Middle Articles

HOSPITAL TOPICS

Clinical Virology: A Year's Experience

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Brit. med. J., 1967, 3, 609-612

Advances in virology over the last decade, mainly resulting from the renaissance of tissue culture techniques and the application of these techniques to the field of diagnostic virology, have led to the discovery of many viruses which have been shown to cause infectious disease.

Hitherto, most diagnostic virology in England and Wales has been performed by the Public Health Laboratory Service. However, the increasing demand for virological investigations has resulted in a few hospitals developing clinical virology laboratories of their own, and it seems possible that this trend may increase. It is important to evaluate such services from time to time in order to see whether or not a useful service is provided, to whom it is of value, and, almost equally important, for both clinicians and virologists to realize its shortcomings and recognize when certain types of investigation are uneconomic or of doubtful value.

This paper presents our first year's experience (21 January 1966 to 20 January 1967) in developing and running a clinical virology laboratory at St. Thomas's Hospital.

Organization and Function of the Laboratory.—The laboratory staff consists of a microbiologist (J. E. B.), assistant microbiologist (E. A. K.), research fellow (J. M. B.), two technicians, a glassware assistant, and a secretary. The function of the laboratory is to provide diagnostic services to the hospital, to conduct research into clinical and epidemiological problems associated with virus infections, and to train both graduates and technicians in diagnostic procedures and research methods. Studies on infection by rubella and herpes simplex viruses have been in progress during the course of the year, the results of which are being presented elsewhere.

Materials and Methods

Selection of Cases.—Patients with possible virus infection were referred for investigation. Before investigations were begun clinical details were discussed; then the microbiologist, whenever possible, visited the wards to see patients and obtain or advise on specimens to be collected. Outpatients were often referred from their respective clinics to the laboratory, where clinical details and specimens were obtained and visits for further specimens, if necessary, arranged.

Identification of Viruses

1. **Isolation.**—Specimens were prepared and processed immediately after arrival in the laboratory (Lenette, 1964), after which they were inoculated into tissue culture. Specimens were then

"snap" frozen and stored at -70°C . Cultures of secondary rhesus monkey kidney (M.R.C. Hampstead) and suspensions of primary vervet (M.R.C. Hampstead) and patas monkey kidney cells (Wellcome Research Laboratories) were supplied regularly. In addition, HeLa, WI-38, and RK13 cell cultures were propagated in the laboratory. Reserves of these cells were kept frozen in liquid nitrogen (Linde LNR-25-B, Union Carbide). Rubella virus was detected by the appearance of cytopathic effect in RK13 cell cultures (McCarthy, Taylor-Robinson, and Pillinger, 1963) and the presence of interference phenomena in patas or vervet cell cultures (Parkman, Buescher, and Artenstein, 1962). Specimens from patients with possible enterovirus infections which failed to show cytopathic effect in tissue culture were inoculated into suckling mice in order to detect the presence of Coxsackie A viruses. The identity of all isolates was confirmed by neutralization tests employing virus-specific rabbit antisera. In addition, fertile hens' eggs and an agar-gel diffusion system were kept readily available for the investigation of patients with possible infection with variola virus.

2. **Serology.**—Sera from patients with respiratory infections were tested against the following antigens by complement fixation: influenza A, B, and C; adenovirus; respiratory syncytial virus; parainfluenza 1, 2, and 3 viruses; *Rickettsia burnetii*; psittacosis; and *Mycoplasma pneumoniae*. Overnight fixation at $+4^{\circ}\text{C}$ was employed (Bradstreet and Taylor, 1962), a microtitre apparatus (Sever, 1962) being used. Antibody titres in sera from pregnant women exposed to or contracting rubella were determined by neutralization (Dudgeon, Butler, and Plotkin, 1964) and complement-fixation tests (Sever *et al.*, 1965). Sera from patients from whom enteroviruses were isolated were, whenever possible, tested for significant rises (fourfold or greater) in antibody titre to the particular virus isolated. Serological evidence of mumps infection was detected by complement fixation, and measles by both complement fixation and haemagglutination inhibition. During the time that influenza was epidemic (January and February 1966) patients with possible influenza virus infection who failed to show significant rises in complement-fixing antibody titre were tested by haemagglutination inhibition (A2Eng. 1964).

Results

From 306 patients referred for virological investigations 741 specimens were obtained. The following patients were excluded: those who were subsequently shown to have disease which was non-viral (13); those from whom adequate specimens were not obtained—for example, because of inability to obtain "convalescent" sera when a serological diagnosis was to be attempted (11); and those referred for estimation of antibody to rubella virus when exposed to possible rubella in early

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pregnancy (17). Attempts at diagnosis were completed on 262 patients. An aetiological agent was identified in 96 (36.6%) of these cases. A diagnosis by virus isolation alone was made in 56 (58%) of the 96 cases, by serology in 19 (20%), and by both serology and virus isolation in 21 (22%). However, serum was not obtained from 38 of these patients who attended the departments of venereology and dermatology with vesicular lesions of the skin or mucous membranes caused by herpes simplex virus, nor from five infants with acute respiratory infection from whom respiratory syncytial virus (2 cases), adenovirus (2 cases), and parainfluenza 3 virus (1 case) were isolated.

Table I lists the viruses detected and the principal clinical syndromes with which they were associated. The number of patients referred by different hospital departments and the results of virological investigations on them are shown in Table II. The high proportion of patients referred by the venereology department was partly caused by a survey on patients with herpes genitalis. Herpes simplex virus was isolated from 28 (38%) of 73 patients with clinical herpes genitalis, no other virus being isolated. Though the clinical diagnosis of herpes genitalis may be simple enough when typical vesicles are present, once ulceration has occurred other conditions have to be differentiated. The most important of these is syphilitic chancre, but such diseases as chancroid, lymphogranuloma venereum, candidiasis, and Behcet's syndrome may also have to be considered. Concomitant serological tests (Wassermann reaction and Reiter's protein complement-fixation test) did not reveal any cases of primary syphilis in this series. The detailed results of this investigation are being published elsewhere.

Fifty-two members of the hospital staff, most of whom were nurses, were referred for virological investigation. The high incidence of infectious disease in general, and of acute respira-

tory and exanthematous disease in particular, is characteristic of communities of young adults living in close quarters. It was possible to obtain specimens from most of these patients within 72 hours of the onset of symptoms, this probably contributing toward the high diagnostic rate (46%) in this group. Rubella was prevalent among the nursing staff from March to April 1966. Since rubella often presents atypically and because of the relation between maternal rubella and congenital malformation, it was thought to be important to confirm the diagnosis of rubella virologically in nurses, not only for their own future reference, but also in order to exclude them from places where they might have contact with pregnant women (for example, children's wards, mothercraft clinics, etc.) until after the period of virus excretion—up to 14 days from the onset of rash (Krugman and Ward, 1964; Green *et al.*, 1965).

The paediatric department has 32 medical beds, but is also responsible for the care of newborn babies. A diagnosis was made on 20 (44%) of 45 cases investigated. The admission rate for acute respiratory infections, which is frequently high during winter, often coinciding with the prevalence of respiratory syncytial and parainfluenza viruses in the community (Chanock and Parrott, 1965), showed no marked increase during the winter months of the investigation. It is of interest that studies on 15 infants with congenital defects or neonatal infections revealed six cases of congenital rubella, five infants having many of the characteristic features of the rubella syndrome (Table III). These infants also possessed some of the additional features described as part of the "expanded" rubella syndrome noted after the large rubella epidemic in the U.S.A. in 1964 (American Pediatric Society and Society for Pediatric Research, 1965). Five of these six infants were of West Indian origin, there being a history of maternal rubella in only one of these cases. In hospital these infants were

TABLE I.—Principal Clinical Syndromes with Associated Viral Diagnosis

Clinical Category	Influenza A	Influenza B	Para-influenza	Adeno-virus	R.S.V.	M. pneumoniae	E.C.H.O.	Cox-sackie	Polio-virus	Rubella	Measles	Mumps	Herpes Simplex	Total
Acute respiratory infection { Adults .. Children ..	8	1	1 (P 3)	2 (Ad 1)	4	2	2 (E 16)	1 (B 2)					1	15
Exanthema ..							1 (E 16)	*3 (A 16)		13	2			19
Acute C.N.S. infections ..							1 (E 14)		1 (P 1)	6				2
Congenital malformations ..														6
Genital herpes ..													28	28
Non-genital herpetic lesions ..													5	5
Ulcerative stomatitis ..													2	2
Bornholm disease ..							2 (E 1)	1 (B 2)						3
Conjunctivitis/kerato-conjunctivitis ..				2 (Ad 3, 10)									3	5
Mumps ..												4		4
Total ..	8	1	1	4	4	2	6	5	1	19	2	4	39	96

* Hand, foot, and mouth syndrome. Serotypes identified are given in parentheses.

TABLE II.—Departments from which Patients were Referred, Together with Virus Infections Diagnosed

Department	No. of Cases	No. of Diagnoses	Influenza A	Influenza B	Para-influenza	Adeno-virus	R.S.V.	M. pneumoniae	E.C.H.O.	Cox-sackie	Polio-virus	Rubella	Measles	Mumps	Herpes Simplex
Venereology ..	73	28 (38.3%)													28
Staff ..	56	26 (46.4%)	8						2 (E 16)	1 (B 2)		12	1		2
Paediatric ..	45	20 (44.4%)			1 (P 3)	2 (Ad 1)	4		2 (E 14 and 16)			7		2	2
General medicine ..	53	11 (20.8%)		1				2	2 (E 1)	1 (B 2)	1 (P 1)			2	2
Dermatology ..	15	6 (40.0%)								3 (A 16)			1		2
Ophthalmology ..	20	5 (25.0%)				2 (Ad 3, 10)									3
Total ..	262	96 (36.6%)	8	1	1	4	4	2	6	5	1	19	2	4	39

TABLE III.—Principal Clinical and Virological Features in Children with Congenital Rubella

Case No.	History of Maternal Rubella	Age at Investigation	Principal Clinical Features	Virus Isolation		Neutralizing Antibody	
				Site	Duration of Excretion	Baby	Mother
1	—	6 months	Bilateral cataract, V.S.D.	+ (T.S.)	7 months	1:64	1:48
2	—	1 month	Neonatal hepatitis, splenomegaly, low birth weight	+ (T.S.)	2 "	1:10	N.T.
3	—	3 years	Bilateral perceptive deafness	Not tested	—	1:64	1:8
4	+	1 week	Apparently healthy baby	+ (T.S.)	Not known	1:48	1:64
5	—	2 weeks	Hepatosplenomegaly, cloudy corneas, low birth weight, malabsorption	+ (T.S.; C.S.F.; C.S.; urine)	*2 months	1:24	1:48
6	—	6 days	Bilateral cataract, V.S.D. and P.D.A.	+ (T.S.; C.S.)	*1 month	1:48	1:48

* Still excreting virus. C.S. = Conjunctival swab. T.S. = Throat swab. V.S.D. = Ventricular septal defect. P.D.A. = Patent ductus arteriosus.

isolated and tested for virus excretion at regular intervals. When at home, mothers were warned not to expose their infants to women who might be pregnant, until such time as tests showed that excretion had ceased. Nevertheless, one infant (Case 4) infected ten nurses during her stay in hospital. No virus other than rubella was recovered from any of the infants with neonatal infection or congenital malformation.

In relation to the number of beds available (298) a disproportionately small number of patients (53) were referred for investigation by the general physicians. Twenty of these patients had had symptoms for one week or longer, thereby making it difficult to be certain that any virus which was isolated was responsible for the patient's illness. Of the 11 patients in this group in whom serological evidence of infection was found or from whom virus was recovered, three had Bornholm disease, and from their stools E.C.H.O. 1 virus (two cases) and Coxsackie B2 virus (one case) were isolated. However, these patients showed neither rising nor elevated antibody titres to the respective viruses recovered, making the relation between virus isolation and illness tenuous.

A diagnosis was made in 19 patients with exanthematous disease (Table I). Generally, apart from cases of rubella, virus studies were not attempted on patients in whom clinical diagnosis was obvious, but only on those who presented a diagnostic problem; examples being a woman of 63 with a respiratory infection, high fever, and maculopapular rash who developed a significant rise in measles antibody titre during the course of her illness, and a child aged 6 months with fever and maculopapular rash from whose throat E.C.H.O. 16 virus was isolated.

Studies were conducted on 17 women exposed during early pregnancy to cases of possible rubella. Four of them were investigated within seven days of exposure, two having been exposed to a child with rubella while visiting their children in hospital. All of these patients were found to have neutralizing antibody to rubella virus, indicating previous infection. They were therefore immune to subsequent infection (Brody *et al.*, 1965; Green *et al.*, 1965). All but one of the remaining patients had neutralizing antibodies ranging from 1:8 to 1:32. However, these women presented at intervals ranging from four to eight weeks after exposure, making the interpretation of results difficult, since it was impossible to say whether their neutralizing antibody titre was acquired as a result of recent or of past infection. More recently, complement-fixation tests were performed in conjunction with neutralization tests, the presence of high titres of complement-fixing antibody being suggestive of recent infection (Sever *et al.*, 1965). Of four patients tested by both neutralization and complement fixation, none had complement-fixing antibody, suggesting that their neutralizing antibody was not a result of recent infection.

Discussion

In contrast to the position a decade or so ago, a virus diagnostic laboratory may be called upon to undertake investigations on an ever-increasing range of viruses. Thus facilities were provided, for example, for the detection of viruses causing acute respiratory and C.N.S. infections; viruses causing neonatal infections or congenital malformations—for example, rubella, cytomegalovirus, herpes simplex; as well as viruses responsible for exanthematous infections—for example, measles, variola, varicella/zoster, and enteroviruses. Such diagnostic procedures required the maintenance of numerous continuous cell-culture lines and the provision of a variety of serological techniques. Furthermore, the deployment of a small staff in both routine and research and the time involved in both seeing patients and discussing these cases with clinicians often imposed considerable strain on the laboratory's resources. Nevertheless, close co-operation between laboratory staff and clinical personnel proved invaluable, saving work in the long run by

ensuring that good clinical material was selected for study and that correct specimens were obtained in the best possible condition with the minimum delay.

However, these factors necessitated some restrictions in the number and types of cases investigated. The 262 cases were investigated at a cost of approximately £40 per patient. A more economic figure could have been achieved only by carrying out a more limited range of investigations. An increase in the number of patients investigated or extension of services to neighbouring hospitals in our group would certainly have necessitated the provision of greater space and more personnel, or a regular supply of cell cultures ready for use from a commercial source. These factors would have entailed much greater expense.

Our experience over the past year demonstrated that the laboratory provided a useful service to certain hospital departments, whereas to others it was of less value than we had expected.

For example, virus studies which clinicians thought useful were the investigation of maternal and neonatal rubella, the diagnosis of atypically presenting exanthematous disease, and the differential diagnosis of vesicular lesions in patients referred by the dermatologists and venereologists. However, in contrast to many bacteriological investigations, most virological diagnostic procedures take a long time to complete, and the results can therefore seldom influence clinicians in the management of acutely ill patients. Furthermore, we found that investigations on many patients admitted to general medical wards who presented as "interesting" or "puzzling" cases with perhaps an unexplained fever or haematological features "consistent with viral infection" were usually unrewarding, since by the time such patients were admitted to hospital they were long past the acute phase of their illness, making either positive or negative virus isolation or serology difficult to interpret. Consequently, we investigated and obtained diagnoses on proportionately fewer patients referred by general physicians than on those referred from other departments in the hospital.

Most diagnostic laboratories dealing with large numbers of specimens are attached to the Public Health Laboratory Service, to hospitals for infectious diseases, or to children's hospitals, most of which, unlike our laboratory, can obtain large amounts of suitable clinical material from acutely ill patients. We believe that most value can be obtained from a laboratory such as ours, which is also an integral part of a research department of a teaching hospital, not by providing a fully comprehensive diagnostic service but by specializing in certain fields, working on them in depth, and training personnel in both diagnostic and research methods. General diagnostic services should mainly be restricted to cases where infectious disease provides an urgent public health problem or requires definite action (for example, smallpox, poliomyelitis, maternal and neonatal rubella), or is likely to prove genuinely useful to clinicians (for example, differential diagnosis of acute C.N.S. infections and atypically presenting exanthematous infection). Inevitably, in these circumstances the services of other laboratories would be required to perform additional tests which a small laboratory could not undertake.

Though many virological investigations are at present of limited value to clinicians, a clinical virology laboratory may profitably extend its studies to the investigation of the role of different agents in varying communities, thus "pin-pointing" specific public health and epidemiological problems. In addition, combined clinical and virological studies, conducted on carefully selected clinical material, are also likely to be rewarding if performed as well-defined projects. Though viral chemotherapy is not generally available today, the position may alter in the future. In such an event, increased sensitivity and diagnostic speed may well be essential, and clinical virology laboratories are particularly well placed to undertake work in order to achieve such aims.

Thus, in these ways clinical virology provides fields where routine and research work may profitably coexist hand in hand, much rewarding research stemming from properly collected specimens from well-documented cases.

Summary

A diagnosis was made on 96 (36.6%) of 262 patients referred for virological investigations between 21 January 1966 and 20 January 1967. The highest numbers of patients were referred by the departments of paediatrics and venereology and from the physician in charge of nurses' health. The highest diagnostic rates were achieved on those patients referred by the nurses' physician (46.4%) and by the paediatricians (44.4%). Investigations on women exposed to or contracting rubella in early pregnancy, on infants with congenital malformations or neonatal infections, and on exanthematous infections which presented atypically were thought to be among the most useful studies conducted. Difficulties encountered in providing a fully comprehensive diagnostic service are described, and emphasis is placed on the importance of conducting epidemiological, clinical, and virological studies on carefully selected material alongside routine diagnostic investigations.

We are grateful to the St. Thomas's Hospital Endowment Fund Committee and the Wellcome Trust for generous financial contri-

butions; to Dr. C. M. P. Bradstreet, Standards Laboratory, Central Public Health Laboratories, Colindale, for supplying numerous serological reagents; to the Virus Reference Laboratory at Colindale and Epsom for typing various enteroviruses; and to the consultant staff of St. Thomas's Hospital for permission to investigate patients admitted under their care. One of us (J. M. B.) is in receipt of a grant from the M.R.C. in order to undertake research on rubella virus.

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COMPUTERS IN MEDICINE

Notification of Death Occurring after Discharge from Hospital

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Brit. med. J., 1967, **3**, 612–613

Although the fact of death has been recorded in practically all instances in this country since the Registration Act of 1837, and a quarterly alphabetical index of persons dying is maintained at Somerset House by the Registrar General, it may still be difficult for a doctor to discover which of a group of patients treated by him has since died. This paper describes an experiment in the systematic notification of deaths occurring after discharge to the hospitals in the Oxford area by a computer method.

Material and Method

Since the beginning of 1962 data from records of every birth, admission to hospital, and death in the population of Oxford and the surrounding area have been abstracted, coded, punched on cards, and matched into personal cumulative files (Acheson, 1964, 1967). This paper deals with hospital discharges and deaths in the defined population during 1963 and 1964. During these years the electromechanical system of data-processing set up in 1962 was replaced by a computer system.

Two cards are punched for each event—an identification card, containing the name, date of birth, N.H.S. number (if known), and other identifying data, and a statistics card carrying coded diagnostic and administrative information. The data are read on to magnetic tape, and edited. They are then sorted into sequence by using the Russell-Soundex phonetic code, sex, and initial as the sorting items, and each new record is compared with all

records in the equivalent subdivision of the master file. The system of record linkage is based on the principles described by Newcombe, Kennedy, Axford, and James (1959), whereby discrepant records are dealt with by allotting appropriate weighting factors (positive or negative) to agreements and discrepancies.

The product of the record linkage programme is a compound cumulative file which contains one entry for each person encountered by the system, each consisting of identification data and the records of a variable number of the defined events.

The next step was to scan the master file for all patients whose records included a death certificate and to write these records on to a separate magnetic tape. Records were selected from this tape for patients who had died in 1963 or 1964 and who had been discharged from hospital once or more in that period. An item was then formed for each discharge containing the following data: identification of patient, date of discharge, date of death, diagnosis on discharge (hospital record), and cause of death (death certificate).

If the patient died while in a hospital all items relating to that hospital were deleted, as it was assumed that the hospital was bound to be aware of the death. To avoid multiple notifications of a death where a patient had been discharged more than once from a hospital, the record of the final discharge was the only one retained. By means of the appropriate sorting lists of persons discharged from hospital within the two-year period who died at home or in another hospital were then prepared for each hospital and printed in alphabetical order of surname. Two years after the last death in the series had occurred—that is, at the end of 1966—the lists were sent to the medical records officer of each hospital in the area.

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