Laboratory Tests for Organ Transplant Rejection

Effective: October 1, 2025

Next Review: May 2026
Last Review: September 2025

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Laboratory tests have been explored as an alternative or adjunct to biopsy. These laboratory tests are intended to screen for, estimate risk for, detect, and/or to rule out rejection following organ transplantation.

MEDICAL POLICY CRITERIA

I. The use of peripheral blood gene expression profiling tests (e.g., AlloMap) in the management of patients after heart transplant may be considered **medically necessary** when all of the following are met (A. – D.):

A. The patient is at least 15 years old; and

B. The patient is at least 6 months post heart transplant; and

C. There is not documentation of signs and symptoms that are attributed to heart transplant rejection (see Policy Guidelines); and

D. The patient has no history of treatment for heart transplant rejection.

II. The use of peripheral blood gene expression profiling tests in the management of patients before or after organ transplantation is considered **investigational** when Criterion I. is not met or for organs other than the heart.

III. The measurement of volatile organic compounds to assist in the detection of heart transplant rejection is considered **investigational**.

IV. The use of peripheral blood measurement of donor-derived cell-free DNA in the management of patients after organ transplantation, including but not limited to the detection of acute transplant rejection or transplant graft dysfunction, is considered **investigational**.

V. The simultaneous use of peripheral blood measurement of donor-derived cell-free DNA and gene expression profiling tests in the management of patients before or after organ transplantation is considered **investigational.**

VI. The measurement of immune response of recipient lymphocytes to donor lymphocytes in cell culture to assess the likelihood of acute cellular rejection after renal, liver, and/or small bowel transplantation is considered **investigational**.

VII. The use of gene expression profiling tests on biopsy tissue (e.g., Molecular Microscope® Diagnostic System) to estimate transplant rejection risk is considered **investigational**.

VIII. The measurement of urinary CXCL10/IP-10 chemokines to monitor for rejection or determine the need for graft biopsy after renal transplant is considered **investigational**.

IX. The measurement of serum non-HLA autoantibodies in the management of patients after organ transplantation, including but not limited to the detection of acute rejection or transplant graft dysfunction, is considered **investigational.**

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Heart transplant rejection risk includes signs or symptoms that can be attributed to rejection. These may include orthopnea, shortness of breath, paroxysmal nocturnal dyspnea, syncope, chest pain, palpitations, nausea, loss of appetite, weight gain, edema, arrhythmias, oliguria, and hypotension.

The AlloMap tests (CareDx) are gene expression profiling tests that use peripheral blood samples to assess for rejection after heart (Criterion I), kidney, and liver transplant (Criterion II).

The Clarava and Tuteva™ tests (Verici Dx), and TruGraf™ Kidney are gene expression profiling tests that use peripheral blood samples to assess for rejection after kidney transplant. (Criterion II).

The HeartsBreath™ test measures breathe markers of oxidative stress (Criteria III).

AlloSure is a commercially available, next-generation sequencing (NGS) assay which quantifies the fraction of donor-derived cell-free DNA (dd-cfDNA) in renal transplant recipients, relative to total cfDNA, by measuring single nucleotide variants (Criterion IV).

The Prospera test (Natera) is also a dd-cfDNA test for renal transplant rejection (Criterion IV).

The HeartCare (CareDx) test combines AlloMap gene expression profiling test with AlloSure Heart measurement of dd-cfDNA.

The LIFECODES non-HLA antibody kit and LABScreen autoantibody panels are tests designed to detect non-HLA autoantibodies to monitor organ transplant rejection.

LIST OF INFORMATION NEEDED FOR REVIEW

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of gene expression profiling test
2. Relevant billing codes
3. Medical records related to this test
    - History and physical exam
    - Date of heart transplant

CROSS REFERENCES

1. Heart Transplant, Transplant, Policy No. 2

BACKGROUND

HEART TRANSPLANT REJECTION

After heart transplantation, patients are monitored for cellular rejection by endomyocardial biopsies that are typically obtained from the right ventricle. The interval between biopsies varies among clinical centers. A typical schedule is weekly for the first month, once or twice monthly for the following six months, and several times (monthly to quarterly) between six months and one-year post transplant. Surveillance biopsies may also be performed after the first postoperative year; e.g., on a quarterly or semi-annual basis. Due to the low rate of rejection after one year, some centers no longer routinely perform endomyocardial biopsies after a year in patients who are clinically stable.

Endomyocardial biopsy is invasive and carries significant risk of adverse effects. Additionally, while endomyocardial biopsy is considered the gold standard for assessing heart transplant rejection, biopsy may be limited by a high degree of interobserver variability in grading of results and the significant morbidity and even mortality that can occur with the biopsy procedure. Also, the severity of rejection may not always coincide with the grading of the rejection by biopsy, and biopsy cannot be used to identify patients at risk of rejection, limiting the ability to initiate therapy to interrupt the development of rejection. For these reasons, endomyocardial biopsy is considered a flawed gold standard.

Therefore, noninvasive methods of detecting cellular rejection have been explored. It is hypothesized that noninvasive tests will assist in determining appropriate patient management and avoid overuse or underuse of treatment with steroids and other immunosuppressants that can occur with false-negative and false-positive biopsy reports.

Many non-invasive techniques are commercially available for the detection of heart transplant rejection. These include the HeartsBreath™ test which measures breath markers of oxidative

stress, the AlloMap® test which provides gene expression profiling of RNA obtained from peripheral blood samples, and Allosure® Heart, which measures donor derived cell-free DNA in peripheral blood.

Noninvasive Heart Transplant Rejection Tests

HeartsBreath™ Test

The Heartsbreath™ test (Menssana Research, Inc) measures breathe markers of oxidative stress non-invasively and is based on the understanding that in heart transplant recipients, oxidative stress appears to accompany allograft rejection. This rejection degrades membrane polyunsaturated fatty acids and evolving alkanes and methylalkanes, which are excreted as volatile organic compounds (VOC) in breath. The Heartsbreath™ test analyzes the breath methylated alkane contour (BMAC), which is derived from the abundance of C4 to C20 alkanes and monomethylalkanes.

AlloMap® Test

Another approach, the AlloMap® test (CareDx, formerly Xdx, Inc.), focuses on patterns of gene expression of immunomodulatory cells as detected in the peripheral blood. For example, microarray technology permits the analysis of the gene expression of thousands of genes, including those with functions that are known or unknown. Patterns of gene expression can then be correlated with known clinical conditions, permitting a selection of a finite number of genes to compose a custom multi-gene test panel, which can then be evaluated using polymerase chain reaction (PCR) techniques. The test applies an algorithm to the results, which produces a single score that considers the contribution of each gene in the panel. The manufacturer website states that a lower score indicates a lower risk of graft rejection; the website does not cite a specific cut-off for a positive test.[1]

Additional Tests

Other non-invasive laboratory-tested biomarkers of heart transplant rejection have been evaluated. These include brain natriuretic peptide, dd-cfDNA (discussed below), troponin, non-HLA autoantibodies, and soluble inflammatory cytokines. Most of these have had low diagnostic accuracy in diagnosing rejection. Preliminary studies have evaluated the association between heart transplant rejection and micro-RNAs or high-sensitivity cardiac troponin in cross-sectional analyses, but the clinical use has not been evaluated.[2 3]

RENAL TRANSPLANT REJECTION

Allograft dysfunction is typically asymptomatic and has a broad differential, including graft rejection. Diagnosis and rapid treatment are recommended to preserve graft function and prevent loss of the transplanted organ. For a primary kidney transplant, graft survival at one year is 94.7%; at five years, graft survival is 78.6%.[4]

Surveillance of transplant kidney function relies on routine monitoring of serum creatinine, urine protein levels, and urinalysis.[5] Allograft dysfunction may also be demonstrated by a drop in urine output or, rarely, as pain over the transplant site. With clinical suspicion of allograft dysfunction, additional noninvasive workup including ultrasonography or radionuclide imaging may be used. Renal biopsy allows definitive assessment of graft dysfunction and is typically a percutaneous procedure performed with ultrasonography or computed tomography guidance. Biopsy of a transplanted kidney is associated with fewer complications than biopsy of a native

kidney, as the allograft is typically transplanted more superficially than a native kidney. Renal biopsy is a low risk invasive procedure that may result in bleeding complications; loss of a renal transplant, as a complication of renal biopsy, is rare.[6] Kidney biopsies allow for diagnosis of acute and chronic graft rejection, which may be graded using the Banff scale.[7 8] Pathologic assessment of biopsies demonstrating acute rejection allows clinicians to further distinguish between acute cellular rejection (ACR) and antibody-mediated rejection (AMR), which are treated differently.

The Pleximark™ test from Plexision measures the immune response of recipient lymphocytes to donor lymphocytes in cell culture and has been proposed to predict the likelihood of acute cellular rejection after renal transplantation.

The Clarava™ and Tuteva™ tests from Verici Dx, and the TruGraf® test from Eurofins Transplant Genomics are gene expression tests that use peripheral blood to generate risk scores for renal transplant rejection. The Clarava™ test is marketed for use prior to transplantation, while the Tuteva™ and TruGraf® tests are marketed for use following transplantation.

Measurement of urinary biomarkers, such as C-X-C motif chemokine ligand 10 (CXCL10), also known as interferon-inducible protein-10 (IP-10), have been proposed as noninvasive tests for early detection of rejection that may reduce the need for unnecessary biopsies. Other urinary biomarkers that are under study for detection of renal transplant complications include CXCL9, urinary perforin levels, urinary mRNA transcripts, and urinary dd-cfDNA levels.

DONOR-DERIVED CELL-FREE DNA

Cell-free DNA (cfDNA), released by damaged cells, is normally present in healthy individuals.[9] In patients who have received transplants, donor-derived cfDNA (dd-cfDNA) may be additionally present. It is proposed that allograft rejection, which is associated with damage to transplanted cells, may result in an increase in dd-cfDNA. AlloSure®, Viracor TRAC™ dd-cfDNA, and myTAIHEART are commercially available assays which quantify the fraction of dd-cfDNA in transplant recipients, relative to total cfDNA, by measuring single nucleotide variants (SNVs). Separate genotyping of the donor or recipient is not required for some tests. Each test has a list of conditions that make the test not suitable for a given patient, such as receiving a transplant from a monozygotic (identical) twin and pregnancy. There are dd-cfDNA tests available for heart, kidney, and lung transplants.

Tests for Transplant Rejection that Evaluate Biopsy Specimens

The Molecular Microscope® Diagnostic System (MMDX, One Lambda, Thermo Fisher Scientific, Inc.) offers laboratory-developed tests that measure mRNA transcript levels in endomyocardial or kidney biopsy specimens and applies an algorithm to score the results.[10] The MMDx Kidney & Heart tests can help stratify the risk for conditions like T-cell mediated rejection (TCMR), antibody-mediated rejection (ABMR), acute and chronic injury, atrophy fibrosis, and arterial hyalinosis.

REGULATORY STATUS

Both the Heartsbreath™ and AlloMap® tests have received approval from the US Food and Drug Administration (FDA):

- In 2004, the Heartsbreath™ test received approval from the FDA through a humanitarian device exemption. The Heartsbreath™ test is indicated for use as an aid in the diagnosis of grade 3 (significant) heart transplant rejection in patients who have received heart transplants within the preceding year. The test is intended to be used as an adjunct to, and not as a substitute for, endomyocardial biopsy. It is also limited to patients who have had endomyocardial biopsy within the previous month.

- AlloMap® received 510k clearance from the FDA for use in conjunction with clinical assessment to identify heart transplant recipients with stable allograft function. The test is intended for patients at least 15 years-old who are at least two months post-transplant and who have a low probability of moderate/severe transplant rejection.

EVIDENCE SUMMARY

The principal outcomes associated with detection of acute heart transplant rejection or graft dysfunction include hemodynamic compromise, graft dysfunction, and/or death. Outcomes relating to use of laboratory tests (such as Heartsbreath™ or AlloMap®) proposed for adjunctive use in heart transplant rejection are best understood by comparing outcomes of patients receiving endomyocardial biopsy alone to those receiving biopsy with the laboratory test. Data from adequately powered, blinded, randomized controlled trials (RCTs) are required to control for baseline differences between groups and determine whether additional testing provides a significant advantage over the standard of care in the proposed uses of these laboratory tests.

HEARTSBREATH™ TEST

A single non-randomized study was published in 2004 on the use of the Heartsbreath™ test. No subsequent studies that evaluated use of the Heartsbreath™ test to assess for graft rejection have been identified.

The FDA approval of the Heartsbreath™ test was based on the results of the National Heart Lung and Blood Institute-sponsored Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (HARDBALL) study.[11] The HARDBALL study was a three-year multicenter study of 1,061 breath samples in 539 heart transplantation patients. Prior to scheduled endomyocardial biopsy, patient breath was analyzed by gas chromatography and mass spectroscopy for VOCs. The amount of C4 to C20 alkanes and monomethylalkanes was used to derive the BMAC. The BMAC results were compared with subsequent biopsy results as interpreted by two readers using the International Society for Heart and Lung Transplantation biopsy grading system as the "gold standard" for rejection.

The authors of the HARDBALL study reported that the abundance of breath markers of oxidative stress was significantly greater in grades 0, 1, or 2 rejection than in healthy normal subjects. However, in grade 3 (now grade 2R) rejection, the abundance of breath markers of oxidative stress was reduced, most likely due to accelerated catabolism of alkanes and methylalkanes that comprised the BMAC. The authors also reported that in identifying grade 3 rejection, the negative predictive value of the breath test (97.2%) was similar to endomyocardial biopsy (96.7%), and that the breath test could potentially reduce the total number of biopsies performed to assess for rejection in patients at low risk for grade 3 rejection. The sensitivity of the breath test was 78.6%, versus 42.4% with biopsy. However, the breath test had lower specificity (62.4%) and a lower positive predictive value (5.6%) in

assessing grade 3 rejection than biopsy (specificity 97%, positive predictive value 45.2%). Additionally, the breath test was not evaluated in grade 4 rejection.

GENE EXPRESSION PROFILING

AlloMap® Test

Clinical Validity

Kanwar (2021) published data from the Outcomes AlloMap Registry (OAR) indicating that asymptomatic or active cytomegalovirus infection is associated with significantly higher AlloMap scores among heart transplant recipients compared to those without infection, even in the absence of acute rejection, potentially resulting in unnecessary biopsies among surveillance patients.[12] Donor-derived cell-free DNA levels measured by the AlloSure Heart test available for a small subset of samples (5.3%) were not significantly different between groups. The authors concluded that further assessment of the combined use of AlloMap and AlloSure scores is required to determine if this will improve differentiating infection-related from rejection-related immune activation. The combined use of these tests, commercially available as HeartCare (CareDx), is addressed below.

Patterns of gene expression for development of the AlloMap® test were studied in the Cardiac Allograft Rejection Gene Expression Observation (CARGO) study, which included eight U.S. cardiac transplant centers enrolling 650 cardiac transplant recipients.[13] The study included discovery and validation phases. In the discovery phase, patient blood samples were obtained at the time of endomyocardial biopsy, and the expression levels of more than 7,000 genes known to be involved in immune responses were assayed and compared with the biopsy results. A subset of 200 candidate genes were identified that showed promise as markers that could distinguish transplant rejection from quiescence, and from there, a panel of 11 genes was selected that could be evaluated using polymerase chain reaction (PCR) assays. A proprietary algorithm is applied to the results of the analysis, producing a single score that considers the contribution of each gene in the panel.

The validation phase of the CARGO study, published in 2006, was prospective, blinded, and enrolled 270 patients.[13] Primary validation was conducted using samples from 63 patients independent from discovery phases of the study and enriched for biopsy-proven evidence of rejection. A prospectively defined test cutoff value of 20 resulted in a sensitivity of 84% for patients with moderate/severe rejection, but a specificity of 38%. Of note, in the “training set” used in the study, these rates were 80% and 59%, respectively. The authors evaluated the 11-gene expression profile on 281 samples collected at one year or more from 166 patients who were representative of the expected distribution of rejection in the target population (and not involved in discovery or validation phases of the study). When a test cutoff of 30 was used, the NPV (no moderate/severe rejection) was 99.6%; however, only 3.2% of specimens had grade 3 or higher rejection. In this population, grade 1B scores were found to be significantly higher than grade 0, 1A, and 2 scores, but similar to grade 3 scores. The sensitivity and specificity for determining quiescent versus early stages of rejection was not addressed in this study; however, it was addressed in a 2016 study.[14]

Crespo-Leiro (2016) published a reanalysis of the CARGO II data to clinically validate the GEP test performance.[14] Blood samples for AlloMap® were collected during post-transplant surveillance and were obtained at least 55 days post-transplantation; >30 days after transfusion of blood products; >21 days after administration of ≥20 mg/day of prednisone; and

>60 days after treating a prior rejection. Four hundred and ninety-nine patients had 1,579 visits with paired endomyocardial biopsy histopathology rejection grades and GEP scores that met inclusion criteria for the study analyses. The reference standard for rejection status was based on histopathology grading of tissue from endomyocardial biopsy. Results indicated that a GEP test score of ≥34 (patients who are more than six months post-transplantation) corresponded to histology-based grade ≥3A (2R) rejection with a positive predictive value (PPV) of 4.0% at two to six months post-transplantation, and 4.3% at >6 months post-transplantation. The negative predictive values (NPVs) were 98.4% at two to six months post-transplantation and 98.3% at more than six months post-transplantation. In both time windows, the NPVs increased from 98.3 to >99.0% for decreasing threshold values below 34. The corresponding PPVs decreased from 4.3 to 2.1. Post-CARGO clinical observations have also been published.[15] The multicenter work group identified a number of factors that can affect AlloMap® scores, including the time post-transplant, corticosteroid dosing, and transplant vasculopathy.[15 16] Scores of 34 or higher were considered positive. Analysis of data from a number of centers collected post-CARGO showed that at one year or more post-transplantation, an AlloMap® threshold of 34 had a PPV of 7.8% for scores of 3A/2R or more on biopsy and a NPV of 100% for AlloMap® scores below 34. There is insufficient information in this study to determine whether there are potential study biases in this report. These findings were limited due to a very low number of rejection events; only five biopsy samples (2.4%) were found to have a grade of 2R or greater. At one year, 28% of the samples showed an elevated AlloMap® score (>34) even though there was absence of evidence of rejection on biopsy. The significance of chronically elevated AlloMap® scores in the absence of clinical manifestation of graft dysfunction and the actual impact on the number of biopsies performed is currently unknown.

A similar analysis by Fujita (2017) evaluated the longer-term predictive value of AlloMap® in a group of 46 patients from the CARGO II trial who survived at least one year after transplant.[17] Mean AlloMap® scores at 6, 9, 12, and 18 months posttransplant were not significantly different from one another, and there was no significant difference in mortality between those with scores about the median and those below at any time point. The authors also analyzed changes in Allomap® scores between different time points and found that only those with an increase in score between six and nine months posttransplant had higher mortality. Changes at all other times were not significantly associated with mortality. The authors concluded that a nine-month score that is less than 1.02-fold of the six-month score had a NPV of 100%, but that isolated scores at any of the time points were not correlated with survival.

Moayedi (2019) published results from the Outcomes AlloMap® Registry (OAR), a prospective, multicenter observational study, which included 1,504 heart transplant patients age 15 and older.[18] Among these patients, survival at one, two, and five years after transplant was 99%, 98%, and 94%, respectively. No association was seen between GEP score and coronary allograft vasculopathy, non-cytomegalovirus infection, or cancer.

Clinical Utility

Kobashigawa (2015) published results of a pilot RCT evaluating the use of the AlloMap® test in patients who were 55 days to six months posttransplant.[19] The study design was similar to that of the IMAGE RCT described below: 60 subjects were randomized to rejection monitoring with AlloMap® or with endomyocardial biopsy at prespecified intervals of 55 days and 3, 4, 5, 6, 8, 10, and 12 months posttransplant. The threshold for a positive AlloMap® test was set at 30 for patients two to six months posttransplant and 34 for patients after six months

posttransplant, based on data from the CARGO study. Endomyocardial biopsy outside of the scheduled visits was obtained in either group if there was clinical or echocardiographic evidence of graft dysfunction and for the AlloMap® group if the score was above the specified threshold. The incidence of the primary outcome at 18 months posttransplant (composite outcome of first occurrence of death or retransplant, rejection with hemodynamic compromise, or allograft dysfunction due to other causes) did not differ significantly between the AlloMap® and biopsy groups (10% vs 17%, p=0.44). The number of biopsy-proven rejection episodes (ISHLT ≥2R) within the first 18 months did not differ significantly between groups (three in the AlloMap® group vs one in the biopsy group, p=0.31). Of the rejections in the AlloMap® group, one was detected after an elevated routine AlloMap® test, while two were detected after patients presented with hemodynamic compromise. In the AlloMap® group, 29 of 42 biopsies were performed due to elevated AlloMap® scores; four were performed due to signs, symptoms, or echocardiographic manifestations of graft dysfunction; five were performed as part of follow-up assessment for treatment for rejection; and four were performed outside the study protocol. In the biopsy group, 253 biopsies were performed, four of which were performed based on clinical need.

In 2010, results of the Invasive Monitoring Attenuation through Gene Expression (IMAGE) study were published.[20 21] This was an industry-sponsored noninferiority RCT that compared outcomes in 602 patients managed with the AlloMap® test (n=297) or routine endomyocardial biopsies (n=305). The study was not blinded. The study included adult patients from 13 centers who underwent cardiac transplantation between one and five years previously, were clinically stable, and had a left ventricular ejection fraction (LVEF) of at least 45%. To increase enrollment, the study protocol was later amended to include patients who had undergone transplantation between six months and one year earlier; this subgroup ultimately comprised only 15% of the final sample (n=87). Each transplant center used its own protocol for determining the intervals for routine testing. At all sites, patients in both groups underwent clinical and echocardiographic assessments in addition to the assigned surveillance strategy. According to the study protocol, patients underwent biopsy if they had signs or symptoms of rejection or allograft dysfunction at clinic visits (or between visits) or if the echocardiogram showed a LVEF decrease of at least 25% compared with the initial visit. Additionally, patients in the AlloMap® group underwent biopsy if their test score was above a specified threshold; however, if they had two elevated scores with no evidence of rejection found on two previous biopsies, no additional biopsies were required. The AlloMap® test score varied from 0 to 40, with higher scores indicating a higher risk of transplant rejection. The investigators initially used 30 as the cutoff for a positive score; the protocol was later amended to use a cutoff of 34 to minimize the number of biopsies needed. Fifteen patients in the AlloMap® group and 26 in the biopsy group did not complete the study.

The primary outcome was a composite variable; the first occurrence of (1) rejection with hemodynamic compromise, (2) graft dysfunction due to other causes, (3) death, or (4) retransplantation. The trial was designed to test the noninferiority of gene expression profiling (GEP) with the AlloMap® test compared with endomyocardial biopsies with respect to the primary outcome. Use of the AlloMap® test was considered noninferior to the biopsy strategy if the one-sided upper boundary of the 95% confidence interval (CI) for the hazard ratio (HR) comparing the two strategies was less than the prespecified margin of 2.054. The margin was derived using the estimate of a 5% event rate in the biopsy group, taken from published observational studies, and allowing for an event rate of up to 10% in the AlloMap® group. Secondary outcomes included death, the number of biopsies performed, biopsy-related complications, and quality of life using the 12-Item Short-Form Health Survey (SF-12).

According to Kaplan-Meier analysis, the two-year event rate was 14.5% in the AlloMap® group and 15.3% in the biopsy group. The corresponding HR was 1.04 (95% CI, 0.67 to 1.68). The upper boundary of the CI of the HR (1.68) fell within the prespecified noninferiority margin (2.054); thus, GEP was considered noninferior to endomyocardial biopsy. Median follow-up was 19 months. The number of patients remaining in the Kaplan-Meier analysis after 300 days was 221 in the biopsy group and 207 in the AlloMap® group; the number remaining after 600 days was 137 and 133, respectively. The secondary outcome, death from all causes at any time during the study, did not differ significantly between groups. There were 13 (6.3%) deaths in the AlloMap® group and 12 (5.5%) in the biopsy group (p=0.82). During the follow-up period, there were 34 treated episodes of graft rejection in the AlloMap® group. Only six of the 34 (18%) patients with rejection presented solely with an elevated AlloMap® score. Twenty patients (59%) presented with clinical signs/ symptoms and/or graft dysfunction on echocardiogram, and seven patients had an elevated AlloMap® score plus clinical signs/symptoms with or without graft dysfunction on echocardiogram. In the biopsy group, 22 patients were detected solely due to an abnormal biopsy.

A total of 409 biopsies were performed in the AlloMap® group and 1,249 in the biopsy group. Most of the biopsies in the AlloMap® group, 67%, were performed because of elevated gene-profiling scores. Another 17% were performed due to clinical or echocardiographic manifestations of graft dysfunction, and 13% were performed as part of routine follow-up after treatment for rejection. There was one (0.3%) adverse event associated with biopsy in the AlloMap® group and four (1.4%) in the biopsy group. In terms of quality of life, the physical-health and mental-health summary scores of the SF-12 were similar in the two groups at baseline and did not differ significantly between groups at two years.

A limitation of the study was that the threshold for a positive AlloMap® test was changed partway through the study; thus, the optimal test cutoff remains unclear. Moreover, the study was not blinded, which could have impacted treatment decisions such as whether or not to recommend biopsy, based on clinical findings. In addition, the study did not include a group that only received clinical and echocardiographic assessment, and therefore, the value of AlloMap® testing beyond that of clinical management alone cannot be determined. The uncertain incremental benefit of the AlloMap® test is highlighted by the finding that only 6 of the 34 treated episodes of graft rejection detected during follow-up in the AlloMap® group were initially identified due solely to an elevated gene-profiling score. Since 22 episodes of asymptomatic rejection were detected in the biopsy group, it is likely that the AlloMap® test is not a sensitive test, possibly missing more than half of the episodes of asymptomatic rejection. Because clinical outcomes were similar in the two groups, there are at least two possible explanations. The clinical outcome of the study may not be sensitive to missed episodes of rejection, or it is not necessary to treat asymptomatic rejection. In addition, the study was only statistically powered to rule out more than a doubling of the rate of the clinical outcome, which some may believe is an insufficient margin of noninferiority. Finally, only 15% of the final study sample had undergone transplantation less than one year before study participation; therefore, findings may not be generalizable to the population of patients 6 to 12 months post-transplant.

In a follow-up analysis of data from the IMAGE RCT, Deng (2014) evaluated whether variability in gene expression profiling results were predictive of clinical outcomes.[22] For this analysis, the authors included a subset of 369 patients who had at least two AlloMap® tests done before an event or the study end, and at least one endomyocardial biopsy and one echocardiogram. Patients were included from both arms of the IMAGE RCT. AlloMap® test results were expressed in three ways, as an ordinal score from 0 to 39, a threshold score of 1 or 0,

depending on whether the score was 34 or more or not, and as a variability score, the standard deviation of all of the ordinal scores within a patient. The AlloMap® results were entered into a multivariable regression model to predict the composite end point, defined as a patient's first occurrence of: rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. AlloMap® ordinal score and AlloMap® threshold score were not predictive of the composite outcome. AlloMap® score variability was significantly associated with the composite outcome, with a hazard ratio for a one unit increase in variability of 1.76 (95% CI, 1.4 to 2.3). While this study implies that variability in AlloMap® score may be a prognostic factor, clinical application of this finding is uncertain.

Section Summary

The most direct evidence on the clinical utility of the AlloMap® test comes from one large RCT comparing an AlloMap®-directed strategy with an endomyocardial biopsy-directed strategy for detecting rejection, which found that the AlloMap®-directed strategy was noninferior. The high NPV of AlloMap enables the avoidance of surveillance endomyocardial biopsy and its inherent risks for certain heart transplant recipients who are at low risk for transplant rejection.

Additional Gene Expression Tests for Transplant Rejection

There are additional studies that have examined the use of gene expression testing to predict or detect organ transplant rejection, including renal transplant rejection.[23-26] However, these tests have mainly been used in the research setting and there is very limited evidence of clinical validity or utility.

DONOR-DERIVED CELL-FREE DNA TESTING

Knight (2019) published a systematic review of studies that investigated the use of dd-cfDNA post-transplantation.[27] A total of 95 publications representing 47 studies of kidneys (n=18), livers (n=7), hearts (n=11), kidney-pancreas (n=1), lungs (n=5) and multiorgans (n=5) met inclusion criteria. Besides one single case report, the studies were retrospective (n=19) and prospective (n=29) cohort studies. There was heterogeneity in methods for differentiating between donor-derived and recipient cfDNA and in calculating the proportion of dd-cfDNA. Trends from these studies were reported, but no meta-analysis was completed due to low study quality and high heterogeneity.

Renal Transplant

Systematic Reviews

Sebastian (2024) published a systematic review that evaluated dd-cfDNA for monitoring solid organ transplantation, analyzing 16 articles (two heart, three liver, four kidney, and seven lung transplantations).[28] The review reported that dd-cfDNA levels rise immediately post-transplantation, decline to baseline within days to weeks, and remain stable in non-rejection patients, while showing increased levels during rejection episodes. The magnitude of dd-cfDNA increase varied across organs: dd-cfDNA was four-fold higher in rejection versus stable patients for liver transplant, two-fold higher for kidney transplant, and 13 to 15-fold higher for heart transplant. The reviewers concluded that dd-cfDNA shows promise for detecting organ rejection but lacks specificity as a standalone diagnostic marker and requires standardization of protocols and methodologies for clinical implementation.

Xing (2024) conducted a meta-analysis of 22 studies to assess the diagnostic accuracy of dd-cfDNA in detecting kidney allograft rejection, with results analyzed separately for AMR and any type of rejection.[29] For any rejection, the pooled sensitivity was 59% (95% CI, 48% to 69%), specificity was 83% (95% CI, 76% to 88%), and the AUC was 0.80 (95% CI, 0.76 to 0.83). For AMR, sensitivity was 81% (95% CI, 72% to 87%), specificity was 80% (95% CI, 73% to 85%), and the AUC was 0.87 (95% CI, 0.84 to 0.90). Significant heterogeneity across studies was attributed to differences in study design, dd-cfDNA thresholds, and sample sizes. Subgroup analyses highlighted trade-offs in diagnostic performance based on dd-cfDNA thresholds, with a 1% threshold offering higher specificity (85%) but lower sensitivity (57%) compared with a 0.5% threshold, which provided higher sensitivity (78%) but lower specificity (66%). Despite the high study heterogeneity, the authors concluded that dd-cfDNA shows promise as a biomarker for kidney allograft rejection and emphasized the need for further prospective studies to establish an optimal threshold.

Xiao (2021) published a systematic review and meta-analysis which assessed the clinical validity of dd-cfDNA testing.[30] The review included nine observational studies of the diagnostic accuracy of dd-cfDNA as a potential marker of graft rejection following kidney transplantation. The review authors calculated a pooled sensitivity of 0.70 (95% CI, 0.57-0.81; I^2, 65) and specificity of 0.78 (0.70-0.84; I^2, 75) from six studies evaluating the diagnostic accuracy of dd-cfDNA for any rejection episode. The area under the receiver operating characteristics curve (AUC) was 0.81 (95% CI, 0.77 to 0.84; I^2, 65) with an overall diagnostic odds ratio (DOR) of 8.18 (95% CI, 5.11 to 13.09). Similar pooled estimates were calculated for five studies discriminating antibody-mediated rejection. The authors reported a pooled sensitivity of 0.84 (95% CI, 0.75 to 0.90; I^2, 0) and a specificity of 0.80 (95% CI, 0.75 to 0.84; I^2, 4) with an AUC of 0.89 (95% CI, 0.86 to 0.91) and overall DOR of 20.48 (95% CI, 10.76 to 38.99). Overall, the authors found greater value in dd-cfDNA as a biomarker for antibody-mediated rejection in patients with suspected renal dysfunction than in discriminating a main rejection episode and cite the need for more large-scale, prospective research on the topic.

Wijtvliet (2020) reported a systematic review and meta-analysis of dd-cfDNA as a biomarker for rejection after kidney transplant.[31] A total of 14 studies met inclusion criteria for the systematic review, of which nine were included in the meta-analysis. Huang (2019) and Bloom (2017), discussed in detail below, were included. Overall, the quality was rated moderate or high for each included study. Moderate heterogeneity was identified for antibody-mediated rejection versus no rejection (I^2=40.1%) and antibody-mediated rejection versus T cell-mediated rejection (I^2=31.5%). Median dd-cfDNA fractions were significantly higher in patients with antibody-mediated rejection than patients without rejection (n=283 samples; weighted minimum difference to mean 1.89%). Median dd-cfDNA values were intermediate for patients with T cell-mediated rejection and were not significantly different from either the antibody-mediated rejection or no-rejection groups.

Randomized Controlled Trials

Akifova (2025) published an RCT that compared dd-cfDNA-guided biopsy to a control group that received clinician-guided biopsy.[32] Forty kidney transplant recipients, with prevalent donor-specific anti-HLA antibodies (dnDSA) and estimated glomerular filtration rate of 20 mL/min/1.73 m^2 or higher, but without previous biopsy-proven AMR were followed for 12 months. In both groups, dd-cfDNA was assessed at inclusion and at 1, 3, 6, 9 and 12 months. The threshold to perform biopsy in the dd-cfDNA group was greater than 50 copies per mL, and biopsies for clinical indication could be performed at any point during the study period in

both groups. The primary endpoint was time from study inclusion to diagnosis of active or chronic active AMR. Thirty-nine of 40 patients had functioning grafts at study completion, and 26 patients underwent biopsy, 13 in each group. AMR was diagnosed significantly earlier in the dd-cfDNA group [median 2.8 months, interquartile range (IQR) 1.7 to 5.3 versus median 14.5 months, IQR 13.3 to 16.7, p=0.003]. Longitudinal dd-cfDNA monitoring had 77% positive predictive value and 85% negative predictive value for AMR. This RCT is limited by small sample size, short-term follow-up, is limited to recipients with detectable donor-specific antibodies, and lack of assessment of overall survival, morbid events, or hospitalizations.

Nonrandomized Studies

Hogan (2025) published a retrospective study that evaluated dd-cfDNA for detecting kidney allograft rejection in 196 pediatric kidney transplant recipients, who underwent 367 biopsies.[33] Participants underwent dd-cfDNA assessment at the time of allograft biopsy. Dd-cfDNA assessment alone was compared with standard-of-care monitoring which included history of previous rejection, estimated glomerular filtration rate, and DSA. The researchers found that dd-cfDNA levels significantly correlated with the degree of histological inflammation and were independently associated with allograft rejection (odds ratio 1.89, 95% CI 1.40 to 2.60). While dd-cfDNA alone demonstrated fair discriminatory ability for detecting rejection (AUC 0.76, 95% CI 0.69 to 0.81), combining dd-cfDNA with standard-of-care monitoring significantly improved discrimination from 0.80 to 0.84 (p=0.01).

Sellarés (2025) published a prospective, multi-center study of 230 kidney transplant recipients that evaluated the TruGraf gene expression test and and Viracor-TRAC dd-cfDNA test for non-invasive rejection diagnosis.[34] Neither biomarker alone or in combination accurately diagnosed rejection generally (area under the receiver operator characteristic curve [AUROC] <0.65). TruGraf did not associate with any specific allograft injury. High TRAC levels, when combined with DSA in for-cause biopsies, predicted AMR/mixed rejection or microvascular inflammation (AUROC = 0.817; p<0.001), and this combination outperformed traditional markers like serum creatinine and DSA alone. The authors concluded that they were unable to validate TruGraf and TRAC for diagnosing rejection but that TRAC may be useful to noninvasively diagnose AMR, mixed rejection, and or microvascular inflammation.

Aubert (2024) evaluated the association of dd-cfDNA with kidney AMR in a multinational observational study of 2,882 transplant recipients across 14 centers.[35] Among the derivation cohort (n=1,134), dd-cfDNA levels were significantly elevated in patients with active AMR (mean, 1.15% ± 0.15%) and TCMR (mean, 2.03% ± 1.13%) compared with stable recipients (mean, 0.36% ± 0.02%; p<0.0001). The odds ratio (OR) for rejection associated with dd-cfDNA was 2.28 (95% CI, 1.90 to 2.74; p<0.001) in multivariable analysis. The inclusion of dd-cfDNA improved the AUC for rejection prediction from 0.78 (95% CI, 0.74 to 0.81) to 0.82 (95% CI, 0.78 to 0.85; p=0.0011) over standard of care parameters. Validation in the external cohort (n=1,748) confirmed these findings, with an AUC increase from 0.743 to 0.842 (p<0.001). For subclinical rejection, dd-cfDNA levels demonstrated an OR of 2.20 (95% CI, 1.66 to 2.95; p<0.001) and reclassified 66.3% of subclinical cases over standard monitoring (p<0.001). Sensitivity analyses showed dd-cfDNA associated with AMR at different post-transplantation intervals, including early (less than three months OR, 2.37; 95% CI, 1.51 to 3.73; p<.001) and late (greater than one year OR, 2.52; 95% CI, 1.94 to 3.32; p<0.001) time points. This study is limited by a lack of longitudinal monitoring data for a subset of patients, lack of information on drug adherence, and non-experimental study design.

Bromberg (2024) assessed the utility of dd-cfDNA as an early indicator of kidney transplant rejection in a retrospective analysis of the ProActive registry study (NCT04091984), which included 424 patients with 1,013 dd-cfDNA tests conducted within six months before biopsy.[36] Among the cohort, 6.1% of biopsies revealed AMR, 14.6% showed TCMR, and 79.3% indicated non-rejection. The study reported significantly elevated dd-cfDNA levels up to five months before AMR biopsies (median, 1.04%; IQR 0.45% to 1.18%) and two months before TCMR biopsies, compared with non-rejection biopsies (median, 0.24%; IQR 0.12% to 0.69%; p<0.001). Sensitivity and specificity for dd-cfDNA in predicting AMR were 77% and 84%, respectively, with an AUC of 0.88 for all rejection types. Limitations included the retrospective design, variability in biopsy timing, and the absence of standardized protocols for patient management.

Moein (2024) conducted a retrospective cohort study that evaluated the diagnostic performance of transcriptomic kidney profiling and dd-cfDNA testing in detecting kidney allograft rejection.[37] The study included 136 kidney transplant recipients, of which 127 also underwent concurrent dd-cfDNA testing. Biopsy results identified 33 cases of rejection, comprising AMR (42.4%), TCMR (36.4%), and mixed rejection (21.2%). Transcriptomic kidney profile testing demonstrated an 82.35% sensitivity and 85.49% specificity, while dd-cfDNA testing showed a sensitivity of 56.66% and specificity of 85.56%. The combined use of positive transcriptomic and dd-cfDNA tests detected 51.51% of rejections, whereas combined negative tests were observed in 70.21% of biopsies without rejection. The PPV and NPV for dd-cfDNA testing were 54.83% and 86.45%, respectively, compared with 52.83% and 92.77% for transcriptomic testing. The study found moderate agreement between transcriptomic kidney profile testing and biopsy results (kappa, 0.50; p<0.01) and fair agreement between dd-cfDNA and biopsy results (kappa, 0.37; p<0.01). Transcriptomic testing identified 90.47% of AMR cases but only 57.89% of TCMR cases, whereas dd-cfDNA testing identified 66% of ABMR and 36.84% of TCMR cases. Study imitations include potentially limited generalizability due to the single-center design, retrospective design, and small sample size.

Results from the ongoing Trifecta study (NCT04239703) published by Halloran (2023) provide an assessment of combined dd-cfDNA fraction and absolute values for prediction of active kidney allograft rejection.[38] The study reported data from 280 biopsies that were taken from 272 patients. 97 patients were female and 9% were Black or African-American; other race or ethnicity data were not reported. The mean post-transplant time was 1,353 days. The study found that about half of all AMR is donor specific antibody (DSA)-negative. For specimens with histologically proven AMR, 51% were DSA-negative. Of specimens found to have AMR with the Molecular Microscope System, 56% were DSA-negative. In specimens with AMR, the percentage of dd-cfDNA (75%) was higher than DSA-positivity (44%). In cases with no rejection, 18% showed dd-cfDNA positivity, and 10% were DSA-positive. The authors conclude that dd-cfDNA is superior to DSA in predicting AMR, but the best performance was found with predictions that incorporated both dd-cfDNA and DSA tests.

Huang (2023) conducted a retrospective single institution study to evaluate the association of dd-cfDNA surveillance levels in adult renal transplant patients with transplant outcomes.[39] The study included 317 kidney transplant recipients with a median follow-up of 590 days. Participants were divided into three categories based on their baseline dd-cfDNA levels; low (n=239), moderate (n=43) and high (n=35). Patients in the high category were more likely to have had previous kidney transplant. There was no difference in the percentage of participants in each group that developed DSA (p=0.52). There was only one graft loss during the study period and it was in a low category participant. Ten participants died during the study period;

all had functioning grafts and deaths were not associated with dd-cfDNA levels. Rejection was more likely to occur in patients with higher dd-cfDNA levels (p=0.02), but the researchers were unable to determine if high dd-cfDNA levels reflected actual graft injury or the higher immunologic risk related to previous transplant. The authors concluded that the role of routine dd-cfDNA surveillance in kidney transplant needs further study.

Dandamudi (2022) published a study of longitudinal cfDNA levels in pediatric kidney transplant patients.[40] The study used serial sampling of 290 plasma specimens from 57 children who had kidney transplant between January, 2013 and December, 2019 at a single institution. Using a one percent cutoff, and 109 samples with simultaneous biopsy data, dd-cfDNA had a 33% sensitivity (95% CI, 19% to 52%) in discriminating biopsy-proven acute rejection, but specificity was 96% (95% CI, 90% to 99%).

Puliyanda (2021) evaluated the use of dd-cfDNA in pediatric kidney transplant patients.[41] A total of 67 patients who underwent initial testing with dd-cfDNA as part of routine monitoring or in response to clinical suspicion for rejection were included. Two of the seven patients with clinical suspicion of rejection and a dd-cfDNA score <1% showed evidence of rejection on biopsy. Using a dd-cfDNA of >1% as a marker of rejection, sensitivity was 86% and specificity was 100% (Area Under the Curve [AUC]: 0.996, 0.98 to 1.00; p=0.002).

Stites (2020) assessed clinical outcomes in 79 patients diagnosed with T-Cell Mediated Rejection (TCMR) 1A/borderline rejection with simultaneous AlloSure assessment of dd-cfDNA across 11 centers between June 2017 and May 2019.[42] Timing of testing with respect to the date of transplantation was not reported. Elevated levels of dd-cfDNA (≥0.5%) were detected in 42 (53.2%) patients. No statistically significant differences between dd-cfDNA distributions when stratified by protocol versus for-cause biopsies was detected (p=0.7307). Elevated levels of dd-cfDNA were associated with adverse clinical outcomes compared to patients with low levels (< 0.5%), including decline in eGFR (8.5% versus 0%; p=0.004), de novo DSA formation (40% versus 2.7%; p<0.0001), and future or persistent rejection (21.4% versus 0%; p=0.003). The authors hypothesize that the use of dd-cfDNA may complement histological evaluation and risk stratify patients with TCMR 1A or borderline rejection identified on biopsy and propose the use of reference ranges as opposed to absolute dd-cfDNA cutoff thresholds.

Sigdel (2019) evaluated the diagnostic accuracy of the Prospera dd-cfDNA test in a retrospective analysis of 300 biorepository plasma samples from kidney transplant recipients at a single academic medical center.[43] Of the 300 samples (193 patients), 217 were biopsy-matched with 38 cases of active rejection, 72 cases of borderline rejection, 82 with stable allografts, and 15 cases of other kidney injuries. The sample cohort was demographically diverse, including women (42.5%), Hispanic and Latino patients (34.6%), Black or African American patients (14%), and pediatric patients (20%). Indication for renal transplantation was unknown in 45.6% of samples. The majority of samples (72.3%) were drawn on the day of surveillance (n = 114 [52.5%] patients) or clinically indicated biopsy (n=103 [47.5%] patients). Timing of tests with respect to the date of transplantation was not reported. Biopsies were evaluated by a single pathologist according to 2017 Banff criteria and classified as active rejection or non-rejection (i.e., borderline rejection, other injury, or stable allograft status). Median dd-cfDNA levels were significantly higher in biopsy-proven active rejection (2.32%) versus non-rejection subgroups (0.47%; p <.0001). All subtypes of active rejection could be detected, and median dd-cfDNA did not differ significantly between antibody-mediated (2.2%), T cell-mediated (2.7%), and combined subtypes (2.6%).

The 2019 report by Sigdel also assessed the performance characteristics of eGFR, which was calculated as a function of serum creatinine with adjustments for age, sex, and race based on the Modification of Diet in Renal Disease (MDRD) Study equation.[43] At a cutoff threshold of < 60, the sensitivity and specificity for eGFR were lower compared to dd-cfDNA, at 67.8% (95% CI, 51.3% to 84.2%) and 65.3% (95% CI, 57.6% and 73.0%), respectively, with a corresponding AUC of 0.74 (95% CI, 0.66 to 0.83). However, the relevance of absolute eGFR measurements is limited as dynamic changes in laboratory parameters (eg, serum creatinine elevation, eGFR decline) are used to flag impaired kidney function in clinical practice in the transplant population. Separate eGFR estimates in the for-cause subgroup were not reported. Major limitations of this study include its retrospective design and single-center setting. While the dd-cfDNA cutoff was prespecified, it was based on prior studies of the AlloSure test and may not be optimized for Prospera.

Huang (2019) conducted a single center study that recruited 63 renal transplant patients with suspicion of rejection that had AlloSure assessment of dd-cfDNA within 30 days of an allograft biopsy.[44] Median years from transplant to dd-cfDNA measurement was 2.0 (interquartile range, 0.3 to 6.5). Within this population, biopsy found acute rejection in 34 (54%) of patients; 10 (15.9%) were cell-mediated only, 22 (25.4%) were antibody-mediated only, and 2 (3.2%) were mixed cell-mediated and antibody-mediated. In contrast to the study by Bloom (2017) below, the optimal threshold for a positive dd-cfDNA result was identified as ≥0.74%. For the outcome of any rejection (i.e., cell-mediated, antibody-mediated, or mixed), use of this threshold was associated with an overall sensitivity of 79.4%, specificity of 72.4%, PPV of 77.1%, and NPV of 75.0%. Discrimination of rejection differed by biopsy findings, however. For the subgroup of patients with antibody-mediated rejection, the sensitivity was 100%, specificity was 71.8%, PPV was 68.6%, and NPV was 100%. The dd-cfDNA test did not discriminate rejection in patients with cell-mediated rejection, as evidenced by an AUC of 0.43 (95% CI, 0.17 to 0.66). Major limitations of this study are its small sample size and single-center setting.

The multicenter prospective DART study (Bloom, 2017) recruited both patients who were less than three months after renal transplant (n=245) and renal transplant patients requiring a biopsy for suspicion of graft rejection (n=139).[45] For the primary analysis, active rejection was defined as the combined categories of T cell−mediated rejection, acute/active AMR, and chronic/active AMR as defined by the Banff working groups. Only patients undergoing biopsy were considered; further exclusion of biopsies which were not for cause, had inadequate or incomplete collection of biopsies or corresponding blood samples, or had prior allograft in situ resulted in the main study cohort (n=102 patients, 107 biopsies). Within this population, acute rejection was noted in 27 patients (27 biopsies). After statistical analysis accounting for multiple biopsies from the same patient, the threshold dd-cfDNA fraction corresponding to acute rejection was set to ≥1.0%. In the main study group, this resulted in a sensitivity of 59% (95% CI 44% to 74%) and specificity of 85% (95% CI 79% to 81%) for detecting active rejection vs no rejection. Returning to the original data set including all biopsies performed for clinical suspicion of rejection, 58 cases of acute rejection were diagnosed in 204 biopsies (170 patients). This prevalence was used to calculate the PPV (61%) and NPV (84%). Biopsies performed for surveillance (n=34 biopsies) were excluded from analysis in this study as only one biopsy for surveillance demonstrated acute rejection. Limitations of this study include the absence of a validation data set. Additional analyses of the DART study have reported on associations between first-year AlloSure dd-cfDNA fraction or serial variability and subsequent eGFR decline[46], and combined use of dd-cfDNA and DSA testing to diagnose active antibody-mediated rejection[47 48]

A number of other studies have evaluated associations between dd-cfDNA assays and graft injury or rejection after kidney transplantation.[27 47 49-53] For individuals with a renal transplant who are undergoing surveillance or have clinical suspicion of allograft rejection who receive testing of dd-cfDNA to assess renal allograft rejection, the evidence includes small diagnostic accuracy studies. Relevant outcomes are OS, test validity, morbid events, and hospitalizations. The available evidence does not show how the use of these tests can impact patient health outcomes. Larger prospective studies validating the dd-cfDNA thresholds for active rejection are needed to develop conclusions for each test. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Heart Transplant

Jiménez-Blanco (2025) published a multicenter, prospective observational study that evaluated donor-derived cell-free DNA (dd-cfDNA) as a noninvasive biomarker for monitoring cardiac allograft rejection in 206 heart transplant patients.[54] Analyzing 1,090 paired EMB and dd-cfDNA samples during the first post-transplant year, researchers found that EMB samples showing ACR had significantly higher dd-cfDNA levels compared to those without rejection (0.189% vs 0.095%, p=0.013). A dd-cfDNA threshold of 0.10% demonstrated a 97% negative predictive value for ACR, suggesting utility in ruling out rejection in low-risk patients. The study also identified a significant correlation between dd-cfDNA and N-terminal prohormone of brain natriuretic peptide (NTproBNP) levels. Combining both biomarkers improved diagnostic accuracy (area under ROC curve 0.681 versus 0.603 for dd-cfDNA alone), with the combination achieving a 98.1% negative predictive value using cutoffs of 0.10% for dd-cfDNA and 1,000 UI/ml for NTproBNP.

Akabas (2024) published a retrospective study of dd-cfDNA testing as a non-invasive screening tool for acute rejection and donor-specific antibodies in pediatric heart transplant recipients.[55] The researchers analyzed 471 dd-cfDNA samples from 192 pediatric patients, with 199 tests paired with endomyocardial biopsy and/or DSA testing. At a dd-cfDNA threshold of 0.2%, 77 samples (16.3%) had elevated dd-cfDNA levels (median 0.48%, range 0.21% to 11%). Among abnormal samples with biopsy correlation, only one showed acute cellular rejection (grade 2R) while 13 demonstrated antibody-mediated rejection, with a negative predictive value of 97% for antibody-mediated rejection. This study is limited by retrospective design and lack of long-term follow-up.

Khush (2024) evaluated dual testing with GEP and dd-cfDNA for ACR surveillance in heart transplant patients from 2018 to 2021 in the Surveillance HeartCare Outcomes Registry (SHORE).[56] This multi-center, prospective study of 2,077 participants assessed the utility of combined GEP/dd-cfDNA testing compared with single-test approaches. The incidence of biopsy-proven ACR varied by test result, with dual-negative results yielding the lowest rate (1.5%; 95% CI,1.1% to 2.0%), GEP-positive/dd-cfDNA-negative results at 1.9% (95% CI, 1.4% to 2.6%), dd-cfDNA-positive/GEP-negative results at 4.3% (95% CI, 2.8% to 6.6%), and dual-positive results showing the highest rate of 9.2% (95% CI, 7.1% to 11.9%). Sensitivity for detecting ACR was 59.0% (95% CI, 51.3% to 66.3%) for GEP alone, 44.7% (95% CI, 37.3% to 52.4%) for dd-cfDNA alone, and 32.3% (95% CI, 25.6% to 39.9%) for dual-positive testing. Specificity for dual-positive testing was 91.7% (95% CI, 91.0% to 92.4%), yielding a positive likelihood ratio of 3.90 (95% CI, 3.08 to 4.96), which exceeded that of GEP (1.37) and dd-cfDNA (2.91) individually. Follow-up biopsy testing after dual noninvasive surveillance showed that dual-negative results were followed by biopsies in only 8.8% of cases (95% CI, 8.2% to 9.4%), whereas dual-positive results prompted biopsies in 35.4% (95% CI, 32.0% to 38.9%). In

this registry cohort, the frequency of biopsies declined over time, with second-year biopsies decreasing from 1.5 biopsies per patient in 2017-2018 to 0.9 in 2021. Despite the reduction in biopsy volume, there was 94.9% survival at two years with only 2.7% experiencing graft dysfunction. Study limitations include site-based variability in biopsy interpretation, biopsies not performed at a consistent time post-transplantation, exclusion of AMR data from sensitivity analyses, and non-experimental study design.

Richmond (2023) published data on pediatric (n=60) and adult (n=61) heart transplant recipients (median age, 24.3) prospectively enrolled at eight participating centers from August 2016 to October 2017 and followed patients for up to 12 months.[57] All patients had samples from one or more endomyocardial biopsies post-transplantation with Allosure dd-cfDNA testing within 24 hours prior to biopsy. dd-cfDNA level was blinded to participants and investigators over the study period. Median dd-cfDNA was significantly higher in the patients who had biopsy-defined allograft rejection (ACR or AMR) compared with healthy allograft participants (0.21% versus 09%, p<0.0001). An area under the curve (AUC) analysis yielded an AUC of 0.78 using a pre-defined dd-cfDNA threshold of 14% and resulted in a test sensitivity of 67% and a specificity of 79% (NPV = 94% and PPV = 34%), a sub-group analysis satisfying patients into adult of pediatric patients found similar results (AUC of the adult cohort = 0.81; AUC of the pediatric cohort =0.79).

Rodgers (2023) conducted a retrospective study that compared dd-cfDNA testing with Allosure, which examines 405 single nucleotide polymorphisms (SNPs), to Prospera, which evaluates 13,292 SNPs, in 112 heart transplant patients.[58] Participants were enrolled from October 2020 to January 2022 and had a median age of 60 years. Both tests used a dd-cfDNA threshold value of 15%. Testing with Allosure resulted in a low sensitivity (39%) and high specificity (82%) for identification of acute rejection; the Prospera test had similar characteristics with sensitivity at an identical 39% and a negligible difference in specificity (84%). Between-group comparisons showed no difference between the two tests. PPV with the Allosure test was 6.2% compared to 7% in Prospera testing (p=0.7) and NPV was 98% for both tests (p=0.76). This study is limited by small sample size and retrospective design.

Feingold (2023) conducted a single institution study that compared pediatric and young adult heart transplant outcomes after implementation of dd-cfDNA surveillance to previous outcomes based on EMB-surveillance.[59] Heart transplant outcomes (graft losses, mortality, and EMB case volumes) from September 1, 2016 to July 15, 2019 were compared to outcomes from September 1, 2019 to July 15, 2022. Both cohorts had surveillance EMB at 2 weeks, 6 weeks, and 3 months. Then, the earlier cohort continued EMB surveillance at regular intervals and the later cohort had dd-cfDNA tests followed by EMB only if dd-cfDNA levels were elevated. From September 2019, 120 patients had 236 dd-cfDNA assessments. A total of 43 dd-cfDNA results triggered right heart catheterization/EMB, and of those, four patients were diagnosed with acute rejection. EMB volumes decreased after implementation of dd-cfDNA surveillance (p=0.002), and the incidence of graft loss (p=0.17) and mortality (p=0.23) were not significantly different. In addition to the lack of randomization and single institution data, the study is significantly limited by short follow-up time.

Kim (2022), assessed the clinical validity of the Prospera Heart dd-cfDNA test versus endocardial biopsy for prediction of acute heart transplant rejection.[60] The study included 811 samples (703 prospectively collected and 108 retrospectively collected) from 223 heart transplant patients with a planned biopsy from two U.S. centers. The median patient age was 54 years and 27% were female. Race/ethnicity of the study population was: 54% White, 21%

Hispanic, 12% Black, 6% Asian and 5% other race/ethnicity. The majority (91% [737/811]) of reference standard biopsies were conducted for surveillance, and median dd-cfDNA was lower in the surveillance samples (0.04%) than the for-cause samples (0.22%). The time from transplant to biopsy was 10 weeks, and the total prevalence of acute rejection was 9.0%. Median dd-cfDNA % was 0.58% in patients with acute rejection, although fractions varied according to rejection type/grade and were higher in those with antibody mediated rejection (median range 0.44% to 3.43%) than those with acute cellular rejection (median range 0.045% to 0.13%). In patients without acute rejection, dd-cfDNA % was 0.04. Diagnostic accuracy for three dd-cfDNA fractions were explored: 0.12%, 0.15% and 0.20%. At a cut-off off of 0.12%, sensitivity was 86.6%, specificity was 72.0%, PPV was 23.4%, and NPV 98.2%. Corresponding values at a dd-cfDNA cut-of of 0.15% were 78.6%, 76.9%, 25.1% and 97.3%, and 78.6%, 82.1%, 30.3% and 97.5% at a dd-cfDNA cut-off of 0.20%. This resulted in an AUC for detection of acute rejection of 0.86 (95% CI 0.77 to 0.96). The optimal dd-cfDNA fraction for detection of heart transplant rejection has yet to be established. Limitations of the study include potential selection bias, as only patients with a scheduled biopsy were included in the study, and study authors noted that the prevalence of acute rejection in the study cohort was higher than in other cohorts.

Khush (2019) published performance characteristics for the AlloSure Heart dd-cfDNA test as assessed in the Derived Cell Free DNA in Association With Gene Expression Profiling (D-OAR) prospective, multicenter registry study.[61] Patients already undergoing AlloMap testing for surveillance were eligible for inclusion; however following a protocol amendment, dd-cfDNA specimens were only obtained in patients with clinical suspicion of rejection and a planned for-cause biopsy after 2016 through 2018. The majority of dd-cfDNA samples (81%) were drawn in the first-year post-transplant. The D-OAR cohort included 841 biopsy-paired dd-cfDNA results, of which 587 were performed for routine surveillance of rejection. Overall, cell-mediated rejection and antibody-mediated rejection were biopsy-confirmed in 17 and 18 cases, respectively. The AUC for detecting acute rejection was 0.64 (95% CI 0.52 to 0.75). At a 0.2% cutoff for dd-cfDNA, the sensitivity, specificity, PPV, and NPV for detection of acute rejection was 80%, 44%, 8.9%, and 97.1% respectively. For the subgroup of patients undergoing surveillance, the sensitivity, specificity, PPV, and NPV were 38.1%, 84.0%, 8.1%, and 97.3%, with a corresponding AUC of 0.61 (95% CI 0.46 to 0.74). Among for-cause samples, the sensitivity, specificity, PPV, and NPV were 53.8%, 76.1%, 11.6%, and 96.6%, respectively. The study is limited by the protocol changes designed to increase the number of observed rejection events overall and low availability of concurrent dd-cfDNA results with respect to biopsy specimens (58%).

In a study funded by TAI Diagnostics, Inc., North (2020) performed a blinded clinical validation study on 158 matched pairs of endomyocardial biopsy-plasma samples collected from 76 volunteer adult and pediatric heart transplant recipients (ages two months or older, and eight days or more post-transplant) between June of 2010 and Aug 2016 from two Milwaukee transplant centers.[62] Based on acute cellular rejection grade as defined by the 2004 International Society for Heart and Lung Transplantation (ISHLT) classification, Receiver Operating Characteristic (ROC) analysis was performed to evaluate diagnostic accuracy across all possible cutoffs. To maximize diagnostic accuracy, Youden’s Index was used to select the optimal cutoff, found to correspond to a donor fraction value of 0.32%. Using this cutoff, clinical performance characteristics of the assay included a negative predictive value (NPV) of 100.00% for grade 2R or higher acute cellular rejection, with 100.00% sensitivity and 75.48% specificity; AUC for this analysis was 0.842, indicative of robust ability of the donor fraction assay to rule out 2R or greater acute cellular rejection for donor fraction values less

than 0.32%. There was no statistically significant correlation of donor fraction with age. Donor fraction elevation can also be caused by other forms of injury to the donor heart such as acute cellular rejection 1R, acute antibody-mediated rejection (AMR), and presence of coronary artery vasculopathy (CAV), thereby requiring correlation of myTAIHEART results with other clinical indicators.

In study funded by a grant from the National Institutes of Health and TAI Diagnostics, Inc., Richmond (2019) assessed 174 postcardiac transplant patients from seven centers (ages 2.4 months to 73.4 years) days with myTAIHEART testing (before transplant; one, four, and seven days following transplant; and at discharge from transplant hospitalization) using blinded analysis of biopsy-paired samples.[63] All the patients were followed for at least one year. Donor fraction, defined as the ratio of cell free DNA specific to the transplanted organ to the total amount of cell free DNA present in a blood sample was higher in acute cellular rejection 1R/2R (n=15) than acute cellular rejection 0R (healthy) (n=42; p=0.02); an optimal donor fraction threshold (0.3%) was determined by the use of ROC analysis, revealing an AUC of 0.814 with a sensitivity of 0.65, specificity of 0.93, and an NPV of 81.8% for the absence of any allograft rejection.

Agbor-Enoh (2021) reported results of a multicenter, prospective cohort study of heart transplant recipients monitored using dd-cfDNA and EMB. A total of 171 subjects were followed for a median of 17.7 months post-transplant. The primary endpoint was AR defined by international standards as a composite endpoint of ACR or AMR, defined based on individual center histologic readings to be consistent with usual care and included the histopathology grades treated at individual centers. Secondary endpoints were ACR grade ≥2 and AMR grade ≥1. Quantification of dd-cfDNA was conducted using shotgun sequencing. SNPs were identified for each donor/recipient pair using genotype data and %dd-cfDNA was computed as percentage of reads with donor SNPs to total reads for donor plus recipient SNPs. Median %dd-cfDNA levels were highest post-surgery and reduced to 0.13% (interquartile range [IQR], 0.03% to 0.21%) by 28 days. In patients with AR, %dd-cfDNA increased again compared with control values (0.38%; [IQR, 0.31 to 0.83%], versus 0.03% [IQR, 0.01 to 0.14%]; p<0.001). The AUROC for AR was 0.92 and a 0.25% dd-cfDNA threshold had a negative predictive value for AR of 99% and would have safely eliminated 81% of EMB.

Lung Transplant

Arunachalam (2024) evaluated the utility of dd-cfDNA as a noninvasive biomarker for monitoring acute rejection after single lung transplantation in a multicenter analysis of 257 test results from 103 patients across six centers.[64] The study applied a dd-cfDNA threshold of 1.0%, corrected for single-lung transplant, at a median of 233 days post-transplant. The authors observed elevated dd-cfDNA fractions in acute rejection (median 1.80%; IQR, 1.04% to 3.56%), infections (1.10%; IQR, 0.52% to 1.74%), and chronic lung allograft dysfunction (CLAD, 0.96%; IQR 0.75% to 1.06%) compared with stable patients (0.46%; IQR, 0.20% to 0.72%; p<0.0001). Sensitivity for dd-cfDNA in detecting acute rejection was 77.8% (95% CI, 58.3% to 94.1%), with a specificity of 84.6% (95% CI, 76.0% to 92%), a PPV of 38.3% (95% CI, 26.9% to 55.0%), a NPV of 96.8% (95% CI, 94.2% to 99.2%), and AUC of 0.85 (95% CI, 0.72 to 0.95). Limitations include retrospective study design, the relatively small number of samples which prohibited some sub-group analyses, and reliance on histopathologic confirmation, which may miss subclinical events.

Sindu (2024) published a retrospective study of elevated dd-cfDNA as a biomarker for detecting ACR and respiratory infections in 95 plasma samples from 81 bilateral lung transplant recipients.[65] The results demonstrated poor diagnostic performance, with percentage of dd-cfDNA showing low sensitivity for detecting ACR (41.67% at 0.5% threshold, 50% for 70% increase) and area under the curve values of 0.499 for ACR detection and 0.360 for ACR grade A2 or higher. While NPVs appeared high (88.89% and 87.50%), this was primarily due to the low prevalence of ACR (12.6%) rather than true diagnostic utility. For respiratory infections, dd-cfDNA levels trended higher in patients with definite or possible infections compared to those without microbial isolation, but the difference was not statistically significant (p=0.059). The authors concluded that the percentage increase in dd-cfDNA has limited clinical utility as a screening tool for ACR and respiratory infections in lung transplant recipients, with overall poor discriminatory ability for detecting allograft dysfunction.

Rosenheck (2022) assessed the predictive ability of dd-cfDNA testing using the Prospera test for lung transplant rejection.[66] The study included 195 samples from 103 patients, who were predominantly White (93%) and male (60%); mean age was 62 years. Black and Hispanic patients comprised 6% and 1% of the study population, respectively. The median time since lung transplant was 198 days, and most patients (85%) underwent lung biopsy for routine transplant surveillance. Consistent with other dd-cfDNA studies, median dd-cfDNA % was higher in patients with acute rejection (AR), which included acute cellular rejection (1.43%) or antibody-mediated rejection (2.50%), than those who were stable (0.46%). Prevalence of acute rejection was 28% (29/103), and prevalence of CLAD or neutrophilic-responsive allograft dysfunction (NRAD) was 21% (22/103); patients could be included in both diagnostic groups. Using a dd-cfDNA threshold of ≥1% for prediction of acute rejection, sensitivity was 89.1% and specificity was 82.9%, resulting in an AUC of 0.91 (95% CI 0.83 to 0.98). PPV was 51.9% and NPV was 97.3%. For a combined measure that included AR, CLAD/NRAD, and infection, sensitivity was 59.9%, specificity 83.9%, AUC 0.76, PPV 43.6%, and NPV 91.0%. As with other dd-cfDNA studies in lung transplantation, this study was limited by the small sample size though unlike other studies samples were collected prospectively.

Khush (2021) utilized samples from the biorepository derived from the Genome Transplant Dynamics study which included 38 unique bilateral or unilateral lung transplantation recipients 15 years of age or older.[67] A next-generation targeted sequencing assay was used to measure dd-cfDNA and acute cellular rejection was graded in trans-bronchial biopsies. Median dd-cfDNA was significantly elevated in acute cellular rejection samples (0.91%; IQR 0.39 to 2.07%) and chronic lung allograft dysfunction samples (2.06%; IQR 0.57 to 3.67%) compared to the samples from stable healthy allografts (0.38%; IQR 0.23 to 0.87%; p=0.021). The antibody-mediated rejection cohort was numerically but not statistically significantly different from the stable healthy allografts cohort (1.34%; IQR 0.34 to 2.40%), which was also not significantly different from the allograft infection group (0.39%; IQR 0.18 to 0.67%; p=0.56). No diagnostic cutoff for use of dd-cfDNA was proposed.

Sayah (2020) conducted a pilot study investigating the ability of AlloSure dd-cfDNA testing to detect acute cellular rejection.[68] Biopsy-matched biorepository samples from 69 lung transplant recipients who had previously enrolled in the multicenter Lung Allograft Gene Expression Observational (LARGO) Study were evaluated. Diagnostic cohorts included patients with respiratory allograft infection (n=26), normal histopathology without infection or rejection (n=30), and acute cellular rejection without concurrent infection (n=13). Samples were obtained between >14 days and <one1-year post-transplant, and samples associated with

potential concurrent infection with rejection were excluded. Median dd-cfDNA levels were 0.485% (IQR, 0.220 to 0.790) in the normal cohort, 1.52% (IQR, 0.520 to 2.550) in the acute cellular rejection cohort, and 0.595% (IQR, 0.270 to 1.170) in the infection cohort. While dd-cfDNA levels were significantly higher in the acute cellular rejection cohort compared to the normal cohort (p=0.026), samples associated with infection were not significantly different from the normal (p=0.282) or acute cellular rejection (p=0.100) cohorts. The AUC for detection of acute cellular rejection was 0.717 (95% CI 0.547 to 0.887; p 0.025). At a threshold of 0.87% dd-cfDNA and an estimated prevalence rate of 25%, sensitivity for acute cellular rejection was 73.1% (95% CI 52.2% to 88.4%), specificity was 52.9% (95% CI 27.8% to 77.0%), positive likelihood ratio was 1.55, negative likelihood ratio was 0.51, PPV was 34.1%, and NPV was 85.5%. The study is limited by the small sample size and use of archived samples, and raises concerns regarding the ability of AlloSure dd-cfDNA testing to detect antibody-mediated rejection and to discriminate between infection and rejection.

The evidence is insufficient to determine that dd-cfDNA results in an improvement in the net health outcome of patients after lung transplant. Larger and additional prospective studies validating the dd-cfDNA threshold for active rejection are needed to develop conclusions. At present, no studies evaluating the clinical utility for AlloSure or Prospera dd-cfDNA testing were identified.

Liver Transplant

Julian (2025) published a prospective observational study that analyzed levels of dd-cfDNA and microRNAs (miRNAs) in 437 plasma samples from 64 liver transplant recipients over one year.[69] Dd-cfDNA levels increased 3.9-fold during acute rejection episodes, achieving 100% sensitivity and 100% negative predictive value with a diagnostic cutoff of 9.88%. However, a low specificity of 66.7% and poor PPV of 17.5% were reported. The miRNAs, miR-155-5p, miR-122-5p, and miR-181a-5p, were significantly upregulated during rejection and improved diagnostic accuracy when combined with dd-cfDNA. This study is limited by small sample size, observational design, and lack of long-term follow-up. The authors concluded that future large prospective cohort studies are necessary to validate the clinical use of dd-cfDNA and miRNA in monitoring liver transplant rejection.

Jana (2024) published a prospective observational study that evaluated dd-cfDNA as a biomarker for early detection of liver transplant dysfunction among 50 liver transplant recipients.[70] Blood samples were collected for dd-cfDNA analysis at postoperative days one, three, and seven and three months post-transplant. Dd-cfDNA levels were significantly elevated in rejection cases compared with non-rejection cases (12.8% vs 4.3%, p<0.0001). The authors reported that dd-cfDNA demonstrated superior predictive performance compared with conventional liver function tests (AUC 0.86 vs 0.65 to 0.75). A dd-cfDNA threshold >10.2% on postoperative day seven predicted three-month graft health with 93.33% sensitivity and 94.44% specificity. This study is limited by small sample size, limited statistical power due to inclusion of only eight biopsy-confirmed rejection cases, and lack of long-term follow-up.

HEARTCARE

The commercially available HeartCare (CareDx) test combines AlloMap GEP testing with AlloSure Heart measurement of percent dd-cfDNA. The combined use of GEP and dd-cfDNA testing for surveillance of acute rejection was assessed in a single-center, retrospective study conducted by Gondi (2021) between February 2019 and March 2020.[71] Patients (n=153) were

required to be ≥55 days post-transplant, hemodynamically stable, ≥15 years of age, and single-organ recipients. The majority of patients were male (74.5%) and white (78.4%) with an average age of 54.5 years. Patients were assessed once monthly between 2 and 12 months, every three months between 12 and 24 months, and every six months between 24 and 36 months post-transplant. Pre-specified thresholds for GEP scores were ≥30 for patients under six months post-transplant and ≥34 for patients six or more months post-transplant. The pre-specified threshold for percent dd-cfDNA was ≥0.20% based on a prior study of the AlloSure test by Khush (2019),[61] described above. In patients under six months post-transplant, endomyocardial biopsy was performed regardless of test results. For patients six or more months post-transplant who received both GEP and dd-cfDNA testing, endomyocardial biopsy was canceled in patients with dd-cfDNA <0.20% regardless of AlloMap score. In patients with positive AlloMap scores but negative dd-cfDNA, endomyocardial biopsy could be performed or deferred in favor of repeat dd-cfDNA testing. Among 495 samples, overall test result distributions were 59.6% for patients negative on both tests, 12.3% for patients positive by dd-cfDNA only, 22.6% for patients positive by GEP only, and 5.5% positive by both GEP and dd-cfDNA. The combined testing approach resulted in a 12.7% reduction (48 biopsies) in endomyocardial biopsy volume compared to GEP testing alone. Among the 172 biopsies performed, two patients with cell-mediated rejection were identified, with corresponding dual-positive tests. Two patients with antibody-mediated rejection were identified, with corresponding tests that were only positive by dd-cfDNA. The study is limited by its retrospective design, incomplete evaluation of performance characteristics, and lack of reporting on health outcomes.

MOLECULAR MICROSCOPE® DIAGNOSTIC SYSTEM

The Molecular Microscope® Diagnostic System (MMDX) estimates the probability of rejection in endomyocardial or kidney biopsy tissue using microarray gene analysis. As previously described, the MMDX test has been used as a comparator to dd-cfDNA test for detecting renal transplant rejection.[38] Schachtner (2023) evaluated discrepant results between MMDX and kidney histology using 72 biopsies from 51 patients. There was 65% concordance between MMDX and kidney biopsy.[72] In most cases of discordance, MMDX showed no rejection, but histology showed rejection. The authors note that histologic evidence drives treatment decisions.

MMDX testing for heart transplant rejection was evaluated by Alam (2022), who used paired results from heart transplant tests for comparisons. MMDX was paired with endomyocardial biopsy (EMBx), and a different pairing was of MMDX and dd-cfDNA.[73] The study used 228 specimens from 135 patients. Thirty percent of the specimens were associated with clinical concern for rejection. MMDX and EMBx showed 84% concordance. MMDX identified 32 specimens with rejection that were discordant with EMBx results. Five specimens were found to be negative for rejection with MMDX but showed rejection with EMBx. There was 72% concordance between MMDX and dd-cfDNA. Treatment for rejection was initiated in eight patients when MMDX results showed rejection and EMBx did not. These treatment changes were also influenced by clinical suspicion of rejection and/or elevated dd-cfDNA or DSA levels. The evidence is insufficient to determine whether MMDX test results can lead to improved health outcomes after heart or kidney transplantation.

IMMUNE RESPONSE OF RECIPIENT LYMPHOCYTES TO DONOR LYMPHOCYTES

Rohan (2020) evaluated the performance of allo-antigen-specific T-cytotoxic memory cells (TcM) for predicting the likelihood of rejection in renal transplant recipients.[74] A total of 22 adult primary renal transplant recipients were tested for allospecific CD154-positive TcM (Plemixmark™). Frequencies of CD154-positive TcM in recipient blood samples induced by overnight stimulation with donor-HLA-matched (donor) peripheral blood lymphocytes were measured with flow cytometry. The index of rejection was reported as donor-specific CD154-positive TcM expressed as a multiple of those induced by stimulation with HLA-mismatched PBL in parallel co-culture. Of the 22 patients, six experienced biopsy-proven T-Cell Mediated Rejection (TCMR) and one experienced antibody-mediated rejection. Six of the seven rejection patients had an index of rejection predicting rejection and 10 of 15 patients with no rejection had an index of rejection predicting no rejection. These results indicated a sensitivity of 83%, specificity of 67%, positive predictive value of 54%, and negative predictive value of 91%.

A study by Ashokkumar (2017) described the creation and validation of a similar test for predicting the likelihood of rejection in pediatric patients after liver or small bowel transplantation.[75] In this study, allo-antigen-specific T-cytotoxic memory cells were measured in a training set of 158 cryopreserved samples from 127 subjects to set threshold values for samples obtained before or after (within 60 days) transplantation. After the test was standardized for reproducibility, it was run on a validation set of 122 samples from 87 patients. Of these, only 97 samples from 72 patients were analyzable. There were no significant differences in donor-recipient HLA-matching between rejectors and non-rejectors. The sensitivity and specificity of the test in post-transplant samples were 84% and 80%, respectively in the validation set.

URINARY BIOMARKERS

Janfeshan (2024) published a systematic review and meta-analysis to determine the role of urinary CXCL10 in predicting renal allograft injury.[76] Of nine case-control studies, four assessed urinary CXCL10 to serum creatinine (Cr) ratio with and without other biomarkers (e.g., CXCL9). Five studies assessed urinary CXCL10 protein levels. The quality assessment of the included studies was deemed satisfactory using the Newcastle-Ottowa scale, but there was significant heterogeneity. The study groups were too dissimilar to merge results. The authors concluded that assessing CXCL10 protein levels detected graft injury more effectively than measurement of the CXCL10/Cr ratio, but neither type of CXCL10 measurement is effective by itself.

Hirt-Minkowski (2023) performed a RCT that evaluated CXCL10 monitoring in 241 people who were immediate post-renal transplant.[77] Both study arms had CXCL10 testing, but the intervention arm monitored lab values and had triggers for biopsy and subsequent treatment adjustment, while the CXCL10 test results for the control arm were concealed. After one year, there were no significant differences in clinical outcomes, including intention to treat (p=0.80), death-censored graft loss (p=0.62), acute rejection (p=0.39), or chronic active TCMR in one-year surveillance biopsy (p=0.59). The authors concluded that no clinical benefit was demonstrated with urine CXCL10 monitoring.

NON-HLA AUTOANTIBODIES

Panicker (2024) published a systematic review of the role of non-HLA antibodies in monitoring heart transplant rejection.[78] Fifty-six studies were included in the systematic review. Due to significant heterogeneity among study designs and outcome measures, a meta-analysis was not conducted. The majority of included studies were limited by small sample size and

retrospective design. The reviewers reported that the evidence for monitoring heart transplant rejection with serum measurement of anti-major histocompatibility complex class I chain-related gene A (MICA) and anti-angiotensin II type I receptor antibodies (AT1R) does not support the role of these biomarkers in monitoring heart transplant rejection and outcomes. The reviewers concluded that future studies with significant mechanistic and clinical validation are needed to determine whether measurement of non-HLA antibodies can aid in monitoring heart transplant rejection.

Oh (2025) published a retrospective cohort study of 192 heart transplant recipients that examined the association between non-HLA antibodies and graft outcomes across four transplant centers in Korea.[79] Antibodies to vimentin (AVA) and type II collagen (ACA) were significantly associated with reduced one-year graft survival (78.6% vs 92.6% for AVA+, p=0.006; 72.2% vs 91.1% for ACA+, p=0.015). AVA+ patients with elevated DSA experienced worse outcomes (45.5% vs 94.1% survival, p=0.002), and the presence of both antibodies (AVA+/ACA+) was associated with poor survival in both early and late periods. This study is limited by heterogeneity in monitoring and immunosuppression protocols and retrospective design.

Pearl (2024) published a retrospective cohort study of 100 pediatric kidney transplant recipients that assessed the impact of AT1R antibodies and endothelin-type A receptor (ETAR) antibodies over five years post-transplant.[80] While 59% of patients tested positive for these antibodies, and positivity was associated with greater eGFR decline and allograft loss within the first two years, no association was found with rejection, antibody-mediated rejection, or allograft loss over the full five-year period. This study is limited by retrospective design and lack of a comparison group of antibody-negative patients.

Pizzo (2022) published a retrospective cohort study of the impact of elevated pre-transplant AT1R antibodies on pediatric renal transplant outcomes over five years in 36 patients aged 2 to 20 years.[81] Patients were stratified into AT1R antibody-negative (<17 U/ml, n=18) and AT1R antibody-positive (≥17 U/ml, n=18) groups, with serial eGFR measurements and biopsies performed for elevated creatinine, strong HLA-DSA, or AT1R antibodies ≥17 U/ml. While the AT1R antibody-positive group showed slightly lower eGFR at most time points (100.2 vs 111.3 ml/min/1.73m² at six months, 80.9 vs 72.6 ml/min/1.73m² at four years), these differences were not statistically significant. Acute rejection rates were similar between groups (5 of 18 vs 6 of 18). The authors concluded that elevated pre-transplant AT1R antibodies were not associated with significantly reduced graft function or increased rejection in this small pediatric cohort. This study is limited by small sample size and retrospective design.

PRACTICE GUIDELINE SUMMARY

INTERNATIONAL SOCIETY OF HEART AND LUNG TRANSPLANTATION

In 2023, the International Society of Heart and Lung Transplantation published updated guidelines for the care of heart transplant recipients.[82] The guidelines included the following recommendations regarding rejection surveillance:

Immunosuppression and Rejection:

Recommendations for Rejection Surveillance by Endomyocardial Biopsy in Heart Transplant Recipients:

- The standard of care for adult heart transplant recipients is to perform periodic endomyocardial biopsy (EMB) during the first 6 to 12 postoperative months for surveillance of heart transplant rejection. Class IIa, Level of Evidence: C
- The standard of care for adolescents should be similar to adults, including surveillance EMB for heart allograft rejection for 3 to 12 months after HT. In younger children, especially infants, the risks associated with EMB and required general anesthesia may outweigh the surveillance benefit for comparably rare acute rejection; therefore, it is reasonable to use a combination of noninvasive screening methods (echocardiography, ECG, biomarkers) instead. Class IIa, Level of Evidence: C
- After the first postoperative year, it is reasonable to continue EMB surveillance in patients who are at higher risk for late acute rejection. This group includes HT recipients with donor-specific antibodies (DSA), a history of recurrent acute rejection, calcineurin-inhibitor free immunosuppression, reduced immunosuppression due to post-transplant malignancy or chronic infection, African American descent. Class IIa, Level of Evidence: C
- Routine EMB later than 5 years after HT are not recommended. EMB should be performed only for cause in patients with signs or symptoms of cardiac allograft dysfunction. Class III, Level of Evidence: C
- Children receiving ABO incompatible cardiac allografts in the first 2 years of life with isohemagglutinin titers toward the donor blood group below 1:32 and without elevated titers post-transplant do not require more frequent EMB or non-invasive monitoring compared to recipients of ABO compatible organs. Class IIa, Level of Evidence: B

<u>Recommendations for the Noninvasive Monitoring of Acute Heart Transplant Rejection:</u>

- Ventricular evoked responses (VER) monitoring for rejection surveillance is no longer recommended as the technology has become obsolete. Class III, Level of Evidence: C
- Gene Expression Profiling (GEP) (i.e., AlloMap) of peripheral blood can be used in low-risk patients between 2 months and 5 years after heart transplant and to identify adult recipients who have the low risk of current acute cellular rejection (ACR) to reduce the frequency of EMB. Data in children does not allow a general recommendation of GEP as routine tool at present. Class IIa, Level of Evidence: B
- In pediatric patients, echocardiography, especially detailed assessment of diastolic function, shows reasonable correlation with significant acute rejection; however, it should not be considered as a sole surveillance method in patients who have a low risk of EMB complications. In younger children, echocardiographic surveillance represents an alternative monitoring modality to avoid or reduce the frequency of EMB. Class IIb, Level of Evidence B.
- The routine clinical use of electrocardiographic parameters for acute heart allograft rejection monitoring is not recommended. Class III, Level of Evidence: C
- Echocardiography may be an acceptable rejection monitoring strategy in patients at low risk for acute rejection and in whom EMB is not possible (i.e., tricuspid valve replacement or difficult vascular access). Class IIb, Level of Evidence: C
- MRI with gadolinium enhancement may be used as an adjunct modality in patients with unexplained graft dysfunction and low-grade or absent histologic evidence of rejection on EMB. Class IIb, Level of Evidence: C
- It is reasonable to integrate biomarkers such as B-type natriuretic peptide (BNP) and high-sensitivity troponins into a rejection monitoring strategy to identify higher risk patients who may benefit from additional evaluation for ACR, AMR or CAV. Class IIb,

Level of Evidence: C

- Post-transplant monitoring for de novo donor specific antibodies (DSA) should be performed at 1, 3, 6, and 12 months post-operatively and annually thereafter. Sensitized patients should be monitored more frequently. Class IIa, Level of Evidence: C
- The use of systemic inflammatory markers such as C-reactive protein (CRP) for acute heart allograft rejection monitoring is not recommended. Class III, Level of Evidence: C
- In younger children, especially infants, the risks associated with EMB and required general anesthesia may outweigh the surveillance benefit for comparably rare acute rejection; therefore, it is reasonable to use a combination of non-invasive screening methods (echocardiography, ECG, biomarkers) instead. Class IIa, Level of Evidence: C
- Use of immune cell function assay (ImmuKnow) cannot be recommended in adult and pediatric heart transplant recipients for rejection monitoring. Class III, Level of Evidence: B

Recommendation for the Management of Late Acute Rejection:

- After the first year, continued rejection surveillance (using a combination of noninvasive methods, GEP or EMB) is reasonable in patients at higher risk for late acute rejection. Risk factors for rejection include younger recipient age, prior history of acute rejection episodes, presence of donor-specific-antibodies, recipient female gender, rejection events occurring >6 months after transplantation, CNI-reduced or -free immunosuppression, and a history of medication of non-compliance. The optimal frequency and duration of rejection surveillance have not been defined. Class IIa, Level of Evidence: C
- Antibody mediated rejection is more commonly identified in late acute rejection compared to acute cellular rejection and should be considered in the differential diagnosis of HT recipients presenting with signs or symptoms of heart allograft dysfunction. EMB with ISHLT immunopathologic evaluation, as well as measurement of circulating HLA donor-specific-antibodies should be obtained before initiating treatment. Class IIa, Level of Evidence: C

Long-term care of heart transplant recipients: Prevention and Prophylaxis:

Frequency of Routine Tests and Clinic Visits in Heart Transplant Recipients:

In addition to routine outpatient follow-up visits, HT recipients should have more prolonged visits every 1 to 2 years for more detailed clinical assessment. (Class I, Level of Evidence B). The purpose of the follow-up visits is to monitor for rejection and screen for adverse events and may include the following:

1. A complete physical examination;
2. Review of medications and changes to medications based on the results of the examinations;
3. Blood work;
4. Echocardiogram;
5. Coronary angiography. Adjunct Intravascular imaging can be considered if expertise available, as Maximal Intimal Thickening (MIT) > 0.3 mm in the first year has been shown to have prognostic value;
6. Surveillance EMB, and noninvasive rejection monitoring [Gene Expression Profiling (Allomap), DSA, BNP and high sensitivity troponins, donor-derived cell-free DNA]

7. Additional education and/or interaction with members of the multidisciplinary team. Class I, Level of Evidence B

In infants early after heart transplantation, far fewer biopsies are performed due to the need for general anesthesia and the difficulties with venous access and bioptome manipulation in small hearts and vessels. There is no consensus regarding the frequency of EMB. Ancillary noninvasive modalities for the assessment of rejection as surrogates to EMB should be considered. Class I, Level of Evidence B.

KIDNEY DISEASE IMPROVING GLOBAL OUTCOMES

In 2009, the Kidney Disease Improving Global Outcomes issued guidelines for the care of kidney transplant recipients.[83] The guidelines did not address dd-cfDNA or gene expression profile testing.

AMERICAN SOCIETY OF TRANSPLANT SURGEONS

In 2023, the American Society of Transplant Surgeons (ASTS) issued a position statement on the role of dd-cfDNA in kidney transplant surveillance.[84] The following recommendations regarding the clinical utility and decision analysis were issued:

- "The most data have been accumulated in adult transplant recipients, and these recommendations are therefore most applicable to adult patient populations.
- We suggest that clinicians consider measuring serial dd-cfDNA levels in kidney transplant recipients with stable renal allograft function to exclude the presence of subclinical antibody-mediated rejection.
- We recommend that clinicians measure dd-cfDNA levels in kidney transplant recipients with acute allograft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection (ABMR).
- We do not recommend the use of blood gene expression profiling (GEP) in kidney transplant recipients for the purpose of diagnosing or excluding sub-clinical rejection, as adequate evidence supporting such use is still lacking.
- We do not recommend the use of blood GEP to diagnose or exclude the presence of acute graft rejection in kidney transplant recipients with acute allograft dysfunction given the paucity of data to support this practice.
- We recommend that dd-cfDNA may be utilized to rule out subclinical rejection in heart transplant recipients.
- We recommend that clinicians utilize peripheral blood GEP as a non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk, adult heart transplant recipients who are over 55 days status post heart transplantation."

"Caveats and recommendations for future studies:

- None of these recommendations should be construed as recommending one biomarker over another in the same diagnostic niche.
- We strongly recommend ongoing clinical studies to clarify the scenarios in which molecular diagnostic studies should be utilized.
- We specifically recommend that studies be carried out to evaluate the potential role of dd-cfDNA surveillance in kidney transplant recipients to improve long-term allograft survival."

SUMMARY

There is enough research to show that gene expression profiling to predict heart transplant rejection improves health outcomes for patients who have had a heart transplant. Therefore, the use of gene expression profiling, including but not limited to the AlloMap® test, for prediction or detection of heart transplant rejection is considered medically necessary.

There is not enough research to show that gene expression profiling to predict transplant rejection improves health outcomes for patients who do not meet policy criteria. Therefore, the use of gene expression profiling tests in the management of transplant recipients who do not meet policy criteria is considered investigational.

There is not enough research to show that the Heartsbreath™ test or any test that measures volatile organic compounds improves health outcomes for patients that have had a heart transplant. Therefore, the measurement of volatile organic compounds to assist in the detection of heart transplant rejection, including use of the Heartsbreath™ test, is considered investigational.

There is not enough research to show that measurement of donor-derived cell-free DNA (dd-cfDNA) to assess rejection improves health outcomes for patients who have undergone organ transplant. Therefore, the use of dd-cfDNA testing, including the AlloSure® and myTAIHEART tests, to assist in the detection of organ transplant rejection is considered investigational.

There is not enough research to show that simultaneous measurement of donor-derived cell-free DNA (dd-cfDNA) and gene expression profiling to assess rejection improves health outcomes for patients who have undergone organ transplant. Therefore, the use of simultaneous dd-cfDNA testing and gene expression profiling to assist in the detection of organ transplant rejection is considered investigational.

There is not enough research to show that measurement of immune response of recipient lymphocytes to donor lymphocytes in cell culture to assess the likelihood of acute cellular rejection after transplantation improves health outcomes for patients who have had an organ transplant. Therefore, the use of measurement of immune response of recipient lymphocytes to donor lymphocytes in cell culture to assess the likelihood of acute cellular rejection after renal, liver, and/or small bowel transplantation is considered investigational.

There is not enough research to show that heart or kidney transplant risk of rejection estimates using gene expression profiling tests (e.g., Molecular Microscope® Diagnostic System) on biopsy specimens improves health outcomes for patients who have had heart or kidney transplant. Therefore, the use of gene expression profiling tests on biopsy tissue to predict rejection is considered investigational.

There is not enough research to show that measurement of the urinary chemokine CXCL10 improves health outcomes of people who have had kidney transplant. Therefore, use of urinary quantification of chemokine CXCL10 to monitor for rejection or determine the need for graft biopsy after renal transplant is considered investigational.

There is not enough research to show that measurement of serum non-HLA autoantibodies improves health outcomes of patients who have had an organ transplant. Therefore,

measurement of serum non-HLA autoantibodies to detect acute organ transplant rejection or graft dysfunction is considered investigational.

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CODES

Codes	Number	Description
CPT	0018M	Transplantation medicine (allograft rejection, renal), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score
	0055U	Cardiology (heart transplant), cell-free DNA, PCR assay of 96 DNA target sequences (94 single nucleotide polymorphism targets and two control targets), plasma
	0087U	Cardiology (heart transplant), mRNA gene expression profiling by microarray of 1283 genes, transplant biopsy tissue, allograft rejection and injury algorithm reported as a probability score
	0088U	Transplantation medicine (kidney allograft rejection) microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection
	0118U	Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA

Codes	Number	Description
	0319U	Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using pretransplant peripheral blood, algorithm reported as a risk score for early acute rejection
	0320U	Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using posttransplant peripheral blood, algorithm reported as a risk score for acute cellular rejection
	0493U	Transplantation medicine, quantification of donor-derived cell-free DNA (cfDNA) using next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA
	0508U	Transplantation medicine, quantification of donor-derived cell-free DNA using 40 single-nucleotide polymorphisms (SNPs), plasma, and urine, initial evaluation reported as percentage of donor-derived cell-free DNA with risk for active rejection
	0509U	Transplantation medicine, quantification of donor-derived cell-free DNA using up to 12 single-nucleotide polymorphisms (SNPs) previously identified, plasma, reported as percentage of donor-derived cell-free DNA with risk for active rejection
	0526U	Nephrology (renal transplant), quantification of CXCL10 chemokines, flow cytometry, urine, reported as pg/mL creatinine baseline and monitoring over time
	0540U	Transplantation medicine, quantification of donor-derived cell-free DNA using next-generation sequencing analysis of plasma, reported as percentage of donor-derived cell-free DNA to determine probability of rejection
	0544U	Nephrology (transplant monitoring), 48 variants by digital PCR, using cell-free DNA from plasma, donor-derived cell-free DNA, percentage reported as risk for rejection
	0575U	Transplantation medicine (liver allograft rejection), miRNA gene expression profiling by RT-PCR of 4 genes (miR-122, miR-885, miR-23a housekeeping, spike-in control), serum, algorithm reported as risk of liver allograft rejection
	0576U	Transplantation medicine (liver allograft rejection), quantitative donor-derived cell-free DNA (cfDNA) by whole genome next generation sequencing, plasma and mRNA gene expression profiling by multiplex real-time PCR of 56 genes, whole blood, combined algorithm reported as a rejection risk score
	0581U	Transplantation medicine, antibody to non-human leukocyte antigens (non-HLA), blood specimen, flow cytometry, single-antigen bead technology, 39 targets, individual positive antibodies reported
	81479	Unlisted molecular pathology procedure
	81558	Transplantation medicine (allograft rejection, kidney), mRNA, gene expression profiling
	81560	Transplantation medicine, measurement of donor and third party-induced CD154+T-cytotoxic memory cells
	81595	Cardiology (heart transplant), mRNA, gene expression profiling by real-time quantitative PCR of 20 genes (11 content and 9 housekeeping), utilizing subfraction of peripheral blood, algorithm reported as a rejection risk score
	86849	Unlisted immunology procedure
HCPCS	None	

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