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Cerebral aspergillosis in liver transplantation

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Abstract

Nine cases of cerebral aspergillosis were identified in a series of 44 brains obtained at necropsy from patients who had undergone liver transplantation. In two of these there was dual infection with *Candida albicans*. The primary focus of infection was invariably in the lungs. One case of pulmonary *Aspergillus* infection was found with no evidence of cerebral disease. Infection tended to occur in the period soon after transplantation, was associated with high dose steroids, retransplantation, and showed a significant seasonal incidence. Neurological findings were non-specific and only two cases were diagnosed before death.

***Aspergillus* infection soon after transplantation indicates that this organism is a considerable nosocomial hazard, particularly in the winter and spring months. Positive cultures before death are rarely obtained and antifungal treatment should be started on clinical suspicion alone.**

Liver transplantation is increasingly being used for the treatment of end stage liver disease. Recently workers have reported a high incidence of neurological morbidity following liver transplantation.[1–3] In many cases the causes are poorly understood and few detailed neuropathological studies have been undertaken.[4–6] We prospectively studied brains obtained at necropsy according to a standard protocol in an attempt to determine the spectrum of neurological disease that may result.[7]

Systemic aspergillosis is a recognised complication of immunosuppression,[8] and cerebral invasion may result in a variety of neurological signs.[9] Several factors may account for the higher incidence of fungal infections in patients who have undergone liver transplantation than in recipients of other solid organs.[10] Different centres, however, seem to report widely differing incidences.[10 11] Difficulties in establishing the diagnosis[12 13] make it probable that exclusive use of microbiological data obtained during life leads to under recognition of systemic infection by *Aspergillus* spp. We therefore reviewed the cerebral histology in all cases of cerebral mycosis currently identified in our necropsy series to establish the true incidence and pattern of disease.

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Methods

Between January 1982 and May 1989 218 patients received 250 orthotopic liver transplantations at the Queen Elizabeth Hospital, Birmingham. Ninety eight (45%) patients had died at the time of this study and of these, 58 (59%) were necropsied at our centre. In some of the earlier cases brain tissue had not been sampled for histological examination, and in others permission for necropsy of the brain was refused. In four cases only paraffin wax-embedded tissue blocks were available for histological examination. A further 40 entire brains were prospectively collected and sampled for histology using a standard protocol. The total sample, therefore, comprised 44 patients (28 female, 16 male) and represented 44·9% of all liver transplant deaths. The mean age of the group was 40·9 years; 38 were adults (range 21–63 years) and six were children (range 11 months–16 years). These patients had received 55 liver transplants, including nine retransplants, and one patient received a transplant three times.

Gross external findings were described at necropsy and the brains then fixed whole by suspension in 10% formalin for three to six weeks. In each case the brain stem and cerebellum were detached and the cerebrum sliced coronally at 1 cm intervals. Any grossly obvious lesions were described and sampled for histological examination. Routine large blocks of frontal, temporal, parietal and occipital lobes, hippocampus, basal ganglia, thalamus, cerebellum, pons and medulla were also processed through a standard technique to paraffin wax. Sections stained with haematoxylin and eosin were prepared from these and also from the four cases for which only paraffin wax embedded tissue was available in file. Special and immunohistochemical stains were performed where indicated.

All clinical records were carefully scrutinised. Particular note was made of any neurological signs and symptoms, microbiological isolates, and previous antifungal treatment. Immunosuppressive treatment followed a previously described standard protocol.[14] General necropsy findings, including histology, were reviewed.

Results

Invasive cerebral aspergillosis was present in nine brains (20%). Identification of the species was possible in four cases, either through culture of specimens taken before death or necropsy tissue. In two brains invasive candidiasis was also evident. There were no other forms of cerebral mycosis in the series. Mean age and sex ratio of affected patients (44·0 years and 1·25F:1M) did not differ sig-

Table 1 Cerebral aspergillosis in liver transplant recipients: summary of clinical details

Case No	*Age*	*Sex*	*Indication for transplantation*	*Survival (days) after transplantation**	*Organism identified*	*Diagnosis before death*	*Anti-fungal treatment†*	*High dose steroids within two weeks of death††*
20	36	M	α_1-antitrypsin deficiency	15	*Aspergillus* sp, *Candida albicans*	No	No	Yes
36	28	M	Acute Budd-Chiari syndrome‡	27	*Aspergillus fumigatus, Candida albicans*	Yes	Amphotericin (IV: 1 day)	Yes
40/42	59	F	PBC‡	17,5	*Aspergillus fumigatus*	Yes	Amphotericin (IV: 1 day)	Yes
64/76	21	M	Chronic active hepatitis	130,55	*Aspergillus* sp	No	No	No
90/91/92	53	F	PBC‡	26,11,6	*Aspergillus* sp	No	No	Yes
93/94	44	F	PBC‡	20,14	*Aspergillus flavus*	No	No	Yes
114	61	F	PBC	96	*Aspergillus fumigatus*	No	No	No
124	56	F	Non-A, non-B hepatitis‡	12	*Aspergillus* sp	No	Amphotericin (IV: 6 days)	Yes
220/223	38	M	α_1-antitrypsin deficiency‡	18.9	*Aspergillus fumigatus*	No	Amphotericin (IV: 15 days)	Yes

*Time from first transplant, second transplant etc, until death.
†Excluding oral prophylaxis: 100 mg amphotericin daily; 100 000 Units nystatin four times daily
††200 mg prednisolone or 1 g methylprednisolone per day.
‡Patients in preoperative acute hepatic failure and coma.

nificantly from the group as a whole (40·9 years and 1·75F:1M). Survival after first transplantation ranged from 12 to 130 days. Significantly, seven out of 18 deaths occurring one to four weeks after transplantation were related to systemic aspergillosis ($p < 0.05$, χ^2 test with Yates' correction). Five patients underwent retransplantation at least once, compared with five out of 35 who did not develop cerebral aspergillosis ($p < 0.05$, χ^2 test with Yates' correction).

All patients received standard immunosuppressive treatment based on prednisolone, azathioprine, and cyclosporin A.[14] Seven out of nine were also given high dose corticosteroids (1 g intravenous methylprednisolone or 200 mg oral prednisolone) within two weeks of death. The total number of bolus treatments were significantly higher in the group with aspergillosis at necropsy (median 5·5; range 0–11·5) than in the other 35 patients (Mann Whitney U test: $p < 0.01$). Only one patient (case 36) became severely neutropenic in the period before death. Basic clinical data are summarised in table 1.

Slicing showed that in seven brains there were obvious gross abnormalities—multiple, widely distributed haemorrhagic softenings, some of which showed central cavitation (fig 1). In case 124, however, only a single area of temporal lobe infarction was grossly evident and in case 64/76 an invasive focus was only detected by microscopic examination. Lesions were found in most areas of the cerebrum, generally at the grey white matter junction, and with no tendency, despite previous assertions,[15] to favour areas supplied by the posterior circulation. Brain stem and cerebellum were, in fact, less commonly affected (table 2).

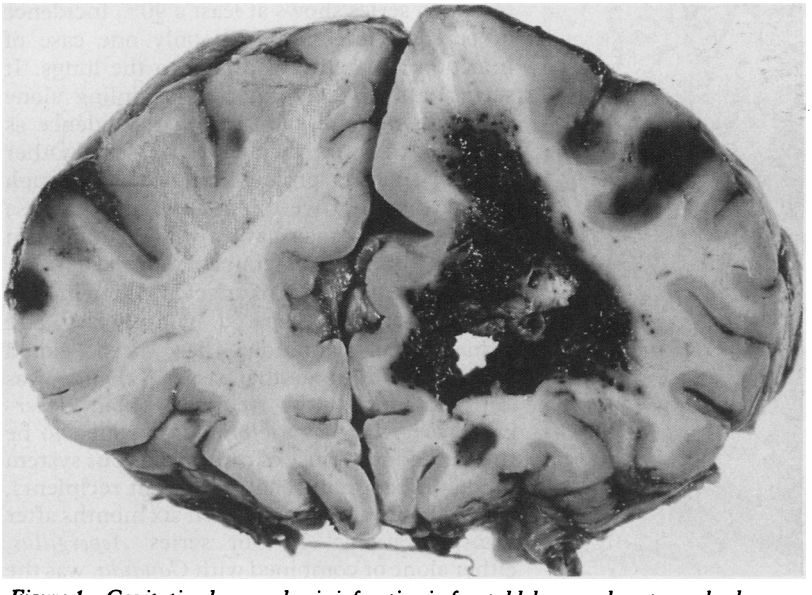

Figure 1 Cavitating haemorrhagic infarction in frontal lobe secondary to cerebral aspergillosis.

Several histological patterns of disease were evident. Most brains showed a predominant pattern of haemorrhagic infarction associated with thrombosis of medium sized vessels. Grocott silver staining showed invasion of vessel walls and surrounding parenchyma by septate fungal hyphae with acute angle branching typical of an *Aspergillus* species (fig 2). In three cases (cases 36, 93/94, and 114), there was also a widespread small vessel vasculitis (fig 3), and Grocott staining of such lesions showed only occasional invasive hyphae. In these brains gross examination underestimated the true extent of infection. Occasional foci of meningeal invasion were noted, but there were no examples of generalised fungal meningitis. Where there was extensive haemorrhage (case 90/91/92), it was difficult to identify organisms and the diagnosis was made from lesions elsewere in the brain. Bleeding was probably due to vessel rupture, resulting from fungal invasion. Inflammatory reactions varied in intensity but were polymorphonuclear in character with no granulomatous reactions.

Cases 20 and 36 showed separate foci of a morphologically different organism including both yeast and pseudohyphal forms. These were obvious only on special staining and were not associated with any noticeable parenchymal damage. In both of these cases blood cultures taken before death had grown *Candida albicans*. Features were interpreted as dual infection with a *Candida* species.

All cases were associated with *Aspergillus* pneumonia confirmed at necropsy. In eight patients, histology of necropsy showed widely disseminated aspergillosis (table 3). In case 124 the brain was the only extra-pulmonary focus. Review of all liver transplant patients at necropsy showed only one further case of

Table 2 Cerebral aspergillosis in liver transplant recipients: distribution of lesions

Case No	Frontal	Occipital	Temporal	Parietal	Basal ganglia/thalamus	Brain stem	Cerebellum
20	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+
40/42	+	+	+	+	+	+	−
64/76	−	−	−	+	−	−	−
90/91/92	−	−	−	+	+	−	+
93/94	+	+	+	+	−	−	+
114	−	−	+	−	−	−	−
124	+	+	−	+	+	+	+
220/223	+	+	+	+	+	+	+

Aspergillus infection (case 12). This was confined to the lungs. The series indicates, therefore, a pulmonary source of infection in all cases, with systemic spread in 90%: the brain was the most commonly affected organ.

Assuming that infection occurred a short time before death, there was a striking seasonal incidence of *Aspergillus* infection (fig 4). All nine patients with cerebral disease and the one case with exclusively pulmonary disease (case 12) died in the six months between November and April. Aspergillus infection was not discovered in the 22 patients who were necropsied in the summer and autumn months, May to October. These differences were significant ($p < 0{\cdot}02$, χ^2 test with Yates' correction).

Neurological signs and symptoms were of little value in the diagnosis of cerebral mycosis: eight patients showed progressive neurological deterioration with encephalopathy, terminal coma, and (in cases 220/223 and 90/91/92) seizures. Case 40/42 showed a more rapid onset of coma accompanied by grand mal fits. Similar features have been noted in other liver transplant recipients who developed neurological complications.[1] Electroencephalogram (EEG) findings in eight cases showed encephalopathy, with evidence of focal lesions in four. Computed tomography scanning was performed in case 90/91/92 and showed a posterior fossa haemorrhage. Neither of these investigations was able to distinguish between infective and non-infective lesions.

In only two patients (cases 36 and 40/42) was the diagnosis of aspergillosis made before death; in both cases sputum culture results were only available within 48 hours of death, allowing little time for treatment. In a further three patients results only became available after death: culture of skin vesicle fluid from case 93/94 grew *A flavus*, sputum culture from case 114 grew *A fumigatus*, and cytological examination of bronchial aspirates from case 124 showed an *Aspergillus* sp. In this latter case and also in case 220/223 amphotericin treatment had already been started on clinical and microbiological suspicions of systemic candidiasis. Chest *x*-ray pictures were generally unhelpful for specific diagnosis of *Aspergillus* pneumonia. Pleural effusions were noted in three patients and five showed areas of consolidation. Only one case (case 124) showed evidence of cavitation. In contrast to the difficulties in diagnosing aspergillosis, blood cultures had grown *Candida albicans* in both the cases (cases 20 and 36) of mixed cerebral mycosis.

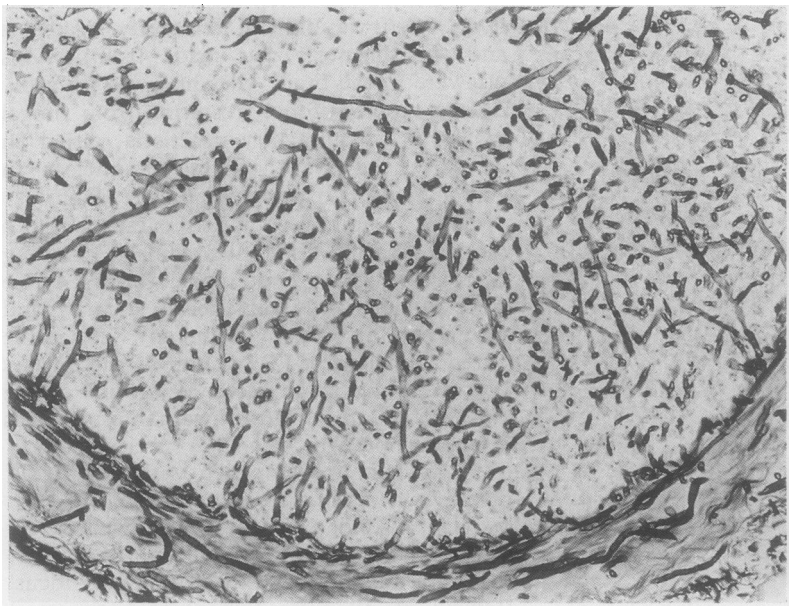

Figure 2 Aspergillus within lumen and invading the wall of a thrombosed vessel (Grocott stain).

Discussion

Cerebral aspergillosis is a recognised complication of immunosuppression, including that associated with organ transplantation. Primary infection in the lungs is almost invariable and the brain is the organ most commonly affected by haematogenous dissemination.[15] The incidence of spread to the brain has been previously reported as between 10 and 50%.[15–17] Our own series shows at least a 90% incidence of cerebral infection with only one case of infection apparently confined to the lungs. It seems unlikely that rigorous sampling alone could account for our observed incidence as most cases showed obvious gross disease. Other factors, such as prolonged survival through intensive supportive treatment and high dose corticosteroids, may have given the organism an opportunity to disseminate more widely.

A higher incidence of fungal infection in liver transplantation compared with heart and kidney transplantation, has been noted previously,[18] but in that series *Candida* was detected much more frequently than *Aspergillus*. *Cryptococcus neoformans* is stated to be the commonest cause of central nervous system fungal infection in renal transplant recipients, generally occurring more than six months after transplantation.[17] In our series *Aspergillus*, either alone or combined with *Candida*, was the only observed form of cerebral mycosis. The incidence of aspergillosis in liver transplanta-

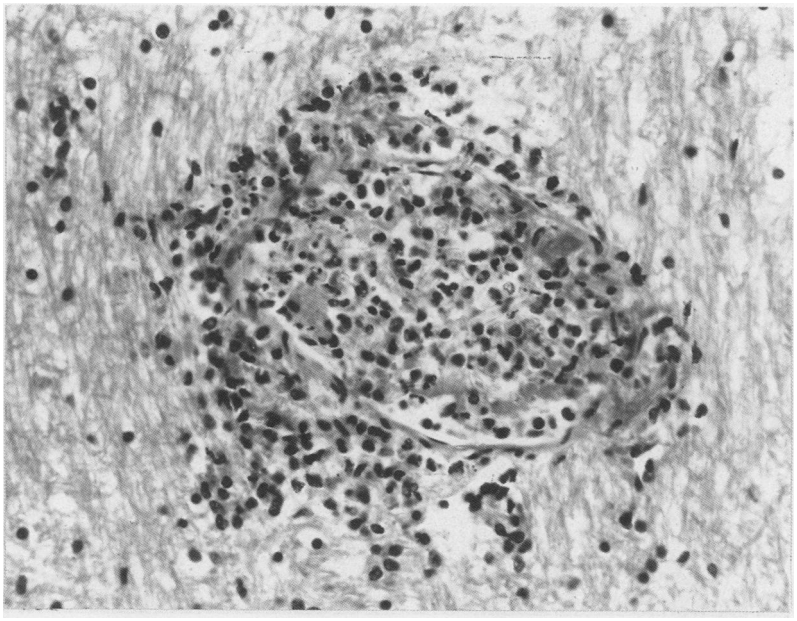

Figure 3 Necrotising small vessel vasculitis in the brain: Grocott staining showed occasional invasive fungal hyphae.

tion seems to vary among centres. The Cambridge group reported very few cases, but did not state their criteria for diagnosis.[10] Although our series was clearly weighted in favour of deaths occurring soon after transplantation, the 23% incidence of pulmonary or systemic aspergillosis in necropsy cases (10% of all deaths in transplant recipients) is similar to the Denver[11] and Pittsburgh[4 18] series.

Aspergillus infections may be sporadic or clustered, the latter reflecting a heavily contaminated environmental source.[15] All but two of our cases died within four weeks of transplantation, which is said to indicate the presence of an excessive nosocomial hazard.[17] No obvious environmental source was identified and the most striking epidemiological feature was a pronounced seasonal variation (fig 4). This has been previously noted[10] and may be due to a higher concentration of airborne spores outside the summer months.[19] Short of restricting transplantation to the summer—a clearly impractical course—it is uncertain how environmental contamination could be reduced. The use of high efficiency particulate air filters does not seem to reduce the incidence of infection.[10]

There are several reasons why liver transplant recipients may have a particularly high risk of developing aspergillosis. Until recently, criteria for the diagnosis of rejection were not well established.[20 21] Consequently, inappropriate anti-rejection treatment might have been given, leading to acquisition, acceleration, or dissemination of pulmonary *Aspergillus* infections. Systemic corticosteroids are a major predisposing factor in aspergillosis of the central nervous system.[22] Seven out of nine of our series received high dose steroids within two weeks of death and the total number of bolus doses were significantly higher in the group with aspergillosis. Liver transplant recipients sometimes require prolonged artificial ventilation after surgery. Those in fulminant hepatic failure may have spent some time preoperatively on a ventilator. The risks of acquiring a pulmonary infection are consequently enhanced. Occasionally a noticeable disparity in size of the transplanted liver may cause respiratory impairment. There is evidence, furthermore, that liver failure in itself may predispose to *Aspergillus* infection, even without immunosuppressive treatment.[23 24] Six of our patients with cerebral aspergillosis were ventilated before surgery for hepatic coma and may have acquired the infection at that time. Many of these factors may have contributed to the significantly increased risk in retransplanted cases.

The clinical diagnosis of cerebral aspergillosis may be difficult, and in most series, including ours, a positive diagnosis is only made at necropsy.[24] The clinical presentation is either acute with focal neurological deficits and fitting or subacute with progressive obtundation.[17] Both patterns were observed in our cases, but similar features were noted in other patients in whom there was no evidence of aspergillosis at necropsy.[1] Likewise, EEG or computed tomography scan evidence of focal lesions may be found, but neither investigation can firmly establish an infective aetiology. Diagnostic problems are compounded in liver transplant recipients: rapid changes in electrolytes, central pontine myelinolysis,[25] drug toxicity,[26] haemorrhage due to coagulopathies, air embolism,[27] watershed infarction[1] and hepatic failure are recognised causes of neurological problems, occurring alone or in combination. Any of these may mimic or mask the onset of central nervous system infection.

The importance of an aggressive therapeutic approach to these patients has been emphasised,[6] but the toxicity of systemic antifungal chemotherapy prohibits indiscriminate prophylaxis. Central nervous system aspergillosis, however, should be strongly suspected in those patients who develop pulmonary infiltrates and focal neurological signs,[22] especially if they have received high dose steroids. Treatment with intravenous antifungal agents should be started even in the absence of positive cultures.

Table 3 Disseminated aspergillosis in liver transplant recipients: organ disease at necropsy

Case No	*Brain*	*Lung*	*Heart*	*Kidney*	*Liver*	*Large bowel*	*Thyroid*	*Oesophagus*	*Pancreas*	*Spleen*	*Other*
20	+	+	+	+	+	−	−	−	+	−	−
36	+	+	+	+	+	+	+	−	+	+	Bladder
40/42	+	+	+	+	−	+	−	−	−	−	−
64/72	+	+	+	+	+	−	−	+	−	−	−
90/91/92	+	+	−	−	−	−	+	+	−	−	−
93/94	+	+	+	+	−	−	−	−	−	−	Skin, stomach
114	+	+	+	+	+	+	+	−	−	−	−
124	+	+	−	−	−	−	−	−	−	−	−
220/223	+	+	−	−	−	−	−	−	−	+	Adrenal

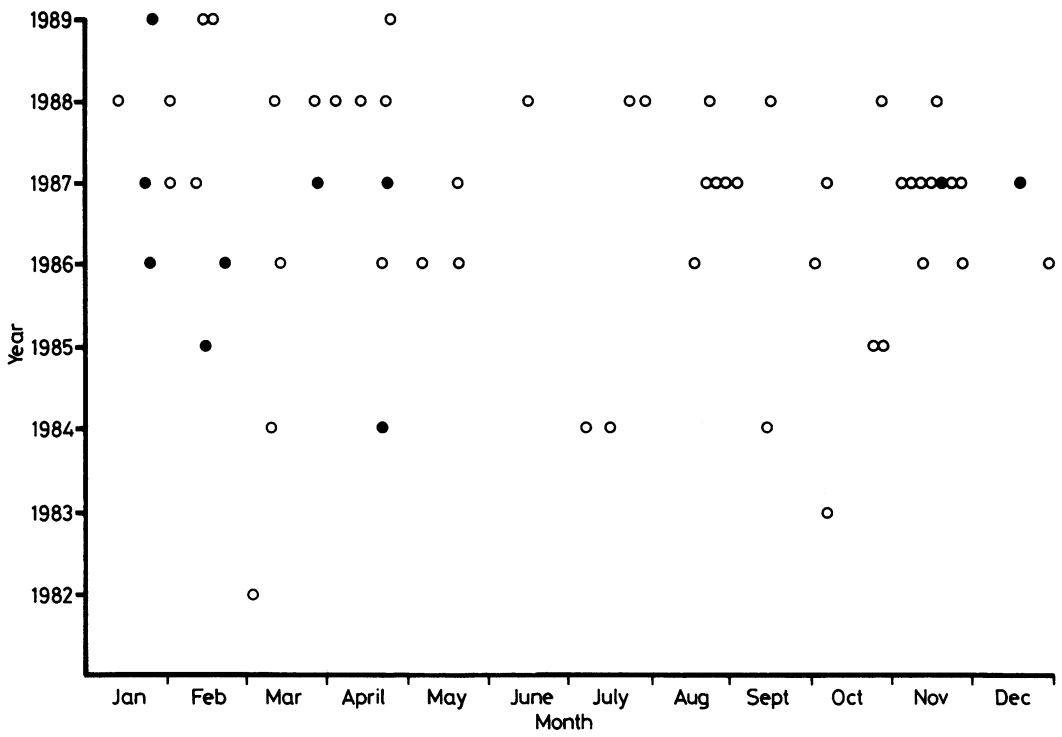

Figure 4 Dates of all liver transplant deaths necropsied at Queen Elizabeth Hospital. Infected cases (including patient without cerebral disease) (●); other liver transplant cases (○).

In view of the poor prognosis of cerebral aspergillosis, early diagnosis and treatment, before dissemination in the central nervous system has occurred, seems to offer the best chance of cure.[17] Despite daily sputum cultures, the organism was identified in sputum or bronchial secretions in only four patients and so late in the course of the disease as to be of no clinical value. Until better serological techniques become available[13] the diagnosis must rely largely on clinical suspicion.

The dermatological manifestations of systemic aspergillosis should not be overlooked.[28] One of our series (case 93/94) developed a "herpetic" rash some days before death. Vesicle fluid was later submitted for fungal culture, resulting in growth of *Aspergillus flavus:* unfortunately, the patient died the day before results were available. The skin is more accessible to laboratory investigation than any other organ, and in disseminated fungal infection, a biopsy specimen of an unusual rash may be the only means of establishing an early diagnosis.

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