Le tecniche molecolari nella diagnosi di infezione: proposte di protocolli diagnostici

Le tecniche molecolari nella diagnosi delle infezioni respiratorie

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Acute respiratory infections are a leading cause of acute illnesses worldwide.

Herman Goossens BMJ 2006
While upper respiratory tract infections are very frequent but seldom life-threatening, lower respiratory tract infections (LRTI) are responsible for more severe illnesses.

Herman Goossens BMJ 2006
Lower respiratory tract infections are among the most common infections of adults and children.

Murdoch DR. APMIS 112: 713–27, 2004
The populations most at risk for developing a fatal respiratory disease are the very young, the elderly, and the immunocompromised.

Herman Goossens BMJ 2006
LRTI: a challenge for diagnostic microbiology

The number of pathogens involved in lower respiratory tract infections (LRTI), with various susceptibilities to antimicrobials, is large constituting an enormous challenge for diagnostic microbiology.

Loens K et al. J Clin Microbiol 2009
LRTI: a challenge for diagnostic microbiology

During recent years a considerable number of previously unknown respiratory agents were discovered whose in vitro culture is very slow or even unrealized.

Ieven, M. J Clin Virol. 2007
Etiological diagnosis: the real problem

While the **clinical diagnosis** of LRTI is usually relatively straightforward, determining the **etiological diagnosis** can be much more difficult due to the limitations of conventional diagnostic tests.

Murdoch DR. APMIS 112: 713–27, 2004
Etiological diagnosis: the real problem

At present there is still a great deficit in the etiologic diagnosis of LRTI. In most studies more than 50% of cases remain without an etiologic diagnosis.

Ieven, M. J Clin Virol. 2007
Etiological diagnosis: the real problem

In general, in only 50% of cases is an etiologic agent detected. Documented infection is uncommon in community-managed infection and is usually only defined in 25 to 50% of hospital-managed infections.

Loens K et al. J Clin Microbiol 2009
At the beginning of the 21st century, a high proportion of diagnostic tests are still performed according to methodologies pioneered by Pasteur at the end of the 19th century, i.e. methods based on culture, microscopy, and serology.

Bissonnette L and Bergeron MG. CMI 2010.
In the 1960s and 1970s, physicians embraced an empirical approach to the management of many infectious diseases, favouring overuse of antibiotics.

Bissonnette L and Bergeron MG. CMI 2010.
Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe
In Europe 90-95% of antibiotic use occurs outside hospitals, and community acquired lower respiratory tract infections are the leading reason for prescribing antibiotics.

Herman Goossens BMJ 2006
The overall **objective** of GRACE is to combat antimicrobial resistance through integrating centres of excellence for studying the **application** of genomics to community-acquired lower respiratory tract infections (CA-LRTI).

https://www.grace-lrtn.org
The goals of GRACE is to enroll 3,000 LRTI patients and matched controls to study the etiology of LRTI in the community by applying conventional and NAATs to detect the causative organisms.

GRACE should therefore help identifying the optimal microbiological method for diagnosis of CA-LRTI.
Traditional bacterial culture and serological testing for detection of pneumonia microorganisms have low sensitivity, are time-consuming, take several days and focus only on a few of the large number of aetiologic agents.

To evaluate the usefulness of NAATs in the diagnosis of acute respiratory infection, the objectives of an etiologic diagnosis in this condition should be remembered.

ieven, M. J Clin Virol. 2007
Objectives of an etiologic diagnosis

1. To avoid empirical start of antibiotic treatment and to allow narrow spectrum targeted antibiotic treatment;
2. to allow appropriate use of antiviral drugs;
3. to allow cohorting of patients in case of hospitalization, preventing nosocomial spread;
4. to provide more accurate epidemiological information to formulate preventive and therapeutic recommendations;
5. to decrease duration of hospital stay and to reduce management costs.

Ieven, M. J Clin Virol. 2007
To answer the first three objectives, diagnosis should be available rapidly, preferably within about 4 h.

The traditional NAATs are unable to fulfill this requirement, but recent technical progress has brought NAATS to age, through the development of multiplex PCR and real time (RT) PCR, coupled with automatic NA extraction.

Ieven, M. J Clin Virol. 2007
Seeplex®
RV 15 ONE STEP

Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Adenovirus A/B/C/D/E
Coronavirus 229E/NL63

Coronavirus OC43
Rhinovirus A/B/C
Influenza A/B/C
RSV A
RSV B

Bocavirus 1/2/3/4
Influenza B virus
Parainfluenza virus 4
Enterovirus

Dual Priming Oligonucleotide technology (Seegene)

<table>
<thead>
<tr>
<th>M. pneumoniae</th>
<th>C. pneumoniae</th>
<th>L. pneumophila</th>
<th>S. pneumoniae</th>
<th>H. influenzae</th>
<th>B. pertussis</th>
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Reevaluation of serological tests

The availability of the very sensitive NAATS has in recent years also put the often-used serological tests in their right perspective.

Serologic tests can never offer an early diagnosis and are therefore rather an epidemiological than a diagnostic tool.

leven, M. J Clin Virol. 2007
Optimization of laboratory strategy

Strategies will have to be developed adapting the evolution of the technology of the NAATs, the population of patients served (children, elderly, and immunocompromised patients) the resources available (infrastructure, staff, full-time service or service limited during some hours of the day, or some days of the week), the number and nature of the agents that can be covered.

Ieven, M. J Clin Virol. 2007
L’organizzazione e le competenze

Nella dichiarazione congiunta di Copenhagen (1993), l’European Council of Legal Medicine (ECLM) e l’Organizzazione Mondiale della Sanità (OMS) definiscono che la Medicina di Laboratorio è rappresentata da 5 discipline: Clinical Chemistry, Clinical Haematology, Clinical Immunology, Clinical Microbiology e Clinical Pathology.

Le Polmoniti: proposta di un percorso condiviso per la diagnosi microbiologica

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Pneumonia in immuno-competent adults

National Standard Method

- Clinical history / Patient group
  - Mild pneumonia
  - Mild pneumonia admitted to hospital due to other reasons eg comorbidity
  - Not pyrexic
  - Pyrexia (T>38°C)

- Sample type
  - Blood
  - Sputum

- Primary testing
  - No microbiological investigation required

- Secondary testing
  - Blood culture (BSOP 37)
  - Microscopy, Culture and Sensitivity (BSOP 57)
  - PCR for Legionella (BSOP 47)
  - HSV and CMV quantitative PCR

- Severe pneumonia
  - Blood
  - Respiratory sample
  - Urine
  - Sputum
  - BAL
  - Pleural fluid
  - Process as for severe pneumonia with the addition of sputum / BAL investigation for Mycobacteria APP (BSOP 40)

- Serumology
- Isolation

- NHS

- Health Protection Agency

- Association of Medical Microbiologists
- Association of Clinical Lung Biopsy Technicians
- Scottish Microbiology Forum
- UK Clinical Virology Network
Pneumonia in immuno-competent adults

National Standard Method

Clinical history / Patient group

- Mild pneumonia
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Sample type

- Not pyrexic
- Pyrexia (T>38°C)

Primary testing

- Blood
- Sputum

Secondary testing

- Blood culture (BSOP 37)
- Microscopy, Culture and Sensitivity (BSOP 37)

No microbiological investigation required

= PCR
= Serology
= Isolation
Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe

https://www.grace-lrti.org
Pneumonia in immuno-competent adults
National Standard Method

Severe pneumonia

- Blood
  - Blood culture (BSOP 37)
  - Mycoplasma spp.
  - Chlamydia spp.

- Respiratory sample
  - Viral PCR screen
  - Mycoplasma spp.
  - Chlamydia spp.

- Urine
  - Urine antigen test for *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae*

- Sputum
  - Microscopy, Culture and Sensitivity (BSOP 57)
  - Legionella culture (BSOP 47)

- BAL
  - Microscopy, Culture and Sensitivity (BSOP 57)
  - Investigation for Mycobacteria spp. (BSOP 40)

- Pleural fluid
  - Microscopy, Culture and Sensitivity (BSOP 57)
  - PCR for *Legionella* (BSOP 47)

Multiplex Real Time PCR

- HSV and CMV quantitative PCR
An acute respiratory tract infection is one of the common causes for hospital admissions of children.
Eziologia infezioni respiratorie ad eziologia virale in età pediatrica a Pordenone: novembre 2008-gennaio 2010
Influenza virus type A and B, parainfluenza virus type 1, 2, 3, respiratory syncytial virus (RSV) type A and B, and adenovirus are major causes of lower respiratory tract infections in infants and young children under 5 yr old.

Human metapneumovirus, also identified in children with respiratory infection, rhinovirus, and coronavirus are known as causative agents of the common cold.

The availability of a rapid viral diagnostic assay will enable physicians to make more accurate treatment decisions, reduce unnecessary antimicrobial agent use, and shorten hospital stays for patients.

Pneumonia in immunocompromised adults

National Standard Method

Clinical history / Patient group

Sample type

Primary testing

Secondary testing

Mild / Severe pneumonia

Blood

Blood culture (BSOP 37)

CMV PCR screen

Mycoplasma Chlamydia spp

Specific Aspergillus / Cryptococcus Investigation

Respiratory sample

Viral PCR screen

Mycoplasma Chlamydia spp

Urine

Urine antigen test for Legionella pneumophila and Streptococcus pneumoniae (BSOP 47)

Microscopy, Culture and Sensitivity * (BSOP 57)

Investigation for Pneumocystis jirovecii

Mycology (BSOP 47)

Legionella culture (BSOP 47)

PCR for Legionella (BSOP 47)

Sputum or Induced sputum

SpO2 and Induced sputum

Microscopy, Culture and Sensitivity * (BSOP 57)

Investigation for Mycobacterium spp (BSOP 49)

PCR for Legionella

HSV and CMV quantitative PCR

BAL *

Nocardia Investigation

Specific Aspergillus / Cryptococcus Investigation

= PCR

= Serology

= Isolation

= ELISA
Pneumonia in immunocompromised adults

National Standard Method
For the molecular diagnosis of respiratory infections the preferred clinical specimens are nasopharyngeal aspirates (NPA) and sputum as well as bronchoalveolar lavage specimens, if available. The superiority of NPA for the detection of all viruses was clearly illustrated in the study by Gruteke et al. (2004).

Ieven, M. J Clin Virol. 2007
Prospects

Where available, PCR tests are an extremely useful addition to the diagnostic armamentarium and have the advantage of being rapid (relevant on occasions for both clinical and infection control purposes) and sensitive, and so are to be preferred over serological tests.

Prospects

When molecular tests can detect within hours (or even minutes) many pathogens in nasopharyngeal swabs, throat swabs, nasopharyngeal aspirates, and sputum, more focused and efficient management of patients with LRTI will become possible.

Entrance into the diagnostic cycle (0–6 hours):
patient arrival, triage, primary evaluation, questionnaire and physical examination by physician, presumptive diagnosis, physician laboratory analysis request(s), clinical sampling, transfer to laboratories, etc.

Conventional diagnostics

Molecular diagnostics in Microbiology and Virology

Emergency & POC Microbiology Lab

- Molecular (multiparametric?) POC diagnostic devices
- Identification and/or drug susceptibility
- Biomarkers (Procalcitonin, cytokines, proANP, Copeptin, proAdenomedullin..)

Immediate healthcare decision

Results of analyses and healthcare decision process (hours to days):
transmission of results, interpretation, patient management, therapeutic intervention, confirmatory testing, treatment adjustment, etc.

Community acquired pneumonia in primary care

Doctors cannot target antibiotics and reduce resistance until new diagnostic tests prove feasible and affordable at the point of care

Herman Goossens BMJ 2006
Prospects

A change in culture without culture...

Bissonnette L and Bergeron MG. CMI 2010.
Prospects

The process of diagnosing infectious diseases is gradually entering an era where a physician is in the position to obtain valuable information on a time-scale comparable to those in other fields of diagnostic medicine.

Bissonnette L and Bergeron MG. CMI 2010.
GRAZIE PER L’ATTENZIONE

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