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The beginning of a new era: systematic testing for pathogens causing acute respiratory tract infections (ARI) in children

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The problem

On average, humans get sick ten times per year. About six times, the illness is due to an acute respiratory tract infection (ARI). Morbidity is especially high in children, since

- they usually encounter the offending organism for the first time in their life;
- the lack of immunity results in shedding of the offending organisms in high numbers of prolonged time as compared to adults;
- their airways are smaller than those of adults and thus the inflammatory response leads to a more significant narrowing of the airways resulting in more severe disease;
- on average, they have a high number of social contacts and also a more intimate contact with peers and caregivers alike resulting in a higher attack rate;
- they display an age-dependent lack of appropriate hygiene measures.

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In poor countries, ARI are one of the leading causes of death (Table 1).[1] Optimal medical management of ARI is, therefore, of the highest importance everywhere in the world. The utmost importance of ARI in children is in sharp contrast to the little knowledge we have about the etiology, epidemiology, and clinical consequences such as development of asthma following respiratory infections. While ARI are comparatively simple to diagnose clinically by investigating the history of the patient and by physical examination, clinical findings alone do not allow to identify the offending microorganism in an individual case. We regularly encounter the peak of the RSV season in the middle of the influenza season; and often – based on only partial knowledge of the epidemiological situation and the spectrum of diseases caused by both organisms –

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Table 1 - Annual number of deaths by cause for children under five years of age in some WHO regions, estimates for 2000-2003[1]

					Europe		The Americas			
	All WHO member states		Africa		Member states with low mortality		All		Member states with low mortality	
	616,764,000		110,944,000		22,050,000		77,885,000		22,978,000	
	Total (x 1000)	%	Total (x 1000)	%	Total (x 1000)	%	Total (x 1000)	%	Total (x 1000)	%
Total deaths	10,596	100	4,396	100	25	100	439	100	50	100
HIV/AIDS	321	3	285	6	0	0	6	1	0	0
Diarrhea	1,762	17	701	16	0	0	51	12	0	0
Measles	395	4	227	5	0	0	1	0	0	0
Malaria	853	8	802	18	0	0	1	0	0	0
ARI	2,027	19	924	21	0	2%	54	12%	1	2
Neonatal	3,910	37	1,148	26	14	55	195	44	29	58
Injuries	305	3	76	2	2	7	23	5	5	10

unspecific ARI reporting misleads the public and treating physicians alike:[2] the lack of appropriate diagnostic tools for ARI and thus the failure to identify ARI-pathogens results in a failure to treat patients optimally. While we know that at least 70% of lower ARI are caused by viruses, "blind treatment" with antibiotics has become the standard of care in most cases of pneumonia and even in many cases of bronchitis in many institutions all over the world. More so, since we do not know the frequency of different ARI-pathogens, new and specific treatments are not developed.

What we currently know

From a principal point of view, ARI-organisms can be divided into:

- **colonizers** like *Streptococcus pneumoniae*; these are usually sensitive to betalactams, and
- **non-colonizers** including bacteria (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis and B. parapertussis*; usually sensitive to macrolides) and viruses (rhino-, entero-, metapneumo-, parainfluenza-, influenza-, adeno-, corona-, boca-, polyoma-, reo- and RS-virus; others).

With the rare exception where a blood culture turns out positive for *S. pneumoniae,* it is still impossible to date to prove that a pneumococcus is the causative agent of pneumonia. Definitive prove of such "colonizers" to be the etiology of lower respiratory tract infection (LRTI) would require lung puncture, in order to avoid contamination in the oral/pharyngeal cavity. For psychological reasons only, this procedure is hardly ever performed – although it is probably safe and indeed would definitely prove the etiology of the respective episode of ARI. In contrast to the situation with colonizing agents, detection of a non-colonizing ARI-pathogen in a nasopharyngeal specimen, especially in a child with first ARI-encounters, usually means that the pathogen detected in the upper airway is the cause of the LRTI.

Solutions

In their paper in this journal,[3] Thomazelli et al. use eight separate PCRs to screen for the presence of ARI-pathogens. They then used fragment length analysis with GeneScan for definite identification of PCR-products. Studying patients from a large pediatric hospital for 1 year, they most commonly found RSV followed by metapneumovirus, parainfluenzavirus 3, adenovirus, and influenzavirus. Dual infections were seen in about 7% of children.

These results are in accordance with our observation.[4] Starting in 1996, we developed a multiplex-RT-PCR to detect nine[5,6] and then 19 different ARI pathogens (work in progress). Over a 10 year period and examining more than 20,000 specimens to date, rhinoviruses are most commonly encountered in children hospitalized for LRTI. RSV causes only

Table 2 - Value of systematically testing for ARI-pathogens

Individual value
Know the etiology of the disease
Less interventions (X-ray, blood tests)
Optimal therapy (no antibiotic or betalactam or macrolide)
Less side effects/cost/pain from interventions
Public health value
Know the epidemiological situation
Teach/educate physicians
Warning system for epidemics
Reduce hospitalization
Reduce use of antibiotics
Predict epidemics
Research value: some options
Describe (molecular) epidemiology
Cohort studies (e.g. development of asthma)
Immunological studies (type of immunity followed by infection)
Modeling of spread of different organisms
Genetic susceptibility to ARI

half as many cases as rhinoviruses, and influenzaviruses are detected in only about 7%. Clearly, these simple findings should make us start developing effective interventions against the most common ARI-microorganisms, not just against influenza. Moreover, using multiplex-PCR as a routine diagnostic tool has reduced the use of antibiotics in our institution; it has taught us the clinical course and the large variability in the clinical presentation of ARI-organisms; and, during epidemics, it has reduced the use of diagnostic interventions such as chest-x-rays or laboratory tests. In many instances, specific therapies can be given, e.g. for mycoplasma or influenza. Additional advantages are summarized in Table 2.

We have created a "web based warning system", , where we publish the number of each ARI-pathogen detected during the preceding week, we predict the activity of each organism for the upcoming epidemiological season (July 1st to June 30th the following year), and we give general information on ARI.

This allows pediatricians in Germany e.g. to tailor the use of RSV-immunoglobulin: in uneven years, RSV-epidemics start late and run with a high peak. This is followed by continuous RSV-activity the following (even) year, sometimes even throughout summer, to reach again an early peak in autumn/winter.[4] Likewise, rhythms can be detected for metapneumovirus and other paramyxoviruses, whereas adenovirus, enterovirus and rhinovirus are detected throughout the year.[4]

Current problems

Whenever new diagnostic tools come along, they need to be validated and this is especially true for PCR based techniques. Contamination must be avoided at all times from the minute the specimen is taken; e.g. negative control samples should occasionally be collected at the bedside of patients (like sterile saline or buffer) to prove that medical staff did not shed "their" pathogen into the patient specimen. Besides the use of strict methods to avoid contamination in the laboratory, validation of the multiplex-testing should be established for each organism by comparison to current standard techniques like cell culture.[6]

Whatever "test-system" is used, its limitations must be kept in mind and need to be communicated to the treating physicians.

The future

It is clear to us that systematically testing for ARI pathogens is the way forward. Technical problems will be solved within a few years. More tests will become standardized and validated. Currently, testing is too expensive for the individual. We ask for € 396.- per test if ordered commercially (www.arti-st.de). Therefore, today public health institutions should use the method for local surveillance. It is in the interest of the public to know, which microorganisms are responsible for the highest morbidity (and perhaps even mortality) in the population. Furthermore, the public must be interested in emerging pathogens like H5N1. These can be included in the multiplex test systems, and this alone should be worth the systematic approach as compared to not meeting the challenges caused by ARI at all or with insufficient and systematically misleading methods. With new technical developments on the horizon, the price for systematically testing for ARI can be substantially reduced in the near future and "acute respiratory infection – systematic testing (arti-st)" will then hopefully become the standard of care for each individual child.

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