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A six-year study of coxsackievirus B infections in heart disease

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SUMMARY

Virological examination of 385 patients with suspected heart disease and 26 with Bornholm disease over a period of 6 years suggested that Coxsackie group B virus infections were associated with at least half the cases of acute myocarditis and one third of the cases of acute non-bacterial pericarditis. Complement-fixation tests revealed only a few cardiac illnesses associated with other infections (influenza and *Mycoplasma pneumoniae*). No evidence of infection was found in chronic cardiac disease.

INTRODUCTION

Although viruses of Coxsackie group B have been those most commonly implicated in heart disease, good evidence as to their causative role is hard to find. The problem arises partly from difficulties in establishing the specific virological diagnosis. Since these viruses can be isolated from the throat for a few days, from faeces for a week or two (longer in children), or from pericardial fluid or myocardial biopsy tissue, virus isolation provides the quickest and simplest method of virological diagnosis. Often, however, the infection may have been present long enough for virus excretion to have ceased by the time faeces have been collected for testing, and few cases are seen early enough for virus to be detected in the throat. Less is known about the duration of infection in pericardial fluid or myocardium, but these specimens are rarely available. Serological tests of paired sera provide an alternative method of diagnosis, but are expensive and not technically simple. Detection of a fourfold or greater rising titre of neutralizing antibodies to one or more of the six viruses provides clear evidence of infection, but again the first blood has often been taken too late for the rise to be demonstrable. Since coxsackievirus infections are common and most persons possess antibodies from past infection, in the absence of a rising titre it becomes necessary to attempt to interpret the significance of the particular titres observed: the higher the titre, the greater the probability of recent infection, but the variable response of different individuals prevents certainty in the interpretation of the titres.

These tests have been applied to specimens from patients with suspected cardiac disease received in this laboratory for many years. Since the beginning of 1966, the introduction of an economical microneutralization test for antibodies allowed a more liberal attitude to the testing of sera (Bell & Grist, 1970). Preliminary reports of our findings have appeared elsewhere (Bell & Grist, 1972; Grist, 1972;

Bell & Grist, 1973). The present paper correlates the virological and serological findings over the period 1966–71 with the clinical diagnoses of the patients studied.

MATERIALS AND METHODS

The virological methods and sources of specimens and clinical information were described previously (Bell & Grist, 1970). It must be emphasized that our clinical classification of patients was based on information provided by many different clinicians when submitting specimens, supplemented by standard follow-up enquiry and where necessary by further correspondence. Patients were allocated to one of five groups: (*a*) Bornholm disease, without cardiac features; (*b*) acute non-bacterial myocarditis, including cases with evidence of both myocarditis and pericarditis; (*c*) acute non-bacterial pericarditis; (*d*) other cardiac diseases; (*e*) non-cardiac disease investigated because of initial suspicion of cardiac disease. This classification sufficed for our analytical purposes but cannot provide a detailed clinical profile of the illnesses associated with coxsackievirus infections.

RESULTS

Table 1 summarizes the findings in the different diagnostic groups according to the results of virological tests. Seven virological categories are distinguished – a finer breakdown than was used for the smaller figures in our preliminary reports (Bell & Grist, 1973). Virological categories I-IV carry the higher diagnostic significance and include 65 % of cases of Bornholm disease, 49 % of myocarditis, 30 % of pericarditis, and 10 % of other cardiac and non-cardiac disease groups (Table 2). Antibody titres of 128 (category V) and less (category VI) carry no diagnostic significance and are predominantly associated with 'other cardiac' and non-cardiac illnesses in the series. The relation between diagnostic groups and virological categories are illustrated in another way in Fig. 1 which shows the distribution of diagnoses associated with each virological category. Of patients in category I (virus isolation plus rising antibody titre) 64 % had Bornholm disease, which is known to be caused by coxsackieviruses or occasionally by echoviruses, the associated viruses being Coxsackie B type 2 (two cases), 3 and 4, echovirus type 6 (two cases) and 19; 27 % had acute myocarditis (Coxsackie A1, B2, B3); the only other patient in this group had rheumatic fever and carditis associated with echovirus 19 infection and ASO titre of 833. Almost equally strong evidence of current or very recent infection is provided by categories II and III, which are distributed similarly between diagnostic groups and are combined in Fig. 1: only 9 % of these patients had Bornholm disease but 44 % had acute myocarditis and 26 % acute pericarditis. Titres of 256 (category IV) provide probable but less certain evidence of recent infection, and the diagnostic profile of this category is shifted even further to the right in Fig. 1, intermediate between the previous categories and the non-significant categories V and VI which have been combined in the Figure. Category VII contained very few cases and was associated with all diagnostic groups except Bornholm disease; the virus isolations unsupported by serological evidence indicated faecal excretion but gave no indication of the duration or pathogenic signifi-

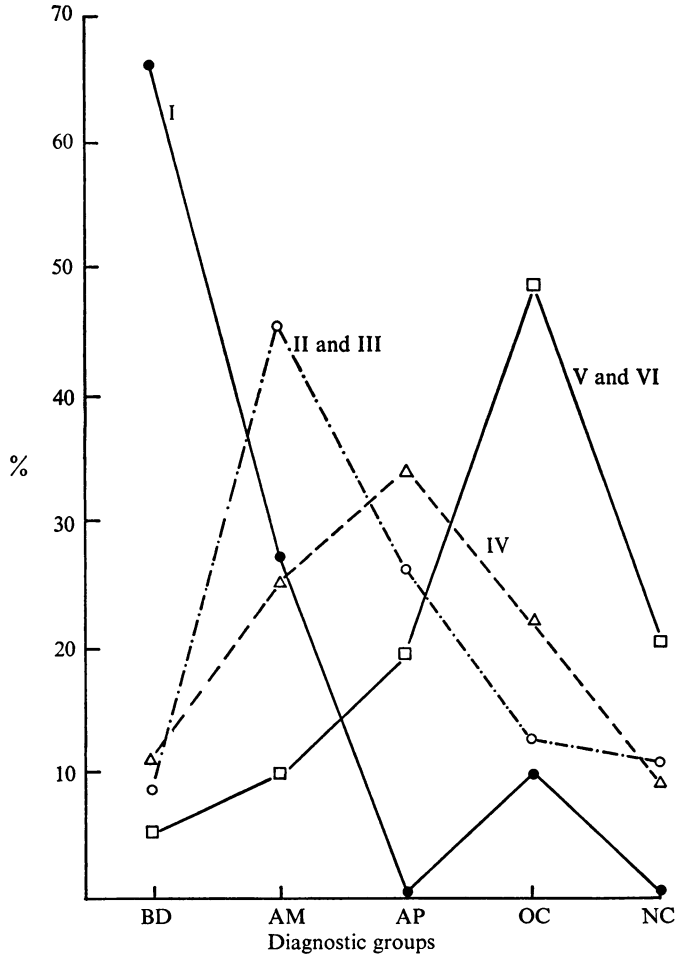

Fig. 1. Distribution of diagnostic groups among virological categories I–VI (see Table 1). BD, Bornholm disease; AM, acute myocarditis; AP, acute pericarditis; OC, other cardiac disease; NC, non-cardiac disease.

Table 1. *Distribution of patients by clinical diagnosis and virological category*

	Virological category							
Diagnostic group	Virus isolation and rising ab.* titre (I)	Rising or falling ab. titre (II)	ab. titre ⩾ 512 (III)	ab. titre 256 (IV)	ab. titre 128 (V)	ab. titre ⩽ 64 (VI)	Virus isolation only (VII)	Totals
Bornholm disease	7	2	2	6	2	7	0	26
Acute myocarditis	3	7	12	11	8	24	2	67
Acute pericarditis	0	3	8	15	12	47	1	86
Other cardiac disease	1	1	4	10	18	125	1	160
Non-cardiac disease	0	0	4	3	8	56	1	72
Totals	11	13	30	45	48	259	5	411

* ab. = antibody.

Table 2. *Percentages of virologically 'positive' (categories I–IV) cases in each year of study*

Diagnostic group	Positive in years indicated (%)						No.		
	1966	1967	1968	1969	1970	1971	Tested	Positive	(%)
Bornholm disease	0	67	86	67	50	40	26	17	(65)
Acute myocarditis	0	67	56	44	20	73	67	33	(49)
Acute pericarditis	0	9	23	37	9	56	86	26	(30)
Other cardiac and non-cardiac diseases	0	0	5	16	10	11	232	23	(10)
Total tested	24	35	76	99	74	103	411	99	(24)

Table 3. *Numbers and proportions of virologically 'positive' cases grouped by age and diagnosis*

Diagnostic group	Age-groups (years)			
	−10	−30	−50	51+
Bornholm disease	5/5* (100%)	8/11 (73%)	4/8 (50%)	0/2 (0%)
Acute myocarditis	6/11 (55%)	10/21 (48%)	12/23 (52%)	5/12 (42%)
Acute pericarditis	3/4 (75%)	5/23 (22%)	11/31 (35%)	7/27 (26%)
Other cardiac and non-cardiac diseases	3/13 (23%)	7/51 (14%)	8/86 (9%)	5/80 (6%)
Totals†	17/33 (52%)	30/106 (28%)	35/148 (24%)	17/121 (14%)

* Number 'positive' (categories I–IV)/total in group.
† Ages of 3 patients are not known.

cance of this – in fact, the types of virus were such as to suggest that most were coincidental and irrelevant to the associated illnesses: in myocarditis, echovirus 22 and poliovirus 3; in pericarditis, echovirus 8; in 'other cardiac disease' (Wolf–Parkinson–White syndrome with tachycardia), coxsackievirus A8; in non-cardiac disease, echovirus 30 (probably the cause of the aseptic meningitis in this case). The same rank order of virological positivity was shown by the diagnostic groups in each year of the investigation, with trivial exceptions (Table 2).

In order to evaluate the possibility of bias from unequal age-distribution in the diagnostic groups, the virological results were analysed by diagnosis and age-group. Table 3 shows that the frequency of infection was, as expected, highest in the youngest and lowest in the oldest patients, but in each age-group evidence of infection was more frequent in acute myocarditis and pericarditis than in other conditions (except Bornholm disease).

Table 4 shows the findings in cases classified as 'other cardiac diseases'. The same criteria of virological positivity have been used (categories I–IV). In none of these illnesses did the percentage with evidence of infection significantly exceed that in non-cardiac disease (7/72 = 10%); all three cases of acute rheumatic carditis had elevated ASO titres indicating streptococcal aetiology.

Although not part of the planned study, many of the sera were also tested by complement fixation with other antigens for routine diagnostic purposes. In view

Table 4. *Virological classification of cardiac cases other than acute myocarditis and pericarditis*

Diagnostic group	No. tested	No. 'positive' (virological categories I–IV)
Acute myocardial infarction	48	7 (15%)
Acute rheumatic carditis	14	3 (21%)
Other acute diseases	12	1 (8%)
Myocardial ischaemia	46	1 (2%)
Other chronic diseases	40	4 (10%)

Table 5. *Results of complement fixation tests for other infections*

Antigens	Numbers of patients tested per diagnostic group (numbers 'positive'* in parentheses)		
	Acute myocarditis	Acute pericarditis	Other cardiac diseases
Influenza A	34 (1)	44 (1)	74
Influenza B	31	37	71
Influenza C	22 (1†)	25	52
Adenovirus	39 (1)	51	88
Chlamydia group	47	68	124
R. burneti	50	69	131
M. pneumoniae	36 (1†)	50 (1)	85 (1)

* Titres $\geqslant 256$ or fourfold or greater rising or falling titres.
† Same case.

of interest in the possible aetiological significance of other viruses and mycoplasmas in cardiac disease, the main results are summarized in Table 5. The few serologically significant results were as follows:

Case 1. Forty-five-year-old woman diagnosed 'acute myocarditis': paired sera gave falling influenza A antibody titres of 32, < 8 indicating recent infection; Coxsackie B and other serological tests negative.

Case 2. Forty-six-year-old woman diagnosed 'influenza with acute pericarditis': paired sera gave rising influenza A antibody titres of 32, 512+; other complement fixation tests negative, but significant titres (256, 256) of coxsackievirus B1 and B2 neutralizing antibodies.

Case 3. Twenty-eight-year-old man diagnosed 'acute myocarditis': paired sera gave rising influenza C antibody titres of 32, 128 and high titres (256, 256) to *Mycoplasma pneumoniae*; Coxsackie B and other serological tests negative.

Case 4. Twenty-five-year-old man diagnosed 'acute pericarditis': paired sera gave falling CF antibody titres of 64, 16 with *M. pneumoniae* antigen; other tests not significant.

Case 5. Seventy-five-year-old man diagnosed 'myocardial infarction': paired sera gave rising CF antibody titres of 8, 32 with *M. pneumoniae* antigen; other tests not significant.

Case 6. Female infant diagnosed 'acute myocarditis': paired sera gave high

adenovirus group antibody titres of 256, 256, and adenovirus type 17 was isolated from stool; other complement fixation tests negative but rising titres (32, 128) of coxsackievirus B3 neutralizing antibody.

DISCUSSION

Virological tests can, with certainty or varying probability, demonstrate the presence of virus infection. They normally provide only partial and circumstantial evidence that the infection is responsible for associated illness, and absolute proof of aetiological relevance may be impossible in individual cases although it may be clearly demonstrable for groups of cases by epidemiological analysis. The high aetiological significance of the classical rising antibody titre (categories I and II of Table 1) arises from its demonstration of a close temporal relationship between the infective episode and the associated illness. Once elevated, coxsackievirus antibody titres decline variably over months or years, often with periodic anamnestic boosts from infections with other antigenically related enteroviruses, making interpretation of particular titres difficult: our data are presented in Table 1 in sufficient detail to allow readers to recalculate using different thresholds if desired. The serologically unsupported finding of an enterovirus in faeces provides unequivocal evidence of infection but does not distinguish coincidental, silent gut infection from an aetiologically significant condition. Because of these difficulties the present study is based on analysis of group data. Cases of serologically unsupported virus isolation (category VII) have not been counted as 'virologically positive' for the present analysis, though they were so included in our preliminary reports – they were few and their exclusion makes little difference to the outcome.

If a more rigorous threshold of positivity is applied to the analysis of data in Table 1, counting only categories I, II and III as positive, the percentages of cases above the threshold are 33 % for myocarditis (similar to 42 % for Bornholm disease, a known enteroviral disease which provides a 'positive control'), 13 % for pericarditis, and 4 % and 6 % respectively for 'other cardiac diseases' and non-cardiac diseases which provide almost 'negative controls'. Although the exact proportion cannot be defined, it is clear that Coxsackie B viruses make a major contribution to acute myocarditis and a significant one to acute pericarditis. This is true in each year of the study and in all age-groups (Tables 2 and 3). Cases occurred in all seasons of the year with lowest incidence in the first quarter and highest incidence of cases with rising antibody titres in the second and third quarters of the year corresponding to the seasonal distribution of enterovirus infections (Bell & Grist, 1973). Koontz & Ray (1971) reported similar findings from Seattle, with evidence of Coxsackie B virus infection in 44 % of myopericarditis cases and 11 % of non-cardiac illnesses but with no seasonal variation. Rising antibody titres were relatively less frequent in our cardiac cases than in Bornholm disease, suggesting that symptomatic cardiac involvement is a somewhat later complication of coxsackievirus infection than that of skeletal musculature.

Recent reviews by Lerner & Wilson (1973) and Abelmann (1973) show that while viral carditis is usually benign the illness may be serious and sometimes

severe, occasionally fatal and sometimes complicated by persisting myocardial inflammation, late sequelae including impaired heart function, E.C.G. abnormalities, and occasionally constrictive pericarditis. Speculation that coxsackievirus infection might initiate chronic cardiomyopathy and even valvular heart disease was strengthened by immunofluorescent demonstration of coxsackieviral antigens in human and experimental cardiac tissues (Burch *et al.* 1967; Burch *et al.* 1968; Burch & Colcolough, 1969). Our own serological studies did not suggest active or recent coxsackievirus infection in chronic cardiac diseases, nor did we find elevated Coxsackie antibody titres in five other cases of hypertrophic obstructive cardiomyopathy tested for Professor J. F. Goodwin. However, persisting high antibody titres would not be expected many years after an infection of average short duration if the chronic disease resulted not from persisting virus infection but from some other continuing process, possibly immunological, initiated by the virus infection.

Other viruses may also cause myopericarditis (Abelmann, 1973), including arboviruses which are common in many tropical areas where they may be important causes of acute, relapsing and chronic heart disease (Obeyesekere & Hermon, 1972). The serological results reported in our present paper suggest that, compared with Coxsackie viruses, the contributions of influenza, adenovirus, chlamydia, Q fever and *Mycoplasma pneumoniae* to myocarditis and pericarditis are small. One of the two patients with evidence of influenza (case 2) and the child with adenovirus type 17 infection (case 6) also showed serological evidence of coxsackievirus infection as an alternative cause of heart disease. Of the three patients with evidence of *M. pneumoniae* infection, one (case 4) also had serological evidence of influenza C, while in case 5 the illness was myocardial infarction. The lower frequency with which coxsackievirus infection was associated with pericarditis as compared with myocarditis in our series suggests that agents other than coxsackievirus might be implicated more often in pericarditis, though this was not supported by the relative frequency of infections diagnosed by complement fixation in myocarditis and pericarditis (Table 5).

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