MARBURG AND EBOLA VIRUS INFECTIONS: A Guide for their Diagnosis, Management, and Control

D. I. H. SIMPSON

Department of Medical Microbiology
London School of Hygiene and Tropical Medicine,
London, England

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FOREWORD

Marburg virus infection was unknown until 1967, when it appeared in circumscribed outbreaks among laboratory workers in two countries of Europe, only to disappear until it re-emerged briefly in 1975 in South Africa. Ebola virus infection, caused by a virus that has been found to be similar but antigenically distinct, appeared in 1976 in extensive and almost simultaneous epidemics in two areas of Africa a long way one from the other. Since then, apart from an accidental laboratory infection in England, neither disease is known to have affected anyone elsewhere, but there is no reason to suppose that they may not do so at any time or that some other new virus causing haemorrhagic fever may not appear and give rise to equally puzzling and dangerous episodes.

Both diseases have obscure origins and virus reservoirs; both are difficult to differentiate clinically from a number of other febrile haemorrhagic diseases and from each other; and both are highly virulent and very readily transmitted from person to person - indeed, not even Lassa fever outbreaks have attained the intensity of those of Ebola virus, particularly among the personnel of dispensaries and hospitals. It is therefore essential to be prepared to deal with these diseases should they recur or with outbreaks of similar haemorrhagic fevers of suspected viral origin.

Under the aegis of the World Health Organization international medical teams were rapidly despatched to conduct thorough investigations of the 1976 outbreaks of Ebola virus infection in collaboration with the governments concerned. The leader of one of these teams, Dr D.I.H. Simpson of the London School of Hygiene and Tropical Medicine, kindly agreed to prepare the present work, in which he has distilled the experience gained by scientists from many countries in the diagnosis of Marburg and Ebola virus infections, their transmission and control, and the care of patients and contacts.

This work does not purport to answer all the questions that these diseases raise; to many of them there is no answer yet and to some the answers are the well-known ones applicable to almost any epidemic of a highly communicable disease. It is presented, however, in the hope that it will prove a practical guide to those in ministries, in hospitals, and in the field who may be faced by the problems created by such highly lethal virus infections as those described in the following pages.

The World Health Organization operates an emergency aid scheme to provide personnel and equipment for assistance in outbreaks of haemorrhagic fevers of suspected viral origin. Requests for assistance under this scheme should be addressed to WHO headquarters or regional offices or to WHO representatives in individual countries.

Dr P. Brès
Chief Medical Officer, Virus Diseases
Division of Communicable Diseases
Geneva, Switzerland
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1. HISTORY AND EPIDEMIOLOGY

Marburg virus infection, causing an acute febrile illness accompanied by severe haemorrhagic manifestations, was first recognized in 1967, when it caused three simultaneous outbreaks among laboratory workers in the Federal Republic of Germany (Marburg and Frankfurt am Main) and Yugoslavia.[1] The virus is known to have been introduced into both countries in consignments of African green (vervet) monkeys (Cercopitheceus aethiops) imported from the same source in Uganda within a few days of each other. The virus was isolated from the blood and tissues of a few of these monkeys. In all, there were 25 primary human infections, with 7 deaths, and 6 secondary cases, with no deaths. The primarily infected patients were exposed to Marburg virus while working with the monkeys or monkey tissue (autopsies, nephrectomies, cell culture preparation). The route of infection has not been completely established but may have been through intact skin, although respiratory and conjunctival infection cannot be ruled out. The secondary, nosocomial infections involved two doctors, a nurse, a post-mortem attendant, and the wife of a veterinarian. All the secondary patients had had direct contact, usually blood contact, with a primary patient. Both doctors infected themselves by accidental pricks with a hypodermic needle used for withdrawing blood from patients. The veterinarian's wife was infected during the acute stage of her husband's illness by blood contact. An additional secondary infection occurred in the wife of one patient 83 days after the onset of his illness, and is believed to have been acquired through sexual intercourse; virus was demonstrated in the husband's semen despite the presence of circulating antibody.

Studies in the Lake Kyoga region of Uganda, where the vervet monkeys had been collected, revealed no evidence of an epizootic nor was any illness detected among monkey trappers. Some workers have claimed that naturally occurring antibodies to Marburg virus were demonstrated in some African primates but this has not been generally accepted. Experimental laboratory infection of several primate species produced uniformly fatal infections and caused an illness similar to that seen in man.

No further cases of Marburg virus infection were reported in the world until February 1975, when an acutely ill patient was admitted to hospital in Johannesburg, South Africa, where he died

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[1] Martini, G.A. & Siegert, R., ed. Marburg virus disease, Berlin, Springer-Verlag, 1971

soon after admission.[1] He was a young Australian man who had been touring in Southern Rhodesia, frequently sleeping in the open. Shortly after his death his travelling companion also became ill, and some time later a nursing sister, who had attended both patients, also developed the illness. Both women recovered from their secondary infections although the travelling companion developed acute pancreatitis during her illness and the nurse developed a painful uveitis during late convalescence. Marburg virus was subsequently isolated from the anterior chamber of the affected eye.

Between July and November 1976, two very extensive and almost simultaneous epidemics of a similar disease occurred about 1000 km apart in Southern Sudan and northern Zaire. Secondary and tertiary person-to-person spread of the infection was a distinct feature of these outbreaks, particularly among hospital staff. In some cases in Sudan as many as eight "generations" of infection were found, but this was unusual. There are believed to have been over 300 cases, with 151 deaths, in Sudan, and in Zaire some 237 cases, with 211 fatalities, have been documented; the actual numbers may be greater. In one Sudanese hospital 76 members of a staff of 230 were infected and 41 died. Throughout the Zaire epidemic and during the earlier stages of the Sudanese outbreak the case-fatality rate was of the order of almost 90%, leading to fear and panic in the local populations. These alarming figures emphasize the tremendous public health importance of this disease.

Virus strains isolated from patients in Sudan and Zaire were shown to be structurally very similar to Marburg virus strains isolated in the Federal Republic of Germany and South Africa but to be antigenically quite distinct.[2,3,4] The name Ebola virus has been proposed for the prototype strain, after a river in Zaire.

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1 Gear, J.S. et al. Outbreak of Marburg virus disease in Johannesburg. British medical journal, 4: 489-493 (1975).

2 Johnson, K.M. et al. Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. Lancet, 1: 569-571 (1977)

3 Bowen, E.T.W. et al. Viral haemorrhagic fever in southern Sudan and northern Zaire. Lancet, 1: 571-573 (1977)

4 Pattyn, S. Isolation of Marburg-like virus from a case of haemorrhagic fever in Zaire. Lancet, 1: 573-574 (1977)

The outbreak in Sudan is thought to have begun in the first week of July 1976, with the illness of a cloth-room storekeeper in a cotton factory in Nzara (Western Equatoria Province). Two weeks later a second storekeeper also became ill, followed a further two weeks later by another cotton factory employee. One of his contacts introduced the disease to Maridi, some distance east of Nzara. The source of the original infection has still not been determined. The infection spread swiftly but only through close and prolonged household contact with an active case. Health personnel in particular were involved through contact with patients' blood, and Maridi hospital acted as an amplifier of the disease. When good nursing techniques, supplemented by the use of protective clothing, were introduced the number of contact infections fell dramatically.

In Zaire the first recognized case occurred during the first week in September 1976 and is thought to have originated at a small mission hospital in Yambuku, just north of Yandonge, Equateur Region. It is thought that parenteral injections may have played a role in transmission. Patients infected in the hospital environment probably then carried the infection back to their villages, setting up new pockets of infection in their homes. The source of the infection in Zaire remains unknown, but it may have been introduced to Yambuku by a patient presenting at the outpatient clinic with a nonspecific febrile illness.

That vervet monkeys were directly involved in the transmission of Marburg virus under well-defined conditions is undeniable, but it is less clear that they are the natural reservoir of the virus, although ecological investigations into the possibility of other animals being involved as reservoirs or vectors of Marburg and Ebola viruses have not yet yielded positive evidence. However, the number of specimens of arthropods (insects), rodents and other animals so far examined is small and the probability remains that one or more species may play a role. It should be recalled that a peridomestic rodent, the multimammate rat (Mastomys natalensis), has been found to be a reservoir and vector of Lassa fever in Africa and that other rodents are known vectors of the viruses of Argentine and Bolivian haemorrhagic fevers.

2. INFECTIOUS AGENT

Both Marburg and Ebola viruses contain ribonucleic acid; under the electron microscope their structure is virtually identical and it is quite distinct from that of any other animal viruses, although some relationship to the Rhabdoviridae has been suggested. The structure is seen as an elongated or filamentous particle, often coiled or branching, and sometimes blister-like structures can be visualized.

3. DIAGNOSIS

3.1 Clinical features

In the earliest stages of the infection the symptomatology is nonspecific and a clinical diagnosis is difficult until more characteristic features of the illness become apparent or when several similar cases occur during an epidemic.

Incubation period. The Marburg outbreaks in the Federal Republic of Germany had an incubation period ranging from 3 - 9 days while in South Africa the period of incubation was 7 - 8 days. In the far larger Ebola virus epidemics there were incubation periods ranging from 4 - 16 days with an average of 7 days.

Onset. The illness in every outbreak began abruptly with severe frontal and temporal headache and severe malaise. There were generalized aching pains, most marked in the lumbar region, and several patients noted that their eyes were extremely sensitive and painful on pressure.

Acute phase. A high fever was generally apparent on the second day of illness and patients became progressively more debilitated over the first few days of their illness. A severe watery diarrhoea, abdominal pain and cramping, nausea, and vomiting began about the third day and the diarrhoea often persisted for a week. In Sudan knife-like chest or pleuritic pain was an early symptom and many patients complained of a very dry, rather than a sore, throat, accompanied by a dry cough. The throat discomfort was sufficiently severe to make patients disinclined to eat or drink. On white skins a characteristic maculopapular rash appeared between days 5 and 7. Beginning on the face and trunk, it soon spread centrifugally to involve the arms and legs. It was often apparent against a pronounced erythema on the back, face and arms. The rash was not itchy and was followed after 3 - 4 days by a fine desquamation. On black skins the rash, often described as "measles-like", was not so obvious and it was often only recognized later with the appearance of skin desquamation. Skin desquamation occurred in all patients with a rash and started 4 - 5 days after the appearance of the rash. Conjunctivitis and enanthem of the palate accompanied by tapioca-like lesions on the tonsils were reported from the Federal Republic of Germany, but the enanthem was not a feature in South African cases. In Sudan, pharyngitis was noted and the throat was found to be extremely dry and was accompanied by fissuring and open sores on the tongue and lips. On average, hospital admission in Sudan was on day 5 and the patients' typical appearance at that time was of "ghost-like" drawn features, deep-set eyes, expressionless faces, and extreme

lethargy. Anxious, sullen expressions were a feature of the illness in Marburg and Frankfurt. Rapid cachexia and dehydration were seen in all patients. There was also a relative bradycardia and some lymphadenopathy.

A very high proportion of patients developed severe haemorrhagic manifestations between days 5 and 7, and fatal cases always had some form of bleeding, often from multiple sites. The gastrointestinal tract and the lungs were most frequently affected. Haematemesis and melaena and sometimes the passage of fresh blood in the stools were often accompanied by bleeding from the nose, gums and vagina, and subconjunctival haemorrhages were common. Abortion and massive metrorrhagias were frequently seen among pregnant women in Zaire. Petechiae, haematuria and bleeding from needle puncture sites were more commonly seen in German patients. In Zaire almost every patient had some form of severe bleeding. This was true also of the earlier cases in Sudan; cases seen later in the Sudan epidemic displayed fewer frank haemorrhages, although many had melaena. In both epidemics death occurred between day 7 and day 16 - in most patients between days 8 and 9 - usually preceded by severe blood loss and shock.

Central nervous system involvement was evident and the severity of the disturbances was often a reflection of the severity of the illness. Paraesthesia, lethargy, confusion, irritability, stupor, aggression, and signs of meningeal irritation were sometimes seen. Bizarre and violent behaviour was seen in a few patients.

Other signs. Hepatitis was a feature in the South African cases and in some German patients but clinical jaundice was not observed. Oliguria accompanied by proteinuria was often seen and some German patients developed ascites and oedema and swelling of the face. Haematuria was not common. One patient in South Africa had an acute pancreatitis, and several German patients had scrotal dermatitis. Signs of myocarditis were quite common. Complications included bacterial pneumonia, orchitis, and testicular atrophy.

Convalescent phase. The acute febrile phase generally lasted for 14 - 16 days and was followed in surviving patients by a slow, protracted recovery accompanied by periodic headache, chest pain, abdominal cramping and marked fatigue. Extreme cachexia and a stooping gait were noticeable features and anorexia often continued for 3 - 4 weeks after recovery. Loss of hair was a fairly frequent complication during convalescence in the German outbreak and some patients were left with prolonged psychotic disturbances.

3.2 Clinical and differential diagnosis

Because Marburg and Ebola viruses are readily transmitted from person to person, particularly to medical and nursing staff and those caring for patients, early diagnosis and isolation of the patient are essential. The diagnosis must be considered in all febrile patients in or travelling from areas in Africa where these viruses are known or suspected to be endemic.

The sudden onset of fever, headache, and malaise, soon followed by chest pain, diarrhoea and vomiting, and rapid cachexia, should alert physicians to the possibility of Marburg or Ebola virus infection. The history, physical examination and epidemiological background should then be carefully assessed. The appearance of a characteristic maculo-papular rash and the rapid onset of cachexia may raise the suspicion of Marburg or Ebola virus infection and the onset of haemorrhagic manifestations may confirm this.

The differential diagnosis may be difficult. Lassa fever must be considered but the onset is generally more insidious and a sore throat, pharyngitis and, in the later stages, facial oedema are more characteristic of Lassa fever.[1]

Malaria generally presents with fever and headache. Blood smears should be examined for malaria parasites but the presence of parasites does not exclude concurrent viral infection. Anti-malarials should be administered routinely as a therapeutic trial (see also Section 5.4).

Typhoid fever may present with fever, headache, rash, gastro-intestinal symptoms, often with lymphadenopathy, relative bradycardia, cough and leukopenia, and sometimes a sore throat. It is perhaps the most difficult infection to distinguish from either Lassa or Marburg or Ebola virus infections. A therapeutic trial with chloramphenicol or tetracycline may serve to differentiate the disease (see also Section 5.4). Blood culture, if it can be performed during the first few days of illness, is often successful in demonstrating the causative bacteria.

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[1] Monath, T.P. & Casals, J. Diagnosis of Lassa fever and the isolation and management of patients. Bulletin of the World Health Organization, 52: 707-714 (1975)

Haemorrhagic complications can result from yellow fever virus and other flaviviruses such as dengue and from the Crimean-Congo haemorrhagic fever group of viruses. Careful epidemiological investigation may reveal a pattern of disease indicating transmission by mosquitos or ticks; by contrast, Ebola virus infection has exhibited a clear pattern of person-to-person transmission. Virus isolation and serological investigation will distinguish these viruses. A history of previous yellow fever vaccination may also serve to eliminate a diagnosis of yellow fever. South American haemorrhagic fevers, such as those caused by Junin[1] and Machupo viruses, may also have to be taken into consideration.

Bacterial infections other than typhoid fever should also be taken into consideration in a differential diagnosis and a search should be made for possible sites of bacterial infection. Blood smears and cultures should be examined and the blood picture determined. The presence of a leukocytosis will often serve to distinguish bacterial infections from those with Lassa, Marburg or Ebola viruses, in which a leukopenia is a constant finding.

Viral hepatitis, leptospirosis, rheumatic fever, typhus and mononucleosis are other diseases which produce signs and symptoms which may be confused with those caused by Marburg or Ebola virus in the early stages of infection.

3.3 Laboratory findings

Clinical laboratory studies have necessarily been limited because of the risk to laboratory personnel. As mentioned above, a leukopenia early in the course of the illness has been a constant feature, together with a low erythrocyte sedimentation rate. An alteration in the granulocyte series has been noted with the appearance of atypical plasmacytoid lymphocytes, and the Pelger-Huët anomaly of neutrophils has been recorded. South African patients had severe disseminated intravascular coagulation, probably resulting from the failure by the liver to synthesize coagulation factors and from depression of bone marrow. These features were not evident in German patients, although there was a fall in thrombocytes while prothrombin and thrombin times, partial thromboplastin times, and fibrinogen levels were not obviously affected.

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[1] Maiztegui, J.I. Clinical and epidemiological patterns of Argentine haemorrhagic fever. Bulletin of the World Health Organization, 52: 567-575 (1975)

Also a constant finding was a noticeable elevation of the levels of serum aspartate aminotransferase (glutamine oxaloacetic transaminase) and alanine aminotransferase (pyruvic transaminase) levels, the former being consistently high and serving as a diagnostic aid. Serum amylase was also raised in some patients, and especially in one in South Africa with acute pancreatitis. Several patients had hypoproteinaemia, which resulted in varying degrees of oedema.

Pathology. Marburg and Ebola viruses are pantropic and produce lesions in almost every organ, but the liver and spleen were most conspicuously affected. Severe degeneration of lymphoid tissue, spleen and liver resulted in large accumulations of cellular and nuclear debris. The pattern of disease was that of stimulation of the reticuloendothelial system, inhibition of the lymphatic system, and vascular changes leading to vascular occlusion and the formation of thrombi and haemorrhages.

Macroscopically, both the liver and spleen were enlarged and dark in colour. On section the spleen revealed no follicles and the pulp was soft and mushy. The liver was extremely friable and blood poured out freely on section, leaving the organ a light yellow colour.

Histologically, severe congestion and stasis were obvious in the spleen. There was proliferation of reticuloendothelial elements in the red pulp and the formation of large numbers of macrophages. Necrosis of the red pulp was accompanied by destruction of lymphoid elements. In the Malpighian bodies lymphocytes were markedly depleted.

There was widespread degeneration and necrosis of liver cells and hyaline changes were frequently seen. Hyaline-necrotic-eosinophilic bodies similar to the Councilman bodies of yellow fever were seen on several occasions. Kuppfer cells were swollen and bulging and full of cellular debris and red blood cells. Sinusoids were also full of debris, and mononuclear accumulations were seen in the periportal spaces. Even at the height of the necrotic process in the liver there was evidence of regeneration of liver cells.

Mononuclear transformation of lymphoid tissue, as well as necrotic lesions, was found not only in the liver and spleen but also in the pancreas, gonads, adrenals, hypophysis, thyroid, kidneys and skin.

The lungs showed few lesions except for circumscribed haemorrhages and evidence of endoarteritis especially in the small arterioles.

Neuropathological changes were confined mainly to glial elements scattered throughout the brain. No lymphocytic reaction was observed but multiple haemorrhages into the brain substance were seen. Glial lesions were either proliferative in the form of glial knots, nodules and rosettes or degenerative in the form of nuclear pyknosis or karyorrhexis. All glial elements were affected, including astrocytes, microglia and oligodendroglia. Cerebral oedema was found in all the human brain material examined.

3.4 Virological and serological diagnosis

Specific diagnosis requires isolation and identification of the virus or evidence of antibody development between paired serum samples.

Attempts to isolate the virus must be carried out only in high-security laboratories with optimum biocontainment facilities.[1,2]

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1 See, for instance:
Hellman, A. et al., ed. Biohazards of biological research, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1973);
Ad Hoc Committee on the Safe Shipment and Handling of Etiologic Agents. Classification of etiological agents on the basis of hazard, 4th ed., US Department of Health, Education, and Welfare, Center for Disease Control, Atlanta, GA (1974).

2 The following laboratories have indicated that they have suitable biocontainment facilities:

Institut de Médecine tropicale Prince Léopold
Nationalestraat 155
Antwerp B-2000
Belgium

Head, Special Pathogens Unit
Microbiological Research Establishment
Porton Down
Salisbury, Wilts., SP4 OJG, United Kingdom

Chief, Special Pathogens Branch
Virology Division, Center for Disease Control
Atlanta, Georgia 30333 USA
(Shipments for the Center for Disease Control should be addressed to: Officer in charge, United States Public Health Service, Room 2339, International Arrivals Building, Kennedy International Airport, Jamaica, NY 11430, USA)

Institute of Poliomyelitis and Virus Encephalitides
P.O. Institute of Poliomyelitis
Moscow Oblast 142 782
USSR

Collection of specimens for rapid diagnosis. Three types of specimen should be collected:

(a) Acute-phase whole blood obtained from patients within 7 days of the onset of illness, the operator taking due precautions for his or her own protection (i.e., wearing gown, gloves, mask, goggles or visor, cap, overshoes). Blood should be collected in sterile, tightly sealed receptacles and despatched in liquid nitrogen (which requires special plastic containers) or in dry ice, or kept chilled with refrigerant packs ("cold dogs") or wet ice. The separation of acute-phase sera from blood clots is not recommended unless facilities are available to protect laboratory workers from infectious aerosols.

(b) Convalescent sera collected from patients at least 14 days after the onset of illness. Whereas paired serum samples from the same patient are desirable, single convalescent serum samples are often valuable and should be obtained whenever possible. Convalescent sera should be separated from blood clots and despatched as above, preferably frozen. Owing to the uncertainty as to the infectivity of the blood, the same precautions as above should be observed when handling the serum.

(c) Liver specimens taken post mortem with a biopsy needle. Liver biopsy of sick patients is strongly contraindicated since it may cause serious haemorrhages in the liver. Each post-mortem specimen should be divided into two; one being placed in 10% buffered formalin and the other handled as for acute blood samples with the precautions already mentioned.

In addition, urine and throat swabs (or washings) collected during the first few days of illness may also be of some value for virus isolation and should be handled as for acute blood samples.

On all occasions when specimens are sent for diagnosis as much relevant information as possible should be included with the specimens: name of the patient, locality, age, sex, date of sample, date of onset of illness, summary of clinical findings and pertinent epidemiological information (e.g., number of similar cases, history of similar cases, history of contact, and occurrence of disease in hospital staff).

Specimens for shipment to high-security laboratories should be packed and despatched in compliance with international regulations.[1] These laboratories should receive prior warning by cable giving details of the airway bill, flight number (connecting flights if necessary) and the estimated time of arrival. A copy of the cable should be sent to the World Health Organization[2] in order to ensure that measures may be taken for international epidemiological surveillance and safety.

Serological diagnosis. Conversion from negative to positive in paired serum samples in a fluorescent antibody test is the most reliable serological test. Complement fixation tests have been much less reliable, and no satisfactory neutralization test has yet been devised for these viruses.

Generally it is not feasible to establish viral diagnostic facilities under field conditions at the site of the outbreak, but fluorescent antibody tests were successfully carried out during the Zaire outbreak.

Virological diagnosis. Positive diagnosis by virus isolation generally takes a minimum of 5 - 7 days. Inoculation of acute samples into Vero cell cultures and intraperitoneal inoculation into guinea-pigs are the most useful methods. Cytopathic changes are not generally observed in any tissue culture system but intracytoplasmic accumulations of viral antigen can be detected by immunofluorescence and histopathological staining. Electron microscopic examination of culture fluid often reveals typical virus structures. Guinea-pigs develop a severe febrile illness and virus can be detected by electron microscopy in their liver and blood taken during the febrile stage. (Early passages of human material in guinea-pigs, while producing a febrile illness, do not necessarily kill the animals; repeated passages may be necessary to produce uniform fatalities.)

Some limited success in the rapid detection of virus has been achieved by electronmicroscopic examination of ultracentrifuged acute serum collected from patients in the early stages of illness.

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1 In this connexion consult: Madeley, C.R. Guide to the collection and transport of virological specimens, Geneva, World Health Organization, 1977.

2 It should be addressed to: Chief Medical Officer, Virus Diseases, World Health Organization, 1211 Geneva 27, Switzerland, (telegraphic address: UNISANTE, GENEVA).

4. ISOLATION OF THE PATIENT

As soon as the diagnosis of Marburg or Ebola virus infection is suspected the patient must be isolated to prevent secondary infection by direct or airborne spread of the virus. Strict barrier nursing must be instituted immediately and all diagnostic specimens, patient's excreta, and any other materials having had contact with the patient must be considered infectious and should be handled or decontaminated accordingly.

Medical centres with adequate facilities for providing isolation and strict barrier nursing should follow the methods described in 1974 in the WHO Weekly Epidemiological Record for Lassa fever,[1] or in Isolation techniques for use in hospitals.[2]

In most regions of tropical Africa highly specialized isolation facilities are not available; the methods described here therefore take likely local conditions into consideration.

4.1 Isolation room

A separate building should be used whenever possible to ensure complete isolation of patients with Marburg or Ebola virus infection from other patient areas. If this is not possible, a private room or isolation ward to which access can be strictly limited should be used. There should be no cross-circulation of personnel or materials from other hospital areas. Good ventilation, provided through screened doors and windows, is essential. Fans, which may raise and circulate dust and droplets should be avoided. Biological hazard warning notices should be prominently displayed.

4.2 Personnel

Care of patients should be restricted to a limited group of trained nursing staff who have been fully instructed in isolation techniques and who have a basic understanding of the clinical features and epidemiology of Marburg and Ebola virus infections.

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1 Weekly epidemiological record, 49: No. 41, pp. 341-343 (1974)

2 United States Department of Health, Education, and Welfare; Center for Disease Control, Isolation techniques for use in hospitals, Washington, DC, US Government Printing Office, 2nd ed., 1975 (DHEW publication No. (CDC) 76-8314).

No untrained staff should be employed in the isolation area. The head nurse is responsible for strictly controlling access to the isolation area. No visitors should be admitted to the isolation area; if any exception proves indispensable the person admitted should be subjected to the containment measures for contacts described in Section 7 below.

4.3 Protective clothing

As has already been mentioned, gowns, gloves, masks, goggles or visors, caps and overshoes are essential and should be worn by all hospital staff. Ideally they should be disposable items, used once only and then discarded into a covered receptacle lined with a large plastic bag on leaving the isolation facility. If disposable protective clothing is not available, the items should be used only once and then sterilized and laundered before reuse. Where supplies of protective clothing are limited, modification of the procedures may be necessary but potentially contaminated items should remain in the isolation area after use. Contaminated items should be removed from the isolation facility in well-closed plastic bags and taken for sterilization by autoclaving or boiling or for incineration.

4.4 Hand washing

Hands should always be washed after contact with a patient or contaminated materials. Disinfectant and washing facilities should be conveniently placed just outside the isolation area. Hands may be rinsed in disinfectant and then washed in soap and water. Mechanical soap dispensers and disposable paper towels are recommended. If a sewage system is not available, washing water should be disposed of in latrines.

4.5 Instruments and dressings

Each patient should have an individual thermometer labelled with his or her name and kept in a receptacle containing disinfectant (alcohol-iodine solution) at the bedside. Stethoscopes and sphygmomanometers should remain in the isolation area. The stethoscope and the sleeve of the sphygmomanometer can be decontaminated between use by rinsing in disinfectant solution. Other reusable instruments should be placed after use in a bowl of disinfectant and regularly removed for sterilization.

4.6 Linen

All bedding and other linen should be placed in plastic bags and removed for sterilization by autoclave or boiling before being laundered.

4.7 Disposal of excreta

Separate toilet facilities should be provided for the isolation area. If this is not possible patients should be allowed to use a bedside commode or bedpan.

Bedpans should be labelled with the patient's name and not be used by other patients unless first sterilized. A used bedpan must not be left in the isolation facility. It should be immediately filled with disinfectant and placed in a plastic bag; after allowing time for the disinfectant to act, the bedpan should be removed inside the plastic bag and emptied in a toilet or latrine separate from other patient areas. The bag and bedpan should then be thoroughly rinsed with disinfectant and returned to the isolation area. The bag should not be reused but placed in a convenient receptacle for incineration. If available, disposable bedpans should be used which can be incinerated after use. Vomitus containers should receive equivalent treatment.

4.8 Supply of food

Whenever possible, relatives should not prepare food for patients within the hospital. The hospital should make its own arrangements to supply food and drink for patients occupying the isolation facility.

Eating utensils, including dishes, should be used only by the patient. They should be washed and treated with disinfectant within the isolation area. Uneaten food should be regarded as infectious and disposed of accordingly.

4.9 Laboratory specimens

All specimens from cases and suspected cases for virological, serological or clinical laboratory investigations must be regarded as infectious. Specimens should be collected in tightly sealed, screw-cap containers which should be dipped in disinfectant before leaving the patient's room, and then be placed inside plastic bags within a closed receptacle for transfer to the laboratory.

4.10 Charts and records

No charts, notes or clinical records should be taken into the isolation room.

4.11 Disinfectant solutions

Several different disinfectants may be used. Commonly available solutions include:

- Sodium hypochlorite (1:500 aqueous);
- Phenol (2% aqueous). Add 0.5% sodium bicarbonate to prevent corrosion of metal instruments;
- Iodophor (0.045% available iodine). Add 0.2% sodium nitrate to prevent corrosion;
- Formalin (2% solution) incorporated with a 2% cetrimonium bromide solution may also be used when other disinfectants are not available.

4.12 Period of isolation

Ideally patients should be isolated until virological studies show that they are free of virus. Demonstration of antibodies by complement fixation or fluorescent antibody tests does not mean that virus excretion has ceased. When laboratory test results are unavailable, decisions about the duration of isolation need to be based on clinical recovery and a regard to possible prolonged periods of virus excretion. A minimum period of at least 7 days without fever and a total of 21 days from onset of illness should be obligatory before a decision on discharge is taken. It is worth remembering that Marburg and Ebola viruses have been detected in seminal fluid and in the anterior chamber of the eye from 3 convalescent patients, 61, 80 and 83 days respectively after the onset of their illness despite the presence of circulating antibodies in their blood.

4.13 Post-mortem procedures

If a post mortem is carried out extreme precautions must be observed. The pathologist, those assisting and anyone else present in the room should wear full protective clothing - gown, respirator,[1] gloves, cap, boots or rubber overshoes. Flushing of body cavities or rinsing of tissues should be avoided; body fluids should be collected on absorbent material, which should be left in body cavities. Pieces of tissue removed for histological examination should be immediately immersed in fixative (formalin, Zenker's solution, or, for electronmicroscopy, glutaraldehyde).

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[1] There is a serious risk of contamination by aerosols, and a bacteriological respirator will provide more protection than a surgical mask and goggles or visor.

Specimens for virological study should be put straight into screw-cap containers, which should then be washed in formalin and removed immediately from the room.

After the post mortem has been completed the room should be thoroughly washed with hypochlorite or phenol solution and, if possible, left unused for 3 or 4 days with maximum ventilation.

Corpses should be wrapped in sheeting soaked in disinfectant solution and then further wrapped in plastic sheeting prior to burial. The hospital authorities should take complete responsibility for burials, and relatives and friends should not be permitted any contact with the deceased's remains.

5. CLINICAL MANAGEMENT

5.1 Supportive measures

Bed rest in hospital should be encouraged as early as possible in the course of the illness. Movement of patients should be minimal and any measures should be directed towards preventing fatigue. Sedative drugs, particularly those without hypotensive effects, may be useful. Hydroxyzine has been used both as a sedative and as an antiemetic with good effect. Other suitable drugs include diazepam, chlordiazepoxide and barbiturates. Body temperature may be lowered by tepid sponging or the use of an antipyretic such as paracetamol to reduce the metabolic rate of seriously ill and toxic patients.

As severe headache and myalgia are constant findings early in the illness an analgesic is recommended. Pentazocine or dextropropoxyphene have been found useful in this respect.

Nausea, vomiting and profuse diarrhoea are universal symptoms in this disease and measures must be instituted as soon as possible to relieve distress and discomfort as well as to reduce the dehydration and electrolyte imbalance. Oral intake should be avoided and losses should be corrected intravenously. Antiemetics, especially those with low hypotensive side-effects (e.g., prochlorperazine, hydroxyzine), may be required. Nasogastric intubation and suction should be instituted for patients with severe nausea, vomiting and abdominal pains.

5.2 Fluid balance

Rapid loss of body fluid is a distinctive feature of this disease and the correction of abnormal losses and the maintenance of fluid balance are essential. As most hospitals do not have adequate laboratory support facilities the type and volume of

intravenous fluid to be prescribed have to be determined on clinical observation. Patients with obvious dehydration (decreased skin turgor, sunken eyeballs, postural hypotension, oliguria, raised haematocrit) should be given saline in sufficient quantity to correct these abnormalities. Where vomiting and diarrhoea are severe there may be a depletion of potassium, which should be replaced with the usual caution. Lactate solutions should not be used because of the risk of acidosis.

If signs of oedema or effusions or shock appear saline replacement should be reduced and treatment diverted towards maintaining the circulation and reducing salt retention.

5.3 Vital function recording

Temperature, pulse, blood pressure, intake and output should all be recorded regularly. With appropriate simple laboratory facilities, baseline and serial measurements of the specific gravity, protein and other constituents of urine can be monitored within the isolation area. The same applies to haematocrit estimations, which can be useful in estimating renal damage and haemoconcentration. Where good laboratory facilities are available blood electrolyte, serum enzyme and other estimations are of great value but it is doubtful if such tests should be carried out in view of the grave risk to laboratory staff in handling potentially infectious materials.

5.4 Antibiotic and antimalarial therapy

In view of the difficulty of differentiating between Marburg or Ebola virus infection and typhoid fever or malaria in the early stages of illness a therapeutic trial using chloramphenicol (50 mg/kg/24 h in 4 divided doses) or chloramphenicol in combination with erythromycin or penicillin given intravenously may be warranted if typhoid fever is suspected. Blood and other cultures should be made before starting on antibiotic treatment. Such therapy should continue for a minimum of 3 - 5 days.

Where malaria is suspected, intramuscular chloroquine should be given. It should be noted that malaria parasites were frequently found in blood films of patients with Ebola virus infection in Sudan, indicating that a parasitaemia does not necessarily indicate malaria as the primary cause of illness.

5.5 Management of severe illness

Haemorrhage. Profuse bleeding, especially from the intestinal tract, has been a consistent finding in severe Marburg and Ebola

virus infections. An increase in the severity of the bleeding was a direct cause of death in many cases. A marked thrombocytopenia was recorded in German patients suffering from Marburg infection and no unequivocal evidence of disseminated intravascular coagulation was elicited.

Fresh blood, platelet concentrates, fibrinogen, vitamin K and aminocaproic acid were all used to treat the bleeding tendency. Concentrates of prothrombin and Factors VII, IX and X were successfully used in a number of cases. However, caution must be exercised, as in South Africa there was evidence of disseminated intravascular coagulation. Heparin was given prophylactically to prevent the development of severe consumptive coagulopathy. A loading dose of 2000 International Units of heparin was given intravenously, followed by a constant infusion of 10 000 IU over 24 hours. The dose thereafter was monitored according to the partial prothrombin time.

It must be emphasized that heparin therapy should not be considered if coagulation factors cannot be monitored. Transfusion of fresh platelet concentrates or even fresh blood may be used provided cross-matching facilities are available.

Other complications. Several complications were recorded in the outbreaks of both Marburg and Ebola virus infections. These included acute pancreatitis, bronchopneumonia, pleurisy with effusion, pericarditis, oliguria and anuria and required specific treatment which will not be dealt with here.

6. SPECIFIC TREATMENT

6.1 Use of convalescent plasma

Plasma containing Ebola virus specific antibodies has only been administered to one proven case of Ebola virus infection. Viraemia levels dropped considerably within 12 hours of administration. Further trials are required before serotherapy in this disease can be fully evaluated.

However, plasma should be administered to a patient in whom the diagnosis is reasonably certain and the severity of illness indicates that such a measure is advisable. Owing to the antigenic differences between Ebola and Marburg viruses, only the specific convalescent plasma is active in each disease; antigenic characterization of the causative virus is therefore a prerequisite to the use of plasma. Plasma should be given as early as possible in the course of illness. It should be given as a rapid intravenous infusion of 250-500ml, depending on available supplies. If fever and other symptoms persist repeat transfusion may be indicated.

6.2 Supply of convalescent plasma

Over 100 litres of human Ebola convalescent plasma were collected in Zaire and Sudan. Only a few litres of Marburg convalescent plasma remain in store. All the stocks have been tested for the absence of virus, for antibody titre, and for the presence of hepatitis B antigen or its antibody. The plasma stocks are at present held frozen at -20°C.[1] For easier storage, lyophilization or fractionation are envisaged but may result in loss in potency.

6.3 Selection of donors

In an epidemic situation the use of convalescent plasma with undetermined potency and safety may be considered. The greatest hazard, and one not to be underestimated, is the transfusion of plasma still containing free virus to a patient suspected on clinical grounds alone of having the disease. The duration of viraemia in cases of Marburg and Ebola virus infection has not been determined but virus excretion by other routes may persist for up to 3 months. As a guiding principle, plasma for use in transfusion therapy should never be collected before a period of 5 - 6 weeks after onset has elapsed.

6.4 Isolation of patients following plasma transfusion

Patients who have received convalescent plasma may continue to excrete virus, and isolation measures should continue until virus cannot be detected in blood or urine. Virus may persist in seminal fluid for several weeks but patients so affected need not necessarily be strictly isolated but warned of the dangers to partners should there be sexual intercourse.

6.5 Use of interferon

One patient was given 80 million units of human interferon over a period of 14 days, administered intramuscularly twice daily in doses of 3 million units. This patient also received immune plasma. It is therefore not possible to conclude whether interferon contributed to the patient's recovery.

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[1] Well-justified requests for convalescent plasma, within the limitations of the quantities available, may be addressed to: Chief Medical Officer, Virus Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

7. EPIDEMIOLOGICAL INVESTIGATIONS AND CONTAINMENT MEASURES

The early disclosure of disease activity and of its extent and prompt reporting in order to carry out appropriate containment measures are of crucial importance. Surveillance teams, consisting of locally recruited staff trained on the spot and supervised by one or more epidemiologists, should be rapidly set up. All team members must be fully informed of the measures they should take to protect themselves, and they should be provided with protective equipment. Their task consists in finding cases and their contacts. They should collect the appropriate specimens to send to laboratories for confirmation of a suspected diagnosis and should prescribe emergency isolation measures. They should be provided with standardized forms for case and control evaluation and for village surveillance reports. Adequate transportation and rapid means of communication are essential for the investigation and control of outbreaks.

For containment measures to be effective a clear definition of cases and contacts must be applied, as follows:

(a) confirmed case: a person with acute clinical symptoms from whom the virus has been isolated and/or in whom the presence of specific antibodies has been demonstrated;

(b) probable case: a person having for 3 days high fever, headache, lumbar pain, nausea, vomiting, abdominal pain, diarrhoea and haemorrhage without any other specific diagnosis and no sedation after antimalarial and antibiotic therapy; knowledge of a contact with a confirmed case or another probable case is essential;

(c) possible case: a person with 3 days of fever and headache without any other diagnosis and not responding to treatment as above and with a contact with a confirmed or probable case within the 3 previous weeks;

(d) contact: a person, even without symptoms, having had direct contact with (a), (b) or (c) - i.e., having shared the same room as or cared for a patient or participated in a burial - either 2 days before onset in that patient, during the disease or immediately after death.

Primary contacts should be isolated and their temperatures taken twice daily. Secondary contacts (i.e., those in contact with a primary contact) need not be so confined. They should be instructed to report to designated medical centres if fever develops; and those medical centres should be aware of the

identities of secondary contacts.

7.1 Active surveillance

Surveillance is a prerequisite for rational measures to be taken at four levels:

(a) Area isolation should be enforced for a duration of two estimated incubation periods (twice 14 days) after the last case. In view of the economic consequences this measure should be regularly reviewed and adapted to the situation. The measure is never absolutely effective but cuts down long-distance travel significantly.

(b) Village isolation should be combined with health education aimed at limiting contact with patients. Bringing patients to hospital is not necessarily the best measure; depending on local conditions, household confinement may be preferable. If hospital isolation is considered necessary it must be very strict and no visitors may be admitted.

(c) Household confinement consists of the isolation of a patient in one house or room, only one person caring for the patient; that person should be treated as a primary contact (see above). Protective clothing should be offered to those caring for patients.

(d) International measures need only be applied if cases occur in cities with international transport facilities and if primary contacts are not identified and isolated.

Individual isolation should last for a period of 14 days; this takes into account the fact that the incubation period may exceed the average period of 7 days.

Precise instructions must be given for the burial of patients who have died (see Section 4.13).

8. EVACUATION OF PATIENTS AND CREATION OF TREATMENT CENTRES IN SUSPECTED ENDEMIC AREAS

Since adequate treatment of patients with Marburg or Ebola virus infection requires adequate isolation facilities and the resources of a well-equipped medical centre, evacuation from rural primary-case centres may be desirable. Regional hospitals should be selected as specialized treatment centres. They should then be suitably equipped and the staff trained in isolation techniques.

9. INTERNATIONAL MOVEMENT OF PATIENTS

Proven or suspected patients should not, in general, be moved out of endemic areas. Inevitably, however, there may be occasions when they may have to be transported internationally. Special flights must be arranged or the use of specially designed isolators should be considered. The prior agreement of the receiving country's health authorities must be obtained before evacuation of this nature is undertaken. Contingency planning on the part of receiving centres is strongly recommended.

10. IMMUNIZATION

No vaccine is at present available against either Marburg or Ebola virus.