

Coronavirus Infections of Man Associated With Diseases Other Than the Common Cold

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About 14,000 paired sera, from patients with various types of acute infectious diseases with suspected viral origin, were screened by complement fixation against a wide set of viral antigens, including coronavirus OC43. A significant change in OC43 antibodies was recorded in 33 cases and a constant high titre, defined as a titre occurring in the respective age group in less than 1% of all sera examined, was found in 45 cases. On the basis of careful retrospective analysis of hospital case records it was concluded that in 28 cases with an increase of OC43 antibody titres, and in two with titre decrease, a disease could be associated with an acute coronavirus infection. In 16 cases the disease was dominated by respiratory symptoms. Eight of these patients, four children and four adults, had pneumonia. Three of the eight pneumonia patients had, however, another concomitant infection, too. Four patients had neurological symptoms, one had severe perimyocarditis, and in five cases fever was the only symptom recorded. Among the patients with a statistically significant high titre of OC43 antibodies, there were 14 cases where a suggestive association with a disease could be envisaged on the basis of hospital records. Five of these patients had pneumonia.

These results suggest that human coronaviruses, so far considered only as one group of causative agents of the common cold, may also be associated with other and more severe diseases in all age groups.

Key words: human coronaviruses, viral diseases, common cold, pneumonia

INTRODUCTION

The coronaviruses are a group of RNA-containing agents that have been associated with only minor respiratory illnesses of man but are known to cause a number of much more severe diseases in both laboratory and domestic animals. Members of the coronavirus group cause infectious bronchitis in chickens, neonatal diarrhea in calves, hepatitis in mice, and encephalomyelitis in swine, etc [McIntosh, 1974]. In man, coryza and nasal discharge have been the most prominent clinical symptoms associated with both natural and experimental coronavirus infections [Hendley et al, 1972; Higgins, 1974; Kaye and

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Dowdle, 1975; McIntosh et al, 1973]. Lower respiratory tract infections have rarely been described. Coronavirus strain 229E was recovered from nasal washes of two children with acute phase pneumonia [McIntosh et al, 1974]. During an outbreak of coronavirus infections among recruits, four cases with pneumonia showed seroconversion to OC43 virus antigen [Wenzel et al, 1974].

Problems in diagnosing human coronavirus infections are well known, namely the failure of most strains to grow in regular cell cultures used for virus isolation as well as the technical difficulties in preparing sufficient amounts of antigen other than OC43 for routine serological screening. Hence it is probable that our understanding of the real clinical significance of human coronavirus infections is still limited and superficial. Indeed, electron microscopic studies have revealed coronavirus-like particles in patient tissues or secretions in a variety of nonrespiratory disorders of man, such as acute hepatitis [Holmes et al, 1970], chronic active hepatitis [Zuckerman et al, 1970], endemic nephritis [Apostolov et al, 1975], acute nonbacterial gastroenteritis [Caul and Clarke, 1975], and lymphatic neoplasia [Arnold, 1978]. It is not known, however, whether these associations really reflect a causal relationship between the coronaviruses and the indicated clinical diseases.

In the routine diagnostic laboratory of this department more than 4,000 paired sera, from patients with various infectious diseases of suspected viral origin, are annually screened by the complement fixation test against a standard set of viral antigens. By using a wide set of antigens it is theoretically possible to discover new disease associations for viruses included in the set. We have had coronavirus OC43 antigen in this screening test since March 1977 and report in this paper a review of the clinical cases so far associated with serologically documented OC43 infections.

MATERIALS AND METHODS

Patients and Sera

From March 1977 to March 1980 paired serum samples from about 14,000 patients were screened at the routine diagnostic laboratory of this department. The patients in question were living mainly in the southern part of Finland, and in a great majority of cases they had been referred to a hospital because of a moderate or severe illness with suspected viral origin.

All sera were screened by the complement fixation (CF) test against the following agents: adenovirus, coronavirus OC43, coxsackie virus type B5, cytomegalovirus, herpes simplex virus type 1, influenza viruses A and B, measles, mumps, parainfluenza types 1 and 3, poliovirus type 2, respiratory syncytial virus, rotavirus, and varicella-zoster as well as chlamydia, mycoplasma pneumoniae, and toxoplasma.

Serological Tests for OC43 Antibodies

A crude OC43 virus preparation grown in mouse brain [Riski et al, 1977] was used as antigen in the CF antibody test carried out by a standard microwell method [Palmer and Casey, 1969; Hovi et al, 1979]. A titre of 1:8 or greater was considered positive. All of the serum pairs with a significant change or a high level of OC43 CF antibodies were subsequently tested with a control antigen prepared from uninfected mouse brain, in order to detect possible false-positive reactions. Most of the serum pairs with an antibody increase in the CF test were also tested with OC43 haemagglutination inhibition (HI) [Hovi, 1978] and with the haemolysis-in-gel (HIG) test [Riski et al, 1977]. Results ob-

tained with CF and HIG tests were in good correlation, whereas several cases showing a definite increase of CF antibodies had a constant low HI-titre.

Patients With Documented Coronavirus Infections

During the screening period all cases with significant (fourfold or greater) changes in OC43 CF antibody titre as well as those with a constant high titre were recorded. The limit for the high titre, 64 or 128, was determined separately for each age group by a computer analysis of the titre distribution of 8,448 serum samples tested at this department in 1978 [Ukkonen et al, in preparation]. Titres seen in less than 1% of all sera tested in the respective age group were considered high in this study (≥ 128 or ≥ 64).

The symptoms of 78 clinical cases thus collected were retrospectively carefully analysed according to case records obtained from the hospitals. Sera from patients with a constant high titre were included in the material only if the first sample had been taken later than 2 weeks after the beginning of the symptoms. Sera from high-titre patients with immunomodulating disease or treatment, such as chronic liver disease or kidney transplantation and steroid treatment, respectively, were excluded from the material. Neither were those high coronavirus titres included where evidence was obtained of another, more probable cause for the disease, eg, bacteremia in a case of pneumonia, nor those where high titres were measured against more than one other antigen. After this critical evaluation, sera from 14 cases with a constant high titre were accepted in the material. Because the temporal coincidence of the infection and the disease in these cases, as well as in cases showing titre decrease, cannot be proven, they are listed separately from those showing a titre increase.

RESULTS

Age and Sex Distribution

Patients showing an increase of coronavirus OC43 antibodies were distributed throughout the different age groups (Table I). However, there was a remarkable accumulation of cases in the groups below 7 years of age. More than one-third (11/28) of the titre increases but only 12% of serum pairs studied came from patients in these age groups. High titres that we considered significant, however, showed a different age distribution with only one patient younger than 7 years (Table II).

A great majority (20/28) of the patients with an increase of OC43 antibodies were male (Table I). Because of the low total frequency of documented coronavirus infections in the material and the way of collecting the material, this uneven sex ratio should not be taken as evidence for differential susceptibility to coronavirus infection-associated clinical disease.

Clinical Symptoms Associated With Coronavirus Infections

A majority of the cases in both antibody-increase and high-titre groups were respiratory infections (16/28 and 12/16, respectively). About half of these were common colds and most of the other patients had pneumonia. In two pneumonia cases there was a concomitant increase of mycoplasma antibodies, and in one case there was a rise of influenza B antibodies. All the pneumonias associated with coronavirus infections were, on the clinical grounds, "atypical," ie of suspected viral origin, and all the patients recovered rapidly. Most of the pneumonia patients had been healthy before. Coronavirus infections were, however, also seen in association with exacerbations of chronic respiratory diseases such as bronchial asthma and chronic bronchitis (Tables I and II) [see also Henigst, 1974].

TABLE I. Acute Symptoms in Patients With an Increase of OC43 Antibodies

No.	Acute symptoms	Age (years)	Sex	OC43 antibodies	Concomitant infection	Other diseases
Mainly respiratory						
1.	Common cold	0.5	M	<8- 32		
2.	Fever + otitis media l.a.	2	M	<8- 32		
3.	Common cold	4	M	<8- 32	Pulmonary tuberculosis	
4.	Common cold	4	M	<8- 64		Lymphoblastic leukaemia
5.	Common cold	13	M	8- 32		
6.	Common cold	19	M	8- 32		
7.	Common cold	54	F	<8- 16		
8.	Common cold	57	M	<8- 32		Chronic bronchitis and emphysema
9.	Pneumonia	2	M	8- 64		Spastic bronchitis
10.	Pneumonia	4	M	<8- 32		
11.	Pneumonia	9	M	8- 32	Mycoplasma pneumoniae 128-1024	
12.	Pneumonia	15	F	8- 32		Bronchial asthma
13.	Pneumonia	30	M	16-128		
14.	Pneumonia + pleurodynia	37	M	<8- 32		
15.	Pneumonia	61	M	8-128	Mycoplasma pneumoniae 16-256	
16.	Pneumonia	65	M	8- 64		Influenza B 8-32
Neurological						
17.	Meningitis serosa	3	M	<8- 32	Chlamydia <8-64	
18.	Common cold + convulsions	4	M	16-256		
19.	Fever and headache	17	F	8-128		
20.	Vertigo	51	F	32-128		
Other						
21.	Fever	1	F	8- 64	Parainfluenza 1 <8-32	Rheumatoid arthritis
22.	Fever	2	M	8- 64		
23.	Fever	2	F	<8- 16		
24.	Gastroenteritis acuta	9	F	8- 32		
25.	Fever	24	F	8- 32		
26.	Pericarditis acuta	33	M	8-128		
27.	Fever	35	M	8- 32		Diabetes mellitus
28.	Acute pancreatitis	59	M	16- 64		

TABLE II. Acute Symptoms in Patients With a High or Decreasing Titre of OC43 Antibodies

No.	Acute symptoms	Age (years)	Sex	OC43 antibodies	Concomitant infection	Other diseases
Mainly respiratory						
29.	Common cold	20	M	128-128	Pulmonary tuberculosis	
30.	Common cold	43	F	64-64		
31.	Common cold	49	F	64-128		
32.	Common cold	59	M	256-32		Chronic bronchitis and emphysema
33.	Pleurodynia and common cold	10	M	64-32		
34.	Acute bronchitis	55	F	128-128		Bronchial asthma
35.	Acute bronchitis	67	F	256-256		Bronchial asthma
36.	Pneumonia	9	M	128-64	Mycoplasma pneumoniae 512-256	
37.	Pneumonia	22	M	64-128		
38.	Pneumonia	54	F	128-128		
39.	Pneumonia	58	F	128-123		
40.	Pneumonia	72	M	128-128		Diabetes mellitus
Neurological						
41.	Fever and convulsions	4	M	128-128		Febrile convulsions 2 years before
42.	Fever and polyradiculitis	34	F	64-8		
Other						
43.	Thyreoiditis	25	F	128-128		
44.	Fever	34	F	128-128	Parainfluenza 1 128-128	

Six patients had neurological symptoms together with signs of an acute infection (Tables I and II). Like the pneumonias, neurological symptoms disappeared rapidly resulting in complete recovery of the patients. Some of the cases are described in more detail in the following.

Case 17. A 3-year-old boy had a typical serous meningitis with a moderate increase of CSF leukocyte counts (max 348 per mm³). Sixty-eight to ninety-four percent of the cells were lymphocytes. Cultures for bacteria from CSF specimens were negative. The neurological symptoms disappeared within 5 days under conventional care. This patient was found to have a concomitant increase in chlamydia CF antibodies, but did not receive antibiotics. The specificity of both corona and chlamydia antibodies was verified by tests using adequate control antigens.

Case 19. A 17-year-old female was referred to a hospital because of high temperature and severe headache. Meningitis was excluded, however, by absence of neck stiffness and normal findings on examination of CSF specimens. The patient recovered within a week.

Case 18. A 4-year-old boy who had been generally healthy before had not had acute infection-associated convulsions previously. After 2 days with typical symptoms of common cold he now had an attack of convulsions and unconsciousness and subsequent hemiparesis for a few hours. Electroencephalography after the attack showed mild, nonfocal changes. The patient recovered fully in a few days.

Case 21. This patient had a relatively severe perimyocarditis with typical signs and symptoms. Recovery was relatively slow with the patient still complaining of tiredness 3 months after the onset of the disease.

DISCUSSION

These results indicate that coronavirus infections associated with diseases other than the common cold are not rare. On the basis of the available data it is, however, very difficult to judge if the documented infections really caused the associated diseases. Infection by another unidentified agent concomitantly with the serologically documented coronavirus cannot be fully excluded, which, of course, is always a problem in the search for specific diagnosis for a disease or symptom with several potential etiological agents. Our goal in the present study has been to record all possible symptoms and diseases coinciding with documented coronavirus infection whatever the cause-and-effect relation of the two phenomena is. With this goal in mind it is not possible to use any control material — for instance, sera from healthy persons collected during the same time period — because subclinical coronavirus infections might have occurred in them as well. Concomitant virus isolations from the patients could have given further evidence for the possible cause-and-effect relation, but samples for virus isolation were taken from these patients only exceptionally and, furthermore, it would not be possible in practice to carry out attempts for coronavirus isolation in a material of this size. More straightforward approaches would be necessary to establish the potential role of human coronaviruses in the etiology of the observed diseases.

The “new” disease associations were, however, not unexpected. Coronavirus infection-associated pneumonia in man has been described before both in children [McIntosh et al, 1974] and in young adults [Wenzel et al, 1974]. Eight out of the 13 pneumonia cases (including both titre increases and high titres) in the present study were adults. Double infections were seen in four out of 13 pneumonia patients. Both mycoplasma and influenza B can cause pneumonia without concomitant coronavirus infection, and it remains unresolved what role, if any, the observed coronavirus infections had in the pathogenesis of these four pneumonia cases. Interesting were two cases with pleurodynia-like symptoms, so far almost exclusively associated with coxsackie virus infections, as well as a case with relatively severe perimyocarditis.

Neurological symptoms are associated at a certain rate with practically any viral infection and also comprised the other major group of symptoms in this study. No typical pattern of neurological symptoms could be ascribed to these cases. It seems possible, however, that a fraction of mild meningitis and radiculitis cases would be caused by coronaviruses.

It is also worth mentioning that in seven of 44 cases prolonged fever was the only symptom noted in the patients. In four cases no specific symptoms were recorded for the period showing OC43 titre increase. These cases may represent subclinical infections or, more probably, possible symptoms of common cold were not recorded as the records were kept for other reasons, eg, for follow-up after a surgical operation.

In conclusion, these observations suggest that human coronaviruses, apart from causing a considerable part of common colds in man, may also be involved in the etiology of more severe diseases in all age groups. These results also prove the usefulness of the wide set of viral antigens in serological diagnosis of viral diseases, as compared to a strategy of using only "the most probable viruses" as antigens. It is unlikely that before these studies a coronavirus antigen would have been included in a "pneumonia virus collection" or, even less probably, in a set used in cases of suspected viral infections of the central nervous system.

REFERENCES

- Apostolov K, Spasić P, Bojanic N (1975): Evidence of a viral aetiology on endemic (Balkan) nephropathy. *Lancet* 2:1271–1273.
- Arnold W (1978): Identical viral infections in four cases of malignant lymphoepithelioma. *Archives of Oto-Rhino-Laryngology* 220:287–289.
- Caul EO, Clarke SKR (1975): Coronavirus propagated from patient with non-bacterial gastroenteritis. *Lancet* 2:953–954.
- Hendley JO, Fishburne HB, Gwaltney JM Jr (1972): Coronavirus infections in working adults: Eight year study with 229E and OC43. *American Review of Respiratory Disease* 105:805–811.
- Henigst W (1974): Vorkommen von Antikörpern gegen Coronavirus (OC 43) in der gesunden Bevölkerung und bei Respirationstraktkranken. Occurrence of antibody against coronavirus (OC 43) in the healthy population and in patients with a disease of the respiratory tract. *Zentralblatt für Bakteriologie und Hygiene. Erste Abteilung Originale, Reihe A* 229:150–158.
- Higgins PG (1974): Viruses associated with acute respiratory infections 1961–71. *Journal of Hygiene* 72:425–432.
- Holmes AW, Deinhardt F, Harris W, Ball F, Cline G (1970): Coronavirus and viral hepatitis. *Journal of Clinical Investigation* 49:45a.
- Hovi T (1978): Nonspecific inhibitors of coronavirus OC43 haemagglutination in human sera. *Medical Microbiology and Immunology (Berlin)* 166:173–176.
- Hovi T, Kainulainen H, Ziola B, Salmi A (1979) OC43 strain-related coronavirus antibodies in different age groups. *Journal of Medical Virology* 3:313–320.
- Kaye HS, Dowdle WR (1975): Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. *American Journal of Epidemiology* 101:238–244.
- McIntosh K (1974): Coronaviruses: A comparative review. *Current Topics in Microbiology and Immunology* 63:85–129.
- McIntosh K, Ellis EF, Hoffman LS, Lybass TG, Eller JJ, Fulginiti VA (1973): The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *The Journal of Pediatrics* 82:578–590.
- McIntosh K, Chao RK, Krause HE, Wasil R, Mocugatt E, Mufson MA (1974): Coronavirus infection in acute lower respiratory tract disease in infants. *Journal of Infectious Diseases* 130:502–507.
- Palmer DF, Casey HL (1969): "Standardized Diagnostic Complement Fixation and Adaptation to Microtest." Atlanta: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, pp 1–56.
- Riski H, Hovi T, Väänänen P, Penttinen K (1977): Antibodies to human coronavirus OC43 measured by radial haemolysis in gel. *Scandinavian Journal of Infectious Diseases* 9:75–77.
- Wenzel RP, Hendley JO, Davies JA, Gwaltney JM Jr. (1974): Coronavirus infections in military recruits. Three-year study with coronavirus strains OC43 and 229E. *American Review of Respiratory Disease* 109:621–624.
- Zuckerman AJ, Taylor PE, Almeida JD (1970): Presence of particles other than the Australia-SH antigen in a case of chronic active hepatitis with cirrhosis. *British Medical Journal* 1:262–264.