Reviewer's report

Title: 2-aminoacetophenone as a potential breath biomarker for Pseudomonas aeruginosa in the cystic fibrosis lung

Version: 2  Date: 30 April 2010

Reviewer: Craig Winstanley

Reviewer's report:

General comments:

The paper describes a novel approach to the issue of diagnosis of infection of cystic fibrosis patients with P. aeruginosa by detection of a volatile biomarker. The authors report that their assay was specific for P. aeruginosa (20 P.a. strains versus one each [?] of a selection of species representing mostly respiratory pathogens). The assay can be used on patient breath samples. In a limited study (33 patients) it proved positive in 93% of P. aeruginosa-infected patients, but also positive for 30.7% of CF patients not infected with P. aeruginosa.

To be an improvement on current diagnostic tests (eg. Culture of the bacteria) any new test would have to show equivalent specificity and better sensitivity. New tests would be especially applicable to children, where early identification of P. aeruginosa can prompt aggressive therapy and pathogen clearance. Once established as a chronic infection (when mucoid colonies of P. aeruginosa are observed) the infection is never eradicated. Hence, tests with improved sensitivity, and tests that are more effective than cough-swabs for non-sputum producers, would be desirable. Any new test needs to be better than sputum culture, which is relatively cheap, to merit any additional costs.

When applied to a limited number of clinical samples, the test fared reasonably well on sensitivity (but failed to identify one positive patient) but the specificity was disappointing. Almost one-third of the small number of non-infected CF patients tested was positive.

The authors have demonstrated that they can discriminate between infected and non-infected patient groups by showing a significant difference in 2-AA levels. Hence, there may be potential for this approach. However, the work presented is very preliminary and the methodology requires considerable improvement and more rigorous testing before it could be considered as useful clinically.

Major Essential Revisions:

1. The microbiology in the paper is disappointing. The choice of strains for comparison seems rather random and unsatisfactory. This needs to be justified in the paper. There is no information about the sources of these isolates or on what basis they were chosen. How many of these were CF isolates? Why were fungi (especially Aspergillus) not included? The logical choices would be those
bacteria/fungi most frequently reported as colonising CF patients. This can also include anaerobes. It is important to note that CF isolates often have mutations. If you only pick one isolate per species, you may be picking an isolate defective in 2-AA production. It is also important to say which media were used to grow which bacteria. Were they all tested on each of the three media mentioned on page 7? Different growth conditions will lead to different bacterial genes being expressed. It has been suggested that in CF infections the bacteria experience near anaerobic conditions. You are not testing this at all. In my opinion, as a minimum requirement to be convincing, the panel should include more than one isolate of important species (those frequently associated with CF).

2. The isolate named as Burkholderia cepacia is almost certainly not really a B. cepacia. There is now an extensive body of literature describing the “Burkholderia cepacia complex”, especially in relation to CF infections. B. cepacia itself is only very rarely associated with CF. B. multivorans and B. cenocepacia are the most common in CF. “Standard techniques” wouldn’t be enough to identify the correct designation for this strain. The strain should be typed, or a properly typed alternative should be used (preferably at least representatives of B. multivorans and B. cenocepacia). On page 14 the authors refer to 2-AA detection in “all four S. aureus cultures”. This leaves me confused about whether multiple isolates / strains were compared and if so how many.

3. Control subjects (p8) were adults. I could not find information on whether the CF patients were children or adults or a mixture in this section. Some data are given in Table 1 indicating a mixture. Hence, the control group differs somewhat from the test groups. Some justification of this would be helpful.

4. Page 13 – was the 2-AA test positive for any of the healthy control samples? If not, how is the difference between the healthy control group and the non-infected CF control group to be explained? Could this indicate that CF patients, or perhaps younger CF patients, are more likely to cause false-positive results? Could it also be interference from S. aureus infections? The discussion on p15 seems to discount the latter possibility, so what is the explanation? The authors admit that they do not know (p16) but this is a potentially serious limitation. To be useful for early detection of P. aeruginosa infections, the test would have to be sensitive to low levels. If it is only useful for patients chronically infected and with high population numbers in their sputum, then it would be of limited use because conventional sputum culture can be used on those patients.

Minor Essential Revisions:
1. I couldn’t understand why the legend for Figure 1 should appear in the middle of the Introduction section.
2. P14, final line – Figure 2 should be in brackets?
3. P15 – Aspergillus should be in italics

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests