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The Investigation of Endomyocardial Biopsy Results, Plasma pro-BNP Levels and Non-invasive Parameters for Diagnosing of Acute Rejection in Patients Who Undergo Cardiac Transplantation

Kalp Transplantasyonu Yapılan Hastalarda Akut Rejeksiyon Tanısında Endomiyokardiyal Biyopsi Sonuçları, Plazma pro-BNP Seviyeleri ve Non-invazif Parametrelerin Araştırılması

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ABSTRACT

Aim: Heart failure is a disease with high mortality and morbidity, reducing the patient's quality of life. Each year 10% of heart failure patients progress to end-stage heart failure. Cardiac transplantation is the gold standard treatment method in these patients, however acute rejection is the most important factor affecting the success of this treatment. In this study, it was aimed to evaluate endomyocardial biopsy results, serum pro-BNP and non-invasive parameters in patients with acute rejection following cardiac transplantation.

Patients and Methods: Twenty patients who underwent cardiac transplantation in our center were included in the study. The patients were divided into two groups, namely acute rejection (n: 10) and without rejection (n: 10). Echocardiography, electrocardiography (ECG), endomyocardial biopsy results, serum reactive proteins (CRP), sedimentation rate and serum pro-BNP levels, were evaluated among the patients and compared between the groups.

Results: Endomyocardial biopsies obtained from patients with acute rejection revealed grade 1 rejection in 6 (60%) patients, grade 2 in 3 (30%) patients and grade 3 rejection in 1 (10%) patients. CRP and sedimentation rate were found to be similar between the groups (p> 0.05). High pro-BNP levels were found in patients with rejection (4843.20 ± 6690.10 pg / ml) when compared to the control group (496.30 ± 216.20 pg / ml) (p: 0.001). In addition, higher pro-BNP levels were detected with progressing of rejection grade (p: 0.03). The highest pro-BNP level was found in a patient with Grade-3 rejection (15211 pg / ml, p: 0.000).

Conclusion: Our results show that serum pro-BNP levels are associated with acute rejection. In addition, higher pro-BNP levels were found to be associated with advanced rejection levels.

Key Words: Cardiac transplant, acute rejection, endomyocardial biopsy, pro-BNP

ÖZ

Amaç: Kalp yetersizliği, mortalite ve morbiditesi yüksek olan hastanın yaşam kalitesini düşüren bir hastalıktır. Kalp yetmezliği hastalarının her yıl %10'u son dönem kalp yetmezliğine ilerlemektedir. Bu hastalarda kalp nakli altın standart tedavi yöntemidir. Ancak nakil hastalarında akut rejeksiyon tedavinin başarısını etkileyen en önemli faktördür. Bu çalışmada kalp nakli sonrası akut rejeksiyon olan hastalarda endomiyokardial biyopsi sonuçları, serum pro-BNP ve non-invazif parametrelerin değerlendirilmesi amaçlanmıştır.

Hastalar ve Yöntem: Merkezimizde kalp nakli yapılan 20 hasta çalışmaya dâhil edil-erek, akut rejeksiyon gelişen (n:10) ve gelişmeyen (n:10) olarak iki gruba ayrılmıştır. Hastalar arasında ekokardiyografi, elektrokardiyografi (EKG), endomiyokardiyal bi-yopsi sonuçları, serum reaktif protein (CRP), sedim ve serum pro-BNP seviyeleri değerlendirilmiş ve gruplar arasında karşılaştırılmıştır.

Bulgular: Akut rejeksiyon gelişen hastalardan alınan endomiyokardiyal biyopsilerde 6(%60) hastada grade 1, 3(%30) hastada grade 2, 1(%10) hastada grade 3 rejeksiyon bulguları, CRP ve sedimantasyon oranı gruplar arasında benzer olarak saptanmıştır (p>0.05). Rejeksiyon olan hastalarda (4843,20 ± 6690,10 pg / ml) kontrol grubu ile karşılaştırıldığında (496,30 ± 216,20 pg / ml) yüksek pro-BNP seviyeleri tespit edilmiştir (p:0,001). Bunun yanı sıra rejeksiyon grade ilerledikçe daha yüksek pro-BNP seviyelerine ulaşılmıştır (p:0,03). Bu bağlamda grade-3 rejeksiyon olan hastada en yüksek pro-BNP düzeyi bulgusu elde edilmiştir (15211 pg / ml, p:0,000).

Sonuç: Bulgularımız serum pro-BNP düzeylerinin akut rejeksiyon ile ilişkili olduğunu göstermiştir. Ayrıca daha yüksek pro-BNP düzeyleri ile rejeksiyon seviyeleri ilişkili olarak bulunmuştur.

Anahtar Kelimler: Kalp nakli, akut rejeksiyon, endomiyokardiyal biyopsi, pro-BNP

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INTRODUCTION

Heart failure (HF) disrupts quality of life and progresses to higher mortality and morbidity rates. Despite the advanced medical treatment strategies and recently developed assisting devices, the prognosis of HF is still quite negative [1,2]. HF is a progressive disease that has an undesirable clinical course that cannot be recovered to previous condition, and every hospital consultations with acute exacerbation result in a decreased survival rate. Timely diagnosis and controlled treatment may delay quick progression and the bridging of treatment strategies can provide additional time to heart transplantation, which is the gold standard treatment for end stage HF. However, in the end, these patients require transplantations [2-4].

Heart transplantation provides effective cardiac functions if patients pass through the postoperative period uneventfully. The main issue following heart transplantation is acute organ rejection, which occurs during the first year of transplantation. The next process following the first year is related to immunosuppressive treatments, which can result in infections, hypertension, renal failure, malignancies and transplant vasculopathy. Five-year survival rates were reported at 70–80% in patients with heart transplantation that received a triple immunosuppressive regimen [2]. The endomyocardial biopsy sample evaluations, echocardiographic, electrocardiographic examinations and serum blood parameters are important for follow up, during the postoperative first year [5,6].

The natriuretic peptides were reported as a pathological neurohormonal activation of various disorder, also especially in disrupted cardiac functions. B-type natriuretic peptide or brain natriuretic peptide (BNP) was reported as a biomarker for acute cardiac events. Acute loading condition related with increased ventricular wall tension, leads to pro-BNP releasing. Therefore, this biomarker was investigated for different kinds of cardiovascular diseases such as pulmonary hypertension, cor pulmonale, ventricle hypertrophy, overload situations in circulation, hypertension, acute coronary syndrome, atrial fibrillation, etc. [7].

In this study we aimed to evaluate the echocardiography, electrocardiography (ECG), endomyocardial biopsy results, serum reactive protein (CRP), sedimentation rate, serum pro-BNP and biochemical parameters in heart transplant patients with acute rejection. Thereafter we compared the results with heart transplantation patients undergoing a healthy progression.

MATERIAL and METHOD

Study Design and Patient Selection

After obtaining an ethical approval from the Human Ethical Committee of Akdeniz University (Approval No.2011,06-82-107/87), a retrospective analysis for cardiac transplantation cases was designed. Thirty patients who underwent cardiac transplantation in cardiovascular surgery clinic of Akdeniz University School of Medicine were recorded retrospectively. Patients who had regular periodical endomyocardial biopsies, simultaneous echocardiography and electrocardiography follow-up records and regular blood parameters follow-up records [cytomegalovirus (CMV) antigen test, sedimentation, C-reactive protein (CRP) test, troponin and pro-BNP (Brain natriuretic peptide) tests], were included in the study. Patients without regular control tests and under treatment due to an active infection, were also excluded from the study.

Following the inclusion and exclusion criteria, a total of twenty patients were included in the study. Demographic findings, echocardiography and electrocardiography results as well as endomyocardial biopsy reports, were evaluated. Thereafter, patients were divided into two groups, based on biopsy reports of 6th month endomyocardial biopsy samples, as the tissue rejection (study) group (n: 10) and the normal biopsy group (n: 10).

Clinical Management

All cardiac transplant patients received triple immune-suppressive treatment in the early postoperative period. This regimen includes calcineurin inhibitors (cyclosporine or tacrolimus) + mycophenolate mofetil (MMF) or Everolimus + oral prednisolone treatments. The drug dosages were determined as follows: Cyclosporine: 2.5

or 5 mg/kg/day (per oral: PO), Tacrolimus: 5 mg twice daily (PO), MMF: 0.5 or 1 gr twice daily, Everolimus: 0.75 or 1.5 mg twice daily (PO). The endomyocardial biopsies (EMB) were taken as follows: one fifteen days during the postoperative 2nd months, one a month during the postoperative 2 to 6th months, one a year after postoperative 6th month. We evaluated the 6th month biopsy results in our study.

The EMB was taken from the right ventricle through the catheterizing right jugular vein. After placement of 8 F introducer sheath to the right jugular vein, the 7 F EMB forceps (Sparrow Hawk®) was progressed into the sheath. After progressing forceps tip (biotom) to the right atrium laterally, biotom was progressed with 90 degrees of rotation for passing through the tricuspid valve and the biotom was directed to the right ventricle apex. When reaching to selected areas 4 to 6 pieces of the sample with a size of 3x2x2 mm were collected with biotom. All collected samples were stored at 10% formalin and 4% buffered formalin solutions and send to pathological examination.

The EMB samples obtained were classified according to 2004 ISHLT (the International Society for Heart and Lung Transplantation) grading guidance, as follows: Grade 0: Non-rejection, Grade 1: Mild rejection, Grade 2: Moderate rejection, Grade 3: Severe rejection [8].

The M-mode and two-dimensional evaluation were made by echocardiography (GE VIVID®). The left ventricle mass and pericardial effusion were evaluated and ejection fraction (EF) and pulmonary artery pressure (PAP) were measured. In the echocardiogram evaluations, the following findings were accepted in favor of rejection: more than 4 mm thickening in the interventricular septum and posterior wall, increased myocardial echogenicity, newly developed pericardial effusion or increment of previous effusion, more than 20% percent decrease in halving time of mitral valve flow pressure, more than 20% decrease in isovolumetric relaxation time of left ventricle (LV), and more than 10% decrease in LV ejection time [9,10].

In the electrocardiogram evaluations, the following findings were accepted in favor of rejection: newly developed arrhythmia, prolonged P wave, more than 20% decrease in QRS voltage of chest derivations, more than 2 mm of ST variances and T wave negativity.

Simultaneous CMV antigen and CRP tests, troponin and pro-BNP were evaluated. The pro-BNP evaluation was made from 10 ml of antecubital vein blood samples stored in EDTA containing tubes. The samples were centrifuged at 1500 rpm for 5 minutes under +4 degree and the top phase plasma levels of the centrifuged samples were taken to another tube. NT-proBNP levels were measured with the electrochemiluminescence immunoassay method and commercially available kits (Roche Diagnostics, Indianapolis, Indiana), by using the Elecsys® 1010 autoanalyzer device from these samples.

Statistical Analysis

The statistical analyses were made using a software program (SPSS for windows v.18). The continuous variables were analyzed using an independent T test, and the categorical variables were compared using the Chi-Square test. The Mann-Whitney U tests were performed for the comparison of groups. The $p<0.05$ results were considered as statistically significant.

RESULTS

Demographical and clinical findings

Twenty patients who underwent heart transplantation were included to the study. The patients were divided into two groups, one with rejection (Study group, n:10) and one without rejection (Control group, n:10). The demographical findings of the two groups were reported in Table 1.

The groups were similar in regards to age, gender, accompanying disease, postoperative drug usage (Cyclosporine, Tacrolimus, Mycophenolate Mofetil (MMF), Everolimus) ($p>0.05$). Similarly, there was no difference between the follow-up periods of the two groups ($p>0.05$).

The higher rejection rates were found in patients with 0 (Rh+) blood groups when compare with control subjects (p: 0.03). The distribution of blood groups in heart transplant patients was summarized in Table 2. The symptoms were found

as follows: 5 (50%) non-specific, 2 (20%) with cardiac irritation, 1 (10%) with low cardiac output complaints, in patients with rejection.

Table 1. The Demographical Findings

Variable	Study Group	Control Group	**p
Gender (male,%)	9(90%)	8(80%)	0.62
Age (mean±SD), years	43.40±6.86	38.20±8.27	0.78
Follow-up, months	11.70±6.96	13.10±6.62	0.08
Diabetes (n,%)	4(40%)	3(30%)	0.70
Hypertension (n,%)	3(30%)	4(40%)	0.70
Cyclosporine (n,%)	6(60%)	5(50%)	0.65
Tacrolimus (n,%)	4(40%)	5(50%)	0.62
*MMF (n,%)	8(80%)	7(70%)	0.84
Everolimus (n,%)	2(20%)	3(30%)	0.71

*MMF: Mycophenolate Mofetil, **p: p<0.05 is considered as statistically significant

Table 2. The Distribution of Blood Groups

Blood Groups	Study Group n(%)	Control Group n(%)	P
0 (Rh +)	6 (60%)	1 (10%)	0.03
A (Rh +)	2 (20%)	4 (40%)	0.68
B (Rh +)	1(10%)	3(30%)	0.81
AB (Rh +)	1(10%)	2 (20%)	0.97

Histopathological Findings from Biopsy Samples

Biopsies performed after the 6th month of transplantation were found as a grade 1 rejection in 6 (60%) patients, a grade 2 rejection in 3 (30%) patients, and one patients (10%) with low cardiac output symptoms had a grade 3 rejection. The histopathological findings are illustrated in Image 1.

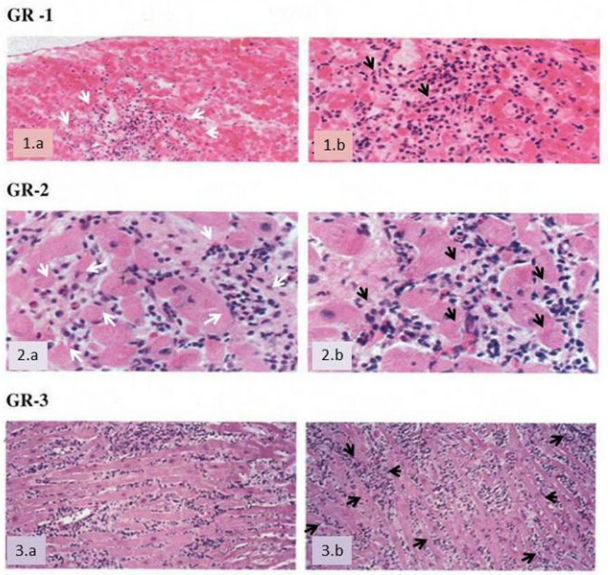
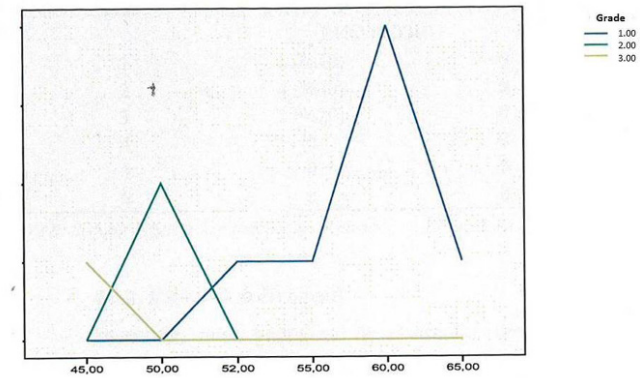


Image 1. Rejection grades in endomyocardial biopsy samples: GR1: Mild rejection; 1.a. Single foci of myocytes injury (white arrows), 1.b.Few perivascular lymphocyte infiltrations (black arrows). GR2: Moderate rejection; 2.a.More than two foci of myocytes injury are associated with at least a moderate cellular infiltrate (white arrows), increased cellular infiltration, 2.b. Increased perivascular lymphocyte infiltrations (black arrows). GR3: Severe rejection; 3 a. The diffuse myocyte injury with erased foci areas, 3 b. Widespread inflammatory cells (black arrows).

Echocardiography

Ejection fractions (EF) were found as 60.00±4.08 % and 55.70±6.27 % in the control and study groups respectively (p: 0.19). The distributions of EF values were presented in Graphic 1. Higher PAP values (38.90±4.97mmHg) were found in the study group when compared with the control group (28.60±2.63mmHg) (p: 0.05). The echocardiography score in the study group were found as score 1 in four patients with increased myocardial echogenicity, score 2 in four patients with increased myocardial echogenicity and more than 4 mm thickening in interventricular septum, score 3 in two patients with myocardial echogenicity, more than 4 mm thickening in interventricular septum and more than 10% decrement in left ventricular ejection time (p: 0.02). There was no pericardial effusion in either groups. Mild-moderate tricuspid insufficiency was detected in 5 patients with rejection.



Graphic 1. The distribution of ejection fraction in regards of rejection fraction in study group

Electrocardiography

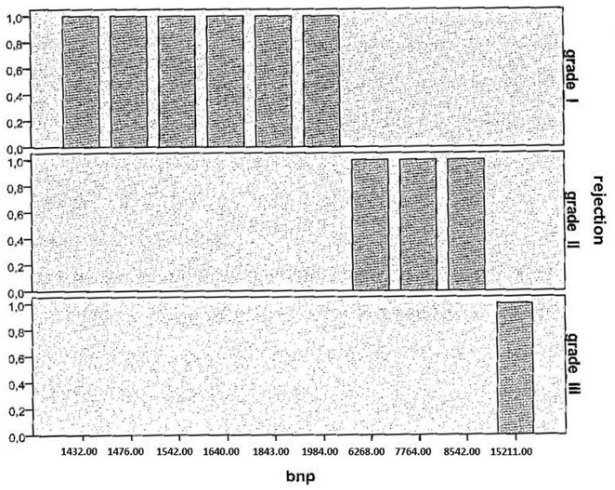
Atrial fibrillation was detected in two (20%) patients who admitted to hospital with cardiac irritation findings. The endomyocardial biopsy findings of these two patients were grade 2 rejections. An amount of 20% decrement in QRS complexes of chest derivations were found in patient with grade 3 rejection who had low cardiac output symptoms.

Additional abnormal electrocardiography finding was not detected in other patients.

Biochemical Findings

The cytomegalovirus tests were negative for all patients. The serum leukocyte numbers were found as 9.65±2.72/mm3and 8.240±1.710/mm3, in the study and control groups respectively (p: 0.19). Erythrocyte sedimentation rates were found as 24.11±8.17 mm/h and 20.32±7.14mm/h in rejection and control groups respectively (p: 0.32). In regards to CRP level, the control group (1.33±0.35 mg/L) and rejection group (3.58±1.18mg/L) were found to be similar (p: 0.11). Only one patient with grade 3 rejection had a more than 12.45 /mm3 white blood cell count (WBC). Additionally, this patient had a 32 mm/h sedimentation rate and higher CRP level (4 mg/L). Although, the troponin levels were found as 0.18±0.02 ng/ml in the rejection group, there was no statistical difference when compared with the control group's (0.09±0.05 ng/ml) troponin levels (p: 0.13).

Markedly higher pro-BNP levels were found in rejection patients (4843.20±6690.10 pg/ml) when compared with the control group's pro-BNP levels (496.30±216.20 pg/ml) (p: 0.001). Furthermore, the distributions of pro-BNP levels in regards of rejection grade in the study group were found to be statistically different (p: 0.03). The highest pro-BNP level (15211 pg/ml) was detected in a grade 3 rejection patient (p: 0.000). The distribution of pro-BNP levels in regards to rejection grades was reported with a bar graph in Graphic 2.



Graphic 2. Bar graph of pro-BNP levels in regards to rejection grades in study group.

DISCUSSION

Our results indicate that pro-BNP levels were found to be higher in acute rejection patients with heart transplantation. Moreover, higher pro-BNP levels were related to an advanced rejection grade. On the other hand, the incremental pro-BNP levels were found to be related with the impaired ventricle functions, although exact discriminative changes were detected with mainly an EMB examination.

The success of heart transplantation is evaluated based on early-mid-long term survival results and quality of life. Although the recent advances that reduce the risk of early complications (early graft insufficiency, infection, rejection) and mortality rates in recent years, the main determinant of a successful transplantation is a one-year survival rate. The early mortality and morbidity risks are related to acute rejection and infections, whereas the late risks are related with coronary artery disease and immunosuppressive treatment associated with renal insufficiency and secondary malignancies [11]. We included twenty heart transplantation patients who underwent heart transplantation and we detected ten acute rejection cases from this group. None resulted in a one-year mortality.

The rejection was classified as mild, moderate and severe in accordance with ISHLT guidelines [8]. The classification is based on EMB sample histopathological evaluations. There were six mild patients, three moderate patients and one severe patient in our series. The acute rejection can usually progress with asymptomatic or nonspecific symptoms. However, cardiac irritation or low cardiac output findings can be detected in progressed cases [12]. In a report from the Turkish population, rejection occurred in 34.2% of heart transplantation cases and EMB samples were used to follow up on progression [13].

Atrial fibrillation (AF) and atrial flutter are the most common arrhythmia types in acute rejection in heart recipient patients, with an incidence of 1% to 2% [14]. The other ECG finding is attenuation of the electrocardiographic QRS complexes for heart failure [15]. Additionally, QRS changes were reported in rejection patients [16]. We

detected AF in two rejection patients with cardiac irritation symptoms and found attenuation of the electrocardiographic QRS complexes in chest derivations of three rejection patients with low cardiac output symptoms. The echocardiographic follow up of heart transplantation depends on evaluation of ventricle functions, strain and mass calculations. Principally, left ventricle (LV) and right ventricle (RV) longitudinal strains are essential for late indication of dysfunction, but not correlating with the rejection grade [17]. Although, myocardial-injury associated graft dysfunction is related to rejection, the grade of damage should confirm with the EMB samples simultaneously. Lack of enhancement in LV ejection fraction and longitudinal strain is associated with poor outcomes after heart transplantation. Ciliberto et al. described the echocardiographic grading for rejection in patients with heart transplantation [9,10]. Additionally, the European Association of Cardiovascular Imaging (EACVI) recommended echocardiographic examination methods of heart recipients for follow up. These methods can be listed as EF, end diastolic and systolic volumes of LV, septal wall thickness, pulmonary artery pressures, pericardial effusion, RV functions, valve regurgitations and longitudinal wall strain evaluations [18]. We did EMB simultaneously with echocardiography during the sixth month. Our findings revealed that EF was similar in patients with rejection and without rejection, although higher PAP values were detected in rejection patients with higher grade. The other findings of our study was the increased myocardial echogenicity: more than 4 mm thickening of interventricular septum and more than 10% decrement in left ventricular ejection time was detected with increment of rejection grade. There was no pericardial effusion in either groups. Mild-moderate tricuspid insufficiency was detected in 5 patients with rejection.

BNP is a pathological hormonal indicator of myocardial response to ventricular stretch. It has a regulatory role for fluid homeostasis and blood pressure by inhibiting to renin–angiotensin–aldosterone system. Thereby, BNP shows cGMP activator, natriuretic, diuretic, vasorelaxant, antifibrotic, and positive lusitropic effects. Therefore, it has been studied as a biomarker in situations that negatively affect heart functions [19,20]. Normal levels of BNP can be affected by hemodynamic alterations and inflammatory reactions. In heart recipients the impaired cardiac functions and allograft vasculopathy leads to incremental serum BNP levels. Despite the higher BNP levels due to increased inflammatory response and disrupted cardiac blood supply, cardiac functions can be found as normal [19,20]. Yin et al. found decreased pro-BNP levels after heart transplantation, however they detected that this decremental values in proBNP levels were reversely affected from early reactions in acute rejection of heart recipients. They suggested that although incremental values can be detected in acute rejection patients, the diagnostic value of proBNP is low [21]. Similarly, Cuppoletti et al. found a lowering in proBNP levels by the duration, after heart transplantation. They found an inverse correlation between acute rejection and proBNP levels and a late increase of NT-proBNP values were observed in their series. Finally, they suggested that isolated proBNP levels are not useful for diagnosing acute rejection after heart transplantation [22]. In our series, proBNP levels were markedly higher in the acute rejection group and the highest proBNP value was detected in a grade 3 rejection patient. However, our sample size was lower than those of previous studies.

Limitations of Study: The main limitation of study is related to low sample size and therefore, the results of our study should be confirmed with larger series. The second limitation is concerning the follow up period. We investigated the EMB biopsies, imaging findings and blood samples at the sixth month. The late response and prolonged systemic response should be investigated after the first year of heart transplantation.

In conclusion, it seems to be that EMB is still the gold standard for evaluation of acute rejection in heart recipients. Although, an alteration of pro-BNP levels seems to be useful for follow up systemic response in acute rejection patients, findings should be confirmed with EMB invasively. The diagnostic accuracy of pro-BNP levels in acute rejection after heart transplantation should be investigated with larger series.

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