AlloSure Test Results Interpretation

TEST DESCRIPTION
The AlloSure test is a clinical-grade, targeted, next generation sequencing (NGS) assay that measures single-nucleotide polymorphisms (SNPs) to accurately quantify donor-derived cell-free DNA (dd-cfDNA) in renal transplant recipients without separate genotyping of either the donor or the recipient. The assay quantifies the fraction of dd-cfDNA in both unrelated and related donor-recipient pairs.

INTENDED USE
The AlloSure test is intended to assess the probability of allograft rejection in kidney transplant recipients with clinical suspicion of rejection and to inform clinical decision-making about the necessity of renal biopsy in such patients at least 2 weeks post-transplant in conjunction with standard clinical assessment.

INDICATIONS FOR USE
AlloSure is indicated for use in renal transplant patients who are 18 years of age or older and at least 2 weeks (14 days) post-transplant.

CONTRAINDICATIONS AND LIMITATIONS (more detail below)
Contraindications (more detail below)
DO NOT USE AlloSure testing for
- Recipients of transplanted organs other than kidney
- Recipients of a transplant from a monozygotic (identical) twin
- Recipients of a bone marrow transplant
- Recipients who are pregnant
- Recipients who are under the age of 18
- Recipients who are less than 14 days post-transplant

Limitations (more detail below)
AlloSure should not be used within:
- 30 days after a blood transfusion that contains white blood cells (washed or leukocyte-depleted RBCs are acceptable)
- 24 hours following a biopsy

Clinical Validity and Clinical Utility (more detail below)
Clinical validity of the AlloSure test was established in single-kidney transplant recipients who were 18 years of age or older and two weeks post-transplant. The multi-center observational DART study demonstrated that AlloSure can discriminate active rejection status. Blood specimens were prospectively collected from kidney recipients at scheduled post-transplant intervals, and concomitantly with clinically indicated or surveillance kidney biopsies performed according to center protocol. dd-cfDNA levels were measured in blood plasma using the AlloSure test and correlated with allograft rejection status ascertained by renal biopsy. An AlloSure test result with high PPV could increase the pre-biopsy probability of detecting active
rejection (antibody mediated rejection or T cell mediated rejection). In association with a high NPV, AlloSure results may reduce the need for biopsy in some cases of elevated serum creatinine.

INTERPRETING ALLOSURE RESULTS

AlloSure test results are available on the CareDx Portal (https://caredx.careevolve.com) and delivered via fax directly to the transplant clinic.

How to Read the AlloSure Report

1. **Recipient information and donor relationship to recipient**
   Recipient information, including date of birth, demographic data, date of blood draw, type of organ transplant and donor relationship to the recipient is included. Donor-recipient relationship is used in the calculation of the AlloSure result. The donor’s relationship to the recipient, as provided to CareDx by the prescriber, is shown here. The accuracy of this information should be verified when interpreting the AlloSure results and any discrepancies reported to CareDx immediately.

2. **Current AlloSure result**
   The current AlloSure result is shown as the percent of donor-derived cell-free DNA (dd-cfDNA) in the total cell-free DNA (cfDNA) in the results box and plotted on the graph along with the numerical value. Results less than 0.12%* (less than 0.18%* for closely related donors) are plotted near the bottom of the graph. Results greater than 16% are plotted near the top of the graph.

3. **Specimen collection date and time post-transplant for the current result**
   The specimen collection date for the current result (day, month, and year), and the time post-transplant on the date the specimen was collected are shown below the graph.

4. **Information to assist with interpretation of the results**
   Important information to assist with interpretation of the AlloSure result is provided directly on the report for convenience. (Detailed information can be found below.)

5. **Longitudinal graph with AlloSure results from the last 12 months**
   Serial AlloSure results within the last 12 months are plotted on the longitudinal graph to assist with evaluating patient results over time.

*Note: The lower end of the reportable range has been updated as of 18Nov2019. The values of 0.12% for unrelated and distantly related recipient-donors pairs, and 0.18% for closely related recipient-donor pairs have been implemented for all specimens tested on or after this date. Between 01Apr2019 and 18Nov2019, the values 0.15% (unrelated) and 0.26% (related) were applied. Prior to 01Apr2019 the lower end of the reportable range was 0.19% (unrelated) and 0.28% (related).
ALLOSURE RESULTS

The AlloSure result is the percent of donor-derived cell-free DNA in the total cell-free DNA present in kidney transplant recipients. When dd-cfDNA levels are so low that a value different from zero cannot be quantified, results are reported as “<0.12%” for patients in which the donor is unrelated and distantly-related or “<0.18%” for patients in which the donor and recipient are closely related (siblings, parent, grandparent, aunt, uncle). Results are reported as “>16%” when they are greater than the established quantifiable range.

Donor-recipient relationship is used in the calculation of the AlloSure result. The donor relationship to the recipient as provided to CareDx is shown on the AlloSure result report. This information should be verified prior to using the result to manage the care of the patient, and any relationship discrepancies should be reported to CareDx immediately. An incorrect donor relationship may affect the calculated dd-cfDNA result. In some cases, the difference in the %dd-cfDNA calculated with a correct relationship compared to an incorrect relationship may have an impact on the clinical interpretation of the result. Refer to Table 1 for example result differences based on donor relationship to recipient.

Table 1. Example differences in AlloSure results based on change in donor relationship to recipient

<table>
<thead>
<tr>
<th>Patient</th>
<th>Incorrect Relationship</th>
<th>AlloSure Result</th>
<th>Correct Relationship</th>
<th>AlloSure Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sibling</td>
<td>1.1%</td>
<td>Unrelated</td>
<td>0.54%</td>
</tr>
<tr>
<td>B</td>
<td>Unrelated</td>
<td>0.19%</td>
<td>Sibling</td>
<td>0.37%</td>
</tr>
</tbody>
</table>

INTERPRETATION OF ALLOSURE TEST RESULTS

- **>1% dd-cfDNA is associated with active rejection (Ref 2)**
  
  dd-cfDNA level greater than 1% indicate a probability of active rejection (antibody-mediated rejection or T cell-mediated rejection). dd-cfDNA levels 1% and below reflect absence of active rejection. For dd-cfDNA greater than 1%, there is a positive predictive value (PPV) of 61% and a negative predictive value (NPV) of 84% for active rejection. The positive and negative predictive values for antibody-mediated rejection at a threshold of 1.0% dd-cfDNA are 44% and 96%, respectively. The reference standard for rejection diagnosis was histological evidence from renal allograft biopsies performed for clinical suspicion.

- **0.21% dd-cfDNA is the median observed in a reference population of stable recipients (Ref 3)**
  
  dd-cfDNA values greater than 1% were above the 96th percentile of all values in a study of stable kidney transplant recipients i.e. outside the normal range for this population. 75% of stable recipients had an AlloSure result below 0.40% dd-cfDNA.

- **>61% increase in dd-cfDNA from a prior sample exceeds the biological and analytical variability observed in the reference population (Ref 3)**
  
  An increase of greater than 61% in consecutive dd-cfDNA results in an individual is greater than the change that may be attributable to normal biological and analytical variation.
EXAMPLE REPORTS

Stable AlloSure Results

The current score in this report is below the reportable range for unrelated donor recipient pairs (0.12% to 16%) and is therefore expressed as “<0.12”. All the prior scores for this patient are very low, each indicating low probability of rejection based on the high negative predictive value (NPV) of 95% at an AlloSure result of 0.21% dd-cfDNA. A change in reportable range occurred April 1, 2019 (from 0.19% to 0.15%), so the first of these low scores was before this date and is reported as “<0.19%”. A second change in the reportable range occurred November 18, 2019 (from 0.15% to 0.12%), so the June 24, 2019 result is reported as <0.15%. 
Change in AlloSure Results to Above Threshold

This report shows a current value of 3.7% dd-cfDNA, which follows several prior low AlloSure results. At prior post-transplant visits, the low (<1%) dd-cfDNA results were associated with a high NPV and low PPV. Using a threshold of 1%, the PPV for active rejection is 61% and the probability of rejection is much higher with the current result of 3.7%.
CONTRAINDICATIONS AND LIMITATIONS OF THE PROCEDURE

Limitations

In cases where a patient receives a repeat or secondary kidney with the original transplanted kidney(s) still in place, the contribution of cfDNA from the prior transplanted organ(s) to recipient’s plasma is minimal (Mehta et al). Data from retransplant patients in the DART study in whom the prior kidney(s) remain in situ suggests that AlloSure can be used in retransplant patients in a manner similar to use in the single-kidney transplant population.

Patients who received transfusions of whole blood or other blood transfusions that contain white blood cell components within one month prior to the AlloSure test may have an inaccurate result. Transfusions of washed red blood cells or leukocyte-depleted, packed red blood cell transfusions do not impact the result.

There are some indications that damage to the graft caused by invasive procedures such as renal biopsy may cause a short-term elevation of dd-cfDNA. Until definitive studies are completed, AlloSure should not be used on patients within 24h following a renal biopsy.

Contraindications

Since the test evaluates genetic differences between the donor and recipient, it is not possible to perform the test for a kidney transplant recipient that is a monozygotic twin to the donor.

When more than two genomes may be present in the recipient plasma (more than recipient + donor), contribution of cfDNA from each genome is not differentiated by the test. This includes pregnancy, due to the presence of fetal genome DNA in the maternal plasma and multiple-organ transplants from different donors since the grafts each introduce a unique genome (e.g. kidney/pancreas) and contribute different basal levels of cfDNA confounding interpretation of the results.

A recipient of multiple transplanted organs that all originated from the same donor presents a situation where elevated levels of dd-cfDNA could have originated from one organ or another or both. If from both, they could be contributing at different basal levels, confounding interpretation of the results. Therefore, AlloSure is not to be used for transplant recipients with multiple transplanted organs from the same donor.

Recipients of allogeneic blood or bone marrow transplant who have received cells with a genome different from the recipient (e.g. non-monozygotic twin) should not receive AlloSure testing.
MORE INFORMATION

Case Study*

Rejection assessment: For-cause AlloSure and Donor-specific Antibody (DSA) testing

The recipient in this case had several low AlloSure results and relatively stable high serum creatinine (1.5 to 1.75 ng/dL). This patient had no DSA detected at day 30 and day 60. For-cause biopsies were performed at day 30 and day 60 because of the early elevated serum creatinine. The two biopsies revealed mild IF/TA and ATN, but no rejection. The nearest AlloSure tests were at day 35 and 44, with results of 0.26% and 0.44%, both well below 1%, which indicates a low probability of rejection. At day 111 the serum creatinine was 1.77 ng/dL and the AlloSure was at its lowest point, below 0.12%. At day 145 the patient’s serum creatinine was 2.06 ng/dL and AlloSure was elevated to 3.7%. DSA testing was positive. A biopsy performed identified acute/active antibody-mediated rejection.

The use of AlloSure may have allowed the biopsies on days 30 and 60 to be avoided since the AlloSure results indicated a very low probability of rejection. Additionally, if an AlloSure had been obtained between day 111 and day 145 the ABMR may have been diagnosed and treated earlier than day 145.

* Patient was a participant in the DART study; refer to Clinical Validity and Clinical Utility section for a description of the study.
Clinical Validation and Clinical Utility: The DART Study

AlloSure in Kidney Transplant Rejection (Bloom et al., 2017)

Accurate and timely detection of allograft rejection and effective treatment are essential for long-term survival of renal transplants. By measuring dd-cfDNA from the circulating plasma, AlloSure is a noninvasive test of allograft cell injury that may enable more frequent, quantitative, and safer assessment of allograft rejection and injury status.

To establish clinical validity of AlloSure in kidney transplantation, CareDx sponsored a prospective sample collection study. The Circulating Donor-Derived Cell-Free DNA in blood for diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study was designed to validate that plasma levels of dd-cfDNA can discriminate active rejection status. 1272 blood specimens were prospectively collected from 384 kidney recipients at 14 clinical sites at scheduled post-transplant intervals, as well as concomitantly with clinically indicated or surveillance kidney biopsies performed according to protocol. Blood was collected at the time of scheduled surveillance visits at months 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, 24; or at the time of each kidney allograft biopsy and up to 2 follow up samples within 8 weeks of the kidney allograft biopsy. Venous blood was collected according to AlloSure protocols and processed at CareDx. dd-cfDNA levels were measured in blood plasma using the AlloSure test and correlated with allograft rejection status ascertained by renal biopsy.

The on-site pathologist’s report was used to determine the diagnosis of rejection in accordance with criteria designated by the Banff Working Groups. The primary analyses combined three subclasses of rejection, T cell-mediated rejection (TCMR), “acute/active” antibody-mediated rejection (ABMR) and “chronic, active” ABMR, because they share common histologic criteria and related cell injury manifestations. The term active rejection is used to describe these pooled classes of rejection and distinguish them from all other biopsy-based diagnoses not associated with active rejection.

The objective of the primary statistical analysis was to determine whether the dd-cfDNA in a recipient’s plasma can discriminate active rejection from no active rejection allograft status. Secondary analyses included comparisons of dd-cfDNA performance in discriminating biopsy-based diagnosis of ABMR from samples that did not have biopsy evidence of ABMR. There were 107 clinically indicated biopsies during the study period that had matched plasma dd-cfDNA results; 27 biopsies from 27 recipients with active rejection and 80 biopsies from 75 recipients without active rejection.

The fraction of dd-cfDNA in blood plasma differed significantly between the groups (Figure 1). The median levels of dd-cfDNA in kidney transplant recipients with active rejection were significantly higher (1.6%) than in the comparator group (0.3%) of biopsy specimens without active rejection (p<0.001). At 1.0% dd-cfDNA, the PPV was 61% and NPV was 84% for active rejection vs no active rejection (Figure 1). At 1.0% dd-cfDNA the PPV was 44% and NPV was 96% for ABMR vs no ABMR (Figure 2).

Median dd-cfDNA was 2.9% (ABMR), 1.2% (TCMR, Types ≥ IB), 0.2% (TCMR Type IA), and 0.3% (controls); p<0.001, ABMR vs controls; p=0.05, TCMR Type ≥ IB vs controls.
These results show that AlloSure measurement of dd-cfDNA level may be used to assess allograft rejection and injury status; levels ≥1% indicate a risk of active rejection (most likely ABMR or TCMR types ≥IB). dd-cfDNA levels below 1% reflect absence of active rejection and may be useful to guide immunosuppressive management. In the DART study, most (204/242) kidney transplant biopsies were clinically indicated, yet only 27% of these clinically indicated biopsies diagnosed active rejection.

Established clinical laboratory tests such as creatinine and viral loads (e.g. BKV) are measured on a monthly or more frequent schedule. As with all laboratory tests, clinical assessment of the patient’s context is important in the interpretation results. Although the dd-cfDNA test may not eliminate the need for biopsy, results with high PPV could increase the pre-biopsy probability of detecting treatable injury, so that biopsy could be made an even more effective diagnostic tool. In association with a high NPV, dd-cfDNA results may reduce the need for biopsy in some cases of elevated creatinine.
AlloSure in Stable Kidney Transplant Recipients (Bromberg et al., 2017)

The biological variation and reference ranges for AlloSure were established in a population of clinically stable renal transplant recipients from the DART study. The biological variability of the dd-cfDNA assay in a reference renal transplant population is relevant to the clinical interpretation of results in allograft recipients, who may undergo serial monitoring of dd-cfDNA to assess the status of the allograft over time.

From 93 DART patients with stable renal allograft function spanning at least three serial visits, 380 blood samples were acceptable for analysis of the reference values of AlloSure. The intra-(CV_i) and inter-individual (CV_G), coefficients of variation, the index of individuality (II), and reference change value (RCV) were all computed.

The dd-cfDNA median value was 0.21%, and the 97.5th percentile was 1.20%. 96% of results were below 1% dd-cfDNA. Biological variability was calculated from recipients in which all values were greater than 0.2% dd-cfDNA and an RCV of 61% was defined.

The reference ranges for dd-cfDNA in this renal transplant population established that values greater than 1% are above the 96th percentile and are outside the normal range for this population. Since the RCV is 61% for dd-cfDNA, we interpret this to be the relative change in serially measured values of dd-cfDNA that exceeds the difference attributable to biological variation.

Clinical Utility

The DART study data have been used to describe the impact of clinical use of AlloSure to inform on the probability of rejection. Seventy-four per cent of clinically indicated biopsies in DART did not have evidence of allograft damage by histopathology. These biopsies exposed recipients to risk of biopsy adverse complications without the benefit of identified pathology. AlloSure may be used to reduce unnecessary biopsies and provide guidance as to when biopsy use is clinically warranted. The data from DART show the impact of determining whether or not a biopsy should be performed based on the results of AlloSure.

Clinical Utility of AlloSure as a Determinant for Biopsy

In the initial analysis of data from the DART study the NPV and PPV were determined for clinically-indicated biopsies performed on single-kidney transplant recipients. The fraction of dd-cfDNA in blood plasma differed significantly between samples drawn at the time of biopsy-confirmed rejection and samples at the time of a no-rejection diagnosis.

The NPV and PPV, along with the rates of occurrence for histopathology-based diagnosis, were used to determine the outcomes in a proposed set of 100 samples from patients clinically indicated for biopsy. Without AlloSure,
100% of the patients will have a renal percutaneous biopsy performed. These patients are exposed to a risk of adverse events (bleeding, infection) that vary in estimates from 1% to 10% (Ahmad, 2004; Redfield et al., 2016; Schwarz et al., 2005). When managed with AlloSure, only a fraction of the patients will receive a biopsy, as described below.

In the DART study, 28% of samples associated with clinically indicated biopsies had an AlloSure score of greater than or equal to 1.0%. When this threshold is used to determine which patients should proceed to have a biopsy, 28 of the 100 patients would have a biopsy and 72 would not. The 28 AlloSure positive may proceed to have a percutaneous renal biopsy to determine the cause of the increased level of dd-cfDNA.

Based on the PPV, 17 of the 28 AlloSure-positive patients will be found to have active rejection as diagnosed by biopsy histopathology. Eleven of the 28 patients will have a biopsy and not be diagnosed with rejection. The yield of these biopsies (rate of positive biopsy) is 61%, whereas the yield of the biopsies without AlloSure is only 27%.

In this set of 100 patient samples, 72 will have an AlloSure score of less than 1%. All of these patients may avoid the risk of morbidity, discomfort, and hardship, as well as the cost of the biopsy procedure. Based on the NPV established from DART, 60 of these 72 patients would not have had any histopathology of rejection if they had been subjected to a biopsy and are the patients with the greatest gain from avoiding biopsy by using AlloSure. These patients originally had an elevated creatinine or other reason for the clinician to suspect rejection, but the cause was not rejection.

Based on the prevalence in DART, if the remaining 12 patients received a biopsy, six would be diagnosed only with a TCMR Type IA, four with TCMR ≥1B, and only two with ABMR. Kidney transplant patients are continuously monitored and ongoing changes in serum creatinine or other indicators of concern regarding kidney function will be available to trigger additional AlloSure and biopsy tests should any negative AlloSure need additional workup.
REFERENCES

AlloSure Publications


   This publication describes the development, analytical validation, and clinical validation in heart transplantation for the AlloSure dd-cfDNA test. The reference materials are described in detail and all of the data from 1117 sample runs are presented to define the accuracy, precision, and performance characteristics of the test.


   The DART study enrolled nearly 400 patients, including 102 patients for which blood was collected at the time of a clinically indicated biopsy and AlloSure results generated. In 107 samples from these patients the performance characteristics of AlloSure to identify active rejection in kidney transplant recipients were defined. A threshold of 1% was chosen to differentiate active rejection from no active rejection. Additionally, AlloSure was even more highly correlated with antibody-mediated rejection and the performance characteristics were defined.


   Some patients in the DART multicenter study did not experience rejection or other signs of allograft injury. These were considered the population of stable transplant recipients and the monthly AlloSure tests were used to define the baseline for quiescent patients (median = 0.21% dd-cfDNA) and month-month biological variation in stable recipients. These data are presented in this publication.


   Patients in the DART study with more than one renal allograft in situ, those who did not have a clinically indicated biopsy at the first visit and had no rejection while on the study (surveillance cohort; n=12), and those with clinically indicated biopsy of the most recent allograft (rejection cohort; n=11) were examined for the impact of biopsy-proven active rejection on dd-cfDNA in repeat transplant patients. In the surveillance cohort, repeat kidney transplant patients had slightly higher, but clinically similar dd-cfDNA levels (median 0.29%) compared to single allograft patients (median 0.19%). The dd-cfDNA levels in repeat transplant patients with biopsy-proven rejection were significantly higher than dd-cfDNA levels in either single or repeat transplant patients without biopsy-proven rejection.

This analysis of the DART study identified the performance of AlloSure when used in conjunction with evaluation of donor-specific antibodies. The PPV of AlloSure for identification of ABMR in DSA-positive patients was calculated.