Experience with cellular and antibody mediated rejection after heart transplantation

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OBJECTIVES: To evaluate celluar and antibody mediated rejection in a group of patients after heart transplantation (HTX), performed in the authors' institution during a six-year period.
METHOD: A retrospective analysis of patients surviving ≥12 months after HTX was performed. Rejection was evaluated from the samples obtained during endomyocardial biopsy, and graft function was assessed using echocardiography. The duration of follow-up ranged from 12 to 72 months.
RESULTS: In the 196 patients evaluated, 65 treated episodes of cellular rejection were recorded in 50 patients, with significant reduction on tacrolimus prophylaxis. Antibody mediated rejection occured in 5.6% of patients, and was complicated by graft dysfunction in 64%.
CONCLUSION: Contemporary immunosuppressive prophylaxis decreases the frequency of cellular rejection, allowing modification of endomyocardial biopsy schedule. Antibody mediated rejection is a relatively rare but clinically important complication after HTX, and necessitates combined agressive therapy.

Key Words: *Endomyocardial biopsy; Graft rejection; Heart transplantation*

Graft rejection is one of the major complications after organ transplantation (1). In the early days of heart transplantation (HTX) programs, this complication was a major obstacle. Widespread clinical use of the method was enabled by the development of new immunosuppresive drugs, particularly calcineurin inhibitors. Owing to the advancements in development, the currently used combination clearly reduces the incidence of cellular forms of rejection (CR). Attention is increasingly focused on antibody mediated rejection (AMR), which is clinically more severe and, moreover, is not adequately controlled by currently used immunosuppressants.

In the present study, we analyzed the occurrence of acute rejection in a group of patients in whom HTX was performed in our cardiology centre during a six-year period. The aim was to evaluate the incidence of graft rejection and further assess the clinical consequences and treatment of this complication.

METHODS

In the period between January 1, 2005 and December 31, 2010, HTX was performed in 247 patients. Thirteen (5.3%) died within 30 days after the surgery, and an additional 38 (15.5%) died during follow-up. Retrospective analysis was performed in the group of 196 patients who survived at least 12 months after HTX; follow-up lasted for 12 to 72 months. Induction prophylaxis with polyclonal antithymocyte globulin was used in all patients; the basis of long-term prophylaxis was a calcineurin inhibitor (cyclosporine A or tacrolimus), typically in combination with mycophenolate mofetil and prednisone.

Rejection was evaluated from samples obtained during endomyocardial biopsy (EMB) from the right ventricle. Banff classifications were used to assess the presence and degree of CR (Table 1) (2); furthermore, the presence of complement fragments C3d and C4d was the basis for diagnosis of AMR (Figure 1) (3,4). Biopsies were performed at regular intervals ('protocolar biopsies'), and a total of 2570 sample series were reviewed. Graft function was assessed using echocardiography performed on the same day as the biopsy. The incidence of rejection was evaluated in the entire group and during two time periods, which varied according to selection of a calcineurin inhibitor (Table 2).

RESULTS

CR

There were 65 treated episodes of CR in 50 (25.5%) patients in the reporting period. One episode occured in 42 patients, two episodes in seven and three episodes in one. Sixty-two episodes were successfully treated with corticosteroids; in three cases, antithymocyte globulin was used. Combination with AMR was detected in two cases only. Episodes of 'pure' CR were never accompanied by the development of heart failure or graft dysfunction.

A significant reduction was observed in the incidence of CR in the second period, during which tacrolimus prophylaxis was more frequently used (Table 3).

AMR

AMR was diagnosed in 11 (5.6%) patients, and graft dysfunction developed in seven. Occurrence and treatment of this complication is presented in Table 4.

DISCUSSION

The main finding of the present study was the decline in CR, which enabled us to reduce the frequency of protocolar biopsies. Rejection after HTX occurs in several forms. Peracute rejection immediately after the operation is extremely rare; subsequently, there may be episodes of CR or AMR. Chronic rejection affects the coronary arteries and is one of the components of coronary graft disease.

For early diagnosis of rejection, EMB is necessary because noninvasive methods are not sufficiently reliable (5). Protocolar biopsies are performed at prespecified time intervals, with decreasing frequency during the time

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TABLE 1
Acute cellular rejection grading according to Banff classification (2)

	Revised Banff (2004)	Original Banff (1990)
No rejection	0 R	0
Focal lymphocyte infiltrates, no myocardial damage	1 R	1 A
Diffuse infiltrates, no myocardial damage	1 R	1 B
Lymphocyte infiltrates, maximum one focus of myocyte damage	1 R	2
Two or more foci of infiltrate with myocyte damage	2	3 A
Diffuse infiltrates with multifocal myocyte damage	3 R	3 B
As 3R + edema, hemorrhage, vasculitis	3 R	4

TABLE 2
Characteristics of patients included in the present study (n=196)

Men/women, n/n	156/40
Age, years, range	19–74
Diagnosis, IHD/DCMP/Other	78/88/30
First period: January 1, 2005 – December 31, 2008	
Total, n	119
CyA/TAC, %/%	64/36
Second period: January 1, 2009 – December 31, 2010	
Total, n	77
CyA/TAC, %/%	4/96

CyA Cyclosporine A; DCMP Dilated cardiomyopathy; IHD Ischemic heart disease; TAC Tacrolimus

TABLE 3
Incidence of acute cellular rejection in the two time periods

First period: January 1, 2005 – December 31, 2008	
Total, n	119
Cellular rejection, n (%)	39 (32.8)
Second period: January 1, 2009 – December 31, 2010	
Total, n	77
Cellular rejection, n (%)	11 (14.3)

First versus second period: P=0.004

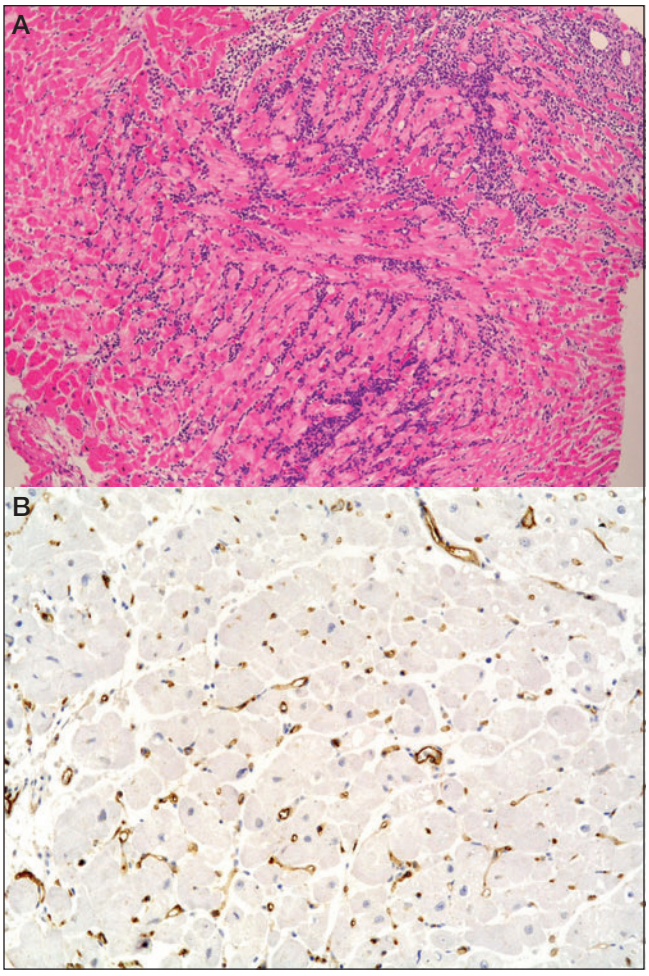

Figure 1) *Photographs of cellular and antibody mediated rejection in endomyocardial biopsy samples.* **A** *Acute cellular rejection, Banff classification 2R. Lymphocyte infiltration with destruction of myocytes. Stained with hematoxylin-eosin.* **B** *Antibody mediated rejection. Diffuse positivity of C4d fragments in capillaries. Immunohistochemical staining using rabbit antihuman C4d polyclonal antibody*

elapsed. According to the classical scheme still used in many centres, 12 to 13 biopsies are performed during the first year. This causes discomfort for the patient and is accompanied by additional risk for complications (6). Based on our findings, we created a new schedule. During the first year, the number of planned biopsies was reduced to eight, and additional biopsies after the first year are performed only in high-risk patients or when there is a clinical need. According to the preliminary prospective evaluation, this approach is safe (7).

Recently, attention has focused on AMR. The basis for AMR diagnosis is EMB findings, the presence of donor-specific antibodies in serum and, in most cases, graft dysfunction. Some histological changes may be present in the biopsy, but the most sensitive finding is the presence of complement fragments C3d and C4d in >50% of vessels encountered in each sample. Thus, immunohistochemical investigation is necessary for this analysis.

According to the literature, AMR occurs during follow-up in 10% to 20% patients (8). Immunized patients – ie, those with the presence of antibodies against a lymphocyte panel before HTX and/or those who developed donor-specific antibodies after HTX – are at increased risk. Another risk factor is cytomegalovirus infection. AMR is a prognostically unfavourable finding that is often accompanied by immediate graft failure, and also by subsequent developement of coronary graft disease (9). In our study, AMR is reported less frequently compared with the literature. This may be explained by the fact that the development of antibodies was not systematically studied; moreover, immunohistochemical test sampling has not always been available. Therefore, we selected only clinically severe episodes or their consequences manifested as graft failure in patients with coronary vasculopathy.

Unlike the cellular form, AMR is not easily treatable. Corticosteroids or antilymphocyte antibodies are not sufficiently effective; it is necessary to eliminate the antibodies present in blood and prevent their further formation (10). The available elimination methods are plasmapheresis and immunoadsorption (11,12), both of which were successfully used in our patients. The elimination is usually completed by administration of intravenous immunoglobulin, which inhibits residual antibodies. An effective way of preventing further antibodies formation is suppression of B-lymphocytes (rituximab) (13) or plasma cells (bortezomib) (14).

Table 4 presents the scenarios in which these treatment options were used. The combination therapy was well tolerated and successful in our patients. Graft dysfunction was eliminated and treated patients had good

TABLE 4
Comprehensive data regarding antibody mediated rejection in 11 patients after heart transplantation (HTX)

Sex/year of birth	Time since HTX, months	Rejection diagnosis	Cause	LV dysfunction*	Treatment	Follow-up, months	Last control (LV EF%)
Male/1953	72	C3d, C4d, CM	Unknown	Yes	IA, IVIG	80	50
Female/1976	0.5	CM	Unknown	Yes	MP, ATG	74	60
Female/1943	13	C4d	Noncompliance	Yes	MP, ATG	58	60
Female/1963	0.5	C4d	Unknown	Yes	MP	40	60
Male/1957	1.5	C4d, CM	↑ PRA	No	IA	30	50
Female/1968	0.25	C4d, DSA	↑ PRA, CM	Yes	PF, IVIG, rituximab, sirolimus	21	55
Female/1944	0.5	C3, Cd4	CM	No	MP	56	60
Female/1966	1	U	Unknown	No	PF, IVIG	26	60
Male/1965	51	CAV	Noncompliance	Yes	ATG	53	SD
Male/1963	10	CAV	Noncompliance	Yes	MP	15	40
Male/1950	13	C4d, CM	Conversion to rapamycin	No	MP, ATG	14	60

**Ejection fraction (EF) ≤40%. ↑ Increase; ATG Antithymocyte globulin; CM Positive cross-match test; CAV Coronary vasculopathy; DSA Donor-specific antibodies; IA Immunoadsorption; IVIG Intravenous immunoglobulin; LV Left ventricular; MP Methylprednisolone; PRA Panel-reactive antibodies; PF Plasmapheresis; SD Sudden death; U Unknown*

prognosis in medium-term follow-up. Recently, we used bortezomib for the first time, which was administered in a young woman who developed high titres of donor-specific antibodies and severe AMR after HTX (15).

CONCLUSION

Retrospective analysis of a relatively large group of HTX patients confirmed that contemporary immunosuppressive prophylaxis decreases frequency of CR. This enables a modification of EMB schedule without negative consequences for the patients. AMR is a relatively rare but clinically important complication after HTX. According to our experience, intensive combination therapy is often successful even in patients developing graft dysfunction.

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