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Plasmapheresis as a rescue therapy to resolve cardiac rejection with vasculitis and severe heart failure. A report of five cases

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Abstract The predominant causes of late graft loss and death after cardiac transplantation are graft rejection and infection. The histopathological classification of acute rejection is based on cellular phenomena such as lymphocytic infiltration and myocyte damage. The adverse prognostic importance of vascular or humoral rejection has been reported, but there is no well-documented treatment available. In our experience, comprising 151 orthotopic transplants, five patients presented with graft rejection characterized by a lymphocytic vasculitis that did not respond to conventional therapy. Because of a deteriorating condition, in spite of vigorous antirejection treatment that included inotropic drugs and circulatory support, plasmapheresis was tried as a last, desperate means to stop the process from developing further. The clinical symptoms rapidly subsided in all five patients after the first couple of plasma exchanges. All of the patients are alive and well after 2–3.5 years of follow-up. Although the mechanism of action is unclear, plasmapheresis was beneficial in these critically ill patients.

Key words Heart transplantation, plasmapheresis, rejection · Rejection, heart transplantation, plasmapheresis · Plasmapheresis, rejection, heart transplantation · Vasculitis, heart transplantation, plasmapheresis

Introduction

Cardiac allograft rejection is not a clearly defined entity. The histopathological diagnosis of acute rejection is based on cellular phenomena such as lymphocytic infiltration and myocyte damage, according to the original classification of endomyocardial biopsy findings [2] with a later modification [4]. There have been several reports recently on vasculitis and the deposition of immunoglobulins in vessels of smaller dimensions [6, 8, 9, 12] that have been interpreted as an expression of vascular (humoral) rejection [8, 11]. Mixed forms of vascular and cellular rejection have also been described [8]. The adverse prognostic importance of vascular rejection in the transplanted heart is well known, and a lower graft survival rate than in that of cellular rejection has been reported [8]. The basic mechanisms responsible for these vascular lesions of the allografted heart are still partially unknown but are thought to be immunological.

Plasmapheresis has been reported as a means of treatment in occasional and desolate cases of vascular rejection, blood group incompatibility, or positive donor crossmatch in heart transplant recipients [7, 8, 12, 14, 15, 17]. This report describes the effect of plasmapheresis in five desperately ill patients with vasculitis in their cardiac allografts, as well as the histopathological development and outcome after long-term follow-up.

Materials and methods

Between 1 January 1988 and 31 December 1993, 151 orthotopic cardiac transplantations were performed in 147 patients. Their age ranged from 13 to 63 years (mean 45 years). There were

Table 1 Specific data on five patients treated with plasmapheresis (*rec* recipient, *don* donor, *IHD* ischemic heart disease, *DCM* dilated cardiomyopathy, *Rej* rejection in transplanted heart)

Case (no)	Sex	Age rec/don	Diagnosis	Ischemia time (min)	Inotropics (days postop)	Immunosuppression	HLA match[a]	Antibody screen	CMV serology rec/don
1	F	58/44	IHD	205	6	Triple + ATG induction	3/1	Pos	Pos/Pos
1	M	59/18	DCM	205	4	Triple	4/1	Neg	Pos/Pos
3	F	17/13	DCM	153	2	Triple	2/1	Neg	Pos/Pos
4	M	47/40	Rej	191	12	Triple + ATG induction	3/1	Neg	Pos/Neg
5	F	60/17	DCM	178	5	Triple	3/1	Neg	Pos/Pos

[a] Number of mismatches in HLA-A,B/DR groups

120 men and 27 women. The disease leading up to transplantation were dilated cardiomyopathy. ($n = 78$), ischemic heart disease ($n = 51$), myocarditis ($n = 6$), and other ($n = 16$).

Immunosuppression

Immunosuppression was administered according to three different protocols. At the start of our program in 1988, the first ten patients (group 1) received high-dose steroids (100 mg/day, tapering over 3 months to 7.5 mg/day), azathioprine, and cyclosporin. After the first ten transplants, the protocol was changed and the patients were divided into two groups according to their preoperative renal function. Group 2 (56 patients) received low-dose steroids (0.2 mg/kg body weight daily, tapering to 0.1 mg/kg body weight daily over 3 weeks), azathioprine, and cyclosporin. This group had normal renal function preoperatively. Group 3 (85 patients) received induction therapy with antithymocyte globulin (ATG); 2.5 mg/kg body weight for 3–5 days), azathioprine, low-dose steroids and, after 2–4 days, cyclosporin. This group had decreased renal function (Cr^{51}-EDTA clearance < 60 ml/min) preoperatively.

The dose of azathioprine varied between 1 and 2 mg/kg body weight, according to white blood cell count ($> 5 \times 10^9$/l). Cyclosporin was administered aiming at a whole blood trough level of 400 µg/l (monoclonal specific measurement), tapering to 200 µg/l after 3 months.

Rejection

Rejection was diagnosed by endomyocardial biopsy and classified according to the criteria of the International Society for Heart and Lung Transplantation (ISHLT) [4]. Routine biopsies were taken weekly for 6 weeks, biweekly for 6 weeks, monthly for 3 months, and then every 3 months. Biopsies were also occasionally obtained when rejection was suspected. Six to eight tissue samples were taken in each biopsy procedure. The standard technique for biopsy procedures was used [5]. When rejection was diagnosed, antirejection therapy was started and control biopsies were taken weekly until the rejection episode was terminated, i.e., when negative findings were obtained in two successive biopsies. Thereafter, the original biopsy routine was followed.

Tissue processing

The endomyocardial biopsy specimens, 1–3 mm in diameter, were fixed in buffered 4 % formaldehyde by continuous shaking on a moving table (Heidolph, Kelheim, Germany) for 45 min. The specimens were rapidly dehydrated in acetone for 15 min and vacuum-embedded in paraffin wax in a tissue processor (Biopsator, Med'Lass, Heidelberg, Germany). Five-micron-thick serial sections at three levels were stained with hematoxylin and eosin, Masson's trichrome, and a combined elastin and van Gieson stain, respectively. Immunohistochemistry was not routinely applied. However, in all five patients who were treated with plasmapheresis, immunostaining was performed at the most severe stage of vascular changes. The ABC immunoperoxidase method of Hsu et al. [10] was performed on paraffin sections in selected cases using commercially available antibodies against various human antigens in order to identify the infiltrating cells as well as immunoglobulins, complement factors, and infectious organisms such as cytomegalovirus, Epstein-Barr virus, and *Toxoplasma gondii.*

Rejection treatment

Rejection treatment was based on the clinical appearance and morphological picture and with regard to the time passed since the transplantation. Rejection grade 1 A–B was not treated. Treatment of rejection grade 2 consisted of pulsed steroids (methylprednisolone, 0.5–1 g daily for 3 days), which would be repeated in the case of persisting rejection in the follow-up biopsy. Rejection grade 3 A–B was treated with ATG (Thymoglobulin, Pasteur-Mérieux), 2.5 mg/kg body weight per day for 3–5 days. In the case of therapy-resistant rejection, monoclonal antibody (Orthoclone OKT3, Ortho-Cilag) was administered, 5 mg/day, for 2 weeks.

Basis for plasmapheresis

Five out of the 147 patients (151 grafts) presented with what was diagnosed as graft rejection with vasculitis not responding to conventional therapy. Their endomyocardial histopathological pictures were complex, but all included some component of vascular process. The cellular rejection component was identified as grade 1 B–3 B. Specific data from these patients are listed in Table 1. Cases 1, 2, 3, and 5 had their first graft, case 4 his second one. Plasmapheresis was used when life-threatening heart failure developed in spite of ongoing antirejection treatment, and when repeated series of traditional antirejection treatment failed to alter the histopathological picture. Plasmapheresis was only used as an ultimate rescue therapy when no other means were judged possible.

The clinical picture of heart failure presented with classical signs, such as oliguria, tachycardia, low systolic blood pressure, elevated venous pressure, low oxygen saturation, and dyspnea. There was no doubt that the histopathological picture (cellular rejection + vasculitis) was the cause of the heart failure since no patient had signs of infection, antibodies directed to the donor, surgical complications, or any other clinical explanation for their development of severe heart failure. The patients' condition had to require inotropic support (cases 1, 3–5) or circulatory support with

extracorporeal membrane oxygenation, ECMO; (case 2) before the decision to use plasmapheresis could be reached.

Plasmapheresis

A polypropylene hollow fiber plasma filter (PF 2000, Gambro AB, Lund, Sweden) with an effective area of 0.38 m^2 and a sieving coefficient of 0.98 for total protein was used. Heparin infusion was given to prevent clotting. The volume of plasma removed per exchange was 3.6 liters, and during each plasmapheresis the plasma volume was replaced with 3.0 liters of normal saline and 0.6 liters of human albumin (Novo Nordisk, Denmark, 200 mg/ml). Temporary vascular access was achieved by percutaneous cannulation of the subclavian or femoral veins. The "basic" series of treatments consisted of three plasma exchanges on alternate days. After that, every patient was individually evaluated and the series was interrupted or prolonged according to the severity of rejection, clinical symptoms, and histopathological findings. The number of plasma exchanges per patient was four in cases 2–4, five in case 5, and eight in case 1. Thus, the decision as to when to stop or continue the series of plasma exchanges was individualized rather than based on fixed rules.

The treatment described in the present report was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all patients gave their informed consent.

Results

All patients underwent plasmapheresis without complications. The clinical symptoms of heart failure rapidly subsided, inotropic drugs and ECMO could be weaned, and the general clinical condition improved in all patients after the first couple of plasma exchanges.

The histopathological endomyocardial biopsy findings from the five patients were initially dominated by vascular phenomena such as interstitial edema and proteinaceous exudate. These were later followed by intimal thickening due to swelling of the endothelial cells (endothelialitis) with nuclear pyknosis and an accumulation of mainly lymphocytes in and, as after plasmapheresis treatment, adjacent to the vessels. When lymphocytes infiltrated the vessel wall, the vascular process was classified as a lymphocytic vasculitis (Fig. 1). A perivascular infiltrate, not invading the vessel wall, was defined as a perivasculitis (Fig. 2).

The number of inflammatory cells within or adjacent to the vessel walls varied considerably between cases but increased with time and usually ended up with a picture of full-blown vasculitis. The biopsy material as such allowed only evaluation of the mural, myocardial microvasculature. The changes were irregularly distributed vertically as well as horizontally in the vascular tree but were most prominent at the level of capillaries and venules (Fig. 1 a, b). Hemorrhages, hyaline thrombi, and arteriolitis were rare findings. Myocyte damage with single cell necrosis/cytolysis was obvious in several biopsies but did not usually dominate the picture. Focal ischemic scarring was seen in late biopsies. Several biopsies in this series also showed acute cellular rejection. This could be seen before (case 3), during (cases 2, 3) and after (cases 2, 3, 5) the vascular episode. Altogether eight episodes of cellular rejection were noted in the five patients and classified as grade 2 ($n = 4$) or grade 3 A ($n = 4$) according to the ISHLT.

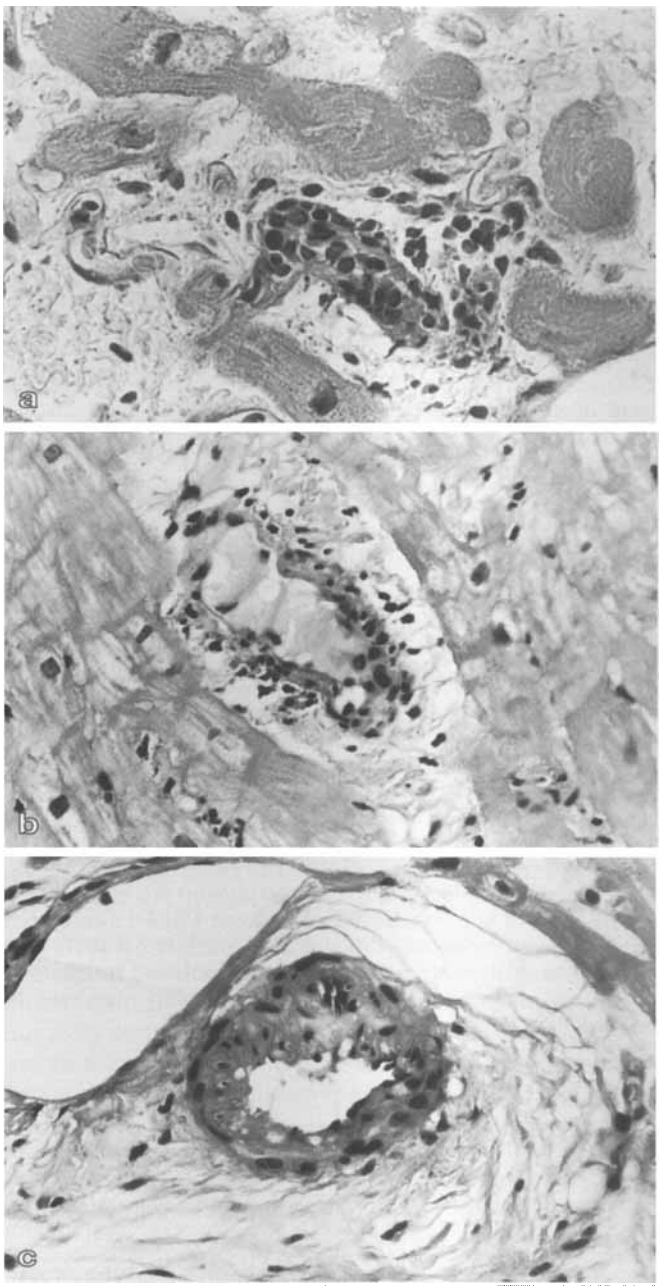

Fig. 1 a–c Light microscopic pictures of vascular profiles showing lymphocytic vasculitis: **a** at a capillary; **b** at a venular level; **c** at an arteriolar level (H & E, medium magnification)

Lymphocytic vasculitis was first seen in the 2nd to 3rd week in three cases (cases 1, 3, 4). Ischemic changes,

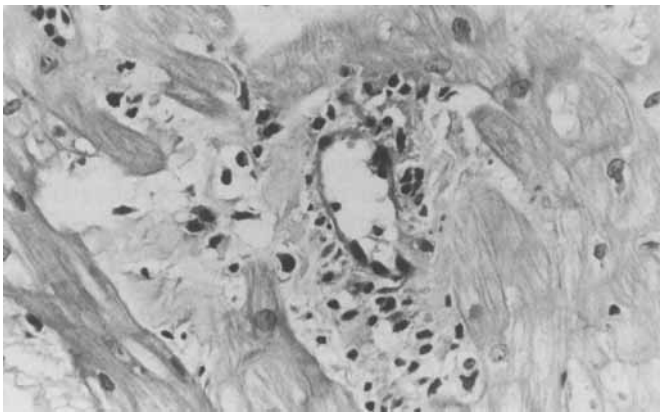

Fig. 2 Light microscopic picture of a vascular profile showing perivascular lymphocytic infiltration diagnosed as perivasculitis (H & E, medium magnification)

normally seen in the 1st and 2nd postoperative weeks, were of great concern as a differential diagnosis in cases 2 and 5, where they were present in successive biopsies for up to 4 weeks, finally merging with an obvious lymphocytic vasculitis (Fig. 3).

After plasmapheresis this inflammatory vascular phenomenon usually regressed and the histological picture showed myocardial repair. Finally, the presence of scarring in the muscle tissue and a perivascular or intramural sclerosis with segmental distribution in the vessels were the only residual changes seen. After several months no lymphocytic vasculitis was observed, but follow-up biopsies, 3–12 months after transplantation, showed focally distributed mild perivasculitis, poor in inflammatory cells in three patients (cases 1, 2, 4).

Immunohistochemistry indicated that T lymphocytes (T-UCHL-1-positive cells) dominated in the inflammatory cell infiltrates and that immunoglobulins (mainly IgM) as well as fibrin were present in areas of exudation. No selective/specific binding of immunoglobulins or complement was found in the vascular structures.

All five patients are alive and have been followed for 2–3.5 years after transplantation. Three patients (cases 2, 3, 5) have been treated successfully with steroids for acute cellular rejection at varying time intervals (weeks to years) after termination of plasmapheresis (Fig. 3). Two patients have developed malignancies (case 2 lymphoma, case 5 adenocarcinoma) in their late follow-up period.

Discussion

Although Ballester et al. reported on successful treatment of vasculitis in four heart transplant recipients with increased immunosuppression [1], there is no known effective treatment for humoral rejection. Several mechanisms of action have been postulated to explain the function of plasmapheresis in this context. One explanation is

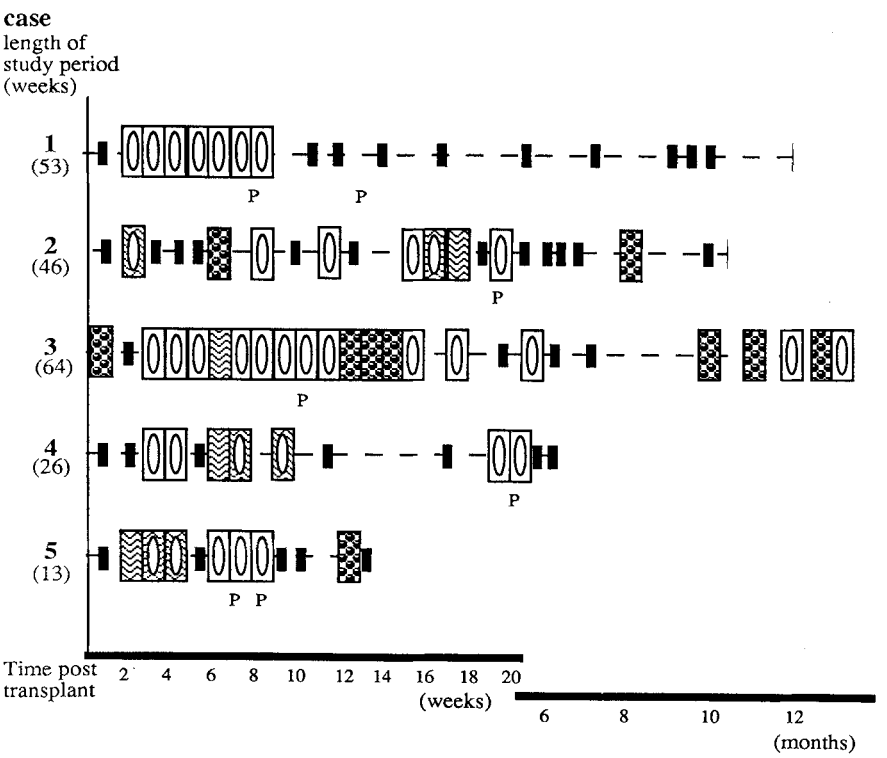

Fig. 3 Diagram showing histological findings in the five cases regarding vasculitis (▯), acute cellular rejection (▩), ischemic lesions (▒), and vasculitis combined with ischemic lesions (▯). (▌ biopsy without any of the mentioned phenomena, *P* plasmapheresis)

that plasmapheresis reduces the number of circulating lymphocytotoxic antibodies and immune complexes. Another possible mechanism would be the removal of circulating lymphokines, such as IL-1, IL-2, and B-cell differentiation factor. Plasmapheresis might possibly also be effective by reducing immunoglobulins and altering the antigen-to-antibody ratio in the recipient.

Since the five patients were not selected for plasmapheresis as part of a true prospective study but rather underwent the treatment because of a life-threatening situation, it was not meaningful to try and construct a control group. Our intention was merely to describe the undeniably beneficial effect of plasmapheresis in our five desperate cases and not to add to the current knowledge about the mechanism of action.

Other authors have been able to correlate different variables, such as CMV infection [13], the postoperative formation of HLA antibodies [16], or specific echocardiographic changes [8], with the development of vasculitis. These correlations were not found in our material, nor were there any correlations to ABO match, the use of inotropic drugs, or preoperative antibody screen. No certain predictors could be identified in the five plasmapheresis-treated patients when compared to the rest of the transplanted patients.

During the period 1988–1993, the incidence of microvascular rejection was 4.2 % in our program. In a recent, prospective study of 36 consecutive cardiac allografts, 7 allografts were reported to have a similar type of vascular rejection [8]. Microvascular phenomena as an indication of rejection have been reported by other authors as well [3, 6, 8, 9, 12]. The relationship between this early process and the later occurring so-called accelerated graft atherosclerosis is not clear at the present time. The mechanism suggested is immunological and mediated by humoral and cellular factors directed specifically at the vascular tissue of the graft. In our five cases the microvasculature was predominantly engaged in the process. The severity of the tissue damage varied over time and between cases. The progress was either slow or became rapidly aggravated with concomitant alarming clinical symptoms. Furthermore, the lymphocytic vasculitis was difficult to perceive when it occurred early together with ischemic alterations due to the effects of preservation and reperfusion (as in cases 1 and 5). The fact that all patients, at some time, had episodes of isolated lymphocytic vasculitis without concurrent evidence of myocyte necrosis and the occurrence of late episodes (after 4–5 months, as in cases 2, 3, and 4) suggests that the picture is an entity separate from preservation/reperfusion-induced changes. In order to catch the lymphocytic vasculitis in the early period after transplantation, the use of immunofluorescence on frozen sections for the demonstration of vascular immunoprotein deposits has been suggested [8]. In our experience, an immunoperoxidase method on paraffin sections may also provide valuable information since an negative result may exclude deposits of immunoreactants (such as immunoglobulins and complement factors).

In three of our five patients, several biopsies showed cellular rejection and lymphocytic vasculitis simultaneously in the same specimen. Whether this reflects a cellular rejection directed toward both myocytes and microvasculature is not clear from our material; however, it does raise the question of whether an isolated lymphocytic vasculitis can reflect a vessel-"oriented" cellular rejection. A mixed vascular and cellular rejection in 9 of 16 cases was also reported by Hammond et al. [8], and it has long been known to occur in renal allografts. After successful treatment with plasmapheresis, three of our patients who, at that time, had no clinical symptoms, showed a pauci-inflammatory microvasculitis in repeated follow-up biopsies, possibly indicating a chronic state of the process (Fig. 3).

This report demonstrates five cases of vasculitis that were successfully treated with plasmapheresis. All patients had severe clinical symptoms that did not respond to conventional antirejection therapy. However, after repeated plasmapheresis, a rapid regression of the histopathological changes occurred and a stable clinical improvement was obtained. Moreover, the vasculitic reaction seemed to have turned into a stage where subsequent cellular rejections were treatable with conventional antirejection drugs and without relapse into severe vasculitis and clinical symptoms. Further work is needed to clarify the mechanisms involved.

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