Usefulness of aetiological tests for guiding antibiotic therapy in community-acquired pneumonia

K. Strålin *

Department of Infectious Diseases, Örebro University Hospital, SE-70185 Örebro, Sweden

Abstract

The goal with antibiotic therapy in community-acquired pneumonia (CAP) is to cure the patient, ideally without causing side effects and without contributing to the further development of antibiotic resistance. Although patients with severe CAP should be treated with broad-spectrum antibiotics, patients with non-severe CAP should preferably receive pathogen-directed therapy. Rapid aetiological tests, such as sputum Gram stain and urinary antigen tests, are useful for targeting initial pathogen-directed therapy. Non-rapid tests, such as cultures, can subsequently support a switch from initial broad-spectrum therapy to narrow-spectrum therapy and direct therapy changes in case of treatment failure. As conventional diagnostic methods often fail to identify the aetiology of CAP, PCR (polymerase chain reaction) tests for respiratory pathogens have become useful and should be further developed. Based on the test specificities, aetiological tests may provide diagnoses with varying reliability, i.e. definite aetiologies (e.g., blood culture and Legionella urinary antigen test), probable aetiologies (e.g., sputum culture and PCR for Mycoplasma pneumoniae), or possible aetiologies (e.g., culture of nasopharyngeal secretions and PCR for Streptococcus pneumoniae). A definite or probable aetiology can often be used to target antibiotic therapy.

© 2007 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

Keywords: Community-acquired pneumonia; Aetiology; Diagnostic tests; PCR; Urinary antigen test; Antibiotics

1. Introduction

Community-acquired pneumonia (CAP) is a common and sometimes severe disease with an annual incidence of about 1% [1] and a mortality rate of 0–30% [2]. Thus, selection of antibiotic therapy in CAP is important for the emergence of antibiotic resistance in society and for the outcome in the patients [3]. Ideally, antibiotic therapy should be directed against the pathogen that is causing the pneumonia. However, as the aetiology is often not known at presentation, patients must initially receive empirical antibiotic treatment.

This review will focus on how aetiological tests can be used to guide antibiotic therapy in adult patients with CAP.

2. Empirical antibiotic treatment in community-acquired pneumonia

In patients with severe CAP, under-use of antibiotics and use of narrow-spectrum antibiotics can be associated with a risk of death [3]. Thus, international guidelines [4–9] recommend treatment with broad-spectrum antibiotics for severe CAP, as defined by the CURB-65 (confusion, urea, respiratory rate and blood pressure – aged 65 or more) score or Pneumonia Severity Index rules. As CAP due to multiple pathogens can occur [10,11], patients with severe CAP should be treated with a broad antibacterial agent(s) for the first 2 days, even if an aetiological agent has been identified.

In patients with non-severe CAP, overuse of broad-spectrum antibiotics can increase the risk of adverse events [12,13] and the development of antibiotic resistance among respiratory pathogens [14] and other bacterial species [15]. Thus, for patients with non-severe CAP, the guidelines from the British Thoracic Society (BTS) [7] and the Swedish Society of Infectious Diseases [8] recommend monotherapy with penicillin or an aminopenicillin, unless atypical pathogens...
are suspected. Penicillin is a reliable therapy for CAP caused by Streptococcus pneumoniae [16], even in cases when the bacterium has reduced in vitro susceptibility to penicillin [17,18]. As S. pneumoniae is the commonest microbiological cause of CAP [2,5,11] and the most commonly identified cause of CAP death [2], it should always be covered by the empirical CAP treatment [8]. The recommendation to treat patients with non-severe CAP with β-lactam monotherapy is supported by two meta-analyses that showed that such treatment was not inferior to treatment with agents with activity against atypical pathogens, unless Legionella was the cause [19,20]. However, the North American guidelines [4,5], recommend routine antibiotic coverage of atypical pathogens for hospitalised patients with non-severe CAP.

3. Prediction of aetiology based on clinical, laboratory, radiological and epidemiological factors

Although single clinical, laboratory and radiological features cannot reliably predict CAP aetiology [11,21], by combining a number of features the possibility of making a correct prediction increases. Acute onset of illness, pleuritic chest pain, high leukocyte count, and lobar chest X-ray infiltrates are associated with pneumococcal aetiology, while young age, gradual onset of illness, and non-productive cough have been associated with mycoplasmal aetiology [22–24]. With a prediction model based on these features, Farr et al. [23] correctly predicted pneumococcal aetiology in 45%, and mycoplasmal aetiology in 77%, of patients who were positive by microbiological testing. Thus, the presentation of pneumococcal pneumonia is variable. This fact further supports the recommendation [8] that S. pneumoniae should always be covered in empirical CAP treatment. Legionella aetiology has been associated with diarrhoea, headache or confusion, liver dysfunction and hyponatraemia [24,25].

Certain risk factors or epidemiological conditions can also indicate that the pneumonia is caused by a certain pathogen. For instance, associations between different conditions and pathogens have been identified as follows: smoking and/or chronic obstructive pulmonary disease – Haemophilus influenzae; alcoholism – S. pneumoniae; exposure to birds – Chlamydia psittaci; recent hotel stay – Legionella; and influenza virus infection – S. pneumoniae, Staphylococcus aureus, and H. influenzae [4].

In a recent study by van der Eerden et al. [12] initial antibiotic treatment, guided by the clinical and epidemiological presentation, was at least as effective as treatment guided by the results of rapid microbiological investigations. Based on clinical criteria, patients were treated with penicillin, erythromycin, amoxicillin/clavulanate, or fluloxacillin ± gentamicin [12]. This study supports the usefulness of the clinical and epidemiological presentation for the selection of antibiotic therapy in CAP, at least when all treatment alternatives are effective against S. pneumoniae.

4. The role of aetiological testing for antimicrobial therapy in CAP

A major role of aetiological testing in CAP is to enable the use of pathogen-directed therapy, and thus reduce the use of broad-spectrum antibiotics. As it is recommended that the antibiotic therapy should be started within 4 h of hospital admission [4,8], rapid tests with a shorter analysis time can be used to influence the choice of first-line antibiotic therapy. Sputum Gram stain, urinary antigen tests, and real-time PCR for respiratory pathogens are examples of such rapid tests. Less rapid tests, such as cultures, conventional PCR for respiratory pathogens, and serology, can provide useful information that may support ongoing antibiotic therapy, support narrowing of broad-spectrum therapy, and support therapy changes in case of treatment failure [3]. Culture of blood and respiratory specimens may be essential for the identification of unexpected or uncommon CAP aetiologies that the empirical treatment does not cover for, e.g., Pseudomonas spp., methicillin-resistant S. aureus, and other highly resistant pathogens. Culture remains a cornerstone of the diagnostic techniques, as it can provide information about antibiotic susceptibility.

In patients who receive narrow-spectrum antibiotics based on clinical prediction of aetiology, identification of the pathogen by an aetiological test is helpful for determining the need for treatment alterations [26].

Another major role of aetiological testing is to survey the spectrum of CAP aetiologies in order to guide future empirical antimicrobial policies.

5. Definite, probable and possible aetiological diagnoses

When an aetiological test is positive, it is important to question the reliability of the result. Depending on the test specificities, positive results of different tests can be categorised to represent definite, probable or possible CAP aetiologies [27]. Based on a classification by Marston et al. [27], a new categorisation of bacterial aetiologies, which includes additional and newer tests, is presented in Table 1. Tests with high specificities can provide definitive bacterial aetiologies and can target safely antibiotic therapy, while tests with low specificities can provide possible aetiologies. When a possible aetiology is identified by a diagnostic test, the result should be related to the clinical presentation, and to the response to initial antibiotic therapy, before treatment alteration should be directed from it. Probable aetiological diagnoses can often be used to target antibiotic therapy.

If microbiological testing provides two (or more) bacterial aetiologies with different reliability in a patient, the aetiology with the strongest reliability is most likely to represent the true aetiology. The role of mixed bacterial aetiologies in CAP is not yet fully understood.
6.2. Sputum culture

Sputum culture is often used for the identification of probable/presumed aetiologies in CAP [11–13,21,27], either singly [13,21] or with the simultaneous identification of the compatible organism in sputum Gram stain [11,12,27]. It is usually recommended that the quality of the sputum samples should be determined by microscopy [4,8]. Samples with a high ratio between the leukocyte concentration and squamous epithelial cells are more likely to originate from the lower respiratory tract than samples with a low ratio [32,33], and should thus be considered more reliable (Table 1). In addition, in order to minimise the impact of contamination from oropharyngeal flora, a bacterial concentration of $10^5$ colony-forming units (CFU)/mL of sputum is often used as the detection limit for a positive sputum culture [34]. The concentration of colonising bacteria in the respiratory tract is probably usually below that limit [35]. It is particularly important to perform sputum culture in cases of necrotising pneumonia, since *S. aureus* aetiology in such cases has been reported to have a high mortality [36]. A negative culture result of good-quality sputum has been suggested to be strong evidence against *S. aureus* aetiology in CAP [4].

Gram-negative enteric bacilli often colonise the respiratory tract of elderly persons and patients who are treated with antibiotics [37]. Thus, sputum cultures positive for such bacteria should be interpreted with caution.

As *Legionella* spp. do not normally colonise the respiratory tract, the isolation of *Legionella* in lower respiratory tract cultures provides a definite diagnosis [38].

In a patient who cannot produce an adequate sputum sample, sputum production can be induced by inhalation of 3% NaCl [39].

6.3. Culture of bronchoscopic-derived samples

In cases of therapy failure, when conventional methods have failed to identify the CAP aetiology, diagnostic bronchoscopy should be considered [6]. Cultures from bronchoalveolar lavage (BAL) and protected specimen brush (PSB) are both sensitive and specific [40]. However, since bacteria from the upper respiratory tract may be introduced into the lower respiratory tract by the bronchoscope [41], false-positive results may occur. Thus, BAL and PSB specimens should preferably be cultured quantitatively [4]. Commonly used cut-offs for positive results are $10^4$ CFU/mL for BAL fluid [11] and $10^3$ CFU/mL for PSB [42].
6.4. Culture of nasopharyngeal secretions

In cases when no sputum sample can be obtained, secretions from the nasopharynx can be obtained for culture [8,28]. Such a culture may identify the pathogen that is responsible for the infection, and may yield useful information about antibiotic susceptibility. For *S. pneumoniae*, nasopharyngeal swab culture has shown sensitivities of 27–58%, and specificities of 82–97% [43,44]. For *H. influenzae*, we [44] found nasopharyngeal swab culture to have a sensitivity of 75% and a specificity of 95%. Among 621 asymptomatic Swedish adults in four studies [28,45–47], culture positivity rates of nasopharyngeal swabs were as low as 2.1% for *S. pneumoniae* and 2.1% for *H. influenzae*. These results support the clinical usefulness of nasopharyngeal culture for identification of these two bacteria in adults.

6.5. Culture of pleural fluid

Diagnostic thoracocentesis should be performed when a significant pleural effusion is present [5]. A positive culture of pleural fluid provides a definite CAP aetiology.

6.6. Sputum Gram stain

The interpretation of Gram-stained sputum samples has been associated with inter- and intra-reader variability [48,49], and the sensitivities and specificities for the detection of pneumococcal pneumonia have varied between different studies [49–52]. However, applied to sputum samples of good quality, Gram stain has been found useful and reliable for targeting pathogen-directed first-line antibiotic therapy in CAP patients [53,54]. In a study by Boerner and Zwadyk [53], 42 of 43 pneumonia patients with a sputum Gram stain showing Gram-positive diplococci responded to penicillin or ampicillin monotherapy. In a prospective study by Roson et al. [54], 93 CAP patients with a sputum Gram stain showing Gram-positive diplococci were treated with a single antibiotic agent with activity against *S. pneumoniae*. Therapy was changed in only three of them.

Sputum Gram stain has rarely been studied for bacteria other than *S. pneumoniae*. Fine et al. [49] found the sensitivity of sputum Gram stain to be less for identification of *H. influenzae* than for the identification of *S. pneumoniae*. However, the study by Roson et al. [54] indicates that when sputum Gram stain identifies Gram-negative coccobacilli, which is usually interpreted as *H. influenzae*, it may be clinically useful information. Thirty of 36 patients with such results were given β-lactam monotherapy and none of them was subjected to therapy change [54].

6.7. Pneumococcal urinary antigen

Among adult CAP patients, the Binax NOW *S. pneumoniae* urinary antigen test has shown sensitivities of 79–87% compared with blood culture [55–57], but only 54%, when cultures of respiratory tract secretions were included in the reference standard [57]. However, the specificity is high. Among adults without respiratory symptoms in three summarised studies [57–59], the false positivity rate was 0.6% (2/336) and it was 8% (1/13) among subjects colonised with *S. pneumoniae* in the nasopharynx. As false positivity may be caused by a recent pneumococcal infection [58,60], a positive result provides a probable, but not a definite, pneumococcal aetiology (Table 1). However, a positive result with a colour intensity of the result line at least as intense as that of the control line is probably more specific [55,57], and may provide a definite pneumococcal aetiology. Concentration of the urine prior to testing will enhance the sensitivity but lower the specificity of the test.

Among 219 young patients with mild pneumonia, Guchev et al. [61] prospectively targeted antibiotic therapy according to the results of the NOW *S. pneumoniae* urinary antigen test. The patients with positive test results (22% of the cases) were treated with amoxicillin, while those with negative results (78%) were treated with claritromycin. The clinical success rates were 90% in the amoxicillin group and 94% in the claritromycin group (non-significant).

However, since the NOW *S. pneumoniae* test has a suboptimal sensitivity [57], it cannot be used to rule out pneumococcal pneumonia. Thus, we [26] studied the clinical course of 152 hospitalised patients (median age 74 years) who were empirically treated with β-lactam monotherapy and divided the patients according to the result of the urinary antigen test, which was positive in 38 patients (25%) and negative in 114 patients. As the analyses were performed at the end of the study, the physicians did not know the results. A successful outcome was noted in 92% of those with a positive test result, and in 76% of those with a negative test result (P = 0.034). Among the 30 patients who did not have a successful outcome, only one patient died, while 29 patients were cured after treatment alteration [26].

These two studies [26,61] indicate that the urinary antigen test can safely target narrow-spectrum β-lactam treatment in CAP. Our study [26] supports the use of β-lactam monotherapy as empirical therapy also in test-negative patients, although physicians should be aware that therapy changes will be needed more frequently than in test-positive patients. Thus, a negative test result motivates additional aetiological testing.

Contrary to cultures, the pneumococcal urinary antigen test often remains positive during antibiotic therapy [56]. This makes the test useful in patients who have received antibiotic treatment prior to sampling.

6.8. Legionella urinary antigen

The Legionella urinary antigen test has a high specificity for *Legionella pneumophila* serogroup 1 [38]. A Dutch study [62] indicates that antibiotic management in Legionnaires disease can be guided by the results of the *Legionella* urinary antigen test. Early adequate therapy did not influence the
outcome among patients with Legionnaires disease who had a negative Legionella urinary antigen test, although it reduced the risk of intensive care unit admission and death by 38% in those with a positive antigen test [62].

6.9. Polymerase chain reaction (PCR) methods

Since conventional diagnostic methods often fail to identify a CAP aetiology, the sensitive PCR technique appears promising for the identification of aetiological agents. However, PCR methods have not yet been standardised for respiratory pathogens other than Mycobacterium tuberculosis and Legionella spp. [63]. While PCR for these two pathogens provides definite diagnosis, PCR for Mycoplasma pneumoniae and Chlamydia pneumoniae can provide probable aetiologies, as they may colonise the respiratory tract of healthy persons [64,65] and persist in the respiratory tract for a long period after an acute infection [66,67].

PCR methods for S. pneumoniae and H. influenzae applied to respiratory tract specimens have rarely been studied. The usefulness of such PCR methods is dependent on the specificity of the DNA target of the method, and on the detection limit of the assay, since bacteria closely related to these pathogens are present in the normal flora of the upper respiratory tract, and since the respiratory tract of healthy adults may be colonised with small amounts of these organisms [44]. Before the clinical use of such methods, they should be evaluated on samples from patients with lower respiratory tract infection and from control subjects. Fig. 1 shows the yield of a multiplex PCR for S. pneumoniae, H. influenzae, M. pneumoniae and C. pneumoniae applied to nasopharyngeal aspirates from CAP patients and controls [28]. Compared with conventional reference standards this assay showed the following sensitivities and specificities: for S. pneumoniae 91% and 85%; for H. influenzae 75% and 86%; for M. pneumoniae 90% and 97%; and for C. pneumoniae 100% and 100%, respectively [28,44].

We correlated the results of PCR for S. pneumoniae applied to nasopharyngeal aspirates to the clinical course in 136 hospitalised CAP patients, who were treated with β-lactam monotherapy [26]. The PCR results were not known to the physicians who treated the patients. Clinical improvement and discharge without treatment change, and survival at day 30, was seen in 93% (n = 53) of 57 patients with positive PCR and in 70% (n = 55) of 79 patients with PCR negative for S. pneumoniae (P = 0.001) (previously unpublished data). These results indicate that PCR for S. pneumoniae may be clinically useful.

We have also studied PCR for respiratory pathogens on BAL fluid from patients with lower respiratory tract infection [68]. The major advantages compared with culture were seen in patients who were treated with antibiotics prior to bronchoscopy. Among 103 such patients, S. pneumoniae was identified by BAL culture in three cases and by BAL PCR in 32 cases (P < 0.001) [68]. As pneumococcal aetiology with a slow response to adequate antibiotic therapy and mycoplasmal aetiology have been reported to be two major causes of therapy failure in CAP [69], PCR for S. pneumoniae and M. pneumoniae may be useful complements to culture of BAL in patients who are subjected to bronchoscopy.

Multiplex PCR assays provide several results in one analysis. If such an assay has a high sensitivity, a negative result may indicate that none of the tested pathogens has caused the pneumonia. Accordingly, negative results for several pathogens support the likelihood that a positive result is truly positive.

Real-time PCR provides a rapid result, which may influence the choice of the first-line antibiotic therapy in CAP. It may also provide quantification of the DNA content of the sample. This enables the selection of clinically useful detection limits that could differentiate between infection and colonisation. Thus, the specificity and reliability of a positive result may be improved (Table 1, footnotes b and c). Well-validated commercial quantitative real-time multiplex PCR assays will probably be valuable aetiological tests.

PCR methods for the identification of bacterial pathogens in blood samples are under development [70] and may become useful tools in the management of CAP [42].

6.10. Rapid tests for viral pathogens

Commercial antigen tests for the influenza virus, based on immunofluorescence or immunochromatographic techniques, have high specificities for the detection of influenza [71,72]. The sensitivities of the tests are also high, although viral culture and PCR have even higher sensitivities [71].

It has been well known for several decades that influenza often precedes severe S. aureus pneumonia [73]. Thus, patients with severe CAP who are positive for the influenza virus by a rapid test should reasonably be treated with antibiotic therapy that includes coverage of S. aureus.

Falsey et al. [74] found rapid influenza virus testing to be of importance for antimicrobial therapy in 166 patients with documented influenza. Patients with positive (n = 86) and negative (n = 80) rapid influenza antigen tests had
similar frequencies of chest X-ray infiltrates (20% and 24%), although antibiotic treatment was given less often to those with a positive antigen test (86% versus 99%; \( P = 0.002 \)). In addition, a positive influenza antigen test resulted in higher rates of discontinuation of antibiotic therapy (14% versus 2%; \( P = 0.004 \)) and the use of antiviral agents (73% versus 8%; \( P < 0.001 \)).

Real-time PCR for viral pathogens was recently reported to double the positivity rate of viral pathogens in patients with lower respiratory tract infection [75]. However, the results caused partial or total cessation of antibiotic treatment in only 11% of the tested patients. The total antibiotic use and cost was not reduced.

### 6.11. Serological tests

Serological testing is useful mainly for systematic surveys for the establishment of frequencies of different CAP aetiologies. In such surveys paired sera should be used. However, a high immunoglobulin antibody titre against \( M. \) pneumoniae, detected by enzyme-linked immunosorbent assay (IgM) or complement fixation test, may be used for the establishment of probable mycoplasmal aetiology [76]. Accordingly, IgM titres of \( \geq 1/16 \) for \( C. \) pneumoniae or \( C. \) psittaci, detected by the microimmunofluorescence test, may be used for the establishment of probable \( Chlamydia \) phila infection [77] (Table 1).

A single elevated antibody titre is too non-specific for the establishment of \( Legionella \) infection [38].

### 7. Experience of first-line antibiotic therapy guided by rapid tests

The usefulness of sputum Gram stain and \( S. \) pneumoniae urinary antigen test for selecting first-line antibiotic treatment has been discussed in Sections 6.6 and 6.7. In a prospective randomised study, van der Eerden et al. [12] compared pathogen-directed therapy with empiric broad-spectrum antibiotic therapy in 303 patients with CAP of different severities. Pathogen-directed therapy was based on the clinical presentation (\( n = 72 \)), or on the results of rapid microbiological investigations (\( n = 62 \)), i.e. Gram stain of sputum or pleural fluid, pneumococcal antigen detection in sputum or pleural fluid, and \( Legionella \) urinary antigen. The rates of therapy failure were similar (~20%) in all sub-groups of the study. However, side effects of antibiotic therapy occurred more frequently in patients treated empirically with broad-spectrum antibiotics than in those treated with pathogen-directed therapy (60% versus 17%; \( P < 0.001 \)) [12].

### 8. Experience of antibiotic therapy guided by non-rapid aetiological testing

Among CAP patients with an identified aetiological agent, therapy changes were carried out according to these findings in 12% in a study by Lidman et al. [13] and in 32% in a study by Ewig et al. [78]. In a study of patients with severe CAP [79], the results of microbiological investigations led to a change in therapy in 42% of cases. The commonest change was simplification of the treatment.

### 9. Economic considerations of diagnostic testing

The main argument against aetiological testing is cost. However, among hospitalised CAP patients, traditional aetiological testing contributes only to a small proportion of the total cost [80]. The largest cost in the management of CAP is the in-patient cost [81]. If the CAP aetiology is known in the early course of disease, optimal therapy with a low risk of adverse events can be given, and the hospital stay can probably be short. In addition, if the aetiology is known, it is possible that the physician will feel more confident to treat the patient as an outpatient or discharge the patient earlier.
This could have positive economic consequences. Studies are needed to evaluate if aetiological testing is cost-effective.

10. Recommended aetiological testing

10.1. Recommendations by international guidelines

International guidelines recommend increased efforts to identify the causative organism in severe CAP[4–8](Table 2), although therapy should not be delayed if there is difficulty in obtaining adequate samples [5]. As the patient is improving, the initial broad-spectrum therapy can be de-escalated or narrowed, according to the results of the diagnostic tests [4,5,8]. However, it is recommended that the therapy should not be narrowed until concerns regarding mixed infection have been appropriately addressed [5,6].

For non-severe CAP, diagnostic testing to identify an aetiology is optional, according to the guidelines of the Infectious Diseases Society of America/American Thoracic Society [4], and should be based on clinical factors, epidemiological factors and prior antibiotic therapy, according to the BTS and Swedish guidelines [7,8].

10.2. Suggested strategy for diagnostic testing

In Table 3, a strategy for diagnostic testing in hospitalised CAP patients is presented. It is based on disease severity and presence/absence of prior antibiotic therapy. Ongoing antibiotic treatment often causes false-negative cultures, although PCR tests [82] and urinary antigen tests [56] often remain positive in spite of the treatment. Thus, the preferred diagnostic techniques could be culture in non-treated patients, and PCR and urinary antigen tests in patients with ongoing treatment. However, in order to identify unexpected pathogens that are not covered by the empirical therapy, blood culture should be performed in all hospitalised CAP patients and culture from respiratory secretions should be performed in all patients with severe CAP, and in cases of therapy failure. This strategy recommends that at least one respiratory tract sample should be collected from all hospitalised CAP patients for culture and/or PCR, in order to enable frequent identification of aetiologies.

11. Conclusion

In order to cure CAP patients without causing unnecessary side effects and without contributing to the development of antibiotic resistance, antibiotic therapy should be carefully selected. Although patients with severe CAP should be treated with broad-spectrum antibiotics, patients with non-severe CAP should preferably receive pathogen-directed therapy. While rapid aetiological tests may be useful for targeting initial pathogen-directed therapy, non-rapid tests may support switch from broad- to narrow-spectrum antibiotic therapy and support therapy changes in the case of treatment failure.

Positive results of the different tests can be categorised to represent definite, probable or possible CAP aetiologies. While a definite or probable aetiology can often be used to target antibiotic therapy, the clinical presentation and the response to the initial antibiotic therapy should be considered prior to therapeutic decisions based on a possible aetiology.

As conventional diagnostic methods often fail to identify the CAP aetiology, PCR tests for respiratory pathogens have become useful and should be further developed.

Funding: The Research Committee of Örebro County Council (Sweden).

Competing interest: None declared.

Ethical approval: Not required.

References


