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HEART TRANSPLANTATION: LOWERED SURVIVAL AND TUMORAL DEVELOPMENT AHEAD OF ACUTE CELLULAR REJECTION

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1. ABBREVIATION LIST

- **ABO** – Blood group system;
- **ACR** – Acute cellular rejection;
- **CAV** – Cardiac Allograft Vasculopathy;
- **CBP** – Cardiopulmonary bypass;
- **CMV** – Citomegalovirus;
- **CNI** – Calcineurin inhibitor;
- **CyA** – Cyclosporine;
- **EMB** – Endomyocardial biopsies;
- **EVR** – Everolimus;
- **HT** – Heart Transplant;
- **MMF** – Mycophenolate mofetil;
- **PRA** – Panel reactive antibody;
- **VAD** – Ventricular assistance device;

2. RESUMO

Introdução e objetivos:

O impacto da rejeição celular aguda durante os primeiros anos após o transplante cardíaco na sobrevida a longo prazo ainda não está bem estabelecido, assim como o seu papel no desenvolvimento da doença vascular do enxerto. Os novos imunossupressores conduziram a uma diminuição da incidência da rejeição celular aguda, mas consequentemente levaram a um aumento do risco de infeções e tumores. O objetivo do nosso trabalho foi analisar o impacto da rejeição celular na sobrevida e a ocorrência de neoplasias, infeções e doença vascular do enxerto em doentes selecionados.

Métodos:

De novembro de 2003 a maio de 2013, 218 doentes foram submetidos a transplante cardíaco. Doentes com menos de 18 anos, sujeitos a outro transplante de órgão prévio ao transplante cardíaco e recetores que faleceram nos primeiros 14 dias após a cirurgia devido a falência do enxerto, foram excluídos. Transplantados com pelo menos um episódio de rejeição celular aguda classificada como 2R ou 3R (Grupo A n=47) foram comparados com recetores livres de episódios de rejeição ou com episódios de rejeição classificados como 1R nos primeiros 3 anos após transplante cardíaco (Grupo B n=171). Os critérios de seleção dos dadores e recetores foram idênticos em ambos os grupos.

Resultados:

A incidência da rejeição celular aguda foi mais elevada nos primeiros 6 meses após transplante cardíaco ($P<0.001$).

Não foram encontradas diferenças estatisticamente significativas na sobrevida a longo prazo (P=0.101) ou na incidência da doença vascular do enxerto (P=0.144) entre ambos os grupos. No entanto, verificámos uma ligeira tendência para a diminuição da sobrevida a longo prazo (61.7 ± 7.3% vs 77.1 ± 3.7%) e sobrevida livre de doença vascular do enxerto (75.9 ± 6.6% vs 86.0 ± 3.5%) no grupo A. As neoplasias *de novo* tiveram uma maior incidência no grupo B (P=0.026) enquanto as infeções foram mais frequentes no grupo A (P=0.036).

Conclusão:

A taxa da rejeição celular aguda na nossa população de estudo verificou-se ser baixa e a maioria dos episódios ocorreram nos primeiros 6 meses após o transplante. O tratamento imunossupressor associado talvez a um estado sobre-terapêutico podem potenciar o aumento da incidência de tumores. Este estudo sugere-nos ainda que pacientes que sofreram de episódios de rejeição celular aguda nos primeiros 3 anos após o transplante têm uma maior tendência a sofrer de doença vascular do enxerto e a uma menor sobrevida a longo prazo, no entanto sem significância estatística.

PALAVRAS-CHAVE:

- Transplante Cardíaco;
- Rejeição celular aguda;
- Biópsia endomiocárdica;
- Terapêutica imunossupressora;
- Infeções;
- Tumores;
- Doença vascular do enxerto.

3. ABSTRACT

Background

The impact of acute cellular rejection (ACR) on long-term survival during the first years after heart transplant has not yet been established, as well as its role on cardiac allograft vasculopathy (CAV). New immunosuppressors have led to a decline of the incidence of ACR and led to increased risk of infections and tumors. We analysed the impact of ACR on long-term survival and considered the occurrence of malignancy, infections and cardiac allograft vasculopathy in the selected patients.

Methods

Between November 2003 and May 2013, 218 heart transplants were performed. Patients under 18-years old, patients undergoing organ transplantation before heart transplant and recipients who died within the first 14 days after heart transplant (HT) due to graft failure, were excluded. Recipients with at least one episode of ACR event graded as 2R or 3R (Group A n=47) were compared with recipients free of rejection events or with an ACR event graded minor than 2R in the first 3 years after heart transplantation (Group B n=171). Patient/donor criteria were selected as identical in both groups.

Results

Incidence of ACR was higher in the first 6 months after heart transplantation ($P < 0.001$).

There was no significant statistical difference in long-term survival ($P = 0.101$) or incidence of CAV ($P=0.144$) between the two groups. A slightly tendency for a lower

long-term survival (61.7 ± 7.3% vs 77.1 ± 3.7%) and survival free of CAV (75.9 ± 6.6% vs 86 ± 3.5%) was verified in Group A. *Malignancy de novo* had an higher incidence in Group B (P=0.026) while infections (P=0.036) were more frequent in Group A.

Conclusion

With this study, we verified that we have a small rate of ACR and mostly occurs in the first 6 months. The effective immunosuppression regimen maybe together with over-immunosuppression may lead to a higher incidence of tumors. This study also suggests that recipients with ACR events are more likely to suffer from CAV and to have a lower long-term survival however with out statistical significance.

KEYWORDS:

- Heart transplantation;
- Acute cellular rejection;
- Endomyocardial biopsy;
- Immunosuppressive therapy;
- Infections;
- Tumors;
- Cardiac allograft vasculopathy.

4. INTRODUCTION

After exhausting all possibilities of medical therapy, heart transplantation has been shown as the most effective therapy for patients suffering from end-stage heart failure. It has demonstrated increase survival and also the quality of life of the recipients.(1)

Since the advent of heart transplant, acute cellular rejection (ACR) has been a major cause of morbidity and mortality. In the mid 1980's cyclosporine (CyA)-calcineurin inhibitor (CNI) - established HT as a therapeutic reality. Nowadays roughly 10% of deaths occur in first year after HT are caused by ACR (2,3) and the decline in mortality decrease has been attributed to a set of parameters such as immunosuppressive induction therapy, maintenance immunosuppressive regimen and an effective surveillance of ACR (4). Despite all the studies that have been carried out to develop a reliable non-invasive method to identify acute rejection, endomyocardial biopsy still remains as gold-standard for rejection diagnostic and grading.(2)

Besides, the incidence of acute cellular rejection decreased significantly, the recipient became vulnerable to a higher risk of infection and malignancy development. Recent data of the International Society of Heart and Lung Transplantation Registry indicates 29.6% patients with malignancies reported within 10-years following heart transplantation.(3)

The most advantageous combination of immunosuppressive drugs is still under research. As the goal of ideal maintenance immunosuppression should be to prevent rejections events without having undesirable side effects, especially major organ toxicity and increased risk for infection and development of tumors.(5)

There is still some controversy and reluctance about the recognition of early ACR events in the long-term survival.

Experimental evidence suggests a correlation between acute cellular rejection and cardiac allograft vasculopathy. CAV can be defined as a chronic rejection event and its ethology is primarily immune.(6) Cardiac allograft vasculopathy is a leading cause of late morbidity and mortality in heart transplant recipients and accounts for 13.7% of 5-10 year mortality.(3)

The objective of this study was to verify whether acute cellular rejection occurring in the first 3 years after heart transplantation affects patient and graft long-term survival. The second end-point was to verify the incidence of tumors, infections and CAV in the follow recipients group without any episode of rejection graded 2R or 3R in the first 3 years after HT.

5. MATERIALS AND METHODS

Patients characterization

The Heart Transplantation Center of the University Hospital – Faculty of Medicine in Coimbra allowed this retrospective study including all 218 HT patients undergoing primary orthotopic heart transplantation, followed up at the Cardiothoracic Cirurgic Center, between November 2003 and May 2013 with a median of 2137 days.

Recipients were selected between 18 and 72 years old at the time of the surgery, when 28 patients were over 65 years.

Exclusion criteria for this study included being younger than 18 years, patients undergoing organ transplantation before heart transplantation and recipients who died within the first 14 days after HT due to graft failure.

Heart transplantation was performed according to the bicaval anastomosis technique, under cardiopulmonary bypass (CPB) and moderate systemic hypothermia (28ºC) with some surgery technical modifications with the aim of decreasing ischemic surgery time

Data was collected by retrospective review of the medical registries organized in the database, inserted in the national registry.

Pre-transplant cross-match was performed in all cases and only patients with negative cross-match were transplanted. PRA (panel reactive antibody) was also performed in all cases and after transplant the result was known and the immunosuppressive regimen adapted accordingly.

Rejection surveillance was performed through right ventricular endomyocardial biopsies (EMB). All EMB were performed by the team cardiologist and the slides were

analysed by the team Pathologist. Routine EMB were obtained following the Center protocol: every 7-10 days in the first month, 10-14th day in the 2nd month, 15-20th days until the 5th month, and then monthly until the end of the first year and once a year after the first year. This schedule would be adjusted whenever it was deemed clinically to do so.

Due to the possibility of byopsing on previous sites and to decrease the probability of false negatives, 2 or 3 myocardial fragments were collected in each moment of biopsy. The 2004-ISHLT-WF defines rejection in 4 grades (OR - no rejection, 1R- mild rejection (1990 Grades 1A, 1B and 2), 2R- moderate rejection (1990 Grade 3A), 3R- severe rejection (1990 Grades 3B and 4). The biopsies graded before 2004 with the old classification were reassigned according to the actual classification.

EMB were analysed only in the first 3-years after HT, with an average of 15 interventions per patient. By time intervals, of [0;6],]6;12] and]12;36] months we analysed the occurrence of ACR, defined as a EMB ≥ grade 2R.(7)

Patients were stratified into 2 groups: A (with at least one episode of ACR) and B (freedom from ACR). We analysed the long-term survival, the occurrence of events such as tumors (except skin cancer but including melanoma), severe infections and cardiac allograft vasculopathy.

We also registered several patient characteristics that could influence the occurrence of acute cellular rejection in HT recipients, likewise age, gender mismatching, ABO mismatching, CMV mismatching, ischaemic time, pulmonary resistance, the need of ventricular assistance device (VAD), and the basic etiology of cardiac disease.

Study regimen and rejection treatment

Before May 2013, induction therapy was administrated to all HT patients. Basiliximab (20 mg/iv, the first dose given intra-OP after reperfusion and the second dose on day 4^{th}-5^{th} post-transplant), methylprednisone intravenously (500mg at the beginning of the surgery and 125 mg every 8 hours for a total of 3 doses, followed by prednisone 0,8 mg/kg/day during the 1st week) and mycophenolate mofetil (1g/oral).

All the patients were treated with a maintenance standard triple immunosuppressive regimen of corticosteroids, calcineurin inhibitor and mycophenolate mofetil (MMF). Only two patients started treatment with an mTOR (Inhibition of the mammalian target of Rapamycin - Everolimus or Sirolimus) instead of MMF, in the first year and both were administered over 6 months after HT.

For the maintenance therapy patients received prednisone with daily doses of (0,2 mg/Kg/ 2-4w PT, 0,15 mg/kg/ 2-6m PT, 0,1 mg/kg/ > 6m) and MMF (initiated before surgery, 2 x 500 mg 1ºw, 2 x 1000mg 2ºw-6º month, 3x 500mg >6º month) in addition to Tacrolimus (initiated between 2º and 7º day after transplant and the dose was adjusted to reach levels of 12-15 ng/mL at 2-4 week, 10-15 ng/mL at 2-6 month and 5-15 ng/mL thereafter) or CyA (initiated between 2º and 7º day after-transplant and the dose was adjusted to reach trough levels of 200-250 ng/mL at 2-4 week, 150-200 ng/mL at 2-6 month and 100-150 ng/mL thereafter).

Intolerance to MMF, raising tumors or evidence of CAV, led to MMF switched to Everolimus (EVR). The dose of EVR was adjusted to reach trough levels of 3-6 ng/ml.

Statistical analysis

Continuous variables non-normally distributed were reported as median and interquartile range (IQR) and were compared using *MANN-Whitney U-test*. Continuous variables normally distributed were compared using independent student T-test. Categorical variables were reported as percentages and were compared using *Chi-square test* or *Fisher´s exact test* when appropriate. Survival and event free survival curves were plotted using using *Kaplan-Meier method* and compared using *long-rank test*. Statistical significance was defined as a two-tailed probability value of $P<0,05$.

Data were analysed using the IBM SPSS Statistics for IOs program.

6. RESULTS

A total of 218 recipients were identified for this study. There were 47 patients who had at least one episode of acute cellular rejection in the first 3 years after heart transplantation (Group A) and 171 patients free of acute cellular rejection events ≥ 2R (Group B). The median age of included patients was 57 (IQR-15) years and 77% were male. The most common indication for HT was ischaemic heart disease (39.4%, n= 89). The median donor age was 35 years old (IQR-18). Recipients and donors were sex matched in 67.7%, ABO identical in 82.7%, and CMV compatible in 73%. The mean ischaemic time was 90.9 ± 37.6 minutes.

Table 1 shows the data for variables related to surgery and patients' characteristics in Groups A and B. There wasn't no significant statistical difference between groups. Although, in Group A the need of mechanical assistance (VAD) was higher (8.5% vs 3.5%).

All patients except one initiated maintenance immunosuppressive therapy with the triple regimen (MMF + IC + CE) but in the first 3 years after HT there was a need in 14.7% (n= 32) to change MMF to EVR. This change was related with CAV 9 patients and with tumor incidence in 5 patients.

There was a significant statistical difference between the incidence of ACR events in first 6 months compared with the second semester and also with two years follow up ($P < 0.001$). (Fig 1; Table 2)

Table 1. Study population characteristics

		ACR 0-3 years			
	Population n=218	**Group A n=47**	**Group B n=171**	**p-value**	
Age of recepient. yr.	57 (IQR-15)	56(IQR-16)	57(IQR-17)	**0.250**	**M.W.U**
Age of donor. yr.	35 (IQR-18)	35(IQR-21)	35(IQR-18)	**0.482**	**M.W.U**
Gender (Female)	23	17	24.6	**0.276**	**χ2**
Sex-mismatching. %					
Compatible	67.7	63.8	67.8	**0.605**	**χ2**
ABO, %					
Identical	82.7	78.7	84.2	**0.375**	**χ2**
Compatible	17.3	21.3	15.8		
Indication for cardiac transplant. %					
Idiopathic	5.3				
Valvular heart disease	10.2				
Ischaemic heart disease	39.4				
Others causes	45.1				
Previous cardiac surgery.%	29.9	24.2	29.2	**0.483**	**χ2**
Ischaemic time, min, mean ± SD	90.9 ± 37.6	92.6 ± 34.3	89.5 ± 38.8	**0.525**	**M.W.U**
CMV mismatch. %					
Compatible	73	78.3	73.0	**0.516**	**χ2**
D+/R-	10.6	6.5	12.6		
D-/R+	14.2	15.2	14.1		
Pulmonary Resistence (WU), mean	3.4 ± 2.3	3.1 ± 1.7	3.5 ± 2.1	**0.386**	**M.W.U**
Ventricular assistence device	4.4	8.5	3.5	**0.147**	**χ2**

ABO – blood group system; ACR- acute cellular rejection; CMV- Citomegalovirus;

P<0,05 indicates statistical significance. P-value not correct for multiples comparisons.

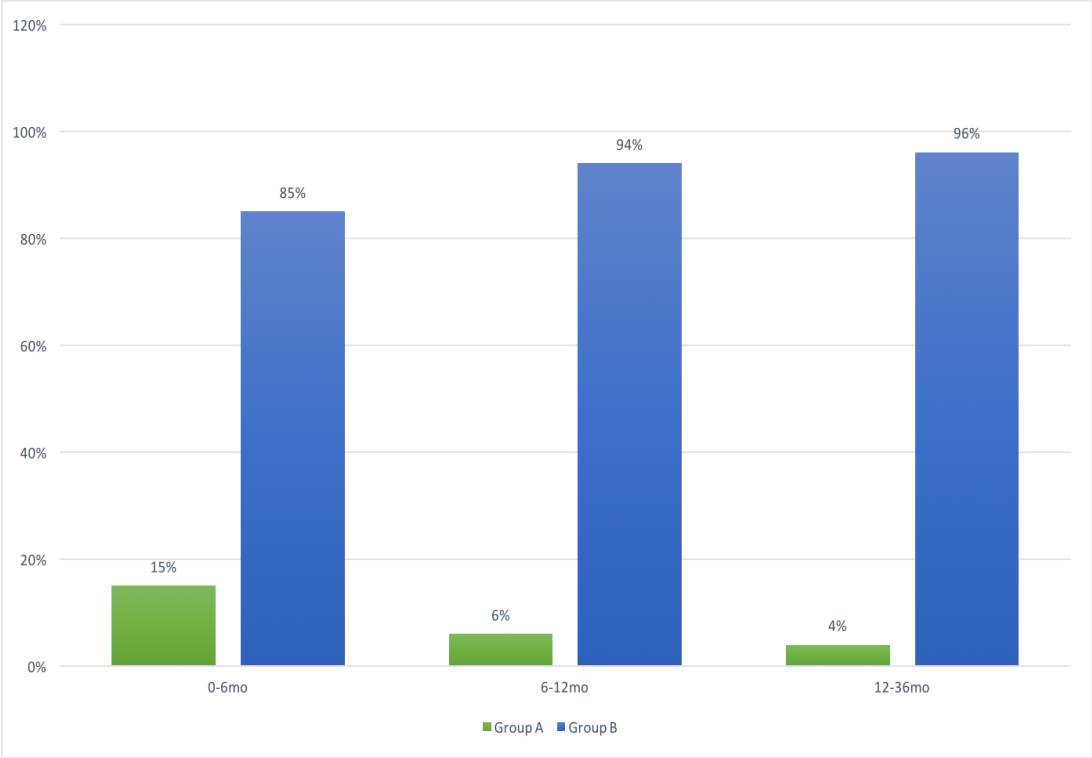

Fig. 1 *Acute cellular rejection events - EMB ≥ grade 2R (Group A).*

Pairwise comparation	
Time intervale	**p-value**
0-6;6-12mo	< 0,001
0-6;12-36mo	0,004
6-12;12-36mo	0,997

Table 2. *Pairwise comparation. P-value < 0.05 indicates statistical significance. Cochran's Q-test.*

HT survival relationship within 3-years incidence of ACR is shown in figure 2.

Overall survival at 1, 5, and 8 years was 88.0 ± 4.2%, 75.3 ± 5.8% and 61.7 ± 7.3% respectively in Group A and 93.0 ± 2.0%, 84.3 ± 3.0%, 77.1 ± 3.7% in Group B. There was a higher survival rate in Group B but with no statistical significance (P=0.101). (Fig 2; Table 3).

Group A mortality was 38.3% (n=18) vs 25.7% (n=44) in Group B. The major cause was infection in both groups followed by neoplasia, vasculopathy (CAV) and cardiac disease.

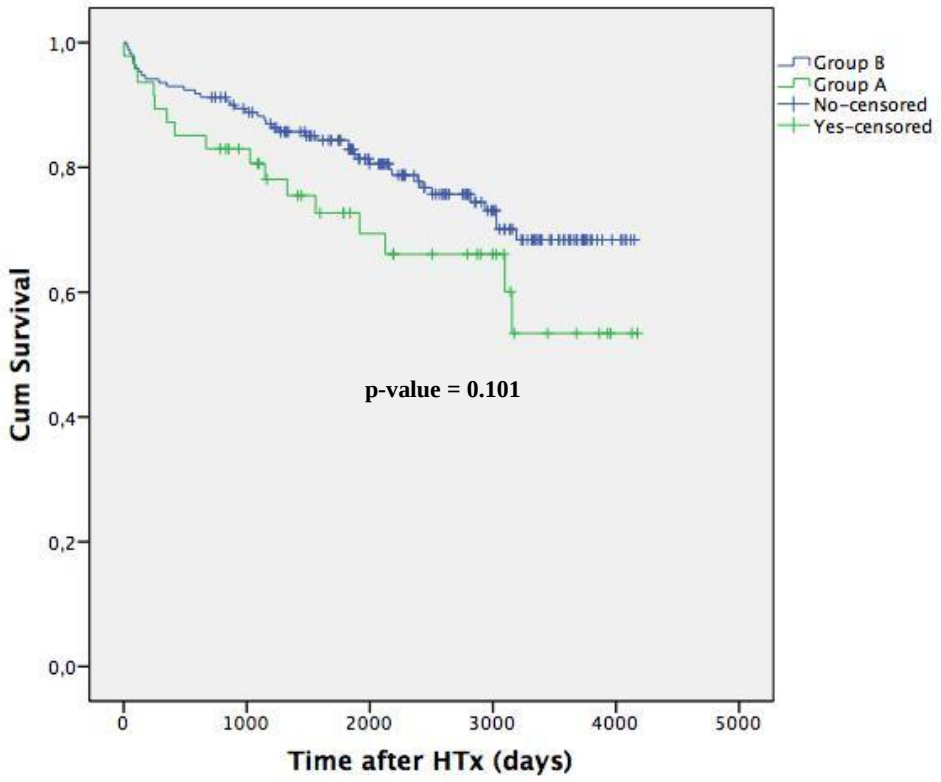

Figure 2. *Effect of acute cellular rejection on long-term survival in transplanted patients. P-value < 0.05 indicates statistical significance.*

	Group A	***Group B***	***p-value***
1-year	88.0 ± 4.2%	93.0 ± 2.0%	0.449
5-years	75.3 ± 5.8%	84.3 ± 3.0%	0.749
8-years	61.7 ± 7.3%	77.1 ± 3.7%	0.111

Table 3. *Survival free of rejection.*

As we subdivided Group B in two sub-groups: Group B1 (N=97) - patients with at least one EMB grade 1R in the first 3-years, and Group B2 (N=74) - patients that during the first 3-years ever had had EMB graded 0R, had no statistical significance between survival curves (P =0.226) compared with Group A; however we can assume a long-term tendency to a higher survival rate in Group B2 compared with Group A and slightly higher with group B1. (Fig. 3; Table 4)

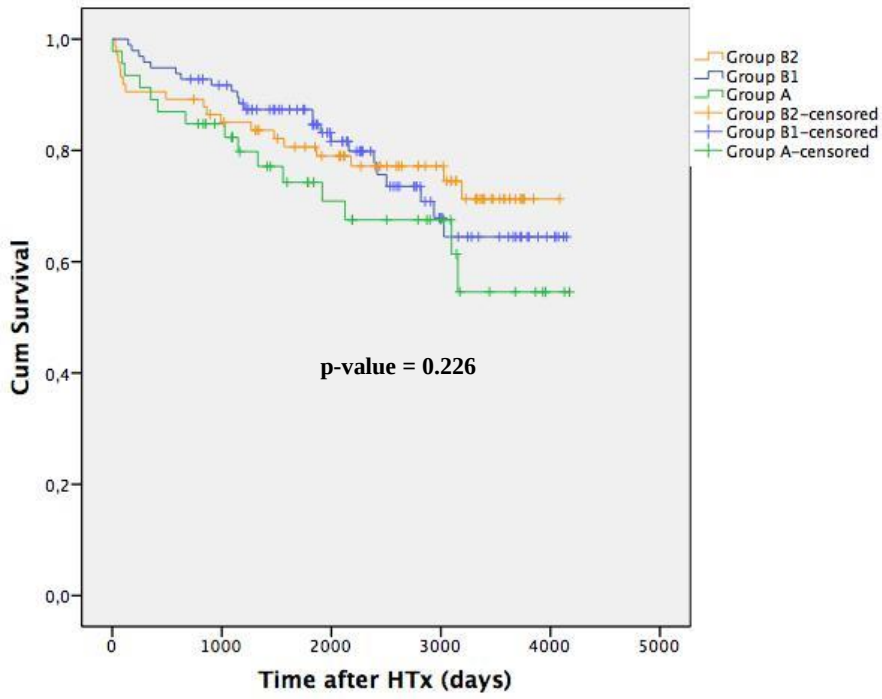

Figure 3. *Effect of acute cellular rejection on long-term survival in transplanted patients. P-value < 0.05 indicates statistical significance.*

	Group A	Group B1	Group B2
1-year	89.1 ± 4.6%	94.8 ± 2.2%	90.5 ± 3.4%
5-years	74.3 ± 6.8%	87.3 ± 3.4%	80.6 ± 4.7%
8-years	67.5 ± 7.7%	70.8 ± 5.9%	77.2 ± 5.1%

Table 4. *Survival free of rejection.*

There was statistical significance in malignancy-free survival between Groups A and B **(P = 0.026).** At 1, 5 and 8 years was 100%, 92.2 ± 3.7% and 89.4 ± 4.6%, respectively, in Group A and 97.5 ± 1.2 %, 84.8 ± 3% and 73.2 ± 4.3% in Group B. As expected, survival free of neoplasia was lower in group of patients free of rejection in the first 3-years. (Fig. 4; Table 5)

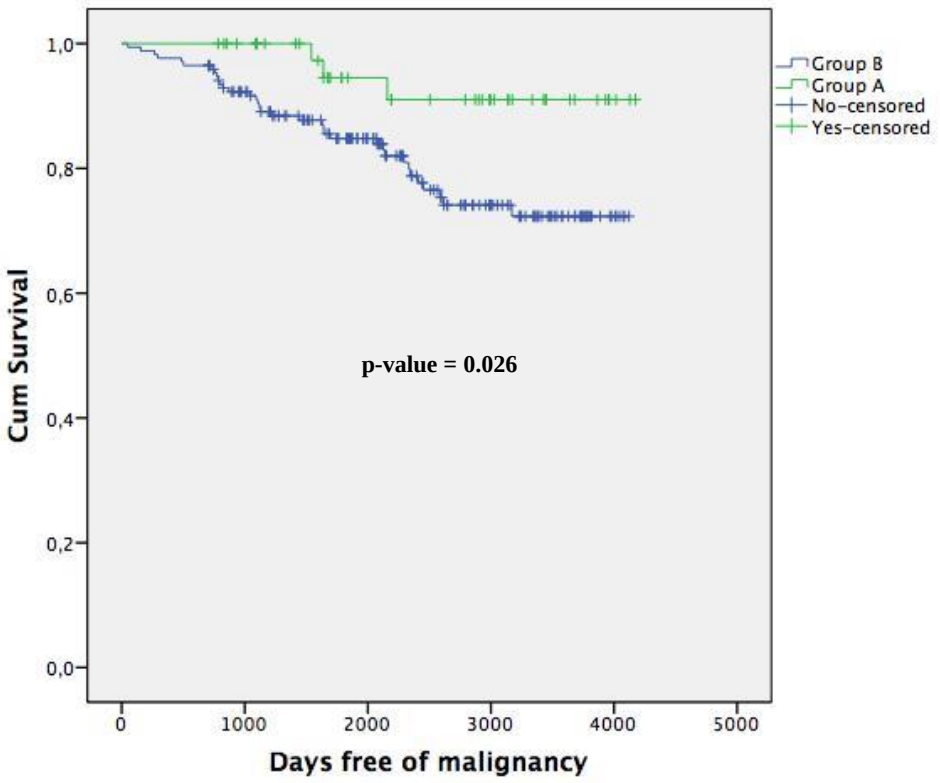

Figure 4. *Time free of malignancy after heart transplantation. P-value < 0.05 indicates statistical significance.*

	Group A	***Group B***	***p-value***
1-year	100%	97.5 ± 1.2%	
5-years	92.2 ± 3.7%	84.8 ± 3.0%	0.009
8-years	89.4 ± 4.6%	73.2 ± 4.3%	0.127

Table 5. *Survival free of malignancy.*

Survival free of serious infections at 1, 5 and 8 years was, respectively, 85.1 ± 5.2%, 71.5 ± 6.7% and 65.5 ± 7.4% in Group A and 93.0 ± 2.0 %, 81.6 ± 3.1%, 68.5 ± 4.2% in Group B, being higher in group free from ACR events (**P = 0.036**). During around a year after 8th-year after-HT survival free from serious infections, was similar between the two Groups. (Fig. 5; Table 6).

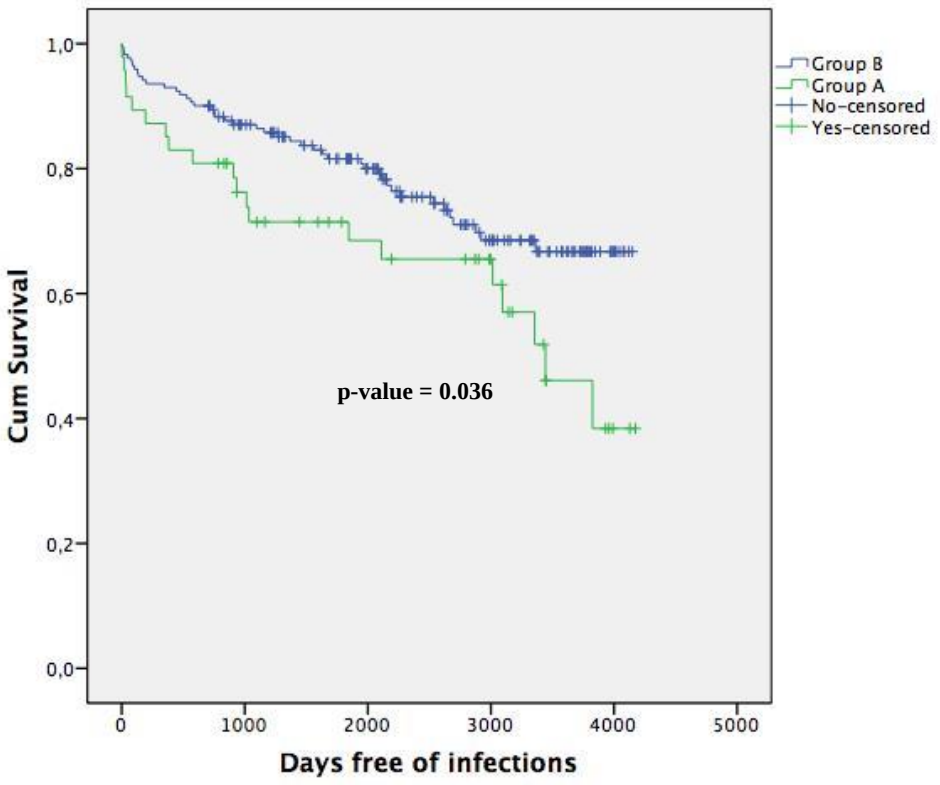

Figure 5. *Time free of infections after heart transplantation. P-value < 0.05 indicates statistical significance.*

	Group A	***Group B***	***p-value***
1-year	85.1 ± 5.2%	93.0 ± 2.0%	0.931
5-years	71.5 ± 6.7%	81.6 ± 3.1%	0.462
8-years	65.5 ± 7.4%	68.5 ± 4.2%	0.042

Table 6. *Survival free of infections.*

Survival free of graft vascular disease at 1, 5 and 8 years was 98.3 ± 1.7% , 89.4 ± 4.1% and 75.9 ± 6.6% in Group A and 98.1 ± 1.1%, 93.0 ± 2.2% and 86 ± 3.5%, respectively, in Group B. Survival free of CAV was slightly higher in Group B since the first year, without reaching statistical significance (P = 0.144). (Fig. 6; Table 7)

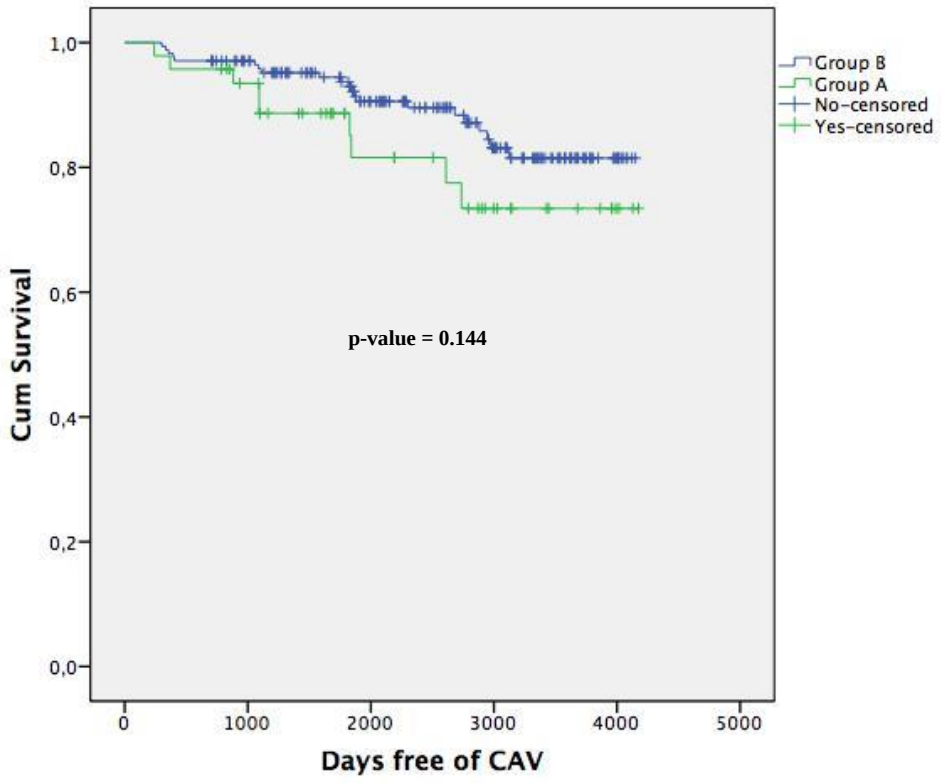

Figure 6. *Time free of cardiac allograft vasculopathy (CAV) after heart transplantation. P-value < 0.05 indicates statistical significance.*

	Group A	***Group B***	***p-value***
1-year	98.3 ± 1.7%	98.1 ± 1.1%	0.083
5-years	89.4 ± 4.1%	93.0 ± 2.2%	0.965
8-years	75.9 ± 6.6%	86.0 ± 3.5%	0.127

Table 7. *Survival free of cardiac allograft vasculopathy*.

7. DISCUSSION

According to the ISHLT data registry, since 2004 there has been worldwide preferential use of ICN in combination with MMF with or without glucocorticosteroids (8). In our Center the prime option of CNI still points to Cyclosporine. The choice of CNI depends of the risk profile of the patient. Tacrolimus is preferred when there is high risk of rejection, co-morbidities like hypertension or hyperlipidemia, for women and children to avoid hirsutism and gingival hyperplasia and in cases of side effects or resistance to CyA. In our study, 198 HT patients initiated treatment with CyA and 20 with Tacrolimus.

There has been an evidence that some risk factors influence the development of acute cellular rejection like female donor and recipient gender, young donor age, black race, long ischaemic time and HLA-DR mismatch status. (9,10) In this study there was not any patient characteristic like gender, age, CMV mismatch, ABO mismatch, ischaemic time or mechanical assistance device (VAD) with statistical significance between the two considered Groups.

In the present study, the incidence of heart transplant patients with at least one ACR $\geq$ grade 2R was higher in the first semester (15%) comparing with second semester (6%) and in the two following years (4%), ($P<0.001$). These results support previous studies where were verify a higher incidence of ACR $\geq$ 2R in the first 6 months after HT. (10,11) Although the immune reactivity and tendency to graft rejection decrease with time, they never disappear entirely, so a reduced number of patients benefited from the prolongation of rejection surveillance through endomyocardial biopsies to identify cases of acute cellular rejection without symptoms and clinically stable.

It has been suggested that heart transplant patients with one or more first year ACR ≥ grade 2R have a lower long-term survival compared with HT patients without first year ACR≥ grade 2R. (2) We verified higher survival curves without statistical difference between the two groups of patients (P=0.101), where the 8-years survival was 61.7 ± 7.3% vs 77.1 ± 3.7%, respectively for Groups A and B. According with previous reports this difference might be due to an higher incidence of CAV in group A. (5)

The most recent data of the International Society of Heart and Lung Transplantation Registry indicates a current 1-year survival of 84.5% and 5-years survival of 72.5%. (3) For Groups A and B, 5-years survival was 75.3 ± 5.8% and 84.3 ± 3.0%, respectively, after our tight schedule to perform surveillance endomyocardial biopsies that permits the diagnosis of early ACR ≥ grade 2R. Detection and also mild ACRs (1R), wiser continuous adjustments of the immunosuppression and awareness of adherence to therapy leading to improved survival in both groups.

In order to study more efficiently the long-term survival in patients who never had an episode of ACR, we subdivide Group B in Group B1 (HT patients with at least one episode of EMB grade 1R) and Group B2 (HT patients always with EMB grade 0R). The long-term survival rates in group B2 isn't statistical different between group A and B1, beside be slightly higher (77.2 ± 5.1% vs 70.8 ± 5.9% vs 67.5 ± 7.7%). We couldn't confirm that acute cellular rejection in the first 3 years affects the long-term survival but we verified a tendency to a lower long-term survival in recipients with at least one episode of ACR ≥ grade 2R (P=0.223).

Graft vasculopathy could be defined as a chronic rejection, that consists on an accelerated form of coronary artery disease that occurs in transplanted hearts. CAV is perhaps the most significant complication and major cause of late death after HT. (5) In

previous studies ACR in the early post-HT period was implicated in the later development of coronary stenosis. The underlying physiologic mechanism may be increased inflammation resulting in plaque development suggesting a relationship between the immunological basis of cellular rejection and CAV. It however still remains as an entity without known genesis and behaviour as a result of multifactorial factors. (6,12,13) This event was not fully supported by our results, as our study showed a slightly increased survival free of CAV at 8-years after HT in patients free of ACR in the first 3-years (86.0 ± 3.5% vs 75.9 ± 6.6%). A randomized study had compared MMF vs EVR and conclude that patients under EVR therapy had a lower incidence and also slower progression of CAV, with incidence of ACR similar between the administration of both drugs. In our Center 14.7% cases (n= 32) changed MMF to EVR and 28.1% cases changed due to CAV. (14–16)

The incidence of neoplasia still remains a leading cause of death 15 years after heart transplant. It remains a matter to debate the better combination of immunosuppression therapy (type, dosage, duration) to determine the cancer risk.

Group A patients had longer time free from malignant events than Group B (P=0.026). Although, both groups have been submitted to the same immunosuppressive maintenance therapy, Group B patients may have a particular immunophenoytpe adaptation, leading to absence of ACR and the higher incidence of tumors. In general, a major role in the development of post-transplant tumors is assigned to the levels of immunosuppression achieved rather than to the number of immunosuppressive drugs used. (13) As known the monitoring of therapeutic enforcement is done by the measure of drug in the blood and this can be influenced by still not known factors.

Infections remains as the leading cause of death after HT predominantly within the first year after transplantation when it causes 30% of related with a higher dosage of

immunosuppression which is required to control receptor immune response early after transplantation. (16,17) Also analysed in this study was the time free of infections between Groups A and B. There was a difference of time free of infections statistically significant between the two groups (P=0.036) with a time free of infections at 5 years after HT in Group A of 71.5 ± 6.7% vs 81.6 ± 3.0% in group B. An episode of rejection leads to an intensification of the immunosuppressive regimen or even treatment with corticosteroids or polyclonal antibodies. Due the treatment and also the stress induced by rejection the recipient may be more immunodepressed favouring these infections.

Study limitation

As a limitation to the present study, it should be noted that in 42 patients where survival was less than 3 years, an average of 15 biopsies no could be performed. This may have led to a slight underestimated of the frequency of 3-years acute cellular rejection.

Besides our retrospective study might be subject to bias, it was originated in a single center and our study population subject to uniform selection criteria, surgical and medical approaches and standardized follow-up.

Furthermore, fortunately the number of patients in Group A is not sufficiently large to compare patient characteristic in relation to Group B.

8. CONCLUSION

With our study we verified the lower incidence of ACR events and the higher number occurred in the first 6 months after HT. We could also see a tendency to a lower long-term survival among recipients with at least one ACR event ≥ grade 2R in the first 3-years after HT, although without statistical significance compared with HT patients free of rejection.

Survival free of cardiac allograft vasculopathy had no difference between the two considered groups. Nevertheless, we found a higher incidence of tumors in patients without any ACR event in the first 3-years after HT and a higher incidence of infections in recipients with history of acute cellular rejection event.

Some of the major causes of mortality after HT nowadays are them due to complications that can be side-effects of over-immunosuppression.

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