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ORIGINAL ARTICLE

Acute cellular rejection the first year after heart transplantation and its impact on survival: a single-centre retrospective study at Skåne University Hospital in Lund 1988–2010

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Summary

Acute cellular rejection (ACR) the first year after heart transplantation (HT) and its impact on survival was investigated. All 215 HT patients at our centre 1988–2010, including 219 HTs and 2990 first-year endomyocardial biopsies (EMBs), were studied. 'Routine' EMBs obtained 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 52 weeks after HT, and 'additional clinically indicated' (ACI) EMBs, were graded according to the 1990-ISHLT-WF. The frequency and severity of first-year ACRs was low, with 6.5% of routine EMBs and 14.1% of ACI EMBs showing ACR ≥ grade 2. Proportionally more ($P < 0.05$) first-year ACRs ≥ grade 2 were found among EMBs in HTs performed during 1988–1999 (9.6%) than 2000–2010 (5.5%), EMBs performed during 16–52 weeks (8.8%) than 1–12 weeks (6.3%) after HT, EMBs in HTs with paediatric (11.3%) than adult (7.1%) donors, and EMBs in sex-mismatched (10.4%) than sex-matched (6.3%) HTs. Five- and ten-year survival was furthermore lower ($P < 0.05$) among HTs with ≥1 compared with 0 first-year ACRs ≥ grade 3A/3B (82% vs. 92% and 69% vs. 82%, respectively). Ten-year survival was 74% compared with 53% in the ISHLT registry. In conclusion, our results indicate that first-year ACRs ≥ grade 3A/3B affect long-term survival. We believe frequent first-year EMBs may allow early ACR detection and continuous immunosuppressive adjustments, preventing low-grade ACRs from progressing to ACRs ≥ grade 3A/3B, thereby improving survival.

Introduction

Issues related to over- and under-immunosuppression are common after heart transplantation (HT). Whereas over-immunosuppression can lead to side effects such as infections, malignancies and chronic kidney disease, under-immunosuppression can result in acute cellular rejection (ACR) [1]. Endomyocardial biopsy (EMB) is still the golden standard for the diagnosis of ACR after HT [2–4]. Whereas 'routine' EMBs are performed according to a pre-planned, centre-specific, schedule, 'additional clinically indicated' (ACI) EMBs may be obtained when symptoms warrant or after previous episodes of ACR.

The International Society for Heart and Lung Transplantation (ISHLT) has published two working formulations (WF) on how to histologically grade EMBs with respect to

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Table 1. The 1990-ISHLT-WF & 2004-ISHLT-WF on grading of ACR.

1990-ISHLT-WF	2004-ISHLT-WF
0 – No ACR	0R – No ACR
1A – Focal, mild ACR	1R – Mild, low-grade ACR
1B – Diffuse, mild ACR	
2 – Focal, moderate ACR	
3A – Multifocal, moderate ACR	2R – Moderate, intermediate ACR
3B – Diffuse, moderate ACR	3R – Severe, high-grade ACR
4 – Severe ACR	

1990-ISHLT-WF, 1990 International Society for Heart and Lung Transplantation working formulation; 2004-ISHLT-WF, 2004 International Society for Heart and Lung Transplantation working formulation; ACR, acute cellular rejection.

severity of ACR: that is, the older 1990-ISHLT-WF [5] and the newer 2004-ISHLT-WF [6] (Table 1). The 2004-ISHLT-WF was developed to resolve inconsistencies between different centres in the use of the 1990-ISHLT-WF. However, the advantages and disadvantages of the two WFs have not been evaluated, particularly not with regard to which scale that provides the best guidance on when to initiate treatment of ACR.

As the risk of death is highest the first year after HT and since 10% of these deaths are caused by ACR [1], our purpose was to study ACR the first year after HT and its impact on survival. Our main hypothesis was that first-year ACRs above a certain 'break point' grade, may affect outcome. We furthermore studied whether the frequency and severity of ACR, during the first year after HT, may be related to the year of HT, the week after HT, and to different recipient and donor risk factors.

Patients and methods

HTs and EMBs

This single-centre study included all 215 HT patients followed at Skåne University Hospital in Lund (SUS-Lund) 1988–2010. 219 HTs were included, of which 214 (98%) were first-time transplantations and five (2%) were retransplantations (three within 7 days, one 175 days and one 18 years after HT). 218 HTs were performed at SUS-Lund. One paediatric first-time transplantation was performed abroad in 2006. One patient, who underwent retransplantation at SUS-Lund in 2002, was originally transplanted abroad in 1984. The mean number of HTs performed at SUS-Lund per year was 9.5 ± 4.3 (0–17). 72 (33%) of the 219 HTs involved patients with prior ventricular assist devices (Fig. 1a). 32 (15%) of the 219 HTs involved paediatric patients below 18 years of age (Fig. 1b).

Among the 215 HT patients followed at SUS-Lund 1988–2010, 2990 first-year EMBs were performed. Out of these, 2635 (88%) were routine EMBs and 355 (12%) were ACI EMBs (i.e. 'additional clinically indicated' EMBs). The numbers of first-year EMBs for each year of, and each week after, HT, are shown in Fig. 1c and d, respectively.

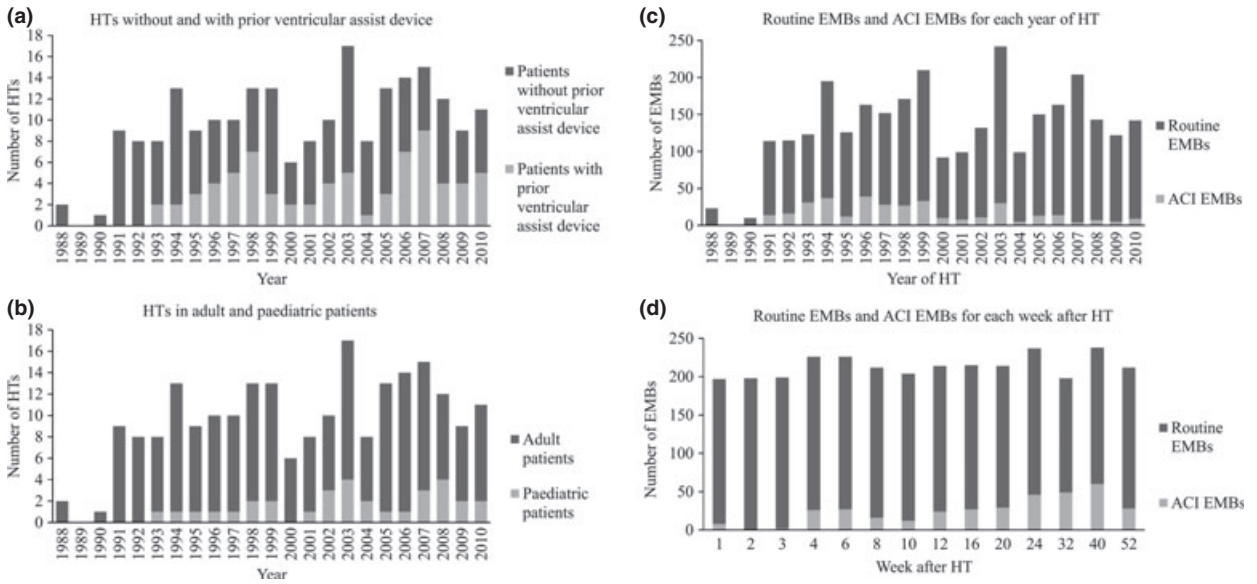

Figure 1 Figure showing the number of HTs per year at SUS-Lund 1988–2010 in (a) patients without and with prior ventricular assist device and (b) adult and paediatric patients below 18 years of age. The figure also shows the number of first-year routine EMBs and ACI EMBs for each year of (c), and each week after (d), HT. ACI, additional clinically indicated; EMB, endomyocardial biopsy; HT, heart transplantation; SUS-Lund, Skåne University Hospital in Lund.

The study was performed with approval from the local ethics board in Lund (Dnr 2011/777, Dnr 2011/368, Dnr 2010/114).

Study population characteristics

Study population characteristics, including recipient and donor age, age difference, gender, sex-matching, AB0-matching and CMV constellation, as well as recipient diagnosis, recipient waiting time, donor heart ischaemic time and immunosuppression, are shown in Table 2. The study population was divided into risk factor categories and groups. For age difference between recipient and donor, recipient waiting time as well as donor heart ischaemic time, four equally large intervals were created, between the highest and lowest values, in order to obtain four risk factor groups within each category.

Induction and maintenance immunosuppressive therapies 1988–1999 vs. 2000–2010

Induction immunosuppression in HTs performed during 1988–2010 (n = 214 of 219; data missing for five HTs), 1988–1999 ('early era', n = 93) and 2000–2010 ('late era', n = 121) is shown in Fig. 2a.

Maintenance immunosuppression in patients alive at discharge, for HTs performed during 1988–1999 (n = 204 of 219; data missing for 15 HTs in which the patient died before discharge), 1988–1999 ('early era', n = 90) and 2000–2010 ('late era', n = 114), is shown in Fig. 2b.

Down-titration of maintenance immunosuppression

Cyclosporine and tacrolimus trough (C0) levels (monitored weekly month 0–6 and every other week month 6–12) were generally targeted, respectively, to 250–300 and 12–15 μg/l month 0–2, 200–275 and 10–14 μg/l month 2–4, 150–225 and 8–12 μg/l month 4–6, 100–175 and 6–10 μg/l month 6–12, and 80–130 and 5–8 μg/l ≥year 1. In adults, daily corticosteroid doses were mostly 20 mg month 0–2, 15 mg month 2–4, 7.5–12.5 mg month 4–6, 5–7.5 mg month 6–12 and 2.5–5 mg ≥year 1. Paediatric patients generally received 10 mg/m^2/day month 0–2, 7.5 mg/m^2/day month 2–3, 5 mg/m^2/day month 3–4, 2.5 mg/m^2/day month 4–5 and 2.5 mg/m^2 every other day month 5–6. Paediatric patients without episodes of ACRs ≥ grade 3A were mostly weaned off corticosteroids after 6 months.

Data collection

The EMB data were collected retrospectively from medical records. Most EMBs were performed at The Haemodynamic Lab, The Clinic for Heart Failure and Valvular Disease, SUS-Lund, and analysed at the Department of Pathology, SUS-Lund. End of follow-up was 30 June 2012. First-year routine EMBs were obtained 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 52 weeks after HT. First-year ACI EMBs were obtained following clinical symptoms, or after previous episodes, of ACR. In general, four to five myocardial specimens were obtained during each EMB. The EMBs had been graded according to the 2004 scale, in complement to the 1990 scale, from 2005 and onwards. However, as the majority of the EMBs obtained 1988–2010 had been graded according to the 1990-ISHLT-WF, data were analysed according to the 1990-ISHLT-WF, instead of the 2004-ISHLT-WF.

Data analysis

The EMB data were analysed with regard to ACRs the first year after HT in relation to EMBs in general, the year of HT, the week after HT, different risk factors, as well as causes of death. We initially aimed to focus on ACRs severe enough to potentially require specific rejection treatment. In the latest ISHLT guidelines from 2010 [7], specific treatment of ACR is generally recommended following asymptomatic ACRs ≥ grade 2R (3A) and symptomatic ACRs irrespective of EMB grade. This strategy is similar to the one used at our centre between 1988 and 2010. Rejection treatment may, however, in some cases, have been administered in asymptomatic patients with early postoperative ACRs of grade 1R (1B-2) or three consecutive ACRs of grade 1R (1B-2) with no tendency to improve. Our initial comparative analyses therefore focused on ACRs ≥ grade 2, rather than ACRs ≥ grade 3A.

As many earlier reports have indicated more first-year ACRs in the 'earlier', compared with the 'later' era of HT [8,9], the proportions of first-year EMBs with ACR ≥ grade 2 were compared between HTs performed during 1988–1999 ('early era') and 2000–2010 ('late era').

As some previous studies have indicated more first-year ACRs in month 0-3 compared with month 4–12 after HT [10–12], the proportions of first-year EMBs with ACR ≥ grade 2 were compared between week 1–12 and week 16–52 after HT, corresponding to month 0–3 and 4–12, respectively.

For the analysis of different risk factors in relation to ACRs, the proportions of first-year EMBs with ACR ≥ grade 2 were compared between each risk factor group, within each risk factor category. The causes of death were also compared between patients with 0 vs. 1 or more ACRs ≥ grade 2. Patients in which survival was too short to obtain EMBs had to be excluded from this comparison.

In addition, as induction and maintenance immunosuppressive therapies have evolved during the last decades, the use of immunosuppression was compared between 1988–1999 and 2000–2010.

Table 2. Study population characteristics and first-year EMBs with ACR ≥ grade 2.

	Study population characteristics				EMBs with ACR ≥ grade 2		
Risk factor category (bold text) and group (normal text)	Mean	SD±	N_1	%	N_2	%	*P*-value
Age of recipient (years) =	**44.6**	**17.2**	**219**				
≤10 = 10 (4.6%), 11–20 = 24 (11.0%), 21–30 = 8 (3.7%), 31–40 = 20 (9.1%), 41–50 = 46 (21.0%), 51–60 = 87 (39.7%), 61≥ = 24 (11.0%)							
Paediatric recipients (below 18 years of age)			32	14.6	38	**9.4**	**0.13**
Adult recipients (18 years or older)			187	85.4	184	**7.1**	
Age of donor (years) =	**40.1**	**15.7**	**219**				
≤10 = 8 (3.7%), 11–20 = 22 (10.0%), 21–30 = 32 (14.6%), 31–40 = 31 (14.2%), 41–50 = 61 (27.9%), 51–60 = 52 (23.7%), 61≥ = 13 (5.9%)							
Paediatric donors (below 18 years of age)			17	7.8	25	**11.3**	**<0.05***
Adult donors (18 years or older)			202	92.2	197	**7.1**	
Difference in age between recipient and donor (years +/−)	**12.4**	**10.8**	**219**				
0–11			127	58.0	134	**7.6**	**0.20**
12–22			49	22.4	38	**5.9**	
23–33			32	14.6	39	**9.3**	
34–45			11	5.0	11	**6.6**	
Gender of recipient			**219**				
Male			149	68.0	73	**7.5**	**1.00**
Female			70	32.0	149	**7.4**	
Gender of donor			**219**				
Male			137	62.6	129	**7.1**	**0.40**
Female			82	37.4	93	**8.0**	
Sex-matching between recipient and donor			**219**				
Sex-matched			161	73.5	138	**6.3**	**<0.05***
Sex-mismatched			58	26.5	84	**10.4**	
AB0-matching between recipient and donor			219				
AB0 identical			184	84.0	188	**7.4**	**0.98**
AB0 compatible			32	14.6	31	**7.4**	
AB0 incompatible			3	1.4	3	**8.3**	
CMV constellation			**204**				
Donor +/Recipient +			107	52.5	101	**6.7**	**0.22**
Donor −/Recipient −			20	9.8	32	**10.0**	
Donor + / Recipient −			28	13.7	25	**7.0**	
Donor −/Recipient +			49	24.0	48	**7.6**	
Recipient diagnosis			**219**				
Arrythmogenic right ventricular dysplasia			3	1.4	2	**7.7**	**0.87**
Dilated cardiomyopathy caused by adriamycin			3	1.4	4	**9.3**	
Dilated cardiomyopathy caused by aortic stenosis or insufficiency			9	4.1	15	**10.8**	
Dilated cardiomyopathy			101	46.1	96	**7.1**	
Hypertrophic cardiomyopathy			10	4.6	11	**7.0**	
Right heart failure with ventricular tachycardia or fibrillation			2	0.9	0	**0.0**	
Ischaemic heart disease			59	26.9	55	**7.1**	
Chronic vascular rejection			1	0.5	2	**11.8**	
Myocarditis			13	5.9	15	**7.1**	
Restrictive cardiomyopathy			6	2.7	8	**8.6**	
Cardiac tumour			1	0.5	1	**12.5**	
Congenital heart disease			11	5.0	13	**8.8**	
Waiting time (days)	**150.4**	**200.7**	**219**				
0–322			192	87.7	187	**7.3**	**0.31**
323–644			19	8.7	29	**9.2**	
645–966			6	2.7	6	**6.7**	
967–1289			2	0.9	0	**0.0**	

Table 2. Continued

Risk factor category (bold text) and group (normal text)	Study population characteristics				EMBs with ACR ≥ grade 2		
	Mean	SD±	N_1	%	N_2	%	*P*-value
Ischaemic time (min)	**185.0**	**63.5**	**216**				
46–126			48	22.2	41	**6.2**	
127–207			84	38.9	86	**7.5**	**0.13**
208–288			78	36.1	91	**8.5**	
289–369			6	2.8	1	**1.9**	
Induction immunosuppression			**214**				
Antithymocyte globulin			201	93.9	212	**7.9**	
Daclizumab			8	3.7	7	**6.6**	**0.87**
No induction			5	2.3	2	**6.9**	
Maintenance immunosuppression in patients alive at discharge			**204**				
Cyclosporine + Azathioprine + Corticosteroids			99	48.5	103	**7.3**	
Cyclosporine + MMF/MPA + Corticosteroids			80	39.2	99	**8.2**	**0.23**
Tacrolimus + MMF/MPA + Corticosteroids			13	63.7	7	**4.0**	
Other combinations			12	5.9	13	**6.8**	

*Indicates statistical significance ($P < 0.05$).

EMB, endomyocardial biopsy; ACR, acute cellular rejection; SD, standard deviation; N_1, number of heart transplantations in each group; N_2, number of EMBs with ACR ≥ grade 2 for each group; MMF, mycophenolate mofetil; MPA, mycophenolic acid; CMV, cytomegalovirus.

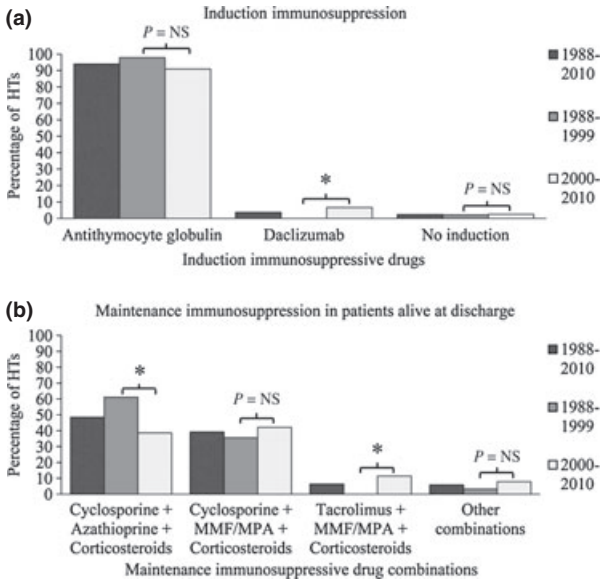

Figure 2 Figure showing induction immunosuppression (a) in HTs performed during 1988–2010, 1988–1999 ('early era') and 2000–2010 ('late era'). The figure also shows maintenance immunosuppression (b) in patients alive at discharge, for HTs performed during 1988–2010, 1988–1999 ('early era') and 2000–2010 ('late era'). *Indicates statistical significance ($P < 0.05$). HT, heart transplantation; MMF, mycophenolate mofetil; MPA, mycophenolic acid.

Finally, survival after HT in relation to ACRs the first year post-HT was also analysed. Patients were divided into five groups based on frequency and severity of first-year ACRs (Table 3). Survival after HT in patients without first-year ACRs ≥ grade 2 (i.e. only ACRs ≤ grade 1A/1B; Group A) was compared to survival after HT in patients with one or more first-year ACRs ≥ grade 2 (Group B). Survival after HT in patients without first-year ACRs ≥ grade 3A/3B (i.e. only ACRs ≤ grade 2; Group C) was compared to survival after HT in patients with one or more first-year ACRs ≥ grade 3A/3B (Group D). Furthermore, to discriminate the impact on survival of ACRs of grade 2 from ACRs ≥ grade 3A/3B, survival in group A was additionally compared to survival in group D, as well as to survival in group E, that is, patients who had one or more first-year ACRs of grade 2, but no first-year ACRs ≥ grade 3A/3B.

Table 3. Groups compared in the analysis of survival after HT in relation to frequency and severity of first-year ACRs.

Group	Frequency and severity of first-year ACRs
A	HTs in patients without first-year ACRs ≥ grade 2 (i.e. only ACRs ≤ grade 1A/1B)
B	HTs in patients with one or more first-year ACRs ≥ grade 2
C	HTs in patients without first-year ACRs ≥ grade 3A/3B (i.e. only ACRs ≤ grade 2)
D	HTs in patients with one or more first-year ACRs ≥ grade 3A/3B
E	HTs in patients with one or more first-year ACRs of grade 2 (but no ACRs ≥ grade 3A/3B)

HT, heart transplantation; ACR, acute cellular rejection.

Statistics

For statistical analysis, SIGMASTAT/SIGMAPLOT version 11.2.0.5 (Systat Software Inc, San Jose, CA, USA) was used. $P < 0.05$ was considered statistically significant. For comparison of ACRs ≥ grade 2, causes of death and immunosuppressive therapies, the chi-squared and Fisher's

exact tests were used. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test.

Results

EMBs in general

The distribution of ACR grades among the first-year routine EMBs (n = 2635) and ACI EMBs (n = 355) is shown in Fig. 3a and b, respectively. ACR ≥ grade 2 was seen in 172 (6.5%) of the first-year routine EMBs and 50 (14.1%) of the first-year ACI EMBs. There were proportionally more ($P < 0.05$) ACRs ≥ grade 2 among the first-year ACI EMBs (14.1%) than among the first-year routine EMBs (6.5%). Notably, among all 2990 first-year EMBs, only two showed ACR grade 3B and only one showed ACR grade 4.

Among the 205 HTs where survival was >25 days and at least one first-year EMB could be performed, ACR ≥ grade 1A/1B were found in 184 of the HTs (89.8%), ACR ≥ grade 2 were found in 114 of the HTs (55.6%), and ACR ≥ grade 3A/3B were found in 78 of the HTs (38.1%).

EMBs each year of HT

The distribution of ACR grades among the first-year routine EMBs (n = 2635) and ACI EMBs (n = 355) in relation to year of HT is shown in Fig. 4a and b, respectively. There were proportionally more ($P < 0.05$) ACRs ≥ grade 2 among first-year EMBs (routine and ACI combined) in HTs performed during 1988–1999 (9.6%) than 2000–2010 (5.5%). This difference was confirmed when separately analysing routine EMBs (8.3% vs. 5.2%; $P < 0.05$), but not ACI EMBs (15.9% vs. 10.3%; P = NS).

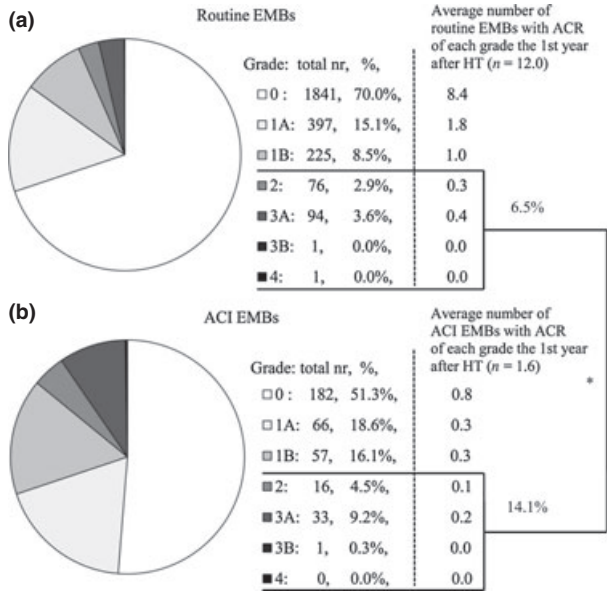

Figure 3 Figure showing the distribution of ACR grades among the first-year routine EMBs (a) and ACI EMBs (b). *Indicates statistical significance ($P < 0.05$). ACR, acute cellular rejection; ACI, additional clinically indicated; EMB, endomyocardial biopsy; HT, heart transplantation.

EMBs each week after HT

The distribution of ACR grades among the first-year routine EMBs (n = 2635) and ACI EMBs (n = 355) in relation to week after HT is shown in Fig. 4c and d, respectively. There were proportionally more ($P < 0.05$) ACRs ≥ grade 2 among first-year EMBs (routine and ACI combined) performed during 16–52 weeks (8.8%) than 1–12 weeks (6.3%) after HT. This difference was confirmed when separately analysing ACI EMBs (16.7% vs. 8.6%; $P < 0.05$), but not routine EMBs (7.1% vs. 6.2%; P = NS).

Different risk factors

The proportions of first-year EMBs with ACR ≥ grade 2 in relation to different risk factors are shown in Table 2.

There were proportionally more ($P < 0.05$) ACRs ≥ grade 2 among first-year EMBs in HTs with paediatric (17 HTs, 11.3%) than adult (202 HTs, 7.1%) donors. Survival analysis, however, showed no difference (P = NS) in 1-, 5- or 10-year survival between the two groups.

Likewise, there were proportionally more ($P < 0.05$) ACRs ≥ grade 2 among first-year EMBs in sex-mismatched (58 HTs, 10.4%) than sex-matched (161 HTs, 6.3%) HTs. Survival analysis, however, showed no difference (P = NS) in 1-, 5- or 10-year survival between the two groups.

No other risk factors, except from donor age as well as sex-matching between recipient and donor, were associated with a significantly higher risk of first-year ACR ≥ grade 2 (P = NS).

Causes of death

In total, 72 of our 215 HT patients (33%) died before the end of follow-up on 30 June 2012. The causes of death were as follows: primary graft failure (PGF; 6%), multiple organ failure (MOF; 6%), rejection (17%), malignancy (26%), infection (8%), chronic kidney disease (6%) and 'other' (32%). After exclusion of 11 patients in which no first-year EMBs could be performed (with PGF and MOF), the causes of death were similar (P = NS) regardless if they had 0 or one or more first-year ACRs ≥ grade 2.

Induction and maintenance immunosuppressive therapies 1988–1999 vs. 2000–2010

As seen in Fig. 2a and b, the immunosuppression differed between the 'early' and 'late' era. Most HT patients at

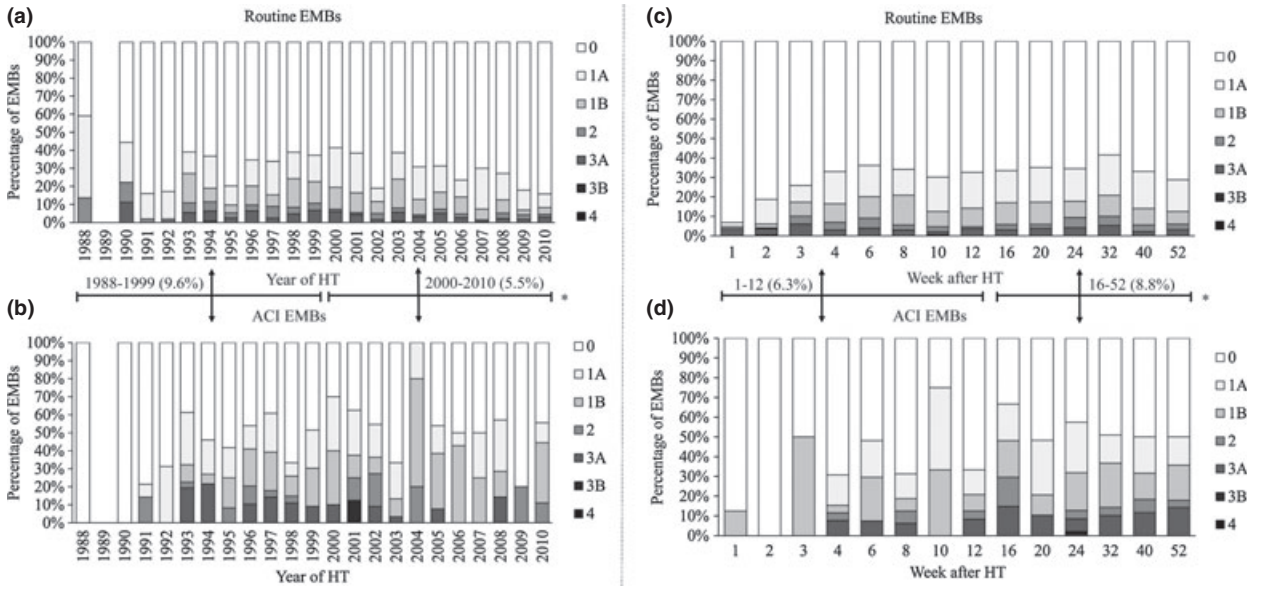

Figure 4 Figure showing the distribution of ACR grades among the first-year routine EMBs (a) and ACI EMBs (b) for each year of HT. The figure also shows the distribution of ACR grades among the first-year routine EMBs (c) and ACI EMBs (d) for each week after HT. *Indicates statistical significance ($P < 0.05$). ACR, acute cellular rejection; ACI, additional clinically indicated; EMB, endomyocardial biopsy; HT, heart transplantation.

our centre 1988–2010 received antithymocyte globulin as induction therapy together with a combination of cyclosporine + azathioprine + corticosteroids as maintenance therapy. Even though antithymocyte globulin, in total 1988–2010, was given more often than daclizumab, the use of daclizumab increased ($P < 0.05$) slightly from 1988–1999 to 2000–2010 (from 0% to 7% of the HTs). Furthermore, even though cyclosporine, in total 1988–2010, was given more often than tacrolimus (91% vs. 9% of the HTs, respectively), from the 'early' era 1988–1999 to the 'late' era 2000–2010, the use of tacrolimus + mycophenolate + corticosteroids increased ($P < 0.05$; from 0% to 11% of HTs) and the use of cyclosporine + azathioprine + corticosteroids decreased ($P < 0.05$; from 61% to 39% of HTs).

Survival after HT

Survival after HT for all 215 HT patients followed 1988–2010, as well as survival after HT in relation to first-year ACRs, is shown in Fig. 5a–c. 1-, 5- and 10-year survival after HT, for all 215 HT patients followed 1988–2010, was 93%, 85% and 74%, respectively.

Long-term survival (5 and 10 years) was lower ($P < 0.05$) among HTs in patients with one or more first-year ACRs ≥ grade 2 (group B) (114 HTs) compared with HTs in patients without first-year ACRs ≥ grade 2 (i.e. only ACRs ≤ grade 1A/1B) (group A) (91 HTs), with 83% vs. 94% and 70% vs. 87% alive 5 and 10 years after HT, respectively (Fig. 5a). Short-term survival (1 year) did, however, not differ (96% vs. 98%, respectively) between the two groups (P = NS).

Similarly, long-term survival (5 and 10 years) was lower ($P < 0.05$) among HTs in patients with one or more first-year ACRs ≥ grade 3A/3B (group D) (78 HTs) compared with HTs in patients without first-year ACRs ≥ grade 3A/3B (i.e. only ACRs ≤ grade 2) (group C) (127 HTs), with 82% vs. 92% and 69% vs. 82% alive 5 and 10 years after HT, respectively (Fig. 5b). Short-term survival (1 year) did, however, not differ (95% vs. 98%, respectively) between the two groups (P = NS).

Furthermore, long-term survival (5 and 10 years) following HT was lower ($P < 0.05$) in group D (one or more ACRs ≥ grade 3A/3B) compared with group A (only ACRs ≤ grade 1A/1B), with 82% vs. 94% and 69% vs. 87% of the patients alive 5 and 10 years after HT, respectively. However, comparing group E (one or more ACRs of grade 2, but no ACRs ≥ grade 3A/3B) with group A (only ACRs ≤ grade 1A/1B) yielded no such difference ($P = 0.13$ at 5 years and $P = 0.12$ at 10 years), with 86% vs. 94% and 72% vs. 87% of the patients alive 5 and 10 years after HT, respectively (Fig. 5c). For neither of these two comparisons, short-term survival (1 year) differed (P = NS) (95% vs. 98% in group D vs. A, and 97% vs. 98% in group E vs. A, 1 year after HT, respectively).

Discussion

The present study describes our centre's complete experience of ACR the first year after HT. Our findings indicate

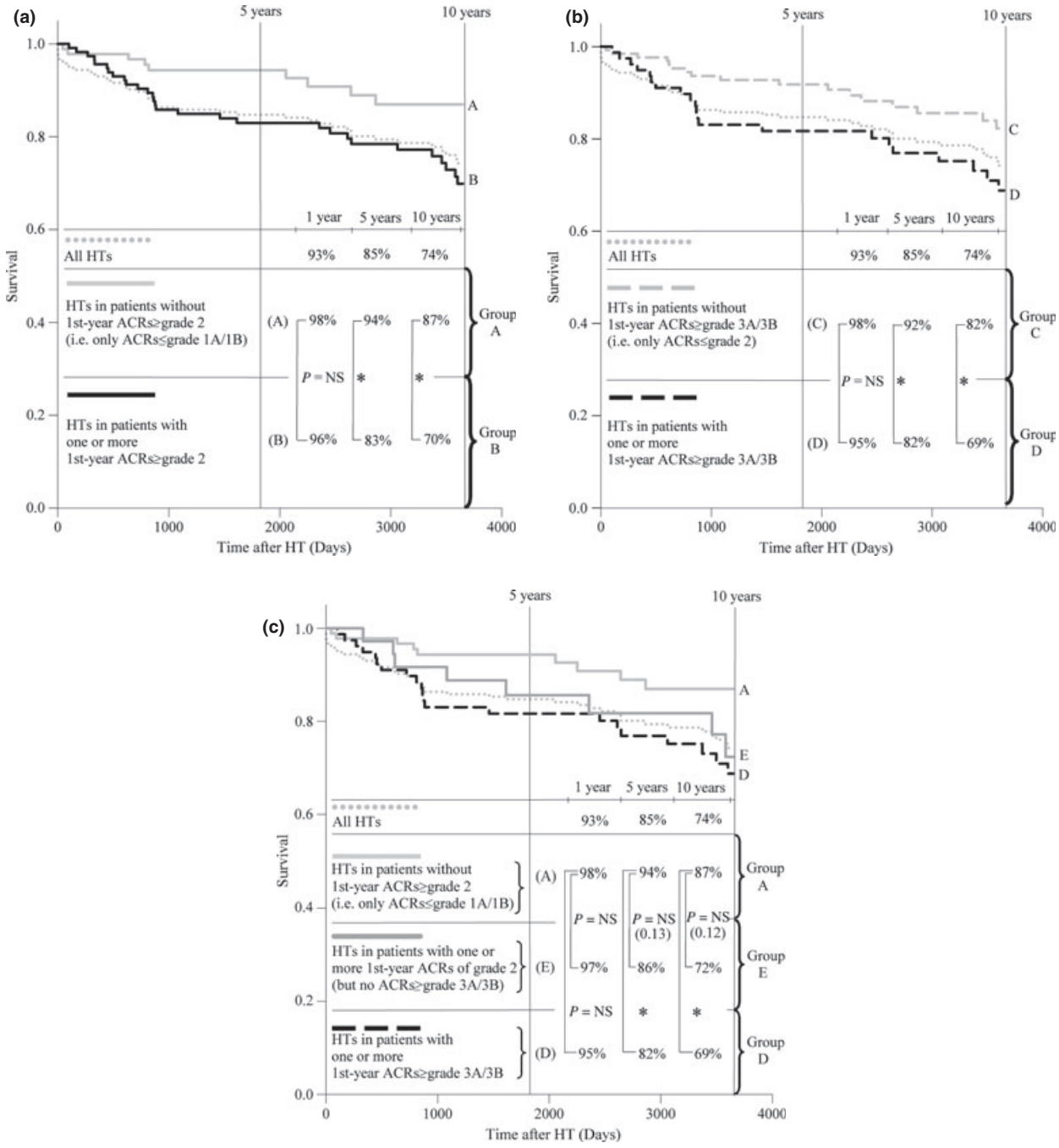

Figure 5 Survival in relation to first-year ACRs as well as survival for all 215 patients followed 1988–2010. The figures show surival in group A vs. B (a), group C vs. D (b), as well as group A vs. D and group A vs. E (c). *Indicates statistical significance ($P < 0.05$). ACR, acute cellular rejection.

that one or more first-year ACRs ≥ grade 3A/3B affect 5- and 10-year survival. Our study additionally shows that there were proportionally more first-year ACRs ≥ grade 2 among EMBs in HTs performed during 1988–1999 than 2000–2010, EMBs performed during week 16–52 than 1–12, EMBs in HTs with paediatric than adult donors, and EMBs in sex-mismatched than sex-matched HTs.

As our findings indicate that first-year ACRs ≥ grade 3A/3B affect 5- and 10-year survival, it is of great importance to prevent low-grade ACRs from progressing to ACRs ≥ grade 3A/3B. We believe our higher 10-year survival rate of 74% compared with 53% seen in the ISHLT registry [1] could partly be explained by our frequently performed EMBs during the first year following HT. These

may have allowed early ACR detection and continuous adjustments of the immunosuppression, leading to improved survival by preventing low-grade ACRs from progressing to ACRs ≥ grade 3A/3B.

Furthermore, in terms of how to prevent low-grade ACRs from progressing to ACRs ≥ grade 3A/3B, the older 1990-ISHLT-WF may offer a better clinical guidance than the newer 2004-ISHLT-WF. The main reason for this is that the 1990-ISHLT-WF has a slightly finer scale for low-grade ACRs, with grade 1A, 1B and 2 in the 1990-ISHLT-WF corresponding to grade 1R in the 2004-ISHLT-WF, making it easier to determine whether there is a low or high risk of progression to ACRs ≥ grade 3A/3B.

Noteworthy, first-year ACRs ≥ grade 3A/3B affected long-term, but not short-term, survival. This could potentially be due to subsequent development of cardiac allograft vasculopathy (CAV). CAV is a major cause of death late after HT [1], and patients with ACR have previously been found to have an increased risk of CAV [13–15]. The suggestion that severe first-year ACRs contribute to late mortality through the development of CAV is, however, not fully supported by our results as the causes of death were unrelated to first-year ACRs ≥ grade 2. However, only 72 patients died, and the data on the causes of death were based on clinical diagnoses rather than autopsies. Patients that died following clinical signs of myocardial infarction were categorized as 'other' (32%), and it is possible that some of these patients died of a previously undiagnosed CAV.

Furthermore, in the present study, proportionally more first-year ACRs ≥ grade 2 were found among EMBs performed during 1988–1999 (9.6%) compared with 2000–2010 (5.5%). This is in consistency with many earlier reports, observing more first-year ACRs in the 'earlier' than 'later' era of HT [8,9]. These results may be explained by improvements in immunosuppression 2000–2010 vs. 1988–1999. Most HT patients at our centre 1988–2010 received antithymocyte globulin as induction therapy together with a combination of cyclosporine + azathioprine + corticosteroids as maintenance therapy. Even though antithymocyte globulin, in total 1988–2010, was given more often than daclizumab, the use of daclizumab increased slightly from 1988–1999 to 2000–2010 (from 0% to 7% of the HTs; Fig. 2). Furthermore, even though cyclosporine, in total 1988–2010, was given more often than tacrolimus (91% vs. 9% of the HTs, respectively), from the 'early' era 1988–1999 to the 'late' era 2000–2010, the use of tacrolimus + mycophenolate + corticosteroids increased (from 0% to 11% of HTs; Fig. 2) and the use of cyclosporine + azathioprine + corticosteroids decreased (from 61% to 39% of HTs; Fig. 2). These differences in induction and maintenance immunosuppression 1988–1999 vs. 2000–2010 may partly have contributed to the differences in first-year ACRs between the 'early' and the 'late' eras.

Moreover, in the present study, proportionally more first-year ACRs ≥ grade 2 were found among EMBs performed during week 16–52 (8.8%) compared with week 1–12 (6.3%). This is in contrast to some previous studies, observing more first-year ACRs in month 0–3 than month 4–12 after HT [10–12]. These results may, however, in our cohort, be explained by the reduced frequency of EMBs performed during 16–52 weeks vs. 1–12 weeks after HT, allowing less frequent adjustments of the immunosuppression. Another reason may be that, during week 16–52 compared with week 1–12, most patients were targeted to lower cyclosporine (80–225 vs. 200–300 μg/l) and tacrolimus (5–12 vs. 10–15 μg/l) trough levels. The doses of corticosteroids were also generally lower during this period (5–12.5 vs. 15–20 mg/day).

In previous studies, a higher risk of ACR has been associated with HLA-mismatching [10,16], young recipients and donors [10,16,17], female recipients and donors [10,16,17], sex-mismatching [18], CMV infection [19,20] and long ischaemic time [10,21]. Other studies have compared ACRs in relation to different combinations of induction [22–35] and maintenance [36–39] immunosuppressive therapies. In consistency with previous studies [17], our study showed that there were proportionally more ACRs ≥ grade 2 among first-year EMBs in HTs with paediatric (11.3%) than adult (7.1%) donors. Likewise, in consistency with earlier reports [18], our study showed that there proportionally more ACRs ≥ grade 2 among first-year EMBs in sex-mismatched (10.4%) than sex-matched (6.3%) HTs. However, none of these two differences had an impact on 1-, 5- or 10-year survival after HT. One reason for this may be that the proportional differences in first-year ACRs ≥ grade 2 were too small. None of the other risk factors, except from donor age and sex-matching between recipient and donor, were associated with a higher risk of ACRs ≥ grade 2, possibly due to the number of patients studied. It should also be noted that the results from the comparisons of different induction and maintenance immunosuppressive therapies in relation to first-year ACRs ≥ grade 2 should be interpreted with caution. The results of these comparisons may not only be affected by the efficacy of the drugs in preventing ACR, but also by risk and history of ACR, which may have influenced which drugs that were used.

As a limitation to the present study, it should be noted that in 14 HTs where survival was ≤25 days, no EMBs could be performed. These HTs could not be included in the analysis of survival in relation to first-year ACRs, and it is possible that this may have led to a slight underestimation of the frequency of first-year ACRs and to a slight

overestimation of survival in relation to first-year ACRs. Moreover, autopsies and coronary angiographies were unfortunately unavailable to the present study, but could have been of value to determine the causes of death and their potential relation to ACR.

In conclusion, our results indicate that first-year ACRs ≥ grade 3A/3B affect long-term survival. We believe the higher 10-year survival rate of 74% in the present study vs. 53% in the ISHLT registry could partly be explained by our frequently performed EMBs during the first year following HT. These may have allowed early ACR detection and continuous adjustments of the immunosuppression, leading to improved survival by preventing low-grade ACRs from progressing to ACRs ≥ grade 3A/3B. Future multicentre studies on ACR are encouraged to further improve survival following HT.

Authorship

CS: study design, data collection, data analysis and writing of the article. JÖ: contributed to study design and reviewing the article. JN, TH, BK and LJ: contributed to data acquisition and reviewing the article. GR: study design, data acquisition, data collection, data analysis, writing and reviewing the article.

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