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Short communication

Co-circulation of human metapneumovirus and SARS-associated coronavirus during a major nosocomial SARS outbreak in Hong Kong

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Abstract

Background: The clinico-epidemiological significance of human metapneumovirus (hMPV) detected during the SARS outbreak is unknown.
Objectives: To characterize a nosocomial hMPV outbreak during the 2003 SARS epidemic.
Study design and methods: All available nasopharyngeal aspirate (NPA) collected from confirmed patients during the first 8 weeks of the SARS outbreak in 2003 were tested for hMPV by a nested RT-PCR assay targeting the F-gene. Clinico-epidemiological information was used to analyze the relationship of hMPV co-infection to specific risk factors (demographics/symptoms/outcomes; status as health-care workers (HCWs)/patients; history of exposure/contact; ward location). Multivariate logistic regression analysis was performed to determine independent risk factors.
Results: An hMPV outbreak occurred during 6–16 March 2003 (first week of the Hong Kong SARS epidemic). hMPV RNA was detected in 31 of 155 (20%) NPAs from SARS patients. HCW status (OR 2.72, 95% CI 1.11–6.68; $p = 0.029$) or epidemiological linkage to the SARS outbreak ward (OR 3.59, 95% CI 1.42–9.05; $p = 0.007$) were independent factors associated with hMPV infection. Symptoms of cough and coryza were more common in co-infected individuals (22.6% vs. 15.9%) but this was not statistically significant. Other clinical manifestations and outcomes were not different in co-infected patients.
Conclusions: A major nosocomial hMPV outbreak involving HCWs occurred during the early SARS epidemic. Patients with dual hMPV and SARS infection were not sicker than those with SARS infection only.

Keywords: Nosocomial outbreak; Human metapneumovirus; SARS

1. Introduction

We previously reported a major nosocomial severe acute respiratory syndrome (SARS) outbreak and its epidemiological findings (Lee et al., 2003; Wong et al., 2004; Yu et al., 2005). Another newly recognized respiratory pathogen, human metapneumovirus (hMPV) (Boivin et al., 2007; McIntosh and McAdam, 2004; Principi et al., 2006), was detected in many early SARS cases worldwide (Kuiken et al., 2003; Poutanen et al., 2003; Tomlinson and Cockram, 2003). Respiratory illnesses caused by hMPV may range from mild ILI (influenza-like illness) to severe pneumonia (Boivin et al., 2007; McIntosh and McAdam, 2004; Principi et al., 2006). Although not the cause of SARS (Ksiazek et al., 2003), its clinico-epidemiological significance during the outbreak remained unknown. Previously we reported the detection of hMPV in SARS patients (Chan et al., 2003), but did not perform detailed clinico-epidemiological studies. We describe a nosocomial outbreak of hMPV infection during the early

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SARS epidemic in Hong Kong, examine risk factors for its acquisition, and hypothesize on its possible interaction with SARS.

2. Methods

All available stored nasopharyngeal aspirate (NPA) from laboratory-confirmed SARS-coronavirus (SARS-CoV) infected adult patients at the prince of wales hospital (PWH), which were collected during the first 8 weeks of the outbreak (March–June 2003), were retrieved for hMPV testing (Lee et al., 2003, 2006). Clinico-epidemiological information in our database (prospectively collected) was used for analyzes (Lee et al., 2003; Wong et al., 2004; Yu et al., 2005). Subjects were categorized into health-care workers (HCWs: doctors, nurses, and medical students), in-patients, and visitors. 'Index ward X' referred to the ward in which the major SARS outbreak ($n > 100$) occurred (Lee et al., 2003).

NPA specimens were tested for hMPV using a nested RT-PCR targeting the F-gene as previously described (Chan et al., 2003). In brief, RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany). The outer primers were 5′-AGC TGT TCC ATT GGC AGC A-3′ for RT and amplification and 5′-ATG CTG TTC RCC YTC AAC TTT-3′ (R = A/G, Y = C/T) for amplification. The reaction was carried out in a single-tube (Superscript One-Step RT-PCR and Platinum Taq; Invitrogen Corp., Carlsbad, CA) by using 0.2 μM of each primer. For the second round of amplification, 0.2 μM of inner primers 5′-GAG TAG GGA TCA TCA AGC A-3′ and 5′-GCT TAG CTG RTA TAC AGT GTT-3′ were used. PCR products were detected by agarose gel electrophoresis.

χ^2- and Student's *t*-tests were used to compare categorical and continuous data, respectively. Significant variables in univariate analyzes were entered into multivariate logistic regression models to determine independent factors for hMPV co-infection. A *p*-value of <0.05 indicated statistical significance. All probabilities were two-tailed. Statistical analysis was performed using SPSS software (version 13.0, Chicago).

3. Results

A total of 155 NPA specimens from SARS patients were tested for hMPV (86 HCWs, 47 patients and 22 visitors). hMPV RNA was detected in 31 (20%) SARS cases. An hMPV outbreak had occurred during 6–16 March 2003, corresponding to the first week of the SARS epidemic. By 16 March, co-infection occurred in 31% of SARS patients. The epidemic curves for both hMPV and SARS-CoV infections are shown in Fig. 1.

Clinical and epidemiological features were compared between SARS patients with or without hMPV co-infection (Table 1). We noted that 77.4% of hMPV co-infections were linked to the index ward X (HCW = 19, patient = 1, and visitor = 4; a few cases had no such exposure—see footnotes of

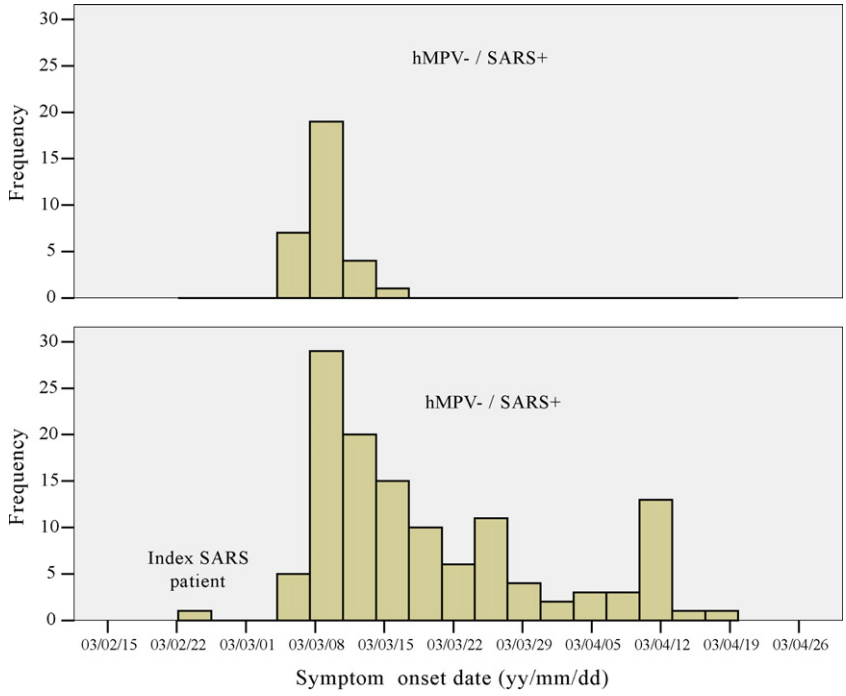

Fig. 1. Epidemic curves for SARS-CoV/hMPV co-infected patients (upper panel), and SARS patients tested negative for hMPV (lower panel). SARS-CoV: severe acute respiratory syndrome-associated cornoavirus; hMPV: human metapneumovirus.

Table 1
Clinical and epidemiological features in SARS patients with ($n = 31$) or without ($n = 124$) hMPV co-infection

Characteristics	hMPV+ (%)	hMPV− (%)	p-Value
Age (year)	35.9 ± 14.2	36.5 ± 13.3	NS
Male sex	48.4	35.5	NS
Co-existing medical conditions[a]	6.5	25.8	0.027
Linkage[b] to SARS index ward X	77.4	48.4	0.004
HCWs	74.2	50.8	0.019
Fever	100.0	100.0	NS
Chills	96.0	92.0	NS
Myalgia	87.1	87.8	NS
Cough and coryza	22.6	15.9	NS
SOB	48.4	49.2	NS
Diarrhoea	19.4	22.0	NS
Use of supplemental O_2	45.2	46.8	NS
ICU admission	19.4	24.2	NS
Death	6.4	7.3	NS
Initial lymphocyte count, mean ± S.D. ($\times 10^9$ L)	0.92 ± 0.45	0.89 ± 0.38	NS
Nadir lymphocyte count, mean ± S.D. ($\times 10^9$ L)	0.30 ± 0.23	0.31 ± 0.23	NS
Peak LDH, mean ± S.D. (U/L)	552 ± 918	615 ± 2385	NS

hMPV: human metapneumovirus; HCW: health-care workers; ICU: intensive care unit; S.D.: standard deviation; LDH: lactate dehydrogenase; NS: statistically insignificant.

[a] hMPV-infected individuals were mostly young, previously healthy HCWs; whereas those without co-infection consisted of a mixed of in-patients and HCWs.

[b] Among the seven hMPV-infected patients who had never visited index ward X, four were linked to the emergency room, and two to different medical wards. The last patient was a deployed nurse who looked after the initial batch of sick health-care workers in a separate cohort ward, and subsequently developed symptoms.

Table 1). Previously healthy HCWs [doctors ($n = 10$), medical students ($n = 7$), and nurses ($n = 6$)] were predominantly (74.2%) involved. Multivariate logistic regression analysis showed that being a HCW (OR 2.72, 95% CI 1.11–6.68; $p = 0.029$) or epidemiological linkage to the index ward X (OR 3.59, 95% CI 1.42–9.05; $p = 0.007$) were independent risk factors for hMPV co-infection, after adjusting for baseline characteristics (age, sex, and major co-morbidity). Clinical manifestations and outcomes did not differ between the comparative groups. Notably, symptoms of cough and coryza were present in 22.6% and 15.9% of patients with and without hMPV co-infection, respectively ($p > 0.05$).

The primary case for the hMPV outbreak could not be identified. Three co-infected individuals (visitors or patient) had travelled to mainland China within 2 weeks preceding symptom onset. Airborne (e.g. use of N-95 respirator in 'high risk' areas), droplets (e.g. use of surgical masks in 'low risk' areas), and contact precautions (e.g. use of gowns and gloves) were instituted beginning on 10 March according to appropriate risk categories, as suggested by CDC (CDC, 2003). No further HMPV cases were detected after mid-March 2003.

4. Discussion

Our results suggest that hMPV co-circulated with SARS-CoV during the first week of the SARS epidemic in Hong Kong. A simultaneous nosocomial hMPV outbreak occurred in the same index ward that housed SARS patients, and HCW were predominantly affected. Although clinical outcomes were not affected, alteration in disease transmission might have occurred as a result of interactions between these two infections.

Our findings are consistent with other reports from Hong Kong (besides PWH), Canada, Singapore, and Vietnam describing the identification of hMPV among patients with suspected or confirmed of SARS during the early outbreak period (Kuiken et al., 2003; Poutanen et al., 2003), with one reported fatality (SARS excluded, patient hospitalized in Hong Kong) (Chan et al., 2004). Although the epidemiological linkages cannot be ascertained, these findings indicate co-circulation of hMPV during that period (Tomlinson and Cockram, 2003). Co-infection by two respiratory pathogens is not uncommon, given similar routes of transmission (e.g. hMPV and RSV) (Manoha et al., 2007; Tomlinson and Cockram, 2003). Efficient transmission of hMPV in health-care facilities, with resulting serious outbreaks, has been reported (Boivin et al., 2007; van den Hoogen, 2007). Involvement of HCWs (as victims and/or vehicles of transmission to other HCWs or patients) in major respiratory infection outbreaks has also been frequently recognized (Brankston et al., 2007; CDC, 2005; Lee and Sung, 2003; Sartor et al., 2002; Sherertz et al., 2001).

hMPV/SARS-CoV co-infection is not associated with significant differences in clinical manifestations and outcomes compared to SARS infection alone (Lazar et al., 2004; Poutanen et al., 2003). However, the simultaneous occurrence of two outbreaks of respiratory infection raises the possibility of significant interaction between them. Upper respiratory symptoms caused by one pathogen may enhance

the dispersal of another through aerosol generation, resulting in 'super-spreading events' (source as 'super-spreaders' or 'cloud' patients—e.g. rhinovirus and *Staphylococcus aureus*) (Bassetti et al., 2003, 2005a,b; Sherertz et al., 2001; Sheretz et al., 1996). Whether hMPV and SARS-CoV interacted similarly is unclear, but co-transmission of hMPV in another major SARS outbreak ($n = 10$) (Poutanen et al., 2003), and the higher percentage of upper respiratory symptoms (e.g. runny nose and cough) reported by early SARS patients at PWH (22%, compared to 2–15% elsewhere), lend support to such a hypothesis (Bassetti et al., 2005a; Christian et al., 2004). In our analysis, cough and coryza were present in a higher proportion (22.6%) of hMPV co-infected individuals (vs. 15.9%), although the difference was not statistically significant. However, the overall scenario is likely to have been complex, as it involved contact/interactions between HCWs, patients, and certain environmental factors (e.g. use of nebulizer) (Lee and Sung, 2003; Lee et al., 2003; Yu et al., 2005).

hMPV might also have been a clinically insignificant 'by-stander'. Further analysis is limited by the retrospective design of the study, small hMPV case number, and lack of viral load data for comparison. Nevertheless, the results indicate that future mechanistic studies on viral–viral/viral–bacterial interactions (e.g. enhanced susceptibility, transmission, and severity) (Bassetti et al., 2003; Brundage, 2006; Sherertz et al., 2001), and studies of the frequency/role of co-pathogens in major infectious diseases outbreaks may be worthwhile (Villena et al., 2003; Yan et al., 2005).

Conflict of interest

None.

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