Nodular Endocardial Infiltrates (Quilty Lesions) Cause Significant Variability in Diagnosis of ISHLT Grade 2 and 3A Rejection in Cardiac Allograft Recipients

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Background: Endomyocardial biopsy is used to guide therapy after heart transplantation. An accurate and reliable diagnosis of rejection is critical for proper patient management.

Methods: A sub-set of 827 biopsies from 273 patients were identified from 8 centers participating in the Cardiac Allograft Gene Expression Observational Study. These included all biopsies graded by local center pathologists as International Society for Heart and Lung Transplantation (ISHLT) Grade 1B or higher and also randomly chosen Grade 0 and 1A biopsies. Each of these cases was reviewed in a blinded manner by 3 study pathologists in the absence of clinical data. The study pathologists were assigned an ISHLT grade and noted nodular endocardial infiltrates (Quilty lesions).

Results: The study pathologists were significantly more likely than local pathologists to diagnose ISHLT Grade 0, 1A and 3B rejection and significantly less likely to diagnose ISHLT Grade 1B, 2 and 3A rejection. Concordance between local and study pathologists was lowest for Grade 2 (17% agreement). Quilty lesions were noted in 3.3% of local Grade 0 cases and in 31% and 37% of local Grade 2 and 3A cases, respectively. Quilty lesions were recognized by study pathologists in 35% of local Grade 2 cases "downgraded" to Grade 0 or 1, but in only 10% of local Grade 2 cases confirmed by study pathologists.

Conclusions: The greatest variability between pathologists in application of the ISHLT grading system is in Grade 2 biopsies, and Quilty lesions are a major contributing factor to the lack of concordance. Accurate application of the ISHLT grading system requires improved recognition and understanding of Quilty lesions. J Heart Lung Transplant 2005;24:S219–S226. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Published in 1990, the working formulation for cardiac allograft biopsy grading[1] was the product of a consensus meeting of pathologists held at Stanford University by Dr. Margaret Billingham. This formulation was a description and classification of the histology of biopsies of cardiac allografts maintained with the prevailing immunosuppressive regimen (usually corticosteroids, cyclosporine and azathioprine). The clinical import of Grade 2 rejection was unknown; a separate grade was assigned to allow multicenter studies to determine the clinical significance of this histology. The grading system, therefore, is not necessarily ordered from least to most "severe" rejection: Grade 1A is focal and Grade 1B is diffuse, but this does not mean that one is more "severe" than another. Grade 0 is the only grade that represents no evidence of acute cellular rejection. Other grades describe patterns of cellular infiltration.

NODULAR ENDOCARDIAL INFILTRATES (QUILTY LESIONS) AND GRADE 2 REJECTION

In 1990, the Stanford group reported finding accumulations of lymphocytes with scattered plasma cells and prominent vascularity in the endocardium (Quilty A), which might extend into the subjacent myocardium (Quilty B).[2] These were named after the first patient in whom they were recognized. These lesions are dense

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Submitted August 11, 2004; revised March 30, 2005; accepted April 1, 2005.

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 1053-2498/05/$–see front matter. doi:10.1016/j.healun.2005.04.001

infiltrates, often without recognizable myocytes in their central portions, containing numerous capillary-sized vessels, and composed of plasma cells and central aggregates of B lymphocytes with a peripheral rim of T lymphocytes. By definition, such lesions connect to the endocardial surface, although in any one section this may not be obvious. They are generally not treated as acute rejection with increased immunosuppression and are not recognized as having prognostic value,[3] although some reports have linked them to subsequent histologic Grades 2, 3A, 3B and 4,[4] and to coronary vasculopathy.[5] However, when these dense infiltrates extend into the myocardium they mimic acute cellular rejection.

Grade 2 histology is defined[1] as "one focus only with aggressive infiltration and/or focal myocyte damage . . . which is sharply circumscribed. Architectural distortion with myocyte damage should be present within the solitary focus." These dense infiltrates can resemble the dense infiltrates extending from Quilty lesions into the myocardium.

To distinguish Grade 2 histology from Quilty lesions, the pathologist must look at additional sections from the specimen to demonstrate that the infiltrate is continuous with the endocardium. One such approach[6] showed that 91% (32 of 35) of Grade 2 biopsies were actually nodular endocardial infiltrates; when untreated, these progressed to Grade 3A rejection only very rarely (1 instance in the subsequent 2 months). Grade 2 histology was also implicated in most of the major discrepancies in biopsy interpretation when biopsies were reviewed by a central panel in conjunction with a trial of a new immunosuppressive regimen. Winters and McManus[7] reported major discrepancies in interpretation between local readers and study pathologists in 20% of biopsies. Grade 2 lesions were involved in 82% of the discrepancies.

THE CARGO STUDY

The Cardiac Allograft Rejection Gene Expression Observational Study (CARGO) was initiated in 2001 to explore the relationship between peripheral leukocyte gene expression and acute allograft rejection and other clinical outcomes. Through this work, gene expression patterns distinguishing acute cellular rejection from quiescence have been identified and are being validated for clinical use. Cardiac allograft recipients from 8 centers were enrolled and followed-up with blood sample and clinical data collection with each post-transplant biopsy encounter. The intent of the study is to show that the correlation of gene expression with acute cellular rejection with the biopsy is the standard for diagnosis of cellular rejection. Therefore, the variability in pathologic grading of the biopsies was of critical importance as the biopsy rejection grades represent an important study end-point. Biopsy slides associated with study patient encounters were obtained and re-read by a panel of 3 study pathologists (G.B., M.B., C.M.) blinded to the clinical information associated with each case. The pathologic data generated from this study provided an opportunity to re-examine variability in the current assignment of ISHLT grades and explore the underlying causes of such variability.

METHODS

Clinical Study

Under institutional review board (IRB)-approved informed consent at each institution, patients enrolled in the CARGO study were enrolled at the time of transplantation and followed during their post-transplant course. At each biopsy encounter, clinical and pathologic data were recorded and a peripheral blood sample was obtained.

Cardiac Biopsy Pathology

For 562 patients from 8 centers, 3,968 biopsy encounters were recorded. A sub-set of 827 biopsies from 273 patients was identified; this sub-set included all biopsies graded by local center pathologists as Grade 1B or higher and also included randomly chosen Grade 0 and 1A biopsies. For each of these cases, endomyocardial biopsy slides were obtained from the participating centers. Slides were blinded with an encrypted identification and distributed to each of 3 study pathologists who assigned ISHLT grades for each case in the absence of clinical data. In all, both the local and study pathologists provided >3,500 readings of the 827 cases. In addition to assigning an ISHLT rejection grade, study pathologists also noted Quilty lesions for each case. In the subsequent analyses, Quilty A and Quilty B lesions are combined.

The 3 study pathologists met once to review, discuss and reach a consensus on cases in which there was significant disagreement on the assignment of ISHLT Grade 2 or 3A. It was agreed that the consensus reading would be done in the absence of clinical data and without knowledge of previous diagnoses by the group. From this review and discussion, a sub-set of 22 cases having putative Quilty lesions that could affect the grading of the case as rejection or no rejection were selected and the tissue block was sectioned onto an additional set of slides for examination. Only the hematoxylin–eosin stain was used; no immunohistochemical stains were performed on these additional sections. Using these serial sections, one of the study pathologists (C.M.) assessed each case to confirm or reject the diagnosis of Quilty lesions by examining the lesions' relationship to the endocardium.

Statistical Analysis

All *p*-values given are 2-sided probabilities. A Yates-corrected chi-square test was used to calculate the probabilities in most analyses in this study. For any analysis involving a contingency table in which the expected number of samples for at least 20% of the cells was <5, the Fisher's Exact test was used.

RESULTS

Distribution of ISHLT Grades According to Local Pathologists

Among the 3,968 biopsies from 562 patients read by local pathologists, the mean time from transplantation was 210.5 days (median 101 days, standard deviation 482.6 days). The clinical characteristics of the 273 patients who provided the 827 biopsies evaluated in the study are shown in Table 1. The distribution of ISHLT grades assigned by local pathologists at the 8 centers is shown in Table 2. Grade 3A and B rejection rates were very low (<3.8% of all biopsies). The vast majority of cases (90.1%) were Grade 0 or 1.

T1 T2

Correlation Between Grades Assigned by Local Pathologists and Grades Assigned by Study Pathologists

The ISHLT grades assigned by the local and study pathologists are given in Figure 1. The frequency of an individual grade by the local pathologists is compared with the average frequency of an individual grade given by the 3 study pathologists. The study pathologists were significantly more likely to diagnose Grade 0, 1A and 3B rejection and significantly less likely to diagnose Grade 1B, 2 and 3A rejection. There was a 3.5-fold decrease in diagnosing Grade 2 by study readers compared with local readers.

F1

Table 1. Patient Demographics

Characteristics	Number	Percentage
Gender		
Male	211	77.3%
Female	61	22.3%
Unknown	1	0.4%
Race		
Asian	3	1.1%
Black	48	17.6%
White	194	71.1%
Hispanic	17	6.2%
Other	5	1.8%
Not specified	6	2.2%
Age at time of transplant (years)		
0–17	9	3.3%
18–50	80	29.3%
51–73	181	66.3%
Unknown	3	1.1%

Total patients: $n = 273$; 827 biopsies read by a local pathologist and 3 study pathologists. Average number of biopsies per patient = 3.03.

Table 2. ISHLT Grade Distribution of 3,968 Biopsies From 562 Cardiac Allograft Recipients

ISHLT grade	Number	Percent
0	1,878	47.3%
1A	1,562	39.4%
1B	133	3.4%
2	247	6.2%
3A	137	3.5%
3B	11	0.28%

The concordance rates for individual study pathologists by local ISHLT grade for each case are shown in Table 3. For this analysis, Grades 1A and 1B were grouped together as the same class as were Grades 3A and 3B. Concordance rates were high for Grade 0 biopsies according to local pathologists. However, concordance for Grade 1A and 1B biopsies was lower for each central reader as were those for Grade 3 rejection. The lowest concordance between local grade and study grading was for local Grade 2 biopsies where study readers agreed only 8% to 26% of the time (average 17%).

T3

Four of the centers (Columbia, Ochsner, Temple and Stanford) contributed 94% of the biopsies. There was no significant difference between these centers in the percentage of local Grade 2 to 3A diagnoses "downgraded" by at least 2 of 3 central panel pathologists to Grade 0 to 1A.

Concordance: Inter-reader Variability for Study Pathologists

Inter-reader variability was assessed between the 3 study pathologists and is shown in Table 4. Concordance rates between the 3 study pathologists were calculated for each local grade of rejection. The concordance rate for study pathologists for the biopsies with local Grade 0 was very high (93%). However, concordance rates dropped markedly for biopsies with local Grade 1 (71%), Grade 2 (60%) and Grade 3 (58%). The overall concordance rate between study pathologists for all cases was 79%.

T4

Frequencies of Quilty Determinations

Quilty lesions may be confused with rejection or might be present in association with particular ISHLT grades. The study pathologists noted for each case whether a Quilty lesion was or was not present. These were analyzed by local ISHLT grade, comparing the observed rates of Quilty lesions to the expected results if Quilty lesions were equally likely to be noted in all ISHLT grades. As shown in Figure 2, significantly more Quilty lesions were reported by central readers in local Grade 2 and 3A biopsies than expected. Quilty lesions were rarely noted by central readers in local Grade 0 or 1A cases. To better understand this association, we focused on local Grade 2 biopsies and asked whether Quilty

F2

	Local Pathologists		Average of Study Pathologists' Results		
ISHLT Grade	No.	%	No.	%	P value
0	416	50.3%	481	58.2%	< 0.01
1A	116	14.0%	211	25.5%	<0.01
1B	58	7.0%	27	3.3%	<0.01
2	159	19.2%	45	5.4%	<0.01
3A	76	9.2%	51	6.2%	<0.01
3B	2	0.24%	12	1.4%	<0.01

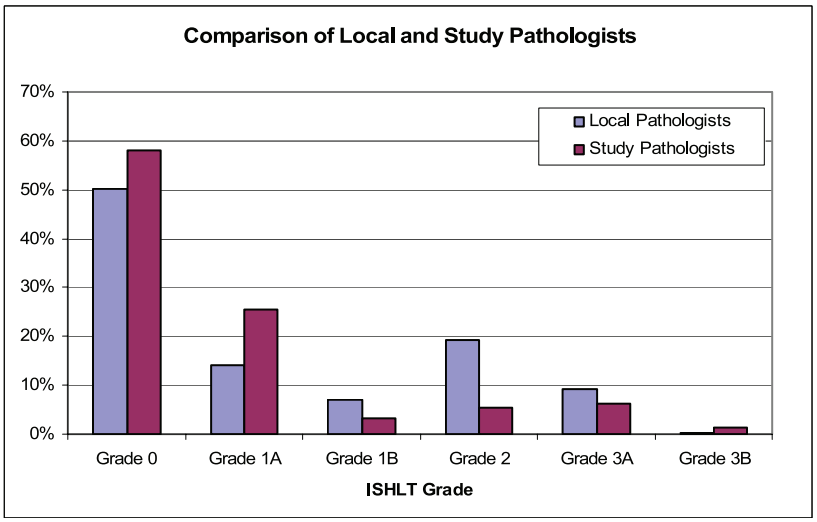

Figure 1. Comparison of ISHLT grade assignments for 827 cases read by local and 3 study pathologists blinded to the clinical data. The study pathologists were significantly more likely than local pathologists to diagnose ISHLT Grades 0, 1A and 3B and significantly likely to diagnose ISHLT Grades 1B, 2 and 3A.

lesions were more likely to be associated with local Grade 2 biopsies "downgraded" to Grade 0, 1A or 1B than with biopsies "maintained" at Grade 2 or "upgraded" to Grade 3A or 3B by study pathologists.

The rates of Quilty annotation by individual study pathologist for the 159 local Grade 2 cases are shown in Figure 3. One central reader was significantly more likely to recognize a Quilty lesion in association with local Grade 2 biopsies "downgraded" to Grade 0, 1A or 1B than in biopsies not downgraded. The other 2 central readers had a non-significant increase in Quilty annotations for those local Grade 2 cases that were downgraded to Grade 0, 1A or 1B, compared with cases retained as Grade 2 or "upgraded" to Grade 3A or 3B.

F3

Consensus Meeting Results

Forty-seven biopsies were examined by all 3 study pathologists at the consensus meeting. Slides were selected based on previous discrepancies between the centralized readers. Most involved Grade 2 and 3A diagnoses, but some Grade 0 and 1 diagnoses were included so that the group would be exposed to the full range of histology. Local grades for these 47 biopsies were: Grade 0, 3 cases; Grade 1A, 4 cases; Grade 1B, 4 cases; Grade 2, 22 cases; and Grade 3A, 14 cases.

In many cases the group was able to identify an infiltrate as Quilty because it was continuous with the endocardium. In other cases, the group was fairly certain that the infiltrate was Quilty, but could not prove it without

Table 3. Concordance Rates Between Local and Study Pathologists, by Local ISHLT Grade

Local pathologist	*n*	Study Pathologist 1	Study Pathologist 2	Study Pathologist 3	Average
Grade 0	416	88%	83%	91%	87%
Grade 1A, 1B	174	55%	53%	37%	48%
Grade 2	159	18%	8%	26%	17%
Grade 3A, 3B	78	18%	46%	55%	40%

Table 4. Study Pathologist Inter-reader Variability, by Local ISHLT Grade

		Number of study pathologists agreeing on ISHLT grade			
Local grade	Number	0	2	3	Concordance
Grade 0	416	3	81	332	93%
Grade 1A, 1B	174	22	86	66	71%
Grade 2	159	33	93	33	60%
Grade 3A, 3B	78	19	41	18	58%
Total	827	77 (9.3%)	301 (36.4%)	449 (54.3%)	79%

additional serial sections to demonstrate linkage to the endocardium. In these cases, the pathologists used their best judgment of what the infiltrate represented (Quilty or rejection), and made a note that a more definitive diagnosis would require confirmation by serial sections.

Of the 14 Grade 3A cases, 12 were confirmed by consensus review, but only 5 of the original local Grade 3A cases were seen as such by the consensus group. Of the 22 local Grade 2 cases, only 2 were considered Grade 2 by the group and 6 were considered either Grade 2 or 3. Of the 16 Grade 2 local diagnoses that were "downgraded," 14 had a Quilty lesion that may have been mistaken for rejection by local pathologists. Of the 9 local Grade 3A cases that were downgraded, 5 had Quilty lesions that may have contributed to the local diagnosis.

Based on the case reviews at the consensus meeting, 22 biopsy cases were selected for serial sectioning to confirm or reject the diagnosis of Quilty lesions. After examination of serial sections, one study pathologist (C.M.) re-assigned ISHLT grades and re-evaluated each case for Quilty A and B lesions. Of 18 local Grade 2 or 3A cases, 17 were downgraded to Grade 0 or 1A after serial sectioning (12 of 12 Grade 2). Of these 17 that were downgraded, 10 had confirmed Quilty B lesions that were the likely cause of the discrepancy.

Clinical Correlations for Local and Study Pathologists Grading

We also looked for clinical correlates for the re-classification of local biopsy grades by study pathologists. Patients with biopsies graded by local pathologists as Grade 2 to 3B and *confirmed* by all 3 study pathologists ($n = 50$) showed worse hemodynamic characteristics at biopsy (defined as cardiac index <2.5 liters/min/m^2, not by echocardiography) than did patients with local Grade 2 to 3B biopsies that were downgraded by all 3 study pathologists ($n = 78$) ($p = 0.038$, relative risk [RR] = 2.18). Local Grade 3A and 3B rejection patients, who were *confirmed* by at least 2 of 3 study pathologists ($n = 54$), were more likely to die within 180 days compared with local Grade 3A and 3B rejection patients who were downgraded by at least 2 of 3 study pathologists to Grade 0 or 1A ($n = 38$) ($p = 0.005$).

DISCUSSION

The variability between pathologists in assigning ISHLT biopsy grades to cardiac allograft biopsies has been noted previously.[8–10] The contribution of the ISHLT Grade 2 histologic pattern to inter-observer variability in biopsy grading has also been recognized.[3,6,11] There has also been, perhaps in response to this variability in diagnosis, a wide variety of clinical responses to asymptomatic Grade 2 biopsy.[6,12–15] It is therefore important for those in clinical management to better understand the origins of variability in histologic diagnosis and develop methods to minimize the extent and impact of this variability.

In this report, the greatest variability between local and study pathologists was in the diagnosis of ISHLT Grade 2 (only 17% agreement with centralized average), with the study pathologists significantly less likely to make the diagnosis of Grade 2 rejection. In a sub-group of 18 cases, the revised diagnosis followed serial sectioning of paraffin blocks to confirm the diagnosis of nodular endocardial infiltrate. The importance of additional sections has been stressed by Fishbein et al,[6] and

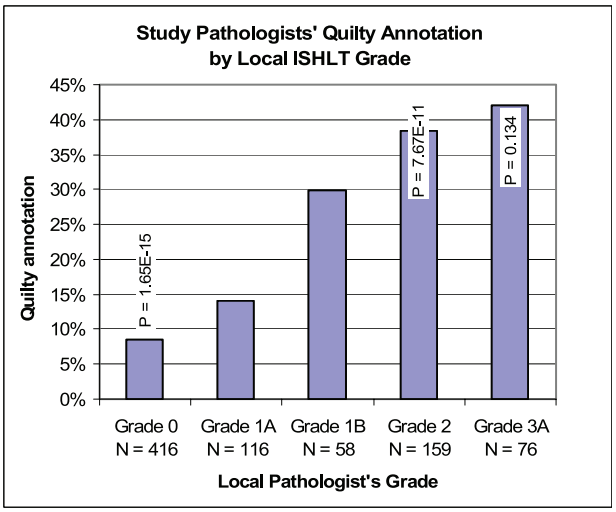

Figure 2. The percentage of cases, by locally assigned ISHLT grade, in which study pathologists made the additional notation of the presence of a Quilty lesion. Significantly more Quilty lesions are reported in local Grade 2 and Grade 3A biopsies than in other grades.

Pathologist	New ISHLT Grade	# of cases	% of cases	Quilty lesions noted #	%	P value
Study Pathologist 1	Grade 0-1B	120	75%	94	59.1%	0.006
Study Pathologist 1	Grade 2-3B	39	25%	21	13.2%	
Study Pathologist 2	Grade 0-1B	128	81%	48	30.2%	0.803
Study Pathologist 2	Grade 2-3B	31	19%	13	8.2%	
Study Pathologist 3	Grade 0-1B	90	57%	25	15.7%	0.262
Study Pathologist 3	Grade 2-3B	69	43%	13	8.2%	
Average of Study Pathologists	Grade 0-1B	113	71%	55.7	35.0%	0.106
Average of Study Pathologists	Grade 2-3B	46	29%	15.7	9.9%	

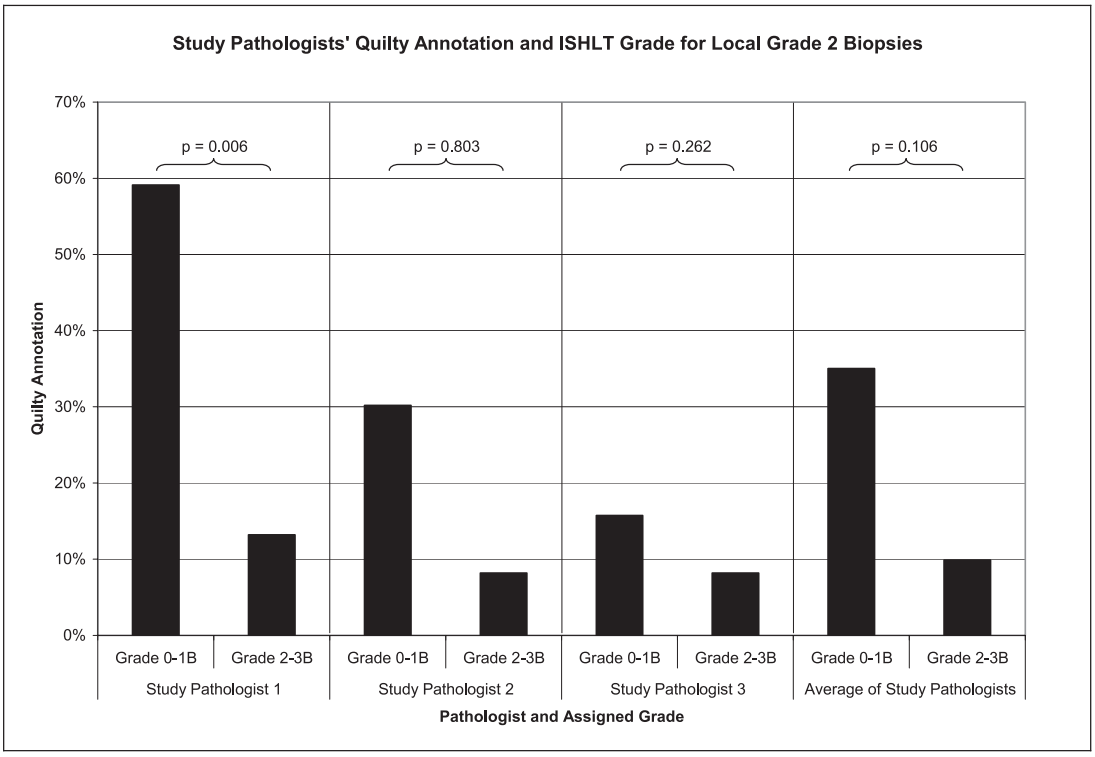

Figure 3. Local Grade 2 biopsies as reviewed by study pathologists. Each biopsy is listed as either "downgraded" to 0, 1A or 1B or "maintained/upgraded" as Grade 2, 3A or 3B. The first study pathologist was significantly more likely to note a Quilty lesion in association with downgraded biopsies than in maintained/upgraded biopsies. The other two pathologists showed a trend but not a significant association.

confirmed by our experience. However, approximately 100 other biopsies, diagnosed by local pathologists as Grade 2, were also "downgraded" to Grade 0, 1A or 1B by study pathologists without review of additional hematoxylin–eosin-stained sections or immunohistochemical studies. The reason for these alternative diagnoses is not clear and merits further attention.

The contribution of Quilty lesions to diagnostic variability is reflected in the strong association of local biopsy grade with the frequency of notation of a Quilty lesion by study pathologists in Figures 2 and 3. Some 71% of local Grade 2 biopsies were downgraded to Grade 0, 1A or 1B by study pathologists and 38% of local Grade 2 biopsies were noted to have Quilty lesions contributing to the diagnostic variability. Similarly, 60% of local Grade 3A or 3B biopsies were downgraded to Grade 0, 1A, 1B or 2 by study pathologists and 67% of these (42% of all local 3A and 3B biopsies downgraded by central readers) were noted to have Quilty lesions.

One additional factor contributing to the downgrades of local biopsy grade by the study pathologists may have been their more strict application of the definition of "Grade 2′" biopsy as "only one focus of inflammatory infiltrate (large aggressive lymphocytes with or without eosinophilia), which is sharply circumscribed."[1] This definition does not include the more diffuse infiltrates

of histologic Grade 1B or 3A. Mistaking the original diagnostic working formulation as a linear scale from 0 to 4 may have led some pathologists to classify diffuse infiltrates, intermediate in density between Grade 1B and 3A, as Grade 2 histology. The study pathologists classified biopsies with this histology as either 1B or 3A or 3B as appropriate.

Another influence on study pathologists may have been experience and familiarity with the literature indicating that most (>90%) Grade 2 infiltrates are actually extensions from Quilty lesions. This knowledge will certainly bias against a diagnosis of Grade 2 rejection.

Recognizing this statistical probability, the study pathologists may have focused more closely on the composition of the dense infiltrate. Localized or circumscribed dense lesions with prominent capillaries, plasma cells and lymphocytes with peripheral clumping of chromatin, suggestive of B-lymphocyte differentiation, were judged to be Quilty lesions rather than Grade 2 even if they did not, in the sections examined, show a connection to the endocardium. The network of vascular channels within Quilty lesions is distinctive. Also, myocytes are infrequently seen within dense Quilty lesions, whereas they are seen more frequently in the infiltrates of rejection.

One additional source of variability between local readers and the study pathologists could be the material available for review. All centers submitted multiple levels from each case on 1 to 11 slides (average = 1.47 slides/biopsy); the average number of pieces per biopsy was 5.4. We were dependent on the local pathologists to send the slide on which their diagnosis was based but this may not have always been the case. It is possible that the study pathologists would have come to a different diagnosis had they reviewed all sections available to the local pathologists. However, as noted previously, having additional sections from a biopsy increases not only the likelihood of recognizing rejection, but also increases the ability to diagnose Quilty lesions. Indeed, as the likelihood of any individual biopsy to contain a Quilty lesion is greater than the likelihood of having Grade 3A, 3B or 4 rejection, having additional levels to study could increase the rate of diagnosis of Quilty lesions relative to rejection.

It is also possible local readers preferred a "false-positive" reading, indicating the biopsy represents rejection, to a "false-negative" reading, indicating no rejection. A false positive results in a steroid boost, which is, by itself, not associated with immediate adverse outcome. A false negative runs the risk of missing rejection, possibly resulting in rapid clinical deterioration of the patient and an adverse clinical outcome.

It is also clear from Tables 3 and 4 that there was considerable variation in biopsy grade assignment by the study pathologists. This variability was modest for local Grades 0, 1A and 1B, but was considerable for Grades 2, 3A and 3B. In light of the contribution of nodular endocardial infiltrates to diagnostic inconsistencies between local and study pathologists, it is reasonable to assume they also contributed to discrepancies between study pathologists. Only in a small sub-set of cases were additional studies (serial sections) performed to more sensitively assess the biopsies for Quilty lesions. Diagnostic consistency may be increased for all pathologists by the use of additional hematoxylin–eosin-stained levels through biopsies as well as immunohistochemical stains on additional slides.[16,17] Further work is necessary to better define the cellular composition of Quilty lesions and cellular rejection to facilitate the histologic distinction of these two entities.

Implications for the ISHLT System—Potential Role of Molecular Testing in Clarification

The post-transplant medical management of cardiac allograft recipients relies on multiple factors, including, but not limited to: physical examination, laboratory studies, catheterization data and the endomyocardial biopsy. None of these tests is sufficiently sensitive and specific to provide a single "gold standard" for the diagnosis of rejection or graft quiescence. The endomyocardial biopsy procedure is expensive, requires considerable time and resources from health-care providers and institutions, and is invasive and inconvenient for patients. Our study has shown that Grade 2 histology and nodular endocardial infiltration contribute to significant variability between pathologists in biopsy interpretation. Indeed, with a diminishing incidence of biopsy-proven rejection, the rate of diagnostic variability exceeds the incidence of biopsy-proven acute cellular rejection. The overdiagnosis of rejection leads to unnecessary therapy for rejection and immunosuppression with the added risk of long-term complications from these therapeutic agents.

The CARGO study was directed toward a peripheral blood–based test using real-time polymerase chain reaction to provide a sensitive and reproducible means to monitor the early events in cardiac allograft rejection, and thereby eliminate the need for some endomyocardial biopsies. These peripheral blood tests may also provide a means to diagnose antibody-mediated rejection, to understand the mechanisms of "biopsy-negative" rejection, to predict future acute cellular allograft rejection and vasculopathy, and thereby guide immunosuppressive therapy. Unfortunately, the variability in the grading of heart transplant biopsies suggests the biopsy itself may not be a true gold standard against which all subsequent tests should be compared; this has clear implications for the evaluation of any new molecular diagnostic test if the only end-point is comparison

with biopsy grade. Multifactorial end-points combining clinical, hemodynamic and biopsy data would provide a better standard. Indeed, the correlation of these multiple factors and peripheral blood gene expression with biopsy histology may provide a basis for further refining of the biopsy grading system by providing insight into the histologic features that best correlate with immunologic status and clinical outcomes.

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APPENDIX

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