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Gene-expression profiling for rejection surveillance after cardiac transplantation

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ORIGINAL ARTICLE

Gene-Expression Profiling for Rejection Surveillance after Cardiac Transplantation

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ABSTRACT

BACKGROUND

Endomyocardial biopsy is the standard method of monitoring for rejection in recipients of a cardiac transplant. However, this procedure is uncomfortable, and there are risks associated with it. Gene-expression profiling of peripheral-blood specimens has been shown to correlate with the results of an endomyocardial biopsy.

METHODS

We randomly assigned 602 patients who had undergone cardiac transplantation 6 months to 5 years previously to be monitored for rejection with the use of gene-expression profiling or with the use of routine endomyocardial biopsies, in addition to clinical and echocardiographic assessment of graft function. We performed a noninferiority comparison of the two approaches with respect to the composite primary outcome of rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation.

RESULTS

During a median follow-up period of 19 months, patients who were monitored with gene-expression profiling and those who underwent routine biopsies had similar 2-year cumulative rates of the composite primary outcome (14.5% and 15.3%, respectively; hazard ratio with gene-expression profiling, 1.04; 95% confidence interval, 0.67 to 1.68). The 2-year rates of death from any cause were also similar in the two groups (6.3% and 5.5%, respectively; $P=0.82$). Patients who were monitored with the use of gene-expression profiling underwent fewer biopsies per person-year of follow-up than did patients who were monitored with the use of endomyocardial biopsies (0.5 vs. 3.0, $P<0.001$).

CONCLUSIONS

Among selected patients who had received a cardiac transplant more than 6 months previously and who were at a low risk for rejection, a strategy of monitoring for rejection that involved gene-expression profiling, as compared with routine biopsies, was not associated with an increased risk of serious adverse outcomes and resulted in the performance of significantly fewer biopsies. (ClinicalTrials.gov number, NCT00351559.)

Advances in immunosuppression after cardiac transplantation have increased the rates of 1-year survival among recipients to nearly 90%. However, acute cellular rejection is still observed during the first year after transplantation (at a rate of approximately 30 to 40%) and occurs at a lower rate thereafter.[1-4] Rejection episodes are associated with an increased risk of allograft vasculopathy and loss.[5-7] Endomyocardial biopsy has remained the primary method of monitoring for rejection, despite the discomfort and the rare but potentially serious complications of the procedure.[8-12]

Quantitative assessment of mononuclear-cell gene expression in peripheral-blood specimens has been explored as a method for detecting the rejection of a cardiac transplant.[13,14] This approach has been investigated as an alternative to an endomyocardial biopsy[13,14] and has led to the development and validation of a commercially available test that has been shown to correlate with the results of an endomyocardial biopsy.[14] Although this gene-expression test has been used at some cardiac transplantation centers to monitor transplant recipients for rejection,[15] it has not been compared systematically in clinical practice with the current standard approach to monitoring for rejection with the use of routine biopsies.

We conducted the Invasive Monitoring Attenuation through Gene Expression (IMAGE) trial to test the hypothesis that a strategy of monitoring for rejection that involves gene-expression profiling is not inferior to a strategy that involves routine biopsies, with respect to a composite outcome of rejection with hemodynamic compromise, graft dysfunction, death, or retransplantation.

Methods

Study Design and Oversight

The IMAGE study was a randomized, event-driven, noninferiority trial conducted at 13 U.S. cardiac transplantation centers from January 2005 through October 2009. The study design has been described previously,[16] and additional details are included in the Supplementary Appendix, available with the full text of this article at NEJM.org. The trial was sponsored by XDx, in which Stanford University owns equity; XDx is the maker of the AlloMap test. The academic investigators initiated and designed the study in collaboration with the sponsor. The trial protocol was approved by the institutional review board at each participating center. The sponsor was involved in the collection and source verification of the data, and the sponsor's biostatisticians performed the analyses with oversight from the study steering committee. The first author wrote the initial draft of the manuscript, and revisions were made by all the authors. Investigators at the core echocardiography laboratory at Stanford University reread all the echocardiograms to calculate the left ventricular ejection fractions that were used in the analyses. An independent end-points committee adjudicated all primary events. A data and safety monitoring board monitored efficacy and safety data. The academic investigators vouch for the accuracy and completeness of the data and of all analyses.

Patients

Patients 18 years of age or older who had undergone a cardiac transplantation between 1 and 5 years previously were eligible for enrollment. Data on cardiac transplantations at participating centers were obtained from the Organ Procurement and Transplantation Network of the United Network of Organ Sharing (http://optn.transplant.hrsa.gov). A protocol amendment on November 27, 2007, expanded enrollment to include patients who had undergone a cardiac transplantation more than 6 months previously, in order to facilitate enrollment. At the time of enrollment, patients were required to be in a clinically stable condition and to have a left ventricular ejection fraction of 45% or greater. Exclusion criteria included a history of severe allograft vasculopathy, antibody-mediated rejection, or the presence of signs or symptoms of heart failure. All participating patients provided written informed consent.

Study Procedures

Patients were randomly assigned, in a 1:1 ratio, to undergo monitoring for rejection by means of gene-expression profiling (gene-profiling group) or routine endomyocardial biopsies (biopsy group). Randomization was stratified according to study center and according to the interval since transplantation (1 year or less, 2 to 3 years, or 4 to 5 years). Monitoring for rejection with the use of the assigned strategy was performed at prespecified intervals in both groups according to the

protocols at the individual transplantation centers (see Table 2 in the Supplementary Appendix). All patients in both groups were also monitored with the use of clinical and echocardiographic assessments. The performance of a biopsy was mandated by the protocol for patients in both groups if clinical or echocardiographic evidence of graft dysfunction was present or, in the case of the gene-profiling group, if the gene-expression profiling score was above a specified threshold. If patients had consistently elevated gene-expression profiling scores and no evidence of rejection on at least two previous biopsies, the protocol did not require further biopsies to be performed in the case of a third or subsequent instance of a score above the threshold.

Gene-expression testing was performed with the use of the AlloMap test (XDx), which evaluates expression levels of 11 informative genes that were shown in previous studies to distinguish between rejection and the absence of rejection. Possible scores range from 0 to 40, with higher scores having a stronger correlation with histologic rejection. In a previous study, a score below 30 had a negative predictive value of 99.6% for histologic evidence of rejection.[14] Therefore, the initial protocol for the current trial specified a score of 30 as a threshold for a mandatory biopsy. However, on November 7, 2005, the protocol was amended to increase the threshold for a mandatory biopsy to a score of 34 in order to minimize the number of biopsies that would be needed in the gene-profiling group. Further details of the test and of the characteristics of the test threshold are provided in the Supplementary Appendix.

Patients were followed for a maximum of 24 months, until they died, or until the study completion date, whichever occurred first. The study ended in October 2009, after the minimum prespecified number of primary outcome events (54) had occurred.

OUTCOMES

The primary outcome was the first occurrence of rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. Definitions of each component of the composite primary outcome are provided in the Supplementary Appendix. Secondary outcomes included death from any cause, the number of biopsies performed, and biopsy-related complications. We also assessed the patients' quality of life and their satisfaction with the method of monitoring for rejection. Quality of life was assessed with the use of the Medical Outcomes Study 12-Item Short Form Health Survey (SF-12). We assessed satisfaction by asking the patients, "How satisfied are you with the current method of detecting rejection?" Responses were scored on an ordinal scale that ranged from 1 (very unhappy) to 10 (very happy).

STATISTICAL ANALYSIS

The trial was designed to test for the noninferiority of gene-expression profiling, as compared with routine endomyocardial biopsies, with respect to the primary outcome. The primary analysis, which was conducted in the intention-to-treat population, was a comparison between the groups of the time to the first occurrence of the composite primary outcome; the comparison was made with the use of the hazard ratios calculated from a Cox proportional-hazards model. The strategy of gene-expression profiling was considered to be noninferior to the strategy of routine biopsies if the one-sided upper boundary of the 95% confidence interval for the hazard ratio with the gene-expression–profiling strategy, as compared with the biopsy strategy, was less than the prespecified margin for noninferiority (2.054). This relative margin was derived assuming a primary-event rate of 5% per year in the biopsy group and the possibility of an event rate of up to 10% per year in the gene-profiling group. This difference, in the view of the investigators, would balance the expected benefit with respect to patient convenience and satisfaction that would result from a reduction in the number of biopsies performed. The study required that a minimum of 54 primary events occur, in order to exclude the inferiority null hypothesis with 80% power, assuming an overall event rate of 5% per year, a rate that was estimated from published observational data.[17,18]

Means and standard deviations for continuous variables were calculated and compared with the use of Student's t-test. Numbers and proportions for categorical variables were compared with the use of Fisher's exact test. Both the Kaplan–Meier method and Cox proportional-hazards models were used to estimate event rates. The effects of an interaction between strategy group and center and between strategy group and interval between transplantation and randomization were tested at an alpha level of 0.15.

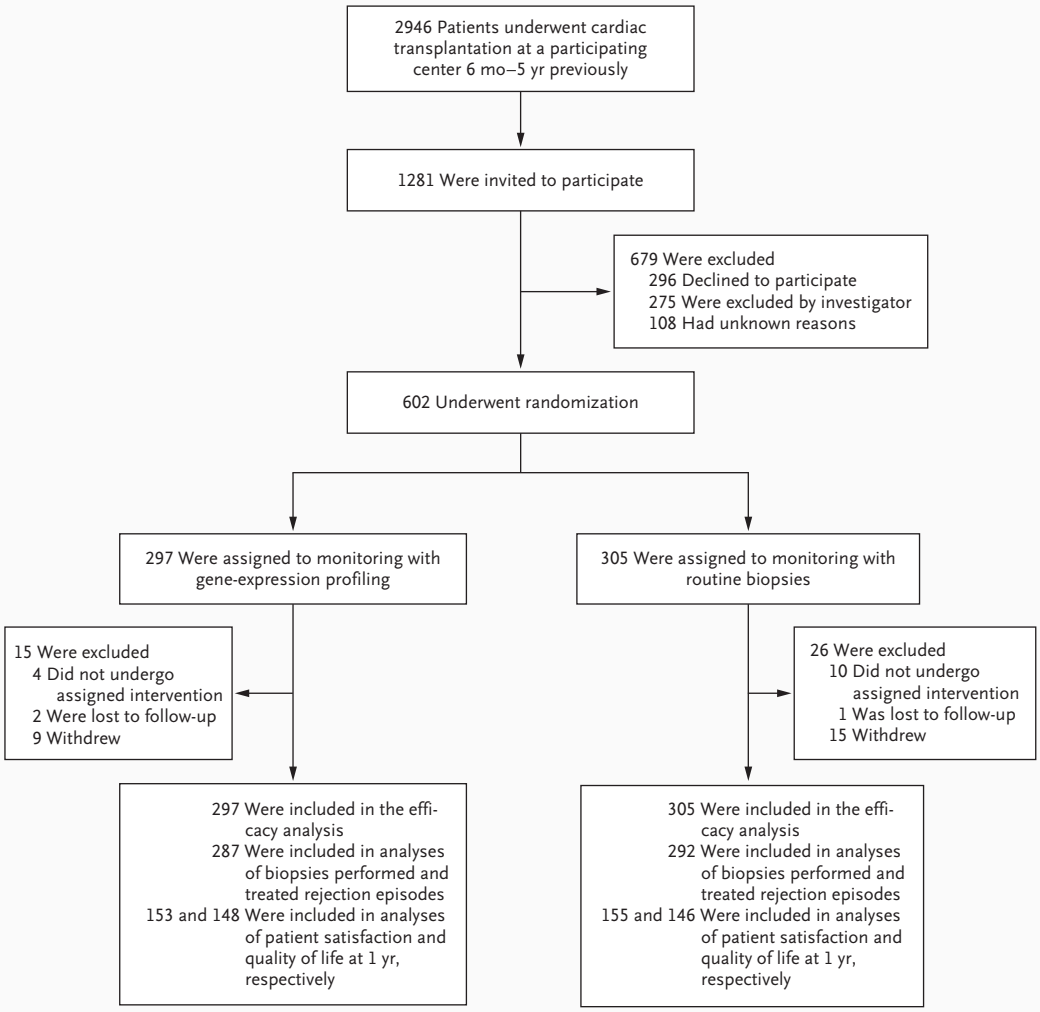

Figure 1. Screening, Randomization, and Inclusion in Analyses.

During the study enrollment period (2005 through 2009), there were 2946 adults who had undergone cardiac transplantation at a participating center between 6 months and 5 years previously, as documented by the Organ Procurement and Transplantation Network of the United Network of Organ Sharing (http://optn.transplant.hrsa.gov). Participating centers were asked to screen all potentially eligible patients for enrollment in the study. A total of 1665 of the 2946 potentially eligible patients (57%) were either not approached for consent or did not meet the eligibility criteria at the time of screening. Details regarding the patients who did not meet the eligibility criteria are not available. The reason that an investigator elected not to enroll a patient was not routinely recorded; however, investigators were encouraged to preferentially enroll patients who were in the early post-transplantation period (<3 years), since data for these patients were expected to be most meaningful. The two other most common reasons for an investigator electing not to enroll a patient were a complicated medical course and the preference of the treating physician to continue with biopsy-based monitoring for rejection. The analyses of biopsies performed and treated rejection episodes included data from patients who completed at least one study visit and who were followed for a minimum of 30 days in the study. Both scheduled study visits and unscheduled outpatient visits were included.

RESULTS

PATIENTS

A total of 602 patients were randomly assigned to be monitored for rejection with the use of gene-expression profiling or with the use of routine endomyocardial biopsies (Fig. 1). The baseline characteristics of the two groups were well matched except that there was a higher proportion of black patients in the biopsy group than in the gene-

profiling group (P=0.01) (Table 1). The interval between transplantation and randomization was 6 through 12 months in the case of 15% of the patients, 13 through 36 months in the case of 68%, and 37 through 60 months in the case of 17%. The median duration of follow-up after randomization was 19.0 months (interquartile range, 9.6 to 23.8).

Table 1. Baseline Characteristics of the Study Population.*

Characteristic	Gene Profiling (N=297)	Biopsy (N=305)	P Value
Age — yr			
Mean	53.9±12.9	54.3±12.8	0.68
Range	18.0–74.0	19.0–78.0	
Male sex — no. (%)	244 (82.2)	249 (81.6)	0.92
Race or ethnic group — no. (%)†			
White	236 (79.5)	232 (76.1)	0.33
Hispanic	22 (7.4)	17 (5.6)	0.41
Black	25 (8.4)	46 (15.1)	0.01
Asian or Pacific Islander	7 (2.4)	6 (2.0)	0.79
Other	7 (2.4)	4 (1.3)	0.38
Indication for cardiac transplantation — no. (%)			0.96
Coronary artery disease	127 (42.8)	130 (42.6)	
Nonischemic cardiomyopathy	152 (51.2)	155 (50.8)	
Valvular heart disease	6 (2.0)	5 (1.6)	
Congenital heart disease	9 (3.0)	9 (3.0)	
Graft vasculopathy or retransplantation	1 (0.3)	3 (1.0)	
Other	2 (0.7)	3 (1.0)	
Interval between transplantation and randomization — no. (%)			
6–12 mo	43 (14.5)	44 (14.4)	>0.99
13–36 mo	205 (69.0)	208 (68.2)	0.86
37–60 mo	49 (16.5)	53 (17.4)	0.83
Cytomegalovirus status — no. (%)			
Donor and recipient positive	128 (43.1)	109 (35.7)	0.07
Donor and recipient negative	44 (14.8)	47 (15.4)	0.91
Donor positive and recipient negative	59 (19.9)	78 (25.6)	0.10
Donor negative and recipient positive	50 (16.8)	58 (19.0)	0.52
Unknown	16 (5.4)	13 (4.3)	
Use of ventricular assist device before transplantation — no. (%)	58 (19.5)	57 (18.7)	0.84
Induction therapy — no. (%)			
Any	168 (56.6)	181 (59.3)	0.74
Muromonab-CD3	4 (1.3)	5 (1.6)	
Antithymocyte globulin	52 (17.5)	53 (17.4)	
Basiliximab	30 (10.1)	43 (14.1)	
Daclizumab	66 (22.2)	63 (20.7)	
Alemtuzumab	12 (4.0)	13 (4.3)	
Other	4 (1.3)	4 (1.3)	

Table 1. (Continued.)

Characteristic	Gene Profiling (N=297)	Biopsy (N=305)	P Value
Immunosuppressive therapy — no. (%)‡			
Cyclosporine	79 (26.6)	83 (27.2)	0.66
Tacrolimus	218 (73.4)	218 (71.5)	0.65
Mycophenolate mofetil or mycophenolic acid	237 (79.8)	250 (82.0)	0.53
Azathioprine	26 (8.8)	15 (4.9)	0.08
Sirolimus	53 (17.8)	65 (21.3)	0.31
Prednisone	132 (44.4)	122 (40.0)	0.28
Medical history after transplantation — no. (%)			
Hypertension treated with medication	247 (83.2)	258 (84.6)	0.66
Diabetes mellitus treated with medication	115 (38.7)	114 (37.4)	0.74
Renal insufficiency§	147 (49.5)	157 (51.5)	0.68
Lipid-lowering drug prescribed	275 (92.6)	283 (92.8)	>0.99
Cancer	38 (12.8)	49 (16.1)	0.30
Left ventricular ejection fraction at first study visit¶	63.2±6.0	63.4±6.1	0.67

* Plus–minus values are means ±SD. Data are for the intention-to-treat population.
† Race or ethnic group was self-reported.
‡ This category includes all medications taken by patients while they were enrolled in the study.
§ Renal insufficiency was defined by a serum creatinine level of less than 1.5 mg per deciliter (133 μmol per liter).
¶ Data for first-visit measurements of left ventricular ejection fraction were missing for 9 patients in the gene-profiling group and 15 in the biopsy group. In the case of five patients in the gene-profiling group and seven in the biopsy group, the left ventricular ejection fraction could not be calculated at the core echocardiography laboratory owing to the poor quality of the echocardiogram. In these cases, the measurement of left ventricular ejection fraction that was obtained closest to the first study visit was used in the analysis.

PRIMARY OUTCOME

The 2-year rate of the composite primary outcome in the gene-profiling group was similar to the rate in the biopsy group (14.5% and 15.3%, respectively; P=0.86) (Fig. 2A). The corresponding hazard ratio was 1.04 (95% confidence interval [CI], 0.67 to 1.68), with the upper boundary falling below the prespecified noninferiority margin. Therefore, monitoring for rejection with gene-expression profiling was noninferior to monitoring with routine biopsies with respect to the prevention of the primary outcome. The results for the individual components of the primary outcome are shown in Table 2. There was no significant interaction with respect to the primary outcome between the assigned group and either the interval between transplantation and randomization (≤12 months vs. >12 months) or the transplantation center (P=0.86 and P=0.99, respectively). Because there was a higher proportion of black patients in the biopsy group than in the gene-profiling group and a higher observed rate of the primary outcome among black patients than among nonblack patients (18.3% vs. 10.2%, P=0.07), we performed an additional analysis adjusting for black race in our Cox model. We found that the results were consistent with those of our primary analysis (hazard ratio, 1.13; 95% CI, 0.70 to 1.84).

DEATHS

The overall rate of survival in our study did not differ significantly according to the method of monitoring (Fig. 2B). The 2-year cumulative rate of death was 6.3% in the gene-profiling group and 5.5% in the biopsy group (P=0.82) (Table 2). The adjudicated causes of death (cardiovascular vs. noncardiovascular) were similar in the groups.

BIOPSIES PERFORMED AND RELATED COMPLICATIONS

A total of 409 biopsies were performed in the gene-profiling group, as compared with 1249 performed in the biopsy group (Fig. 3, and Table 8 in the Supplementary Appendix). The frequency of

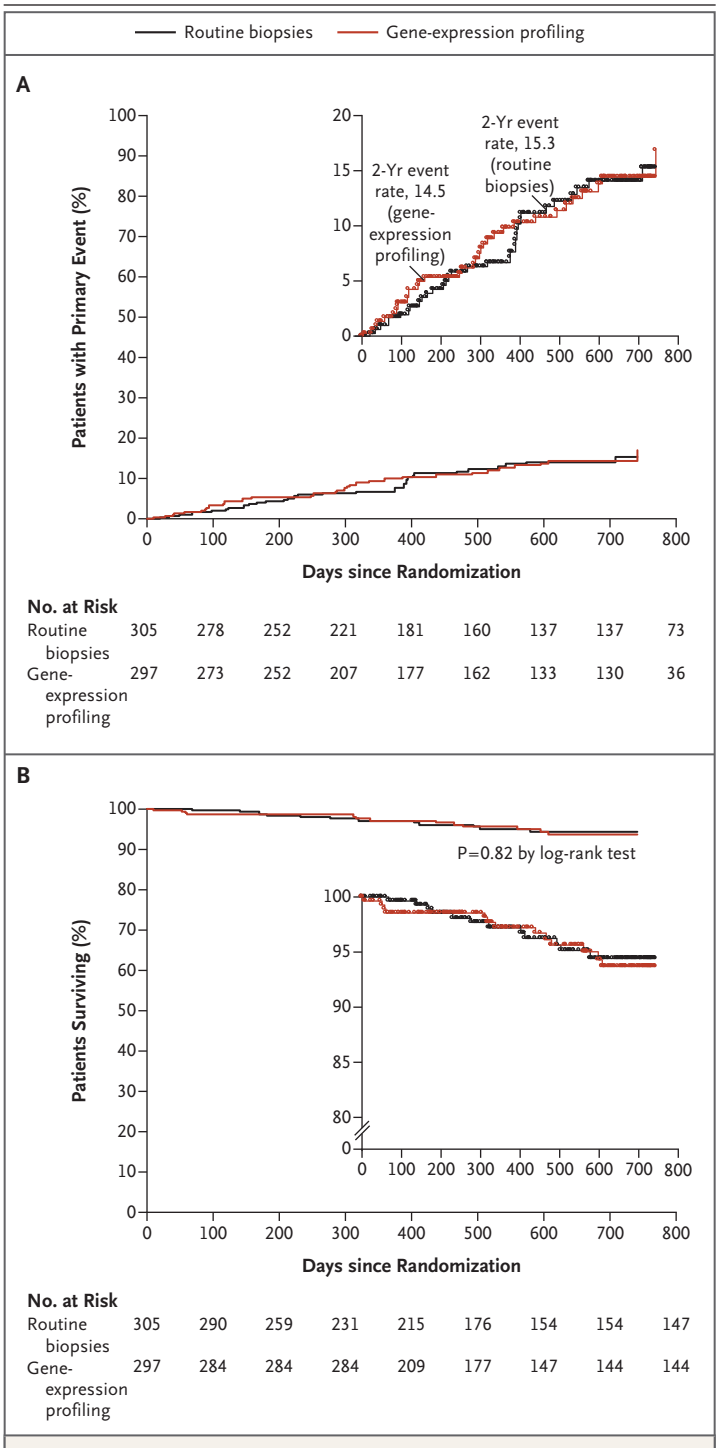

Figure 2. Kaplan–Meier Estimates of the Time to the Composite Primary Outcome and the Probability of Survival.

Panel A shows the time to the first occurrence of any of the following primary events: rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. Only the first event that was part of the composite primary outcome was considered. Panel B shows the probability of overall survival. The inset in each panel shows the same data on an enlarged y axis and on a condensed x axis.

biopsies was 0.5 biopsies per patient-year of follow-up in the gene-profiling group and 3.0 biopsies per patient-year of follow-up in the biopsy group (P<0.001). In the gene-profiling group, 67% of the biopsies were performed because of elevated gene-expression profiling scores; another 17% were performed, per protocol, when signs, symptoms, or echocardiographic manifestations of graft dysfunction were present at the time of a clinic visit, 13% were performed as part of a follow-up assessment after treatment for rejection, and 3% were performed outside the study protocol. In 28 instances (9% of the cases in which there were elevated scores), consistently high gene-expression profiling scores did not result in performance of a biopsy (see the Methods section). Biopsy-related complications occurred in four patients in the biopsy group and in one patient assigned to the gene-profiling group (Table 2).

INTENSITY AND COMPLICATIONS OF IMMUNOSUPPRESSION

The overall intensity of immunosuppression throughout the study was similar in the gene-profiling group and the biopsy group (see the Supplementary Appendix). Despite a higher number of infections among patients monitored with gene-expression profiling than among those monitored with biopsies (53 vs. 43) (Table 7 in the Supplementary Appendix), we found no significant differences between the groups in mean levels of calcineurin inhibitors (Section 2.2 in the Supplementary Appendix). The mean serum creatinine levels during the study were also similar in the two groups (1.42±0.41 mg per deciliter [125.5±36.2 μmol per liter] in the gene-profiling group vs. 1.42±0.59 mg per deciliter [125.5±52.2 μmol per liter] in the biopsy group, P=0.95). Finally, the incidence of any cancer was similar in the two groups (3.7% in the gene-profiling group and 3.3% in the biopsy group, P=0.83).

REJECTION EPISODES

A total of 34 treated episodes of rejection occurred in the gene-profiling group, as compared with 47 episodes in the biopsy group (Section 2.4 and Table 9 in the Supplementary Appendix). In the gene-profiling group, six treated episodes of rejection were initially detected as a result of a biopsy performed because of an elevated gene-expression score. In the biopsy group, 22 treated episodes of rejection were asymptomatic and were detected on routine biopsy alone.

Table 2. Trial Outcomes.

Outcome	Total Events		2-Yr Cumulative Event Rate		P Value	Hazard Ratio (95% CI)*
	Gene Profiling	Biopsy	Gene Profiling	Biopsy		
			%			
Composite primary outcome — no. of events†	34‡	33	14.5	15.3	0.86§	1.04 (0.67–1.68)
Rejection with hemodynamic compromise as first event — no. of events	11	13			>0.99¶	
Cellular, biopsy-confirmed‖	2	7				
Antibody-mediated, biopsy-confirmed**	3	1				
Mixed, biopsy-confirmed	3	2				
Probable, not biopsy-confirmed††	4	3				
Graft dysfunction due to other causes as first event — no. of events	11	14			0.68¶	
Allograft vasculopathy	1	1				
Nonspecific graft failure	11	13				
Death as first event — no.	11	6			0.23¶	
Cardiovascular	7	5				
Noncardiovascular or unknown	4	1				
Death at any time — no. of events‡‡	13	12	6.3	5.5	0.82§	1.10 (0.50–2.40)
Cardiovascular	8	9				
Noncardiovascular or unknown	5	3				
Adverse events associated with biopsy — no. of patients/total no. (%)§§	1/287 (0.3)	4/292 (1.4)				
Tricuspid-valve incompetence¶¶	0/287	2/292				
Symptomatic pericardial effusion‖‖	0/287	1/292				
Bleeding	0/287	1/292				
Other***	1/287	0/292				

* The hazard ratio was estimated with the use of the Cox model, which included study-group assignment as a factor.

† The composite primary outcome was rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. The analysis was performed on the basis of the first occurrence of any of the components. One patient in the biopsy group underwent retransplantation. The event was categorized as a death in the analyses, as specified by the statistical-analysis plan.

‡ One case of graft dysfunction was adjudicated as due to probable rejection (not biopsy-confirmed), allograft vasculopathy, or both. Therefore, this event is listed twice, once in the category of probable rejection and once in the category of allograft vasculopathy.

§ P values were calculated with the use of the log-rank test.

¶ P values were calculated with the use of Fisher's exact test for categorical variables.

‖ Confirmation of cellular rejection on biopsy required that a local pathologist classify the biopsy specimen, according to the International Society for Heart and Lung Transplantation system for grading rejection, as a grade of 2R (according to the 2004 version, in which the grades range from 0 to 3R) or 3A (according to the 1990 version, in which grades range from 0 to 4). Higher numbers indicate more severe rejection.

** Confirmation of antibody-mediated rejection on biopsy required histologic evidence of acute capillary injury or immunopathological evidence of antibody-mediated injury (as assessed with the use of immunofluorescence or immunohistochemical testing).

†† Probable rejection included events that, in the absence of histologic confirmation on biopsy, were considered by the end-points committee to be caused by rejection.

‡‡ This category includes deaths that occurred as the first event, as well as deaths that occurred after a nonfatal primary event.

§§ The total number includes all patients who completed at least one study visit and who were followed for a minimum of 30 days in the study.

¶¶ This category included tricuspid regurgitation with a grade of moderate-to-severe or higher on the basis of the local echocardiography report. One patient had severe tricuspid regurgitation, and the other patient had moderate-to-severe tricuspid regurgitation.

‖‖ Hypotension and presyncope developed in one patient in the biopsy group after a routine heart biopsy; an echocardiogram in this patient showed a new, moderate-grade pericardial effusion that required hospitalization for observation. The effusion resolved without the need for drainage.

*** One patient was inadvertently given subcutaneous formalin instead of lidocaine before venous cannulation, and the wound required local débridement by a plastic surgeon.

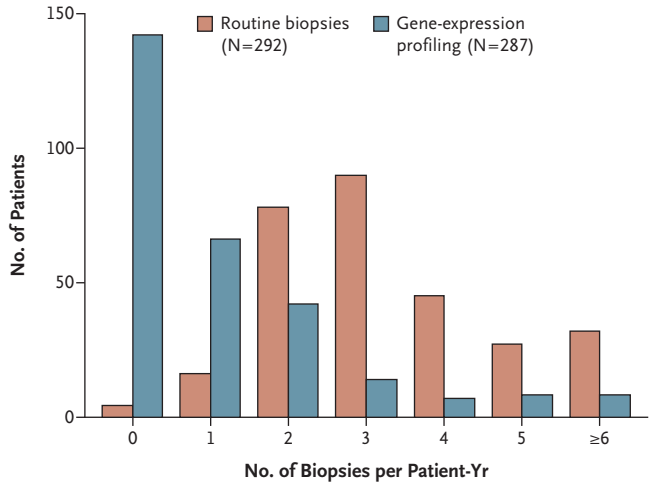

Figure 3. Frequency of Endomyocardial Biopsies Performed.

The distribution of outpatient endomyocardial biopsies performed per patient-year of follow-up is shown for patients in the biopsy group and patients in the gene-profiling group. The majority of patients in the gene-profiling group (88%) underwent two biopsies or fewer per patient-year, and 50% did not require a biopsy during the study.

HEALTH STATUS AND PATIENT SATISFACTION

At enrollment, no significant differences were found between the two groups in the physical-health and mental-health summary scores of the SF-12 (Table 10 in the Supplementary Appendix). The physical-health summary score was higher in the biopsy group than in the gene-profiling group at 1 year (47.3 vs. 44.7, P=0.03), but both the mean physical-health and mental-health summary scores were similar in the two groups at 2 years (physical-health score: 45.1 in the gene-profiling group and 46.2 in the biopsy group, P=0.52; mental-health score: 50.8 and 50.7 in the two groups, respectively; P=0.66). At enrollment, the scores for patient satisfaction were similar in the gene-profiling group and the biopsy group (6.86 and 6.74, respectively; P=0.61). During the course of the study, there was an increase in the satisfaction score in the gene-profiling group, to 8.15 in year 1 and 8.74 in year 2, whereas the scores in the biopsy group remained similar throughout the study to the score at enrollment (6.64 in year 1 and 6.66 in year 2). The differences in patient-satisfaction scores at 1 and 2 years between patients in the gene-profiling group and those in the biopsy group were significant (P<0.001 for both comparisons).

DISCUSSION

In this multicenter study involving patients who had received a cardiac transplant more than 6 months before enrollment and whose condition was clinically stable, the use of gene-expression profiling of peripheral-blood specimens in combination with clinical and echocardiographic assessment, as compared with the use of endomyocardial biopsies according to standard practice, resulted in a significant reduction in the number of biopsies performed and did not result in an excess of adverse outcomes. In addition, patient satisfaction was higher with the gene-expression profiling method of monitoring than with the biopsy method, reflecting the preference of many patients for avoiding an invasive procedure.

For gene-expression testing, a score below 34 was used in the majority of cases (97%) to identify patients who were at low risk for rejection and in whom a biopsy was not needed. Although the use of a higher threshold may further minimize the number of biopsies needed, the results of our trial suggest that a score below 34 represents a prudent threshold to use in clinical practice in the case of patients for whom the interval after transplantation is more than 6 months.

There were fewer treated episodes of rejection in the gene-profiling group than in the biopsy group, and this difference was due primarily to fewer asymptomatic episodes of rejection observed in the gene-profiling group than in the biopsy group (see the Supplementary Appendix). Although gene-expression profiling may not have detected all the cases of asymptomatic rejection, we did not observe an excess 2-year cumulative risk of graft dysfunction, death, or retransplantation in the gene-profiling group. This observation suggests that not all asymptomatic episodes of rejection that occur more than 6 months after transplantation warrant treatment. Some of these episodes may be explained by a misreading on the part of pathologists of benign nodular endocardial infiltrates (Quilty lesions) in biopsy specimens, whereas others may represent a subgroup of histologically defined rejection episodes that resolve without augmentation of immunosuppression.[19,20] Conversely, undetected rejection may lead to long-term graft dysfunction through such mechanisms as progressive myocardial fibrosis or coronary-artery intimal hyperplasia. The late consequences of untreated rejection are poorly understood and may not have been clinically ap-

parent during the follow-up period (a median of 19 months) in our study.

Only 6 of the 34 treated episodes of rejection in the gene-profiling group were detected with the use of the gene-expression test. The other episodes were detected because of the presence of overt symptoms of heart failure or echocardiographic evidence of graft dysfunction. These observations raise the possibility that clinical observation may detect the majority of serious rejection episodes. Some transplantation centers in the United States and many centers worldwide have discontinued the practice of performing routine biopsies after the first year post-transplantation.[21] However, many physicians who treat transplant recipients have been reluctant to adopt this practice until the relative safety of such an approach can be shown in a comparative trial. Therefore, the majority of transplantation centers in the United States continue to perform biopsies beyond the first year post-transplantation, although there is considerable institutional variation in the frequency and duration of monitoring for rejection.[2] There has not been sufficient equipoise to justify a comparison of monitoring by means of clinical observation with monitoring by means of routine biopsies, but our findings may provide the basis for such comparisons in future studies.

The results of our trial must be interpreted in the context of several important limitations. Only patients who had received a cardiac transplant more than 6 months previously were eligible for enrollment. Such patients have a lower risk of rejection and may be at lower risk for adverse outcomes due to undetected rejection than patients for whom the interval after transplantation is 6 months or less. We chose to enroll patients who were at a lower risk for rejection because the relative safety of an approach that minimizes the number of biopsies has not been confirmed, and we did not want to expose the study participants to an undue risk of adverse events. This decision reflects the characteristically conservative approach to the care of cardiac-transplant recipients and the reluctance of clinicians and patients to accept even a small possibility of causing harm.

Only 20% of potentially eligible patients were enrolled in the study. Patients who had received a cardiac transplant less than 3 years previously were recruited preferentially, and a substantial number of eligible patients were not enrolled, owing to patient or physician preferences. Details regarding these patients were not available, but it is likely that patient selection was biased toward the inclusion of low-risk patients, thereby restricting the generalizability of our findings. This limitation should be taken into account by clinicians when they consider the use of gene-expression profiling in the care of their patients.

The low projected event rates and the limited number of available patients necessitated the choice of a wide noninferiority margin. The trial's reduced power was reflected in a relatively wide confidence interval that does not exclude the possibility of a 33% decrease in primary event rates (or 1.8 fewer events per 100 patient-years) or of a 68% increase (3.7 excess events per 100 patient-years) among patients in the gene-profiling group. Our composite outcome was chosen to include both clinically overt rejection and the possible consequences of undiagnosed rejection. Because graft dysfunction, death, or retransplantation may be caused by conditions other than rejection, the inclusion of these end points may have further reduced the trial's power. A more robust test of noninferiority would have necessitated a considerably larger sample than that which was feasible, given the limited number of cardiac transplantations performed worldwide.[22]

Finally, the lack of blinding in the study may have influenced the intensity of immunosuppression in the gene-profiling group. However, we did not observe any significant differences between the groups in mean levels of calcineurin inhibitors throughout the study, in serum creatinine levels, or in the incidence of neoplasms.

In conclusion, our study suggests that gene-expression profiling of peripheral-blood specimens may offer a reasonable alternative to routine biopsies, for monitoring cardiac-transplant recipients for rejection if the interval since transplantation is at least 6 months and the patient is considered to be at low risk for rejection. However, the study had limited power to allow for a firm conclusion to be reached regarding the use of gene-expression profiling as a substitute for the performance of biopsies. A larger trial with a narrower noninferiority margin and a longer follow-up period would be necessary to definitively resolve this issue.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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