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Serology of Pseudomonas

Pseudomonas serology: confusion, controversy, and challenges

P M Farrell, J R W Govan

Contrasting messages on the diagnostic value of *Pseudomonas* serology in CF

The two interesting but contrasting (and possibly confusing) papers on *Pseudomonas* serology in cystic fibrosis (CF) published in this issue of *Thorax* illustrate the controversy and challenge that have become increasingly important as very young patients are routinely diagnosed through newborn screening. Fortunately, such infants at diagnosis are typically free of *Pseudomonas aeruginosa* (PA) infection,[1] unlike about 30% of those diagnosed by traditional methods following signs/symptoms of CF.[2] The potential to eradicate non-mucoid PA, and even to delay transformation to mucoid species,[1] makes ascertainment of the initial PA infection one of the highest priorities in current clinical management. Yet, just as in the diagnosis of CF per se, traditional methods of PA identification (relying on microbiology) leave much to be desired in young children with CF. Thus, more attention has focused once again on the potential diagnostic value of *Pseudomonas* serology. In a recent review Rosenfeld *et al*[3] stated that ''limitations of serologic markers of *P aeruginosa* infection include lack of commercially available standardized assays and lack of specificity to the site of *P aeruginosa* infection (i.e. upper or lower airway)''.

The pioneering studies on *Pseudomonas* serology were published 2–3 decades ago when the research teams of Niels Hoiby and Gerd Doring developed the initial methodology and applied *Pseudomonas* antibody titre determinations to patients with CF.[4–6] The first tests were based on detection of ''precipitating antibodies'' (precipitins) against a pool of sonicated extracts from common 0-antigen serotypes. Their evaluations showed that rising antibody titres correlated with PA respiratory infections. They also showed[6] that, soon after the onset of PA lung infections, the numbers of individual precipitin bands rose and eventually became a sign of ''poor prognosis''. Subsequently, Brett and co-workers[7] published important research describing the rising antibody titres and potential for early identification of PA infections. More recent investigations[1 8] have confirmed the findings of Brett *et al*[7] and support the potential usefulness of *Pseudomonas* serology in children with CF.

When viewed against this background, the investigations of Kappler *et al*[9] and Tramper-Stranders *et al*[10] are significant steps forward, although their contrasting conclusions appear to represent steps in different directions. Our independent reviews of the papers led both of us to recommend that they be published with revisions because we were favourably impressed with the quality of the studies. Not knowing the identity of the other reviewer until commissioned to write this editorial, and judging each manuscript on its own merits, we reached similar decisions for a variety of reasons and appreciate having this opportunity to share our perspectives. However, distinguishing the two studies during the review process was a challenge for each of us. Hopefully, this commentary will alleviate confusion, explain why controversies are currently inevitable regarding *Pseudomonas* serology, and highlight some of the future challenges. We begin with a summary of the articles from Munich and Utrecht, as outlined in table 1.

It is important to emphasise that both studies used the same commercially available ELISA test system, but different cut off values were used to define a positive antibody titre. Specifically, the investigation in Munich by Kappler *et al*[9] simply used the manufacturer's recommended threshold (>1:500) with concomitant quality control mechanisms. In contrast, the investigation in Utrecht by Tramper-Stranders *et al*[10] applied lower titres to discriminate PA antibody positivity after creating receiver-operator curves (ROC) to identify the titre cut off value that maximised sensitivity while preserving specificity. The ROC technique was developed originally in the 1940s to improve operator vigilance for radar based detection of incoming aeroplanes[11] and applied[12] successfully to ''signal detectability and medical decision-making'' in the 1970s. It is a valuable method but depends on having a reliable ''gold standard'' marker—for example, an actually observed aeroplane or a positive PA culture associated with lower respiratory infections. As described below, this requirement is a particular challenge with respiratory secretion cultures involving children. In fact, the ''gold standard'' is tarnished in this situation, and the usually valid assumption that sensitivity is a function of the test and not prevalence may not apply when an unreliable indicator exists—that is, oropharyngeal culture results. The design differences may also partially explain the discrepancy in conclusions. The Munich team had the advantage of repeated measures every 3 months in a prospective assessment. On the other hand, the Utrecht group actually performed two assessments (table 1): (A) a cross sectional evaluation of serological data compared with microbiological results, and (B) a prospective annual examination of the same variables in 4–14 year old children. Although the microbiologically determined PA status appeared somewhat similar, the methods of obtaining respiratory secretions necessarily varied among patients as shown clearly in table 1 in the paper by Kappler *et al*.[9] Of the 183 patients, only 76 (42%) expectorated sputum and thus oropharyngeal swabs were used for 107 patients. Tramper-Stranders *et al*[10] state that ''the sensitivity and positive predictive value of oropharyngeal swabs range between 44% and 83%'', which reflects the results of a variety of studies using concurrent bronchoalveolar lavage and oropharyngeal swabs, as reviewed by Tramper-Stranders *et al*.[13] Table 1 in the paper by Kappler *et al*[9] also exposes another

Table 1 Comparison of studies on *Pseudomonas* serology

	Paper 1	Paper 2	
Authors	Kappler *et al*[9]	Tramper-Stranders *et al*[10]	
Origin	Munich, Germany	Utrecht, The Netherlands	
Timing	2000–2002*	2002–2005	
Design	Prospective: sequential assessment of serology every 3 months for 2 years compared with PA cultures during 2 years	Cross sectional (A) and prospective (B) assessment of microbiology and serology with PA serology evaluated in 2004 or annually in the prospective study of children with CF	
No of CF patients	183	(A) 220	(B) 57
Age (years)			
Range	2–41	0.7–65	4–14
Median	15.7	11.8	7.9
Mean	16.7	14.5	8.1
PA status			
Free	37%	42%	54%
Intermittent	15%	27%	23%
Chronic†	48%	31%	23%
Mucoid	54%	?	?
Methodology	Commercially available ELISA test system (Mediagnost, Germany) measuring alkaline protease, elastase and exotoxin A titres (positive titre defined as >1:500) by Kappler *et al*[9] whereas Tramper-Stranders *et al*[10] used ROC determined cut off values)		

PA, *Pseudomonas aeruginosa*.
*Timing of the serological assessment by Kappler *et al*[9] included correlation of ''the antibody test results, firstly, with known microbiological data from the previous 2 years and, secondly, with microbiological results from the following 2 years'', while Tramper-Stranders[10] determined antibody titres ''at the end of the observation period in 2004'' in the cross sectional study (A) and annually for 3 years ''concomitantly when cultures were taken'' in the prospective study (B).
†Defined by Kappler *et al*[9] as ⩾7 positive of 8 cultures and by Tramper-Stranders *et al*[10] as >50% of all cultures positive.

obvious but very relevant problem—namely, that younger CF patients are more likely to be assessed by oropharyngeal swabs than by sputum, and they are also more likely to be PA-free or intermittently culture positive. In contrast, 60 of 88 patients (75%) with chronic PA infection expectorated sputum for culture. Thus, the very group of young CF patients of greatest interest (and importance) for these studies is the most difficult to evaluate reliably as the ''gold standard'' (PA positive culture) is potentially unreliable. Furthermore, using microbiological results to construct ROC curves with a cross sectional population of CF patients is daunting, although the Utrecht team mitigated this by selecting those with ''chronic colonisation''.

The overall results of the study by Kappler *et al*[9] showed that the combination of determining the three antibody titres and using a >1:500 cut off yielded the best results with a sensitivity of 86%, specificity of 96%, and a positive predictive value of 97%. Not surprisingly, the validity was higher for CF patients in whom sputum cultures were available (sensitivity 95%, specificity 100%, positive predictive value 100%); this again reflects the difficulties associated with oropharyngeal cultures. From comparative analyses of serological and microbiological results, Kappler *et al*[9] concluded that regular determination of serum antibodies is reasonable in CF patients with negative or intermittent PA colonisation, but not in those with a positive PA status. In their judgement, a rising antibody titre indicates probable infection. They stated on the basis of combined serological and microbiological data that ''for CF patients chronically infected with *P aeruginosa* and positive antibody test results, routine follow up antibody determinations are thus of no use'' (that is, they provide no added value). Kappler *et al*[9] further concluded that ''positive antibody results almost prove colonisation with *P aeruginosa* (and) negative test results indicate the absence of *P aeruginosa* with increasing probability as age decreases''. Finally, based on their evidence that a rise in antibody titres indicates probable infection, they recommend PA eradication treatment even in the absence of positive microbiological culture.

On the other hand, Tramper-Stranders *et al*[10] were dissatisfied with the advised cut off values of the Mediagnost kit and used ROC curves to create better cut off values. Applying these new cut off values, they found a sensitivity of 96% and a specificity of 79%. Individually, all three of the antibody titres discriminated reasonably well between the absence and presence of chronic PA colonisation, but their threshold values were surprisingly low, which ''might reflect the relatively high value of the negative control sera from the ELISA kit'' (Tramper-Stranders, personal communication). The sensitivity of individual antibody tests was 87% for elastase, 79% for exotoxin A, and 76% for alkaline protease. However, the first positive culture for PA was preceded by positive serology in only five of 13 patients (38%) studied in the prospective assessment. They concluded that, although serological tests are sensitive for identification of chronic PA, ''the failure of serological tests to detect early colonisation in young patients emphasises the need for continued reliance on cultures''.

There are a number of other possible explanations for the discrepancy in observations and conclusions. One of the difficulties with interpretation of such studies relates to defining the stage of *Pseudomonas* infection in CF. Conceivably, there are at least four stages: (1) PA-free with lung disease (PA susceptible); (2) PA-free by culture but with positive serology (with or without lung disease); (3) PA infected (showing lung disease signs/symptoms) with non-mucoid PA; and (4) PA infected with mucoid PA. The issue of intermittent colonisation with the same or different non-mucoid PA adds an additional complication. It is therefore not surprising that there is uncertainty on the differences between ''colonisation'' and infection. As Kappler *et al*[9] stated, there seems to be a continuum between colonisation and infection. Another variation is in the PA serological test, but this was not an issue in the studies by these two groups although the differing threshold values may account for some variations in data interpretation.

In future, alternative and more sensitive PA serological tests should be developed or re-investigated. These could include the use of more reliable and informative antigens that more accurately reflect the complex and evolving relationship between PA and the CF lung. For instance, panreactive rough lipopolysaccharide (LPS) or panreactive monoclonal antibodies (MAbs) to the core region of PA have previously been shown to detect early non-mucoid PA in respiratory secretions of CF patients.[14 15] Based on the importance of mucinophilic and chemotactic properties in the initial stages of pulmonary colonisation in CF patients[16] and the rationale of flagella based vaccination, it is not surprising that flagellar antigens have also proved capable of detecting an early antibody response to PA colonisation of the CF respiratory tract.[17] Most recent studies by Corech *et al*[18] show that the type III cytotoxin system might be more useful for early detection of ''acute'' PA infections. Specifically, they stated that ''measurement of seroconversion against PopB and/or the combination of ExoS/PopB provides a sensitive and early indication of infection, and when combined with oropharyngeal culturing for *P aeruginosa*, it does not miss CF patients with *P aeruginosa* infections''.

Resolving the controversies in *Pseudomonas* serology will depend on more research in PA-free and initially PA infected children with CF using improved serological methods applied longitudinally with greater frequency. Monitoring PA antibody titres in children with CF diagnosed through newborn screening offers many advantages: (1) they begin PA-free; (2) the titres are initially very low and constant; (3) seroconversion per se indicates PA infection with a host immune response and not colonisation. However, the greatest difficulty in studying young children with CF—that is, the problem of culturing lower respiratory secretions—will continue to plague these investigations. Although either nasopharyngeal/tracheal techniques or oropharyngeal techniques may be used, their sensitivity and reliability can always be challenged when standard microbiological culturing methods are employed. Consequently, interpretation of the data published in the two current papers and all the literature becomes very difficult. For these reasons, non-culture based methods such as serological tests or polymerase chain reaction require further research and evaluation.

To discover the truth about the value of *Pseudomonas* serology in children with CF, we need to have more comprehensive research with better microbiological and serological techniques. We also need to identify an optimal panel of redundant complementary PA antigens that are clinically significant virulence factors. Ultimately, a combination of PA microbiology and serology will probably be used—serology will not replace microbiology. In the meantime, the implications of earlier studies[4–7] which are supported by the data of Kappler *et al*[9] as well as other publications[1 8 14] remain intriguing and interesting.

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Authors' affiliations
P M Farrell, Professor of Pediatrics and Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726-2336, USA
J R W Govan, Cystic Fibrosis Group, Centre for Infectious Diseases, University of Edinburgh Medical School, Edinburgh EH16 4SB, UK

Correspondence to: Dr P M Farrell, Professor of Pediatrics and Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726-2336, USA; pmfarrel@facstaff.wisc.edu

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Chronic cough in children

Diagnosing chronic cough in children

M D Shields

Further scientific evidence for the usefulness of signs and symptoms in predicting a specific cause of chronic cough in children

Problem coughing is common in children and can be produced by almost all the respiratory disorders that affect them. Rather than applying a comprehensive battery of tests to all children, most doctors use clinical pointers in the history and examination to determine the need for and targeting of investigations. Indeed, experienced clinicians usually use more than one single feature or diagnostic test and bring together bits of the history, clinical examination, and selective investigative tests to arrive at a diagnosis. Typical clinical cues recommended to be used in evaluating cough include (1) age of symptom onset, (2) quality of the cough, (3) triggers, periodicity and timing of cough, and (4) associated features such as wheezing.[1]

Recent evidence suggests that parents do not report the frequency or severity of cough accurately.[2 3] However, work from Professor Chang's unit has shown that parental reporting of wet versus dry cough is likely to be accurate.[4] Most of the clinical characteristics or pointers used to predict specific diseases causing cough in children have not been rigorously subjected to assessment as would be required for a diagnostic test[5] and, typically, historical case series have been used. For example, a bizarre loud and honking cough which increases with increased attention and abates at night in an otherwise well child who shows ''la belle indifference'' to the cough suggests a psychogenic origin. This