Interpretation of endomyocardial biopsy after heart transplantation

Potentially confusing factors

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Summary

Histological examination of the myocardium by endomyocardial biopsy is a standard method of monitoring the presence of acute rejection in the transplanted heart. The histopathological consequences of the biopsy procedure itself have been investigated in non-transplanted hearts in the baboon. Organization of thrombus, necrosis of myocytes adjacent to the biopsy site, and mononuclear cells (including T lymphocytes) surrounding the biopsy site appear after biopsy; should a subsequent biopsy be taken from this area, these appearances may be confused with the appearances associated with acute or resolving cardiac rejection. This problem has been encountered in the clinical transplant programme. Observations on the myocardial histopathological changes resulting from brain death and from parasitic infestation, both of which may also lead to confusion in the interpretation of endomyocardial biopsies, are also presented.

Awareness of these factors in patients with heart transplants should lead to caution in the interpretation of the histopathological features and may avoid unnecessary extra immunosuppression early after transplantation. Observations indicate that endomyocardial biopsy should not be the sole method of monitoring for the development of acute rejection.

Histopathological changes at the site of endomyocardial biopsy

In 9 healthy sedated (ketamine 10 mg/kg intramuscularly) non-transplanted Chacma baboons (Papio ursinus), endomyocardial biopsies were performed by a standard technique, an Olympus 19C biopsy being introduced through the right jugular vein. On each occasion, under fluoroscopic control, 5 biopsy specimens were taken from the endomyocardium of the right ventricle. Two baboons were anaesthetized on the day of endomyocardial biopsy and the heart was excised; hearts were excised from the remaining animals on days 1, 4, 7, 14 and 30 after biopsy.

In all animals, the heart was examined macroscopically and the right ventricular biopsy sites were examined histologically. Light microscopic sections were stained with haematoxylin and eosin and by the Martius scarlet blue method. In 3 cases, monoclonal antibodies were used on frozen sections to investigate the presence of lymphocyte subpopulations.

The evolution of the histological changes in these hearts is summarized in Table 1, and examples of the microscopic changes are shown in Figs 1-3. In summary, the biopsy site caused an initial endomyocardial defect which became covered by fibrin-platelet thrombus within 24 hours. Thrombus was more extensive than the original endomyocardial defect, possibly due to stripping off of endocardium at the margins of the biopsy site as well as to extension of thrombus onto the surrounding intact endocardium. Interstitial haemorrhage occurred between the myocytes underlying the biopsy site. Over the next 4 days, a mononuclear cellular infiltration appeared at the biopsy site, with various forms of myocyte necrosis (contraction band necrosis, myocytolysis and coagulative necrosis) in some areas. Biopsy site thrombus underwent organization by granulation tissue; in time, the latter appeared to mimic stromal collapse fibrosis. Haemosiderin-laden histiocytes were also seen. By 14 days, focal heavy infiltrates of mononuclear cells remained. These included numerous cytotoxic-suppressor and inducer-helper T cells which appeared pyroninophilic. Granulation tissue ingrowth into the thrombus was well advanced.

Histological examination of the myocardium by endomyocardial biopsy, introduced by Caves et al. in 1973, has proved invaluable in the management of patients with heart transplants. In patients who have undergone repeated biopsies, there is a distinct possibility of again sampling a previous biopsy site. No information is available about changes in the endomyocardium after endomyocardial biopsy. The histopathological consequences of the biopsy procedure itself were therefore investigated in non-transplanted hearts in the baboon.

In addition, observations on the myocardial histopathological changes which may result from brain death and from parasitic infestation, both of which may also lead to confusion in the interpretation of endomyocardial biopsies, are presented.

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TABLE I. EVOLUTION OF HISTOLOGICAL CHANGES AFTER ENDMYOCARDIAL BIOPSY

<table>
<thead>
<tr>
<th>Day of biopsy</th>
<th>Macroscopic appearance</th>
<th>Microscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Small endocardial pit</td>
<td>Endomyocardial defect with stripped endocardium around the site; scanty surface thrombus, interstitial haemorrhage</td>
</tr>
<tr>
<td>1</td>
<td>Thrombus on and around the biopsy site; local subendocardial haemorrhage</td>
<td>Surface thrombus, interstitial haemorrhage, coagulative necrosis of myocytes adjacent to biopsy site with some neutrophils; thrombus extends onto adjacent intact endocardium</td>
</tr>
<tr>
<td>4</td>
<td>Thrombus firmly adherent to biopsied areas, subendocardial haemorrhage less evident</td>
<td>Moderate early organization of thrombus; mononuclear cell infiltrate extends into adjacent myocardium; various forms of myocyte necrosis present</td>
</tr>
<tr>
<td>7</td>
<td>Persistence of thrombus, disappearance of subendocardial haemorrhage</td>
<td>Organization of thrombus with granulation tissue and an inflammatory response at the base of the biopsy site; abundant mononuclear cells* in adjacent myocardium</td>
</tr>
<tr>
<td>14</td>
<td>Reduction of thrombus with fibrous tissue covering surface of biopsy site</td>
<td>Fibrosing granulation tissue contains a heavy infiltration of mononuclear cells* extending into surrounding myocardium</td>
</tr>
<tr>
<td>30</td>
<td>Focal endocardial scar</td>
<td>Endocardial fibrosis (minimal residual fibrin) and scanty mononuclear cells</td>
</tr>
</tbody>
</table>

* Cytotoxic-suppressor and inducer-helper T cells.

Myocardial histopathology after brain death

In 23 healthy anaesthetized Chacma baboons, brain death was induced by a rapid increase in intracranial pressure brought about by the insertion of a Foley catheter and inflation of its balloon; details of this technique have been reported previously.\(^3\)\(^-\)\(^4\) The clinical criteria of brain death were noted within 20 minutes of this procedure. During the agonal period, electrocardiographic changes occurred which included multifocal ventricular ectopic beats and features indicating myocardial ischaemia. Subsequently, sinus tachycardia persisted for a mean period of approximately 120 minutes, and in some animals ischaemic changes with Q waves were seen throughout this period.\(^3\) These changes were thought to be associated with myocardial endogenous catecholamine release.\(^4\) The baboon was supported haemodynamically for periods ranging from 2 to 24 hours by the infusion of intravenous fluids. At the end of the experiment the heart was excised, and sections of myocardium were examined by light microscopy after staining with haematoxylin and eosin, Unna-Pappenheim stain, and Masson's trichrome stain; lymphocyte subsets were also investigated with monoclonal antibodies.

Of the 23 animals studied 85% showed diffuse or focal myocardial structural changes consisting of various forms of myocyte necrosis (contraction bands, coagulative necrosis, myocyte lysis and stromal collapse). The presence of contraction bands (Fig. 4) in these hearts could not be artefactual as sections were taken at autopsy not biopsy. The areas of myocyte necrosis were surrounded by a mild degree of interstitial oedema. Focal aggregates of mononuclear cells surrounded necrotic myocytes (Fig. 5), especially in areas of coagulative necrosis or myocyte lysis. Capillary endothelial damage could also be seen in the majority of hearts.
Discussion

Two quite separate factors, which might confuse the histopathological picture of endomyocardial biopsy and lead to an erroneous histological diagnosis of acute cardiac rejection, are presented. The inflammatory response to the trauma of biopsy itself (particularly since in clinical practice endomyocardial biopsies tend to be taken from the same area at the apex of the right ventricle) or to the myocardial injury associated with the extreme haemodynamic changes which take place during the agonal period after a sudden rise in intracranial pressure may lead to a histopathological picture which strongly resembles or may be indistinguishable from that of acute rejection. A third possible cause for confusion might be the presence of a parasite within the myocardium, and an example of this is discussed and illustrated below.

Contraction bands are relatively common after biopsy and, if not accompanied by other histopathological changes, may rightly be ascribed to the bioptome. The presence of mononuclear cells may well be part of the inflammatory response to a previous biopsy. The presence of organizing thrombus and/or granulation tissue on the endocardial aspect of the sample should alert the cardiologist to the possibility that this may be a previous biopsy site. Platelets, which are known to act as mediators of inflammation, may play a role in the genesis of the inflammatory cells at the biopsy site.

The possible factors contributing to the marked myocardial structural changes during the agonal period have been discussed elsewhere. Similar changes (to those described in the baboons of this study) of myocardial damage have been seen in 5 patients in whom the donor heart failed to function after transplantation or in whom early donor-heart failure occurred after transplantation. In brief, at autopsy in all 5 patients the donor heart looked normal to the naked eye, except for occasional subendocardial haemorrhages. On light microscopy, however, all hearts revealed various combinations of three forms of acute myocardial necrosis, namely contraction bands (coagulative myocytolysis), coagulative necrosis and colliquative myocytolysis. Focal mononuclear cellular infiltration was also seen in relation to some of the necrotic myofibres (Fig. 6).

It is clear that the changes during and after the development of brain death, almost certainly the result of prolonged release of myocardial endogenous catecholamines, may result in histopathological appearances resembling those of acute cardiac rejection.

In 1 of our patients who underwent heterotopic heart transplantation, three initial endomyocardial biopsies showed prominent areas of mononuclear cell infiltration, suggesting acute cardiac rejection. In the fourth specimen, however, Toxoplasma gondii was seen within the myocytes (Fig. 7) and the mononuclear cell infiltration was attributed to the presence of this parasite. At the time of subsequent biopsies of the transplanted heart, the patient’s own heart was biopsied and also showed the presence of the parasite, together with mononuclear cell infiltration. The recipient heart therefore acted as a ‘control’ for the donor heart, in that both showed effects attributed to the presence of the parasite but only the donor heart showed additional changes attributable to acute rejection. Details of this patient and his subsequent clinical course have been reported previously; evidence suggested that the infection was transferred with the donor heart.

Histologically, toxoplasmosis produces focal interstitial infiltrates containing lymphocytes, plasma cells, histiocytes and a few eosinophils. Because inflammation is not encountered in the vicinity of parasitized myocytes, the inflammatory response is attributed to rupture of the infected myocytes and cyst disintegration rather than to the presence of the parasites within the myocytes. The cellular infiltrate in toxoplasmosis is more mixed (lymphocytes, plasma cells, histiocytes and eosinophils), whereas in early acute rejection the cells consist almost entirely of activated lymphocytes. Unless the organism is
positively identified, the inflammatory changes accompanying the infection could well be interpreted as being due to acute rejection.

A light microscopic appearance histologically identical to *Toxoplasma* infection may be seen with *Sarcocystis* infection. Awareness of the factors influencing myocardial histology in patients with a heart transplant should lead to caution in the interpretation of the histopathological features and may avoid unnecessary extra immunosuppression in patients early after transplantation. Our observations also indicate that endomyocardial biopsy should not be the sole method of monitoring for the development of acute rejection. A second method of monitoring, such as radionuclide scanning, echocardiography or cytological and immunological monitoring, should also be employed to support or refute the biopsy findings.

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