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Additional Diagnostic Yield of Adding Serology to PCR in Diagnosing Viral Acute Respiratory Infections in Kenyan Patients 5 Years of Age and Older

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The role of serology in the setting of PCR-based diagnosis of acute respiratory infections (ARIs) is unclear. We found that acute- and convalescent-phase paired-sample serologic testing increased the diagnostic yield of naso/oropharyngeal swabs for influenza virus, respiratory syncytial virus (RSV), human metapneumovirus, adenovirus, and parainfluenza viruses beyond PCR by 0.4% to 10.7%. Although still limited for clinical use, serology, along with PCR, can maximize etiologic diagnosis in epidemiologic studies.

Serology was until recently the principal method for diagnosing viral pneumonia (1–6). Serology, however, can yield false-negative results if the collection of acute- or convalescent-phase blood samples is delayed or too early (4). Moreover, serology is logistically cumbersome and clinically uninformative, because people must return several weeks after the acute illness has resolved (if they survive) for convalescent-phase blood collection. The advent of nucleic acid-based tests (e.g., PCR) of upper respiratory tract specimens offers sensitive, timely diagnostic alternatives (6). The role of serologic testing in the setting of PCR-based diagnosis is unclear.

We enrolled persons that were ≥5 years old (median, 11.0 years; range, 5 to 81 years) presenting with acute respiratory illness, defined as cough, difficulty breathing, or chest pain and a documented temperature of ≥38.0°C or oxygen saturation of <90%, in rural western Kenya from 1 January 2009 and 28 February 2010 (7). At the time of presentation to the clinic, we collected acute-phase blood and nasopharyngeal and oropharyngeal swab specimens in viral transport media. Patients were asked to return 4 to 6 weeks later for a convalescent-phase serum blood draw. Quantitative real-time reverse transcription-PCR (qRT-PCR) of specimens was carried out for adenovirus, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza A and B viruses, and parainfluenza virus (PIV) types 1 to 3 using singleplex qRT-PCR assay (7, 8). The qRT-PCR results were considered positive if the threshold cycle (C_T) was <40.0. Paired sera were tested for antibodies to influenza viruses using hemagglutination inhibition assays and for antibodies to RSV, hMPV, adenovirus, and PIV using standard methods for indirect IgG enzyme-linked immunoassay, in which all assays were optimized for reagent/diluent composition and concentration and incubation time/temperatures to obtain the highest limit of detection for anti-

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TABLE 1 Results of serologic testing of acute- and convalescent-phase sera and qRT-PCR among patients with acute respiratory infections of ≥5 years of age[a]

Virus	No. of patients with the indicated result(s)/no. of total patients tested (%)				
	With ≥4-fold rise in titer	With ≥4-fold rise in titer and PCR positive	PCR positive	PCR positive and with ≥4-fold rise in titer	Positive by PCR or serology
Influenza A virus	62/232 (26.7)	37/62 (59.7)	70/232 (30.2)	37/70 (52.9)	95/232 (40.9)
Influenza B virus	27/232 (11.6)	5/27 (18.5)	20/232 (8.6)	5/20 (25.0)	42/232 (18.1)
RSV	8/204[b] (3.9)	2/8 (25.0)	17/204 (8.3)	2/17 (11.8)	23/204 (11.3)
Adenovirus	2/203[b] (1.0)	1/2 (50.0)	33/203 (16.3)	1/33 (3.0)	34/203 (16.7)
Parainfluenza virus 1	3/203[b,c] (1.5)	0/3 (0)	0	0	3/203 (1.5)
Parainfluenza virus 2	2/204[b] (1.0)	1/2 (50.0)	8/204 (3.9)	1/8 (12.5)	9/204 (4.4)
Parainfluenza virus 3	5/204[b,c] (2.5)	0/5 (0)	0	0	5/204 (2.5)
Human metapneumovirus	18/204[b] (8.8)	5/18 (27.8)	15/204 (7.4)	5/15 (33.3)	28/204 (13.7)

[a] The study was performed on samples collected from individuals in western Kenya. The samples were collected 1 January 2009 to 28 February 2010. The median number of days between acute- and convalescent-phase sera was 42, and the range was 32 to 72 days.
[b] Although the acute and convalescent-phase sera for 232 patients were tested for all viruses, the serum samples for fewer of these patients had qRT-PCR results for some pathogens, resulting in lower denominators (i.e., 203 or 204).
[c] One patient was positive for both parainfluenza virus 1 and parainfluenza virus 3, which could indicate cross-reactive viral epitopes.

viral IgG antibodies with minimal background binding to control antigen (9–12). Fourfold or greater rises in titers were considered seropositive for acute infection.

Among patients with serology and qRT-PCR performed, ≥4-fold rises in titers ranged from 1% for adenovirus and PIV to 27% for influenza A virus (Table 1). For the three viruses for which at least 10 patients were seropositive, 60%, 28%, and 19% of patients seropositive for influenza A virus, hMPV, and influenza B virus, respectively, were also positive for the same virus by qRT-PCR. Among patients who were qRT-PCR positive for influenza A virus, 53% were seropositive for influenza A virus compared to 3 to 33% seropositivity among those positive by qRT-PCR for other viruses. Serologic testing increased the diagnostic yield beyond qRT-PCR by 0.4% to 10.7%, depending on the virus (Table 1).

We found despite its high analytic sensitivity, qRT-PCR can miss some viral infections detected by serology (4, 6). In Thailand, serology also detected some influenza infections not identified by PCR (13). We also found that the virus to which a patient was seropositive was not always detected in the pharynx by qRT-PCR. Possibly the swab missed the virus in the pharynx. Alternatively, seropositivity might not be specific for symptomatic infection for some viruses. Among 7 viruses tested for by serology in coastal Kenya, only seropositivity for RSV was statistically more common among children with pneumonia than healthy controls (14). Our evaluation was limited by a small sample size, lack of serology data on children, and no serology results for viruses where serology might be more informative of acute infection, like rhinovirus and human bocavirus (15, 16). It is likely that both PCR and serology are needed to fully assess the etiologic contribution of viral respiratory pathogens in epidemiologic studies; additional studies are needed to better define the relationship between the two diagnostic modalities.

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