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Need for Rigor in Design, Reporting, and Interpretation of Transcriptomic Biomarker Studies

We agree with Fu et al. that microRNAs (miRNA) likely regulate the human immune response to *Mycobacterium tuberculosis* (5). However, we are troubled by aspects of the design, reporting, and interpretation of that study.

First, RNA samples from individual subjects were pooled to create a single TB sample and a single control sample. Repeated microarrays of these two pooled samples represented technical rather than biological replicates. Within-group variability is therefore due to an artifact(s) introduced during labeling and hybridization rather than a measure of underlying biological variability. The resulting statistics are not a valid measure of between-group differences (3).

Second, the authors made no adjustment for multiple comparisons. At a *P* value of 0.05, we can expect 5% or 61 of the 1,224 miRNAs detected to be statistically significant by chance alone, suggesting that 66% of the 92 miRNAs identified would be false discoveries (1). We are unable to validate or replicate the analysis because the authors did not provide access to the microarray data set in a public database.

Third, in real-time PCR experiments, the authors normalized miRNA expression to U6 splicesomal RNA. The use of this controversial control should be fully justified by the authors. As Exiqon and others caution (4, 9), U6 should not be used for normalization, as this nuclear molecule is absent in serum unless cell lysis has occurred. Could there have been differential cell lysis among the sick (suffering from tuberculosis [TB]) and well (control) subjects? It is essential that studies examining circulating miRNA evaluate this possibility, because hemolysis may alter miRNA levels up to 50-fold (11).

Finally, the authors' choice to enroll healthy uninfected adults as the control group fundamentally limits the conclusions that may be drawn from this study. Pulmonary TB is a life-threatening infection generally accompanied by extensive tissue destruction and a systemic inflammatory state causing fever and weight loss (8). These drastic physiological changes undoubtedly alter the serum miRNA expression profile of patients with active TB in comparison to that of healthy adults.

As Berry et al. note in their analysis of blood mRNA expression, the critical question is whether the variation in expression represents host responses specific to TB or nonspecific expression such as is observed in a variety of illnesses (2). For example, hsa-miR-29a, the miRNA the authors describe as having "great potential to serve as a marker for active pulmonary tuberculosis infection," is also reported to be a potential biomarker for biliary atresia (6), colorectal liver metastasis (12), clear cell renal cancer (7), breast cancer (13), and irritable bowel syndrome (14), among other conditions. We are concerned that Fu et al. identified a circulating miRNA profile associated with systemic disease rather than TB. A TB-specific biomarker must distinguish TB from clinical mimics such as bacterial pneumonia, cancer, and sarcoidosis (10).

High-throughput biological assays have tremendous potential to advance our knowledge of host-pathogen interactions and identify clinically useful biomarkers. However, to advance the field, it is essential that investigators report data in a manner that ensures analyses can be repeated and confirmed. Biomarker discovery must include a clinically relevant control group in order to identify transcriptional changes specific to the condition of interest.

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