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The routine serological investigation of cases and contacts of rubella

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SUMMARY

The results of testing sera from 111 patients with rubella-like illnesses and 283 contacts of patients with rubella-like illnesses are described. A sensitive haemagglutination-inhibition test was used in conjunction with fractionation of serum proteins when this was indicated. It was concluded that the testing of serum protein fractions for IgM and IgG rubella antibody greatly increased the effectiveness of laboratory diagnosis. Evidence is presented that during the study period subclinical rubella was relatively uncommon in adults and that the accuracy of clinical diagnosis was high.

INTRODUCTION

There is a variety of laboratory methods available for investigating a patient thought to be suffering from rubella. If the patient is a pregnant woman it is of particular importance that the methods used are not only accurate, but also capable of being carried out on such specimens as are likely in practice to be available. Virus isolation or direct immunofluorescent staining of virus antigen in infected cells from the throat may be negative in a significant proportion of cases presenting within a week of the onset (Haire & Hadden, 1972). Serological tests have proved to be of greater value. The rubella haemagglutination-inhibition (HAI) test is the most rapid, the simplest and the least expensive method of measuring antibodies to rubella virus. However, a predictable limitation of this test is that many patients are likely to present at a time when rubella HAI antibodies have already reached their highest titre and therefore diagnostic rises in antibody cannot be demonstrated. We have previously described a gel filtration procedure for detecting rubella specific IgM antibodies (Pattison & Mace, 1973, 1975) which allows early convalescent rubella HAI antibodies to be distinguished from those acquired in the remote past. In this report we describe 3 years' experience of the routine use of the rubella HAI test and the more extensive gel filtration technique for the confirmation of recent rubella infection.

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MATERIALS AND METHODS

Sera

During the entire period of the study (January 1972–August 1974) sera from suspected cases of rubella were submitted from the Middlesex Hospital, London W.1, West Middlesex Hospital, Isleworth, Middlesex, St Ann's General Hospital, Tottenham, Rush Green Hospital, Romford, Essex, Barking Hospital, Barking, Essex, and certain General Practitioners in North West London. During the last 4 months of the study sera were also submitted from patients attending The London Hospital Group.

Serological tests

A sensitive rubella HAI test incorporating overnight reaction of virus and test serum was used throughout (Pattison & Mace, 1975). Gel filtration with Sephadex G-200 was performed according to the techniques of Pattison & Mace (1975). The rubella HAI antibody of early convalescent sera elutes in 2 peaks by this technique and the Peak 1 HAI activity is taken as an indication of the presence of specific rubella IgM antibody. Full quantitative and qualitative validation of this procedure has been previously described (Pattison & Mace, 1975).

RESULTS

During the period of the study 394 patients were investigated in order to confirm or exclude recent rubella. One hundred and eleven of these had presented because of a recent rubella-like illness. The remaining 283 patients presented because of contact with suspected cases of rubella.

Patients presenting with rubella-like illnesses

Of the 111 patients presenting with rubella-like illnesses the diagnosis of recent rubella was confirmed in 103 (Table 1). Diagnostic rises in titre could be demonstrated in only 33 of these cases because in the majority there was already a high titre of specific antibody in the first serum. The ability to demonstrate rising titres of antibody is clearly dependent on the time after the onset of the rash at which the first serum is taken (Table 1). Thirty of the patients presented within 4 days of the onset of the rash and for 27 of them (90 %) it was possible to demonstrate a diagnostic rise in titre. However, such rises could only be demonstrated in 6 of the 31 (19 %) cases who donated their first serum 4–7 days after the onset of the rash. It was not possible to demonstrate rising titres in any of the 42 cases in which the first serum was donated more than 7 days after the onset of the rash.

In the 70 cases in which diagnostic rises in titre could not be demonstrated the clinical diagnosis of rubella was confirmed by fractionating the sera and demonstrating the presence of specific antibodies of the IgM class (Table 1). The reliability of this fractionation procedure as applied to late post-rash sera was confirmed by the finding that antibodies of the IgM class were present in the second sera of all 33 cases in which diagnostic rises had been demonstrated (Table 1) and in none of 100 high titre sera from patients without a history of recent rubella

Table 1. *Results of laboratory investigation of patients who presented with a rubella-like illness*

Days after onset of rash when first serum taken	Recent rubella confirmed by: 4-fold or greater rise in HAI titre	Recent rubella confirmed by: Static HAI titres but rubella specific IgM present	Recent rubella *not* confirmed (rubella specific IgM absent)†
Less than 4	27	3	2
4–7	6	25	3
8–14	0	21	3
15–21	0	7	0
22–28	0	7	0
29–56	0	5	0
57–84	0	2	0
Total	33*	70	8

* Specific rubella IgM was also demonstrated in the second serum samples of all these patients.

† Specific IgM antibody was not detected in sera which had been taken within 3 weeks after the onset of the rash.

Table 2. *Results of laboratory investigation of patients who presented because of recent contact with a rubella-like illness*

Status on presentation	No. of patients	Serological evidence of recent rubella: Rising HAI titres	Serological evidence of recent rubella: Presence of specific IgM alone	Recent rubella excluded by: Rubella HAI titrations alone	Recent rubella excluded by: Specific IgM determinations
Seronegative	35	12*	—	23	—
Seropositive	248	0	2	84	162
Total	283	14		269	

* Specific IgM antibody was detected in the second serum of all these patients.

(Pattison & Mace, 1975). It is of interest that the specific IgM test was able to confirm recent rubella infection in 7 sera that had been taken more than 1 month after the onset of the rash. In each instance the patient had presented to a physician at the time of the original illness when a clinical diagnosis of rubella was made. Laboratory confirmation was only sought when it was subsequently realized that the patients were pregnant at the time of the rubella-like illness.

Patients who presented because of recent contact with rubella-like illness

Serological investigation was carried out on specimens received from 283 individuals who presented because of recent contact with patients who had rubella-like illnesses (Table 2). Only 35 (12 %) were found to be seronegative on initial rubella HAI testing and 12 of them subsequently developed clinical rubella. Second sera obtained from these 12 patients showed the presence of specific rubella antibodies and in every instance some of this antibody was shown to be

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of the IgM class. The remaining 23 individuals were still seronegative one month after contact.

The majority of the patients (248 out of 283) were found to be seropositive on initial testing and to exclude the possibility that they had suffered recent *subclinical* rubella the following procedure was adopted. (N.B. The titres which define each category will vary according to the sensitivity of the rubella HAI test used.)

(1) Patients with rubella HAI titres of 1/20 or greater who presented within 10 days of a non-family contact with rubella were regarded as immune at the time of the contact.

(2) Patients with low titres of antibody (1/160 or less) in a first specimen taken more than 10 days after a non-family contact or any time after a close family contact were requested to donate a second serum sample so that an attempt could be made to demonstrate a diagnostic rise in antibody titre. Specific IgM tests were not carried out on these low titre sera unless an antibody rise was detected.

(3) Sera from patients with high titres of antibody (1/320 or greater) who presented more than 10 days after non-family contact or any time after close family contact were fractionated and tested for the presence of specific rubella IgM antibody.

With the sensitive rubella HAI test used here the majority (164 out of 248) of the seropositive individuals who had had recent contact with a rubella-like illness fell into category 3, and therefore their sera required fractionation. Specific rubella IgM was detected in only 2 of these 164 sera. One of these patients presented to her doctor and donated a serum on the day her 3-year-old child developed a rash which was clinically diagnosed as rubella and the other presented 10 days after being in contact with an isolated case of suspected rubella. Neither patient had any other known contact with rubella or a rubella-like illness in the previous 3 months.

Cases of clinical rubella confirmed by serological testing

Details of the 115 confirmed cases of clinical rubella are shown in Table 3. Not surprisingly the largest group consisted of the 67 pregnant women. The 28 women who were not pregnant were investigated because of the nature of their occupation (7 nurses, 4 medical students and 3 teachers), or because they suffered complications of the disease (3 arthralgia and 2 thrombocytopaenic purpura) or simply because they had presented with fever and a rash for diagnosis (9 patients). Eleven of the patients were adult men aged 20–34 years who were investigated because of their occupation (5 medical students), the occurrence of complications (5 with arthralgia) or in the final case as part of the investigations into the cause of a rash in the patient's pregnant wife. Nine of the patients were children aged 2–9 years who had been admitted to hospital with fever and a rash for diagnosis.

The clinical features of the 115 confirmed cases of clinical rubella are shown in Table 4. Only 2 of the 115 patients did not have a rubelliform rash as part of their clinical illness. These 2 had cervical lymphadenopathy associated with an upper

Table 3. *Cases in which a diagnosis of recent rubella was confirmed*

Category	Age	No. of patients
Pregnant women	19–37	67
Other adult women	16–29	28
Adult men	20–34	11
Children	2–9	9
	Total	115

Table 4. *Clinical features of the* 115 *confirmed cases of rubella*

	No. of patients
Rubelliform rash	113
Cervical lymphadenopathy	88
Upper respiratory tract signs	40
Joint involvement	26
Purpura	2
History of recent contact with a rubella-like illness	24

respiratory tract infection and in both cases other members of their families developed typical rubella with a rash within the next 4 weeks. Other features of clinical rubella were not recorded in case notes as consistently as the presence or absence of a rash. However, cervical lymphadenopathy was known to be present in 88 cases (77 %), involvement of the upper respiratory tract and/or conjunctivitis in 40 (35 %) and joint involvement (most commonly arthralgia of the joints of the wrist and hand) in 26 (23 %). Only 24 of the 115 could give a history of recent contact with a case of suspected rubella.

Subclinical rubella

As described above, during the entire period of the study only 2 cases of subclinical rubella were found during the routine investigation of sera submitted by physicians whose patients reported recent contact with rubella. However, it is possible that susceptible patients might be in contact with rubella without realizing it and in the absence of any subsequent illness would not present to a doctor. Thus, subclinical rubella might pass largely unrecognized. The following investigations were therefore undertaken in an attempt to assess the occurrence of unrecognized subclinical infection in adult women.

One hundred sera with rubella HAI titres of 1/640 or greater were selected from 984 sera submitted for routine rubella antibody screening from ante-natal and staff health clinics and were fractionated and tested for the presence of specific rubella IgM. Not a single case of subclinical rubella was detected. During the same period 7 other patients attending the same clinics presented with a rubella-like illness which was confirmed as being due to rubella virus infection.

A further attempt to find subclinical cases of rubella was made by following up those women who had been reported, on the basis of the absence of rubella HAI antibodies, as non-immune during the first trimester of their pregnancies. Just

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before being given rubella vaccine 2–5 days post-partum they were again screened for rubella antibodies. There was on average a 6-month interval between the paired sera. Two out of the 59 women studied who had titres of less than 1/10 in the first trimester had positive titres (both 1/2560) immediately post-partum. On questioning, both patients gave a history of rubella-like illness within the previous 3 months. One was correctly diagnosed as rubella at the time but in the other the rash was considered to be due to an allergy to mussels. Neither can therefore be regarded as a truly subclinical case although the latter would have escaped attention had this special study not been in progress.

DISCUSSION

In the present series of cases a diagnostic rising titre of rubella HAI antibodies could only be demonstrated in 33 out of the 103 rubella patients who presented initially with a rubella-like illness and in only 45 out of 115 (39%) if the cases who presented initially because of contact with rubella are included. In the remaining 70 cases rubella HAI antibodies were already present to high titre in the first serum specimen examined and confirmation of the clinical diagnosis could only be achieved after testing for specific rubella IgM. Thus it was clearly established that the use of a technique for separating serum immunoglobulins considerably increased the ability of the laboratory to confirm or exclude a diagnosis of recent rubella.

However, measurement of rubella specific IgM should in no way be regarded as capable of entirely replacing accurate clinical and epidemiological assessment of each individual patient. Work still in progress on the follow-up of some of the cases described here indicates that specific rubella IgM may still be detectable in some individuals for 10–12 months after the acute episode. Therefore the mere finding of such activity cannot pin-point the acute episode any more accurately than some time within the previous 12 months. Moreover, since occasionally rubella specific IgM may no longer be detectable in a serum taken as early as 22 days after the onset of the rash (Pattison & Mace, 1975), the failure to find such activity in a serum taken from a patient during the first trimester of pregnancy does not guarantee that rubella virus infection has not occurred during the current pregnancy.

It is usually considered that only half the cases judged to be rubella on clinical grounds alone are confirmed by laboratory testing (Forbes, 1969; Emond, 1971). On the other hand, in the present series the diagnosis of rubella appears to have been much more accurate since serological evidence of recent rubella was found in 93% of the cases presenting with a rubella-like illness. This may have been due partly to the fact that most of the patients studied in this report were adults. Clinically atypical cases however may be overlooked; it was noted that the only 2 cases of rubella without a rash occurred in patients who transmitted the infection to other members of their families in whom the clinical disease was typical. Without these typical cases the 2 atypical ones would never have been suspected.

During the 32 months of the study in which 248 cases of possible contact were

investigated only 2 cases of subclinical rubella were identified, both solely on the basis of the presence of specific IgM in their serum. However, this is not a true reflexion of the occurrence of subclinical rubella in the population since in routine practice it is contact with suspected rubella that initiates a search for such cases. In this series only 21% of cases with confirmed *clinical* rubella gave a positive history of contact, and therefore one would expect to identify only a fifth of subclinical cases if a history of contact with rubella is taken as a starting point for investigation. However, a deliberate search for subclinical cases among women attending an ante-natal and a staff health clinic failed to reveal a single case, indicating that in the population studied the rate of subclinical rubella in 1973 was less than 1 per 1000. In the same population in 1973 there were 7 cases of clinical rubella per 1000, giving a ratio of clinical to subclinical rubella of at least 7 to 1. A figure of 9:1 was found in the 15–21 year age group on St Paul Island which contrasted with a ratio of 1:1 in young children during the same outbreak (Brody, 1966). However, the P.H.L.S. Rubella Working Party found a ratio of only 2 clinical to 1 subclinical case in an adult population (Report, 1968). In spite of this latter report the experience gained from the study described here suggests that subclinical rubella in adult fair-skinned women should be regarded as a rare consequence of reported contact with suspected rubella. If serological evidence of subclinical infection is found the timing of the actual episode of infection may be difficult unless a rising titre of rubella antibodies can be demonstrated. Moreover, a recent report (Peckham, 1974) suggests that the risk to the foetus is considerably less if subclinical as opposed to clinical rubella occurs during the first trimester of pregnancy, although the possible sparing effect of prophylactic immunoglobulin and the emergence of defects which manifest late remain to be assessed. The present series illustrates that the commonest conclusion reached after testing patients who reported a rubella contact was that there had been no recent infection with rubella virus. The importance of measuring specific rubella IgM in this context is that it gives more precise evidence for drawing this conclusion than rubella HAI tests alone, and is particularly useful in patients who present more than 10 days after contact and at that time have high titre rubella HAI antibody.

The procedure we have outlined above for the exclusion of recent subclinical rubella necessitated the fractionation of 164 out of 248 sera from patients who were found to be seropositive after being in contact with a possible case of rubella. Moreover, we have routinely fractionated all early convalescent sera from the 123 patients with possible clinical rubella. This includes those 45 (Tables 1 and 2) in whom a rising titre of antibody was demonstrated since a specific IgM determination provides a valuable confirmatory test especially if the patient is pregnant. In all we fractionated serum specimens from 287 (73%) of the 394 patients and contacts investigated. We feel that these tests were essential to ensure that the combined result of clinical and laboratory evaluation was more accurate than clinical judgement alone.

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