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Strategies for routine biopsies in heart transplantation based on 8-year results with more than 13,000 biopsies

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Abstract. The endomyocardial biopsy (EMB) in heart transplant recipients has been considered the "gold standard" for diagnosis of graft rejection (REJ). The purpose of this retrospective study is to develop long-term strategies (frequency and postoperative duration of EMB) for REJ monitoring. Between 1985 and 1992, 346 patients (mean age 44.5 years, female patients = 14%) received 382 heart grafts. For graft surveillance EMBs were performed according to a fixed schedule depending on postoperative day and the results of previous biopsies. In the first year the average number (no.) of EMBs/patient was 20 with 19% positive for REJ in the first quarter, dropping to 7% REJ/EMB by the end of the first year. The percentage of REJ/EMB declined annually from 4.7% to 4.5%, 2.2% and less than 1% after the fifth year. Individual biopsy results in the first 3 postoperative months had little predictive value. Patients with fewer than two REJ (group 1), vs patients with two or more REJ in the first 6 postoperative months (group 2), were significantly less likely to reject in the second half of the first year (group 1: 0.29 ± 0.6 REJ/patient; group 2: 0.83 ± 1.3 REJ/patient; $P < 0.001$) and third postoperative year (group 1: 0.12 ± 0.33 REJ/patients; group 2: 0.46 ± 0.93 REJ/patient; $P < 0.05$). In conclusion, routine EMBs in the first 3 postoperative months have only limited predictive value, however the number of routine EMBs can be drastically reduced later depending on the intermediate postoperative REJ pattern. [Eur J Cardio-thorac Surg (1995) 9: 592–598]

Key words: Heart transplantation – Endomyocardial biopsies – Biopsy statistics and numerical data – Heart transplantation standards – Immunosuppressive agents therapeutic use

The endomyocardial biopsy (EMB) has remained the gold standard for diagnosis of graft rejection (REJ) in heart transplant recipients for more than a decade. Inherent disadvantages of this invasive diagnostic procedure include the possibility of pneumothorax, hemothorax, pericardial tamponade and injury to the tricuspid valve apparatus. Various diagnostic procedures have emerged in the past years for noninvasive diagnosis of cardiac rejection with limited sensitivity and specificity. These include echocardiography [14], cytoimmunological monitoring [4] and intramyocardial electrograms [10] to name a few. Nevertheless REJ monitoring with the EMB has allowed for a consistent, reproducible and standardized method of graft REJ surveillance and is considered standard in most heart transplantation centers. In order to limit the use of this invasive procedure as much as possible, a careful analysis of general and individual REJ patterns may aid in the development of definite future EMB strategies. Based on the results of more than 13,000 EMB in a single center over an 8-year period, this study attempts to optimize biopsy timing and differentiate patient groups requiring minimal graft surveillance with EMBs.

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Materials and methods

Between April 1985 and October 1992, 346 patients (mean age 44.5 years, female patients = 14%) received 382 heart grafts at the Hannover Medical School. Routine biopsies were used in all patients for graft surveillance and interpreted by one pathologist.

Immunosuppression

Initial immunosuppression in all patients started with an intraoperative bolus of 500 mg methylprednisolone at the time of aortic cross-

Table 1. Scheduled biopsy intervals in weeks. Recommended interval depending on results of previous biopsy (*left column*) and postoperative month (*top line*)

Months postoperatively	2	4	6	8	10	12	14	16	18	20	22	24
A0 = no rejection	4	4	4	4	5	6	6	8	10	12	12	12
A1 = minor rejection	2	3	4	4	5	6	6	8	10	12	12	12
A2 = mild rejection	2	3	3	3	4	4	4	6	8	10	10	12
A3 a = mild to moderate rejection	1	1	2	2	2	2	2	4	4	4	4	4
A3 b = moderate rejection	1	1	2	2	2	2	4	6	6	6	6	6
A4 = severe rejection	1	1	2	2	2	2	2	2	2	2	2	2
R1 = resolving rejection	1	1	2	2	2	3	6	8	8	8	10	10
R2 = resolved rejection	2	3	4	4	5	6	8	8	10	12	12	12

clamp removal. Induction immunosuppression was started with polyclonal antithymocyte globulins (RATG[1], 1.5 mg/kg for 4 days, intravenously), monoclonal antibodies (OKT3[2], 5 mg/day for 7 days) or intravenous cyclosporin A (CsA)[3] for 7 days post-transplant (2 mg/kg per day) on a random basis. Immediately postoperatively methylprednisolone was added at a dose of 125 mg every 12 h for a total of three doses succeeded by oral prednisolone at 0.5 mg/kg per day. The latter drug was tapered to 15 mg/day for the first 3 - months postoperatively and finally to 5–10 mg/day for the remaining time period. Azathioprine was started on postoperative day 2 with 1–2 mg/kg and the dose was adjusted for a minimal white blood cell count of $3.5–5.0 \cdot 10^9$/l not to exceed 3 mg/kg per day. Maintenance oral cyclosporine therapy was commenced between day 0 and day 5 depending on renal function, with target specific CsA through levels for the first year of 250–300 µg/l, for the second year 150–200 µg/l and then 100–150 µg/ml. Dosage adjustments were executed according to creatinine levels, where maximal creatinine levels of 120–150 µmol/l were considered acceptable

Rejection treatment

The first line of REJ treatment in all patients consisted of pulsed methylprednisolone (7–10 mg/kg per day for 3 consecutive days). The success of REJ treatment was controlled a week later by EMB and echocardiographic evaluation of the ventricular performance. Repetitive treatment for ongoing acute REJ was defined as treatment of an acute REJ within 7 days of a previous REJ treatment

Detection and grading of acute REJ

Detection and monitoring of acute rejection was accomplished by standard transvenous EMBs [2] graded according to the Hannover classification [8]. An average of 5–8 biopsy specimens were obtained from different regions within the right ventricle. During the first months post-transplantation the minimal interval between positive biopsies following a rejection was 1 week and this interval was lengthened with increasing duration of the postoperative course [12] By the end of the first year the average follow-up interval was 4 weeks (Table 1). The biopsy schedule had been designed on empirical grounds. Treatment was instituted in patients with moderate or severe REJ (grade A-3b, grade A-4). For this study a cardiac REJ was defined as treated REJ.

[1] C. Biber, Palo Alto, USA
[2] Ortho Pharmaceutical Cooperation, Raritan, New Jersey, USA
[3] Sandimmun, Sandoz, Basel, Switzerland

Data analysis

The frequency of biopsies and the incidence of REJ were determined in relation to time postoperatively. The REJ-free interval (days) between specific EMB results and subsequent REJ was calculated over time for all patients. The distribution of the REJ-free intervals was analysed and the lower 10th percentile determined. This percentile indicated a minimum REJ-free interval for which the probability of overlooking REJ was less than 10%. The impact of induction therapy, type of REJ treatment and standard immunosuppression on the incidence of REJ were evaluated. Subgroups of the study population were defined by cluster analysis to determine a subpopulation with a diminished risk for REJ. Patient group definition was based on the number of REJ episodes within the first 6 postoperative months (group 1: fewer than two REJ per patient/group 2: 2 or more REJs per patient).

Statistical analysis

Patient data were analyzed with the Statistical Program of Social Sciences (SPSSWIN 6.0, Birmingham). Continuous data were expressed as mean ± standard deviation and were compared by a two-tailed unpaired *t*-test. For risk profile evaluation, the study population was divided according to the early incidence of rejection by hierarchic cluster analysis. A P value of less than 0.05 was considered significant.

Results

The study population included all patients with a minimum survival of 30 days postoperatively. In this group of patients the preoperative diagnosis was dilative cardiomyopathy in 211 patients (61%), ischemic cardiomyopathy in 104 patients (30%) and other forms of end-stage heart disease such as valvular disease, or hypertrophic cardiomyopathies in 31 patients (9%). The mean donor age was 26.7 ± 6.5 years with a mean ischemic interval of 153 ± 39 min.

The actuarial survival rate of the study population was 92% at 1 year, 88% at 2 years, 78% at 5 years and 70% at 8 years postoperatively. If the perioperative and early postoperative deaths are included in the analysis, the actuarial survival rate was 80% at 1 year, 76% at 2 years, 70% at 5 years and 61% at 8 years. A total of four patients died perioperatively and 37 patients early postoperatively. The perioperative deaths were related to intraoperative right ventricular failure in two patients and disseminated intravascular coagulopathy in two. The causes of death within

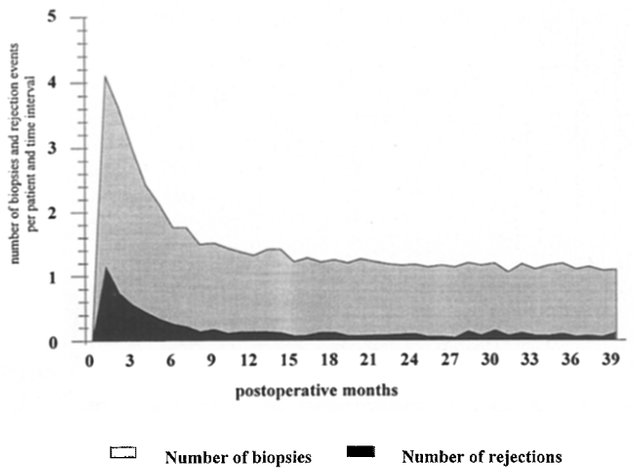

Fig. 1. Average frequency of biopsies and rejections for the total study population calculated on a monthly basis

the first 30 postoperative days were related to infection in 76% and to REJ in 24% almost always resulting in multiorgan failure. Later causes of death (more than 30 days) were acute REJ in 12 patients (17.9%), chronic REJ or sudden death in 31 patients (46.3%), infection or sepsis in 13 patients (19.4%), malignant disease in 6 (9%), cerebrovascular accident in 4 patients (6%) and 1 patient died in a car accident (1.5%).

Transplant vasculopathy (TVP) was diagnosed in 66 patients (20.5%) and the actuarial freedom from TVP was 85% at 1 year and 61% at 5 years postoperatively. The immunosuppressive protocol included cytolytic induction therapy with RATG in 265 patients (77% of study population), OKT-3 in 25 patients (7%) and antilymphocyte globulin (ALG) in 17 patients (5%). A total of 38 patients (11% of study population) were treated with intravenous CsA starting immediately postoperatively without the addition of any kind of cytolytic therapy.

A total of 13,743 endomyocardial (EMB) were taken for a patient-survival product of 19,198 patient/months averaging 0.72 biopsies per patient/month. The average frequency of EMBs performed and average number of REJ over time for the entire follow-up is depicted in Figure 1. There was a sharp decline in the number of EMB performed in relation to the postoperative month. During the first postoperative month 28±18% of the weekly EMB were positive for rejection and in the following months this percentage dropped through 20±12% in the second month to 10±7% at the end of the first year. In the following years the percentage of EMB positive for REJ was 5±3% at the end of the second year and 3±3% at the end of the fourth year. In Table 2 the rejection-free interval (mean and lower 10 percentile) of the study population for specific biopsy gradings are depicted. For no rejection (A-D) the minimum time interval for 90% of all the patients to experience a REJ was 6 days for the first 3 months, 15 days during the second postoperative quarter and 26 days and 36 days for the third and fourth postoperative quarters. This interval continues to increase with postoperative time. At the same time the number of EMB positive for REJ declined drastically. During the first 3 months REJ occurred in 19±8% of all biopsies, during the second quarter of the first postoperative year in 14.9±7% of the biopsies and during the third and last quarters of the first postoperatively year in 9.2±6.5% and 7.3±4.5% of the EMB, respectively. The 10 percentile intervals must therefore always be related to the decreasing incidence of REJ as postoperative time increases.

The interval between two EMB positive for REJ increased from 7 days to 14, 21 and, finally, 29 days for the first postoperative year. Biopsies showing resolving REJ (R-I) were followed by REJ within a minimum of 7 days

Table 2. Biopsy interval statistics. Average percentage of biopsies positive for rejection (percent rejection). Minimum time interval (days) for the lower 10 percentile and average interval of the study population between specific biopsy results (*left column*) and biopsies diagnosed as rejection for different postoperative time periods

Observation period Percent rejection	0–90 days 19±8%		90–180 days 14±7%		180–270 days 9±7%		270–360 days 7±4%	
	10 percentile	Mean	10 percentile	Mean	10 percentile	Mean	10 percentile	Mean
Interval from A0 to rejection	6	48	15	114	26	226	36	301
Interval from A3 to rejection	3	40	7	91	8	221	14	240
Interval between rejections	7	15	14	36	21	62	29	92
Interval from R1 to rejection	7	43	8	93	19	143	21	206
Observation period Percent rejection	Year 2 5±3%		Year 3 4±2%		Year 4 3±3%		Year 5 3±2%	
	10 percentile	Mean	10 percentile	Mean	10 percentile	Mean	10 percentile	Mean
Interval from A0 to rejection	64	342	70	410	84	311	183	576
Interval from A3 to rejection	14	272	13	244	23	415	8	58
Interval between rejection	33	14	35	24	36	27	33	21
Interval from R1 to rejection	23	265	28	213	13	454	86	127

Interval in days

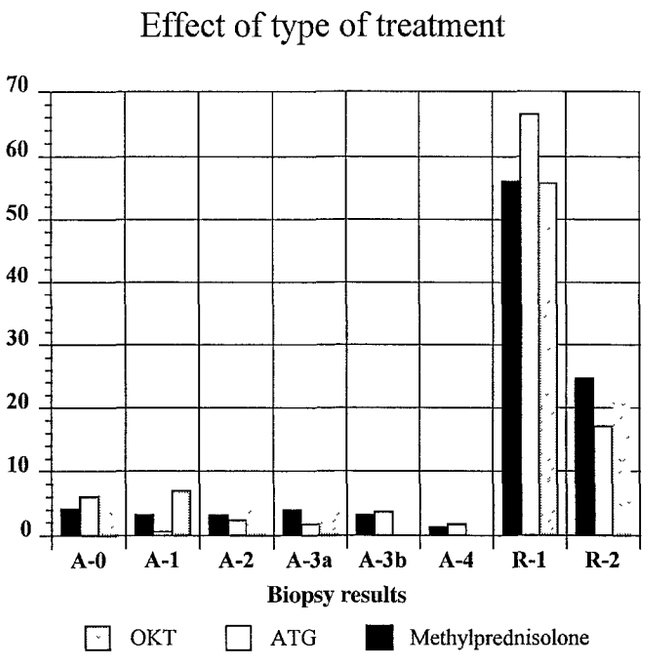

Fig. 2. Results of the control biopsy following rejection treatment after 7 days. The impact of different rejection therapy regimens is depicted in the individual columns

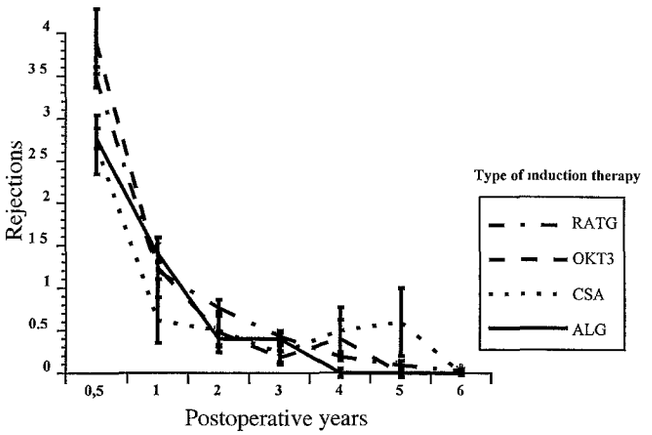

Fig. 3. Influence of induction therapy on incidence of rejection over time

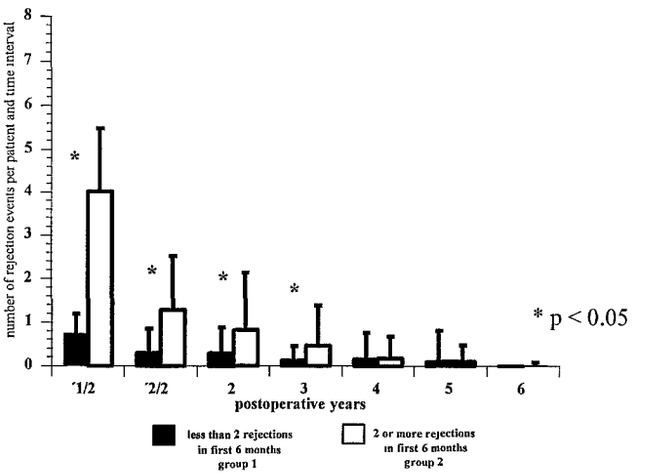

Fig. 4. A comparison of the incidence of rejection for patients with fewer than 2 rejections (group 1) and patients with 2 or more rejections during the first 6 postoperative months

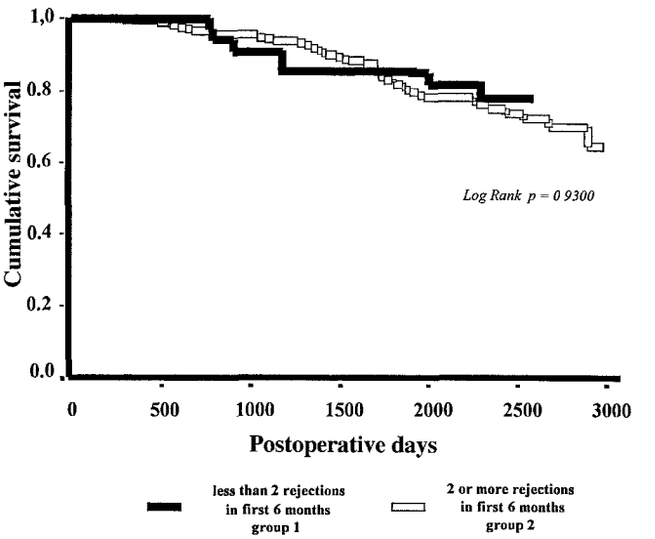

Fig. 5. Actuarial survival for patients with fewer than 2 rejections (group 1) and patients with 2 or more rejections during the first 6 postoperative months

in 90% of the study population during the first postoperative months and this intervals increased with postoperative time as shown in Table 2. For the second and third postoperative year, the intervals between REJs remained stable while the interval between an A-0 and REJ consistently increased.

The results of the immediate control biopsies following REJ treatment are depicted in Figure 2 in relation to the type of REJ treatment exercised as an average over the entire follow-up period. The type of REJ treatment did not effect the control biopsy results. In all subgroups the majority of control EMBs were either R-1 or resolved REJ (R-2). An average of 4–5% of the control biopsies showed ongoing REJ in patients treated with RATG or intravenous steroids. None of the control biopsies in patients treated with anti-CD3 monoclonal antibody (OKT-3) showed ongoing REJ. Figure 3 displays the impact of the type of induction therapy on the incidence of REJ in the first 7 postoperative years. There was no statistical difference between induction therapy using RATG, OKT-3, or no induction antibodies at all (CsA).

Based on an average distribution of REJ episodes per patient and postoperative period the study population was divided into different groups by cluster analysis in order to determine prognostic indicators for later EMB results. Patients with fewer than two REJ during the first 6 postoperative months (group 1: $n=53$; mean rate of REJ 0.74 ± 0.45) were compared to those with two or more REJ

Table 3. Comparison of quality of triple drug immunosuppression between patients with fewer than 2 rejections (group 1) and patients with 2 or more rejections during the first 6 postoperative months.
CsA, cyclosporin A; SD, standard deviation; mg/d, milligram per day; µg/l, microgram per liter

	Group 1 <2 rejections/patient		Group 2 2 or more rejections/patient		
	Mean	SD	Mean	SD	
CsA dose 1st year	338.26 mg/d	154.48 mg/d	374.43 mg/d	109.24 mg/d	p<0.006
CsA dose 2nd year	204.53 mg/d	168.07 mg/d	329.03 mg/d	114.62 mg/d	p<0.001
CsA dose 3rd year	133.79 mg/d	149.87 mg/d	247.68 mg/d	128.62 mg/d	p=n.s
CsA level 1st year	228.49 µg/l	45.89 µg/l	236.51 µg/l	66.24 µg/l	p=n.s.
CsA level 2nd year	196.05 µg/l	49.36 µg/l	200.36 µg/l	63.68 µg/l	p=n.s.
CsA level 3rd year	180.78 µg/l	87.82 µg/l	175.65 µg/l	62.53 µg/l	p=n.s.
Prednisone 1st year	12.89 mg/d	2.02 mg/d	14.58 mg/d	3.07 mg/d	p<0.01
Prednisone 2nd year	6.51 mg/d	2.58 mg/d	8.69 mg/d	3.37 mg/d	p<0.01
Prednisone 3rd year	6.28 mg/d	2.66 mg/d	7.43 mg/d	3.04 mg/d	p=n.s.
Azathioprine 1st year	126.95 mg/d	44.52 mg/d	108.95 mg/d	42.37 mg/d	p=n.s.
Azathioprine 2nd year	110.92 mg/d	51.07 mg/d	110.53 mg/d	54.44 mg/d	p=n.s
Azathioprine 3rd year	94.2 mg/d	59.49 mg/d	108.17 mg/d	54.63 mg/d	p=n.s.

(group 2: $n=203$; mean rate of REJ 4.02 ± 1.46) during the same time period. As shown in Figure 4, patients with few REJ (group 1) proved to have a lower incidence of REJ during the next 3 years of postoperative follow-up. During the second half of the first postoperative year the average rate of REJ was 0.29 ± 0.56 REJ/patient in group 1 and 1.29 ± 1.23 REJ/patient in group 2 ($P<0.001$). During the second year, group 1 patients were likely to experience 0.29 ± 0.60 REJ/patient compared to 0.83 ± 1.3 REJ/patient in group 2 ($P<0.01$). The late (>30 day) postoperative mortality did not correlate with the number of REJ experienced during the early postoperative phase (6 postoperative months). Figure 5 compares the actuarial survival of group 1 and group 2 with a 3-year actuarial survival rate in group 1 of 91.5% and 95.3% in group 2 (n.s.) and a 5-year survival rate of approximately 80% in both groups.

An analysis of the impact of second year results in respect to the rate of REJ showed similar results. Patients with fewer than two REJ during the second postoperative year (group A: $n=215$: average rate of REJ 0.27 ± 0.45 REJ/patient during 2nd year) had significantly fewer REJ episodes during the third postoperative year (group A 0.31 ± 0.67 REJ/patients) than patients with two or more REJ in the same period (group B: $n=41$: 2.98 ± 1.39 REJ/patient during 2nd year; 1.03 ± 1.47 REJ/patient during 3rd year; $P<0.01$). After the third postoperative year the difference between group A and group B was statistically significant.

The amount of triple drug immunosuppression in group 1 and group 2 is outlined in Table 3. Patients with more REJ (group 2) had received significantly higher CsA doses during the first and second postoperative years and also more prednisone.

Discussion

Despite the emergence of a considerable number of non-invasive procedures for cardiac REJ monitoring following heart transplantation [7] the endomyocardial biopsy (EMB) has remained the gold standard [9, 15]. The inherent potential for life-threatening complications caused by this invasive procedure warrants a careful indication of every biopsy performed and preferably its replacement in long-term follow-up by non-invasive diagnostic studies. A number of studies have emphasised the significant impact of EMBs on the development of tricuspid valve regurgitation due to damage of the tricuspid valve structure [6]. In addition, EMBs may result in ventricular-coronary fistula [5], pericardial tamponade and pneumothorax [1].

Echocardiography, with analysis of diastolic function, has become the major alternative to EMBs in this respect [3, 14]. The considerable amount of subjectivity involved in the interpretation of this type of diagnostic study, the occasional occurrence of significant REJ in histological specimens without impairment or alteration of cardiac function and the inability to differentiate between viral myocarditis and REJ are definite limitations of this procedure. In addition, verification of REJ diagnosed by echocardiography often requires the use of the EMB. Graft surveillance is therefore often performed with EMBs, especially in the early postoperative period, despite this method's risk and limitations. The intent of this analysis was to develop strategies to minimize the use of EMBs in heart transplant recipients based on an 8-year experience of more than 13,000 biopsies in nearly 350 patients.

The Hannover Medical School heart transplant program empirically developed a biopsy schedule (Table 1) early in the transplant program, which resulted in a relatively large number of EMBs per patient and year when compared to other centers. The advantage of this routine is a very close surveillance of each patient for REJ and therefore one can hypothesize that, especially during the first year with an average of 20 biopsies per patient, the overall majority of REJ episodes have been detected by EMB. This allows for the determination of an individual REJ pattern of each heart transplant recipient and these data show that the postoperative time is one of the most significantly

altering factors in this analysis. The overall majority of REJ episodes occur during the first 6 months postoperatively (Figure 1). Within this time period initially more than 20% of the EMBs in this study population were positive for REJ. As time progressed this proportion decreased and by the end of the second year an average of only 4% of all biopsies were positive for REJ. Consequently the first 6 postoperative months are quite critical in REJ surveillance. The empirically derived biopsy schedule took this pattern into account with short intervals especially in the early postoperative period (Table 1).

According to the biopsy interval statistics obtained from the results of all EMBs evaluated in this study (Table 2), minimum time intervals, in days, between specific biopsy results and rejection can be calculated. The 10 percentile was used to ensure REJ diagnosis in 90% of all patients. For example, during the first 3 months the interval between an A-0 and a REJ (A-3 b) increased from 7 days to 15 days in the second postoperative quarter and finally to 36 days by the end of the first year. For interpretation of these figures it is important to correlate them with the absolute percentage of EMBs positive for REJ within the same time interval. As REJ became progressively rarer with postoperative time, the significance of the minimum interval decreased substantially. For example, an increase in the time interval in follow-up periods with a low percentage of biopsies positive for REJ had a decreasing impact on the total number of REJ episodes missed. Consequently the interval between EMBs may be lengthened beyond the minimum interval of the 10 percentile without considerably increasing the risk of unobserved REJ. Especially for the first 3 postoperative months, this table demonstrates the limited predictive value of individual biopsy results in terms of possible future REJ events. In the first quarter the 10 percentile minimum interval between an A-0 (no rejection) and REJ is similar to the interval between an R-1 (resolving rejection) and REJ or between two REJs. As time progresses the predictive impact increases.

The type of REJ treatment, using either methylprednisolone bolus treatment, or cytolytic therapy with OKT-3 or RATG, did not affect the results of control biopsies (Figure 2). In the majority of control EMBs the diagnosis was resolving (R-1) or resolved REJ (R-2). In addition, the type of induction therapy does not alter the incidence of REJ in the postoperative period (Figure 3). In this respect the use of cytolytic therapy (OKT-3, RATG or ALG) did not decrease the incidence of REJ episodes when compared to patients treated with standard triple drug immunosuppression from day 1 post-transplantation.

In order to define a subpopulation with a decreased risk for late REJ, and therefore a diminished need for control biopsies, the study population was clustered into two subgroups. Patients with fewer than two REJ during the first 6 postoperative months (group 1) were significantly less likely to reject during the second half of the first year as well as during postoperative year 2 and 3 when compared to patients with 2 or more REJs during the first 6 postoperative months (group 2). The two groups have the same long-term actuarial survival (Figure 5) and the long-term freedom of REJ was not influenced by the standard immunosuppression (Table 3). Patients with more REJ (group 2) had higher levels of triple drug immunosuppression to counteract the higher incidence of REJ.

In order to evaluate the impact of these results on future EMB strategies, one must critically review the validity of EMBs for detection of rejection. In 1992 Sharples et al. evaluated the error rates of EMBs for detection of REJ and then concluded that four biopsies are sufficient for the evaluation in most patients [11]. In this study population the average number of fragments obtained was 6.5 ± 2.1 per patient. Besides the sometimes extensive patchy pattern of REJ in the heart, the interobserver variability of EMBs must be discussed. The incidence of REJ within a general patient population may vary considerably between individual transplant centers depending on the classification used and the pathologists' internal guidelines [13]. Therefore a comparison between centers is often difficult and the recommendation of one center is often not transferable to others. Based on our analysis, we feel that biopsy intervals can be scheduled on an individual basis. The initial postoperative period must be considered the most critical in terms of incidence of REJ with a relatively low predictability of future biopsy results. This characteristic changes significantly with time. It is probably safe to omit control biopsies after the first postoperative year in patients with a low frequency of REJ during the first 6 postoperative months (fewer than 2 REJs per patient). Further experience will help to verify this recommendation based on the resuls of this study and we anticipate a further reduction in the amount of necessary biopsies with advances in noninvasive graft surveillance.

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