSSUM system is the need for preoperative examinations that are not routinely done in many procedures.[3] McCarthy and co-workers' statement that diabetes and cardiac diseases are independent risk factors is not supported by our multivariate analyses. Finally, they claim that outcome data gathered during the hospital stay are inadequate. Although we agree that for a comprehensive assessment of surgical complications, such as incisional hernia, longer follow-up would be needed, the hospital stay during the study period ranged from 6 to 16 days (median) depending on surgical type. Therefore, we believe that the data presented are valid.

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1 Hosmer D, Lemeshow S. Applied logistic regression. New York: Wiley, 1996.
2 Koperna T, Schulz F. Prognosis and treatment of peritonitis: do we need scoring systems? *Arch Surg* 1996; **131:** 180–86.
3 American Society of Anesthesiologists Task Force on Preanesthesia Evaluation. Practice advisory for preanesthesia evaluation. *Anesthesiology* 2002; **96:** 485–96.

## Questions about comparative genomics of SARS coronavirus isolates

Sir—YiJun Ruan and colleagues' analysis (May 24, p 1779)[1] of the comparative genomics of coronavirus isolates from 14 patients with severe acute respiratory syndrome (SARS) is to be welcomed. Two questions, however, are begged by their survey.

The first question concerns genomic evolution of the SARS virus. The single-stranded RNA genome of the SARS virus assures genetic lability under moderate selective pressures and high rates of genetic drift. Droplets of respiratory-tract fluids in nasopharyngeal aerosols have volumes of $10^{-6}$–$10^{-7}$ mL, so that the expected SARS virion population in a single droplet is between 0·1 and 10, even for patients with maximum degrees of viraemia. Thus, most infective doses are probably in the range of 10–$10^3$ virions, whereas a patient's SARS virion-load at peak viraemia is about $10^{12}$. Considering the $10^3$ second effective serum lifetime of a virion, a patient's $10^5$ second viraemic-term may see generation of about $10^{14}$ virions, or about $10^{12}$ infective doses. Even with $10^2$ successfully infective virions sourced per infected cell—a conservative upper-estimate—there are at least half a dozen viral generations per case history, or about 20 viral generations across the three case-history generations studied by Ruan and colleagues. Since the observed per-base replication error-rate of RNA polymerases is about $3\times10^{-5}$ and the SARS viral genome has about 30 000 bases, the expected genome copying error-rate is about one base per viral generation, or about 20 base errors of aggregate genetic drift after 20 generations, roughly congruent with the 16 "observed twice" single nucleotide polymorphisms reported by Ruan and colleagues.

Crucially, however, these 14 case-isolates represent infections during March and early April, 2003, whereas Ruan and colleagues relate that the SARS epidemic began in Guangdong province in November, 2002, so that it has been propagating and mutating at least four—and perhaps as much as five—times longer than is represented by the time-span of all the analysed cases. Where is the four-fold larger genetic drift? Specifically, why is there such close genomic similarity between the Singapore cases and all of the overseas cases? Unless these all trace to the same index case in early March, which seems unlikely, their close genomic similarity is quantitatively inexplicable.

The second question concerns the ease with which the SARS virus propagates in vitro, a quite unusual, if not unique, characteristic for known human coronaviruses. This issue is at best thoroughly puzzling and at worst deeply troubling. How do Ruan and colleagues think that this set of viral propagation peculiarities arose?

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1 Ruan YJ, Wei CL, Ee LA, et al. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. *Lancet* 2003; **361:** 1779–85.

### *Author's reply*

Sir—Lowell Wood raises concerns about our analysis of SARS coronavirus (SARS-CoV) strains, questioning the small number of mutations described. Although Wood is correct in his theoretical calculations, which are based on generalised in-vitro experiments, three explanations can be invoked to address his concerns.

First, despite a high mutational frequency of the SARS-CoV, the ultimate clone that emerges is dependent on positive and negative in-vivo selection; only those clones that have a replicative benefit (even a small advantage) will emerge as the dominant isolate. Since our sequencing method is based on direct analysis of PCR products, the full mutational heterogeneity in a viral population from one individual cannot be estimated. For example, a mutation that is present in only one in 1000 viruses within one isolate will simply not be detected, nor is it likely to be biologically important.

Second, only a fraction of the viral particles present in body fluid is capable of infection, with that fraction highly dependent on the presence of antibodies, the viral load of the



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