



LABORATOIRE SION

BIOLOGICAL ANALYSIS AND MEDICAL IMAGING

Specialized tests – Ultrasound – specimen collection at home or at the office.

SPUTUM CYTOBACTERIOLOGICAL EXAMINATION



SCE is a key, non-invasive test in the management of bacterial pneumonia

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Abstract

Bacterial pneumonia is a frequent reason for consultation. Sputum cytobacteriological examination (SCE), a non-invasive and inexpensive test, allows precise identification of the causative organism so that antibiotic therapy can be adjusted. It also makes it possible to monitor the efficacy of treatment. It is requested when the patient presents symptoms associated with a lower respiratory tract infection, or when he has already been treated for bacterial pneumonia. In a setting where the majority of the population has already received BCG, it is to be preferred over the search for acid-fast bacilli when pneumonia is suspected.

General considerations on pneumonia

Bronchopulmonary infections are lower respiratory tract infections that may involve the bronchi (bronchitis), the parenchyma (pneumonia and pulmonary and pleuropulmonary suppuration) or both at once (bronchopneumonia). The factors that favour the occurrence of pneumonia are: influenza; alcoholism; smoking; asthma; splenectomy; immunosuppression.

The clinical signs of pneumonia are: cough; fever and shaking chills; dyspnoea; pleuritic pain; expectoration (purulent, watery, rusty); digestive symptoms (nausea, vomiting, abdominal pain and diarrhoea) and mental confusion. The findings on clinical examination of pneumonia are: fever (in 80% of cases); tachypnoea >30/min; tachycardia >100/min; crackles (80%); signs of consolidation (present in 1/3 of cases); labial herpes.

Clinical signs are insufficient to establish the microbial aetiology. Comorbidities, the epidemic context and any institutionalisation of the patient are more reliable orienting factors. Blood cultures and sputum culture are also useful. It is customary to distinguish, among pneumonias, community-acquired, nosocomial, immunocompromised-host and atypical pneumonia.

- **Community-acquired pneumonia** is acquired outside hospital or within the first 48 hours of a hospital stay. It is an acute infection of the pulmonary parenchyma, essentially bacterial, whose mortality before the antibiotic era could exceed 50%.

The causative organisms are, in the usual order of frequency: *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae* and, more rarely, *Legionella pneumophila*, *Chlamydia pneumoniae*, and finally *Staphylococcus aureus*, together with enterobacteria in the elderly. Anaerobic organisms are observed in aspiration pneumonia. Following influenza, bacterial superinfection is possible: *Streptococcus pneumoniae* is most often implicated, but also *Staphylococcus aureus*, with a risk of resistance.

- **Nosocomial pneumonia** is acquired in hospital after a hospital stay of more than 48 hours. It is frequent: 10 to 30% of patients on ventilators. It is severe: mortality 20 to 50%. Isolation of the causative micro-organisms is the key to treatment.

The main species responsible are Gram-negative bacilli (GNB) in 60% of cases and staphylococci in 40% of cases. In one third of cases the infection is polymicrobial. The GNB are, in order of frequency: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella*, *Enterobacter*, *Serratia*. The staphylococci are *Staphylococcus aureus* (30%) and also *S. epidermidis* (10%). *Candida* spp are found with increasing frequency, in up to 10% of cases. Among the other species, mention should be made of *S. pneumoniae* and *H. influenzae*, in early nosocomial pneumonia (less than 5 days of hospitalisation).

- **Pneumonia in the immunocompromised patient** is severe and results from the breakdown of the balance between host and environment through failure of the immune system.

The micro-organisms to be sought are: *Pneumocystis carinii*, *Toxoplasma gondii*, CMV, *M. tuberculosis*, *Aspergillus* spp, *Candida* spp, *Cryptococcus neoformans*.

- **Atypical pneumonia** presents with symptoms that may be less pronounced than those of typical pneumonia. For example, some people with pneumonia have neither fever nor chest pain. The pneumonia then suggests a respiratory infection such as bronchitis, sinusitis or influenza.

Among the bacteria that cause atypical pneumonia are *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia psittaci* and *Legionella pneumophila*.

Micro-organisms responsible for bronchopulmonary infections				
	Pneumonia		Bronchial superinfections	Cystic fibrosis
	community-acquired	nosocomial		
<i>S. pneumoniae</i>	■	■	■	■
<i>H. influenzae</i>	■	■	■	■
<i>S. aureus</i>	■	■	■	■
<i>M. catarrhalis</i>	■	■	■	■
<i>K. pneumoniae</i>	■	■	■	■
Other GNB	□	■	■	■
<i>P. aeruginosa</i>	□	■	■	■
<i>Burkholderia cepacia</i>	□	■	□	■
Mycobacteria	•	•	•	□
<i>Nocardia</i>	•	•	□	□
<i>Chlamydia pneumoniae</i>	•	•	□	□
<i>Mycoplasma</i>	•	•	□	□
<i>Legionella</i>	•	•	□	□
<i>Coxiella</i>	•	□	□	□
Anaerobes	□	•	□	□
<i>Candida</i>	□	•	□	□
<i>Aspergillus</i>	□	•	□	•
■ to be sought in all cases				
• to be sought in particular circumstances				
□ to be sought exceptionally				

Microbiological diagnosis by sputum culture

- For some authors, routine bacteriological examination of sputum should be proscribed. It must not delay the initiation of empirical treatment. More than 90% of these infections are due to a limited number of bacterial species. Its results are unreliable because of salivary contamination. First-line antibiotic treatment is prescribed empirically on the basis of epidemiological criteria. SCE is then limited to refractory bronchial suppuration.
- For other authors, the indications are less restrictive. This examination has informative value provided that: 1) the sputum specimen is of good quality; 2) the quality of the specimen is confirmed by microscopic examination; 3) clinical significance is attributed only to the predominant species if it reaches or exceeds the threshold of 10^7 bacteria/ml. If these three conditions are met, it is worthwhile to proceed with antibiotic susceptibility testing of the bacterial strain.

Sputum collection must follow a rigorous protocol: it must be performed in the morning, on waking, after rinsing the mouth and teeth with sterile distilled water and during a coughing effort, assisted by physiotherapy if necessary. Bacteriological examination must be carried out without delay.

Microscopic examination is performed according to the technique described by Bartlett and adapted by Murray and Washington. It makes it possible to assess the degree of contamination by saliva. It consists in examining either a wet preparation or a Gram-stained smear under the microscope at 100x magnification and in counting, as an average over 10 fields, the epithelial cells and the leukocytes per field (after checking the morphology at a higher magnification). The results of microscopic examination make it possible to distinguish 5 classes of sputum: the presence of alveolar macrophages is evidence of the lower origin of the secretions.

Class	Epithelial cells per field	Leukocytes per field
1	>25	<10
1	>25	<10
1	>25	<10
1	>25	<10
1	>25	<10

Class 1 and 2 sputum specimens are heavily contaminated with saliva. They cannot be used for culture. Another specimen must be requested. Class 3 and 4 sputum specimens have a leukocyte count that reflects an inflammatory reaction but are contaminated with saliva. Class 5 sputum specimens are the most appropriate for bacteriological examination. Those of class 4 are acceptable.

After fluidification of the specimen, an appropriate dilution is inoculated, allowing enumeration of bacteria beyond 10^5 CFU/ml, onto: enriched chocolate agar (5 to 10% CO₂) with or without bacitracin, blood agar, and selective agar for Gram-negative bacilli. Identification and antibiotic susceptibility testing are usually limited to one or two bacterial species present in quantities $> 10^7$ bacteria per ml.

This examination comes in addition to, or in association with, the search for mycobacteria by Ziehl-Neelsen staining; the inoculation of media allowing the culture of fungi, or a viral analysis (generally by PCR), may be requested in addition to or in place of routine bacteriological analysis.

Together with the culture of a sputum specimen, a CBC (Complete Blood Count) may be performed to assess the nature and number of leukocytes and to argue in favour of an infection. It may also be combined with a blood culture to diagnose septicaemia, which often goes hand in hand with pneumonia.

Biographies

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3. LEROY, O., GEORGES, H., BEUSCART, C., SANTRE, C., MOUTON, Y., Analyse critique des critères de diagnostic des pneumonies nosocomiales. *Lettre Infect.*, 1994, 9 : 339-343.