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Impact of Routine Surveillance Biopsy Intensity on the Diagnosis of Moderate to Severe Cellular Rejection and Survival after Pediatric Heart Transplantation

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Abstract

Data are lacking on routine surveillance biopsy (RSB) intensity and outcomes after pediatric heart transplantation. Pediatric Heart Transplant Study (PHTS) centers received a survey on RSB practices from 2005 to present. PHTS data were obtained for 2010–2013 and integrated with center-matched survey responses for analysis. Survey response rate was 82.6% (38/46). Centers were classified as low, moderate, and high intensity programs based on RSB frequency (0 - more than 8 RSB/yr). RSB intensity decreased with increasing time from HT. Age at HT impacted RSB intensity mostly in year 1, with little to no impact in later years. Most centers have not replaced RSB with non-invasive methods, but many added ECHO and biomarker monitoring. Higher RSB intensity was not associated with decreased 4-year mortality (p= 0.63) or earlier detection of moderate to severe (ISHLT grade 2R/3R) cellular rejection (RSB-MSR) in the first year (p=0.87). First year RSB-MSR incidence did not differ with intensity or age at HT. Significant variability exists in RSB intensity, but with no impact on timing and incidence of RSB-MSR or 4-year mortality. Reduction in RSB frequency may be safe in certain patients after pediatric HT.

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Disclosure Statement

The authors have no conflicts of interest to disclosure.

Author Contributions

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Ashley D. Osborne – data collection and analysis
James K. Kirklin – manuscript editing
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Introduction

Endomyocardial biopsy (EMB) is widely accepted as the gold standard for diagnosing graft rejection in both pediatric and adult heart transplant (HT) recipients. Despite this indication, the majority of EMB are performed in asymptomatic patients as part of routine post-transplant rejection surveillance protocols. Each protocol is center-specific and reflects the experience and personal preference of the transplant team. Thus, no consensus exists for the optimal intensity of routine surveillance biopsy (RSB) after HT, especially in the pediatric population.

The past 25 years have brought significant advances in immunosuppression, imaging, biomarkers, and diagnostic pathology. The pediatric heart transplant community lacks current data as it pertains to routine surveillance biopsy diagnosed moderate to severe cellular rejection (ISHLT grade 2–3R, RSB-MSR). For this study, we hypothesized a wide variation in RSB protocols amongst pediatric heart transplant centers, as well as no correlation between RSB intensity, incidence of RSB-MSR, and survival. A survey was administered to all Pediatric Heart Transplant Study (PHTS) member centers to investigate the variability of surveillance intensity and ultimately analyze the correlation between PHTS outcome data and RSB intensity.

Methods

A survey was developed by the authors and administered to all participating centers (47 at the time of this study) from June to August of 2014. The goal was to examine variability amongst centers regarding their routine surveillance biopsy protocols for standard-risk patients after heart transplantation. The survey contained nine multiple choice questions with a section for written comments if the protocol had changed over the study time period of 2005–2014. Topics included number of biopsies performed in the first year post transplant, years two through five, and beyond the 5th year. All surveys were completed by either a transplant coordinator or transplant cardiologist. The questions did not initially address age-specific protocols. Centers with age-based surveillance protocols included this data in the comments section of the question. Given the responses, we were able to stratify the data into three age groups: infants (less than 1 year), children (1 to 9 years), and older children/adolescents (10 to 18 years). Responses to questions regarding biopsy frequency were used to stratify centers into three surveillance groups - low, medium, and high intensity (Table 1). The authors did not build the survey with predefined protocol intensities. Surveillance intensity groups were created from the three data clusters seen within the survey responses. In most cases, this classification varied within the time period after transplant secondary to age-based protocol data provided by the center. Each center received a separate classification for the time period after transplant and the associated age group. For example, a center may have been classified as a low intensity program for infants during year 1, but a medium intensity program for children. Furthermore, a center may have been classified as low intensity for infants during year one, but as medium intensity for infants during years 2–5.

The remaining questions addressed use and timing of noninvasive imaging to replace surveillance biopsies, use of biomarker and blood tests [AlloMap (CareDx Inc., Brisbane,

CA, USA) or ImmuKnow (Cylex Inc., Columbia, MD, USA)] for rejection monitoring, changes in biopsy frequency during the era, and whether or not those changes resulted in perceived changes in clinical rejection rates.

Rejection and mortality data from 2010–2013 were acquired from PHTS, a prospectively collected database managed by the University of Alabama at Birmingham. PHTS membership contained 52 centers across three continents at the time of data collection. The 2010 version of the PHTS rejection reporting form was used as it was the first year the database collected information on the indication for biopsy during a rejection episode, including for surveillance purposes. Rejection was defined as a biopsy proven episode of ISHLT grade 2R/3R acute cellular rejection that resulted in an augmentation of immunosuppression. Only moderate to severe cellular rejection (ISHLT 2R/3R) was included in the study to eliminate the interpretive variability surrounding 1R classification. Patients with antibody-mediated rejection (AMR), clinically diagnosed rejection without biopsy data, and a history of multi-organ transplantation were excluded from the analysis. The data set was queried to find all episodes of rejection with “routine protocol” as the biopsy indication that were associated with a documented “rejection therapy.” PHTS data was then integrated with only the current era survey responses to create a unique data set for analysis.

Not all member centers were actively reporting data to PHTS at the time of survey administration. A center was only included in the current study if both survey results and PHTS data were available for analysis.

PHTS does not collect data on negative biopsies, which prohibited us from knowing the exact number of biopsies performed per patient. To perform the integrated analyses, we calculated the number of expected yearly biopsies based on the biopsy frequency by age and time from transplant, as reported by each center on the survey.

Statistical Analysis

All analyses were performed at Washington University in St. Louis School of Medicine. Study-specific variables were extracted from the PHTS limited dataset in order to create a unique dataset for analysis. Prevalence and proportions were estimated with contingency tables and tested for differences with Pearson Chi-square test. Survival and time to rejection were estimated with product-limit survival curves. Group hazard rates were estimated and compared with Cox proportional hazard regression. The covariate was age group (adolescents, children, and infants) for both the mortality and time to first rejection analysis. All analyses were performed with SAS 9.4 (SAS Institute, Cary NC).

Results

Survey Data

Thirty-eight of 46 PHTS centers (82.6%) responded to the author’s survey. Seven centers were excluded from the study due to poor data compliance and five centers did not respond to the survey. Combined survey and PHTS data were available for 34 centers (89.5% of responding centers). Table 1 represents the breakdown of centers by RSB intensity based on

age at transplant and time from transplant. RSB intensity was divided into three categories based on survey responses – low, medium, and high intensity. The definitions of low, medium, and high intensity were different based on time after HT.

Half of the centers reported a medium intensity protocol during the first year after transplant, regardless of age at transplant. The remaining centers followed a low intensity protocol for infants and a high intensity protocol for children and older children/adolescents. In years 2 through 5, nearly half of centers followed a medium intensity protocol regardless of age at transplant. The remaining centers mostly followed a high intensity protocol. Beyond the 5th year, the majority of centers followed a medium intensity protocol regardless of age at transplant. Several centers stated they would adjust RSB intensity for a positive crossmatch, elevated panel reactive antibody (PRA), or history of rejection. One center used patient weight to determine the number of first year biopsies for infants, with one additional center using patient age for children in the first year post transplant. Only one center reported a protocol change that impacted their intensity group during the study period (2010–2013). This center reported changing from a medium to high intensity program beyond the 5th year for all age groups in some patients.

Twenty-two of 34 centers (64.7%) have not replaced routine biopsies with non-invasive imaging (Table 2). Of those that have, 10 replaced the RSB with an echocardiogram (mainly starting before the second year after HT) and one has replaced an RSB with cardiac MRI or stress ECHO (after the 5th year post HT). Centers that replaced biopsies with non-invasive monitoring did not report a perceived increase in clinical rejection. Half of the centers surveyed have incorporated BNP or NT-proBNP levels into their routine follow-up bloodwork. Twenty-seven of 34 centers do not use AlloMap or ImmuKnow as part of their routine surveillance protocols. One center uses AlloMap alone, three use ImmuKnow alone, and two use both. Data were missing for one center. The earliest use was 2009, and one center reported using AlloMap to replace a biopsy in patients with previous correlation between biopsy and AlloMap results.

PHTS Data

The 34 included PHTS centers submitted 797 rejection forms from 2010–2013 using the 2010 version of the rejection reporting form. Of those, 169 records were excluded due to a lack of required data or because the episode did not fulfill the definition of rejection. Overall, 76% (476/628) of rejection episodes were diagnosed on RSB. In all, 470 of the reported 476 rejection episodes diagnosed on RSB reported an ISHLT cellular rejection score: 0R – 48 (10.2%), 1R – 142 (30.2%), 2R – 258 (54.9%), and 3R – 22 (4.7%). The study cohort was 50.9% female, 24.9% African American, and 68.5% Caucasian. Tacrolimus was the primary immunosuppressant in 86.7%, with 78.2% also receiving mycophenolate mofetil and 50.3% receiving prednisone as secondary therapy. The median panel reactive antibody (PRA) at the time of transplant was 0%.

Integrated PHTS and Survey Data

PHTS data were combined with the survey results for further analysis. Some centers reported changing RSB intensity within the study time period. This finding could have

confounded the analysis using the three nominal intensity categories (low, medium, and high) since a center could have belonged to more than one category over the study period. Therefore, the mortality analysis was performed using the number of yearly biopsies as reported by each center in the survey, based on their specific practices for age and time from transplant. A proportional hazard regression analysis was performed to evaluate the association between all-cause mortality over the 4-year study period and RSB intensity. Nine numerical categories were created representing the number of RSB per year – 1, 2, 3, 4, 5, 6, 7, 8, and more than 8. There was no significant difference between the all-cause mortality hazard ratio of the nine RSB categories (p= 0.63) (Table 3) or between the age groups (p=0.23).

During the first year after HT, the time to first RSBMSR was similar (p=0.87) between all 9 numerical categories of RSB intensity (Table 3). The time to first RSBMSR varied significantly based on age at transplant (p=0.0064). Adolescents demonstrated a shorter time to first RSBMSR compared to both infants (HR 2.418, 95% CI 1.364–4.287) and children (HR 1.523, 95% CI 1.005–2.310). The time to first RSBMSR was similar when comparing children to infants (HR 1.587, 95% CI 0.891–2.828). There was no difference between the first-year incidence of RSBMSR for each age group (infants p=0.90, children p=0.33, adolescents p=0.40) based on RSB intensity. In addition, there was no difference in RSBMSR prevalence in years 2–5 for children (p=0.25) and adolescents (0.62). The results for infants were inconclusive due to a small sample size.

Discussion

We found a great deal of variability amongst PHTS centers in regard to RSB intensity. There is no clearly superior regimen shown in the literature.[1–4] The debate over optimal RSB intensity is not new. In the past 20 years, both adult and pediatric authors have questioned the utility of RSB beyond the first 6–12 months after HT.[3, 5–12] Chi and colleagues[13] demonstrated 86% of rejection episodes occur in the first 2 years after adult HT, with a rejection incidence of only 2% on scheduled biopsy in the 3rd year after HT. The adult HT community has questioned the utility of any RSB beyond 5 years after HT.[14] However, a small but significant risk of moderate ACR (ISHLT grade 3A) continues in later years, with an incidence of 3.5–4% in years 2 through 7.[15] It should be stated that this study was published in 2001 and did not incorporate current non-invasive means to diagnose late rejection. Thus, current adult guidelines only recommend RSB in those with “higher risk for late rejection” beyond the first year after HT, and label RSB “optional” beyond the 5th year after HT in both adults and children.[16]

Pediatric RSB protocols have developed from a combination of published data and center-specific experience, with age at HT and time from HT driving variability. Biopsy data[1] from the early 1990s failed to show rejection in asymptomatic infants, while 8.4% of RSB were positive for rejection (ISHLT grade ≥ 2) in older children. The largest single-center report to date (82 patients, 1169 biopsies) demonstrated rejection (ISHLT grade ≥ 3A) on 18% of RSB during the first year, 12% between years 2–5, and 2.9% beyond 5 years after HT. [2] Levi et al. showed only a 0.36% incidence of EMB positive rejection (≥ ISHLT grade 1B) in asymptomatic patients (770 biopsies) on tacrolimus therapy during the first year after

transplant, and no asymptomatic patient had a positive biopsy greater than 1 year after transplant.[3] In contrast, routine surveillance biopsies can detect late episodes of moderate to severe rejection in children (n=269, mean follow-up 5 years), with an 8–10% annual incidence of asymptomatic moderate rejection up to 11 years after OHT.[4] There are currently no studies in the pediatric HT population comparing outcomes in patients managed on rejection surveillance protocols of varying intensity. The current study is rather timely, given the current focus on cost reduction in health care and improvement in patient satisfaction and quality of life.

The survey responses allowed us to separate the centers into three groups: low, medium and high intensity surveillance protocols (Table 1). Not surprisingly, the most common surveillance method was a medium intensity protocol after transplant, regardless of age at HT and time after HT. Most programs favor medium-high intensity surveillance for the first 5 years and low-medium intensity beyond the 5th year post-transplant. A recent report by Godown and colleagues saw a significant decrease in surveillance EMB in adolescents, but not infants, when comparing first year surveillance EMB practices in a 2006 survey to a 2014 survey.[17] Our survey also collected RSB intensity data from 1995–2004, but was excluded as it could not be analyzed with PHTS data. Most programs favored a high intensity protocol until after the 5th year post-transplant when most decreased the intensity to medium. Collectively, there has trend toward reduction of RSB intensity over the past two decades.

Non-invasive imaging and biomarker monitoring are increasingly utilized as part of surveillance protocols. Roughly one third of responding programs have replaced a RSB with non-invasive imaging at some point after HT. Echocardiography can potentially identify those with a low likelihood of rejection, thereby functioning as a risk-stratifying tool for which patients truly require a biopsy and which have a low likelihood of rejection.[18–20] Lunze and colleagues used tissue Doppler imaging (TDI) to predict non-rejection with 100% accuracy in 22 children after heart transplantation.[20] Interestingly, 18/24 rejection events were during the first year after transplant, which may make TDI a reasonable pre-biopsy screening tool. Strain imaging and cardiac MRI have also shown promise in differentiating between rejection and non-rejection in children. Kindel and colleagues recently published a comprehensive review on the scope of noninvasive imaging tools to monitor for rejection after pediatric heart transplantation.[21]

Trending biomarkers such as BNP/NT-proBNP has increased in the current era, with half of the responding centers now incorporating it into their surveillance protocols. Elevated BNP/NT-proBNP has been correlated with decreased ventricular function in heart failure.[22–28] It can serve as a useful screening tool for the presence of ventricular dysfunction in the face of acute rejection, or to support not performing a biopsy in asymptomatic patients with normal non-invasive imaging.[29–30] New biomarkers for identifying acute rejection have demonstrated potential in small studies. Highly-sensitive cardiac troponin T levels rise with acute cellular rejection, and decrease as rejection resolves.[31] Serum SERCA2a (sarcoplasmic reticulum Ca^{2+} - ATPase) levels have been shown to decrease with acute cellular rejection in adult heart transplant recipients, even showing potential to differentiate between mild and moderate/severe rejection.[32] Biomarkers and echocardiography must be

interpreted with caution in the first year after transplant. BNP levels decrease rapidly after HT prior to leveling out at 4 months.[33–34] Similar improvements in echocardiographic parameters occur over the first year after HT as well.[35–36] Therefore, following the trends of these non-invasive surveillance tools is more useful than responding to an isolated value.

Our results indicate no difference in 4-year mortality, time to first rejection, or first year RSBMSR based on RSB protocol intensity. Age does play a role in the time to first rejection independent of RSB intensity, with adolescents diagnosed earlier compared to infants and children. In addition, biopsy frequency did not impact prevalence of RSBMSR during years 2–5 in children and adolescents. There does not appear to be an early advantage associated with higher RSB intensity, suggesting a reduction in RSB intensity could be safe with no impact early mortality. The best approach to answering this clinical question would be to prospectively study the impact of RSB intensity on rejection incidence, prevalence, morbidity, and mortality. The pediatric HT community could then use the data to create rejection surveillance protocols based on a patient's rejection history.

Limitations to the current study were secondary to the nature of a retrospective database review. We could not include 12 centers due to lack of a survey response or because of poor data compliance with PHTS. This additional data could have impacted the analysis and results. Significant amounts of potential data were excluded due to lack of the main study variable. Indication for biopsy was not listed on 101 rejection forms, and 68 additional forms were excluded for failing to fulfill the study definition of rejection. Indication for biopsy was not included in PHTS until 2010, making it impossible to include patients with rejection prior to that date. Furthermore, we could not control for practice variations at each center. Our analysis assumes each PHTS center strictly followed their surveillance protocol. It is possible that variations from the protocol may have impacted the results. We also could not control for alterations in immunosuppressive therapy, which may have impacted rejection frequency and outcomes.

Conclusions

Significant variability exists in RSB intensity among PHTS centers, but with no impact on timing and incidence of first year RSBMSR or 4-year all-cause mortality. The data are reassuring that a reduction in RSB intensity may be safe in certain populations, and could be used to design a prospective trial evaluating the impact of RSB intensity on the incidence of RSBMSR and outcomes after HT. The information gained from such a trial could lead to more tailored RSB protocols based on the patient's post-transplant clinical course.

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Appendix A

Survey of Routine Surveillance Protocol Intensity

**** Please respond to the following question for the time period of 2005-present***

Question 1:

How many routine surveillance biopsies does your center perform in the first year post transplant?

- **a.** 4
- **b.** 5
- **c.** 6
- **d.** 7
- **e.** 8
- **f.** more than 8

If your practice changed during this time period, please state when the change was made and describe the change:

Questions 2:

How many routine surveillance biopsies does your center perform in the second to fifth year post transplant?

- **a.** 1
- **b.** 2
- **c.** 3
- **d.** 4
- **e.** 5 or more

If your practice changed during this time period, please state when the change was made and describe the change:

Question 3:

How often does your center perform routine surveillance biopsies beyond 5 years post transplant?

- **a.** Yearly
- **b.** Every 2 years
- **c.** Every 3 years

If your practice changed during this time period, please state when the change was made and describe the change:

Question 4:

Does your center's protocol include noninvasive imaging (ECHO, MRI) in place of routine surveillance biopsies?

- **a.** Yes, ECHO
- **b.** Yes, MRI
- **c.** Yes, both
- **d.** Yes, other
- **e.** No

If your practice changed during this time period, please state when the change was made and describe the change:

Question 5:

If so, when does your protocol start using noninvasive imaging (ECHO/MRI) in place of routine surveillance biopsy?

- **a.** Year 2
- **b.** Year 3
- **c.** Year 4
- **d.** Year 5
- **e.** Beyond year 5

If your practice changed during this time period, please state when the change was made and describe the change:

Question 6:

Does your center routinely check NT-pro BNP levels to monitor for acute rejection?

- **a.** Yes
- **b.** No

If you answered YES, please state when your center started using this test, and the frequency of use:

Question 7:

Does your center incorporate blood tests such as AlloMap or ImmuKnow into your protocol to evaluate for acute rejection?

- **a.** Yes, AlloMap
- **b.** Yes, ImmunoKnow
- **c.** Yes, both
- **d.** No

If you answered YES, please state when your center started using the test, and the frequency of use:

Question 8:

Has your center reduced the number of routine surveillance biopsies in your post-transplant rejection monitoring protocol during this time period?

- **a.** Yes, but without adding non-invasive imaging or blood tests
- **b.** Yes, and replaced the eliminated biopsy with non-invasive imaging
- **c.** Yes, and replaced the eliminated biopsy with blood tests
- **d.** No

If your practice changed during this time period, please state when the change was made and describe the change:

Question 9:

If you answered YES to Question 8, have you seen an increased incidence of rejection requiring a change in medical therapy or additional therapies?

- **a.** Yes
- **b.** No

If your practice changed during this time period, please state when the change was made and describe the change:

**** Please respond to the following questions for the time period of 1995–2004***

Question 1:

How many routine surveillance biopsies does your center perform in the first year post transplant?

- **a.** 4
- **b.** 5
- **c.** 6
- **d.** 7
- **e.** 8
- **f.** more than 8

If your practice changed during this time period, please state when the change was made and describe the change:

Questions 2:

How many routine surveillance biopsies does your center perform in the second to fifth year post transplant?

- **a.** 1
- **b.** 2
- **c.** 3
- **d.** 4
- **e.** 5 or more

If your practice changed during this time period, please state when the change was made and describe the change:

Question 3:

How many annual routine surveillance biopsies does your center perform beyond 5 years post transplant?

- **a.** 0
- **b.** 1
- **c.** 2

If your practice changed during this time period, please state when the change was made and describe the change:

Question 4:

Does your center's protocol include noninvasive imaging (ECHO, MRI) in place of routine surveillance biopsies?

- **a.** Yes, ECHO
- **b.** Yes, MRI

c. Yes, both

d. Yes, other

e. No

If your practice changed during this time period, please state when the change was made and describe the change:

Question 5:

If so, when does your protocol start using noninvasive imaging (ECHO/MRI) in place of routine surveillance biopsy?

a. Year 2

b. Year 3

c. Year 4

d. Year 5

e. Beyond year 5

If your practice changed during this time period, please state when the change was made and describe the change:

Question 6:

Does your center routinely check NT-pro BNP levels to monitor for acute rejection?

a. Yes

b. No

If you answered YES, please state when your center started using this test, and the frequency of use:

Question 7:

Does your center incorporate blood tests such as ImunoKnow into your protocol to evaluate for acute rejection?

a. Yes

b. No

If you answered YES, please state when your center started using the test, and the frequency of use:

Question 8:

Has your center reduced the number of routine surveillance biopsies in your post-transplant rejection monitoring protocol during this time period?

a. Yes, but without adding non-invasive imaging or blood tests

b. Yes, and replaced the eliminated biopsy with non-invasive imaging

c. Yes, and replaced the eliminated biopsy with blood tests

d. No

If your practice changed during this time period, please state when the change was made and describe the change:

Question 9:

If you answered YES to Question 8, have you seen an increased incidence of rejection requiring a change in medical therapy or additional therapies?

a. Yes

b. No

If your practice changed during this time period, please state when the change was made and describe the change:

THANK YOU FOR TAKING PART IN OUR SURVEY.

Table 1

Intensity of Routine Surveillance Biopsy Protocol amongst PHTS Centers from 2005 to 2014

	Low	Medium	High
YEAR 1			
Infants	0–4 (n=11)	5–7 (n=17)	8 or more (n=6)
Children	0–4 (n=7)	5–7 (n=15)	8 or more (n=12)
Older Children/Adolescents	0–4 (n=6)	5–7 (n=16)	8 or more (n=12)
YEARS 2–5†			
Infants	0–3 (n=6)	4 (n=16)	5 or more (n=12)
Children	0–3 (n=6)	4 (n=16)	5 or more (n=12)
Older Children/Adolescents	0–3 (n=5)	4 (n=17)	5 or more (n=12)
BEYOND 5 YEARS			
Infants	None - every 2 or more years (n=10)	Annually (n=20)	Biannually (n=4)
Children	None - every 2 or more years (n=10)	Annually (n=20)	Biannually (n=4)
Older Children/Adolescents	None - every 2 or more years (n=10)	Annually (n=20)	Biannually (n=4)

† Intensity is based on total number of biopsies performed between year 2 and year 5 after heart transplantation. Values do not represent the number of annual biopsies during this time period.

Table 2

Use of Non-Invasive Studies for Rejection Monitoring

	Number of Centers
Non-invasive imaging in place of biopsy	
No	21
Yes - ECHO only	10
Yes - MRI only	1
Yes – Both	1
Yes – Other	0
No response/Unknown	1
NT-proBNP or BNP use in rejection monitoring	
No	16
Yes	16
No response/Unknown	2
AlloMap or ImmuKnow use	
No	27
AlloMap only	1
ImmuKnow only	3
Both	2
No response/Unknown	1

Table 3

Time to First RSBMSR by Number of Yearly RSB and Hazard Ratios by Number of RSB per Year Compared to No Yearly RSB

RSB Rate per Year	Median Time to First RSBMSR, years	First Rejection Hazard Ratio	Mortality Hazard Ratio
0	0.31	--	--
1	0.47	0.511	0.000
2	--	0.650	0.000
3	0.35	1.362	0.686
4	0.50	0.601	0.220
5	0.18	0.497	0.342
6	0.42	0.498	0.308
7	0.08	0.745	0.070
8	0.17	1.020	0.144
More than 8	0.20	0.600	0.171
p-value	--	0.87	0.63

RSBMSR – routine surveillance biopsy diagnosed moderate to severe cellular rejection; RSB – routine surveillance biopsy