Propuesta de Taller Nacional para estudio multicéntrico

Resumen de la propuesta

DATOS DEL PROYECTO
Título del estudio: Evaluación del beneficio diagnóstico del uso de AND libre circulante derivado del donante (dd-cfDNA) en trasplante renal
Investigador principal: Grupo Español de trabajo en Histocompatibilidad e Inmunología del Trasplante (GETHIT)
Lugar de realización: Centro de Transfusión de la Comunidad Valenciana
Resumen del proyecto La presencia de ADN libre circulante derivado del donante (dd-cfDNA) en el contexto del trasplante renal ha sido recientemente demostrada que se encuentra elevada en pacientes trasplantados que presentan un daño inicial del injerto, pudiéndose utilizar su monitorización como biomarcador temprano de la función renal y predictivo de la pérdida de injerto. El objetivo de este estudio es evaluar la correlación entre el dd-cfDNA medido en plasma y los resultados obtenidos de la biopsia renal para su implementación como método no invasivo en el diagnóstico de rechazo agudo. En este estudio controlado multicéntrico y transversal se pretende evaluar los beneficios diagnósticos de la cuantificación del dd-cfDNA para su uso como biomarcador diagnóstico de rechazo agudo. Para ello se pretenden incluir un total de 40 pacientes que hayan sido trasplantados renales y que presenten necesidad de realización de biopsia por sospecha clínica de rechazo agudo. La valoración del dd-cfDNA se llevará a cabo mediante secuenciación masiva o PCR-NGS mediante un panel comercial de marcadores STR (AlloSure®, CareDX, USA). La cuantificación del dd-cfDNA se realizará previa a la realización de la biopsia, pero en el momento justo de la sospecha clínica de rechazo. Se estudiarán diferentes grupos de pacientes atendiendo al tipo de rechazo que se diagnostique por biopsia (rechazo mediado por células T, rechazo mediado por anticuerpos, rechazo mixto y rechazo subclínico). El estudio de pacientes con sospecha de rechazo renal permitirá obtener información valiosa sobre los diferentes niveles de daño renal agudo. El análisis del dd-cfDNA será estudiado a nivel nacional por los miembros del grupo GETHIT en un taller nacional intercomparativo cuyos resultados se expondrán en la reunión anual del mismo durante el 43 Congreso de la Sociedad Española de Inmunología.

Study Proposal

Executive Summary

Title of the Study: Assessment of the diagnostic benefit of donor-derived cell free DNA (dd-cfDNA) for renal transplantation
Principal Investigator and Sponsor: GETHIT Group
Study Site(s) and Location(s): GETHIT Group Sites
Condition/intervention to be studied: To assess the correlation between dd-cfDNA measured in blood plasma and histopathology reporting, based on "for cause" renal biopsy
What is/are specific research question(s) aim(s) to be addressed within the proposed study design? In this cross-sectional, multi-center, controlled study, we will evaluate the diagnostic benefit of quantifying dd-cfDNA in diagnosing graft rejection. We will include recipient patients who underwent renal transplantation and show the need for graft biopsy due to changes in clinical and/or laboratory parameters. dd-cfDNA will be quantified at the time of biopsy. The primary objective is to assess the correlation between dd-cfDNA measurements in whole blood and histopathology reporting. We will assess dd-cfDNA measurements in detecting active rejection (borderline rejection, t-cell mediated rejection, antibody-mediated rejection, or mixed rejection). Further, the benefit of dd-cfDNA in predicting long-term graft loss or graft outcome in addition to standard of care parameters will be evaluated.
What is/are hypothesis(es)? By studying patients presenting for a cause biopsy will allow to collect an enriched cohort of samples that would have different diagnosis and may present with different levels of renal allograft injury. The analysis of dd-cfDNA in these samples will be helpful for the GETHIT Group for hands-on experience and own assessment by a workshop format study on how the biomarker may deliver relevant additional information which when combined with histology and other information, it may inform clinicians of events, which may be detrimental to graft

survival.

Identify and define the primary outcome and when the outcome will be measured:

- At diagnose when renal biopsy is indicated as cause of suspected allograft dysfunction
- Correlation of dd-cfDNA with diagnosis:
    - Transplant biopsies will be interpreted by histopathologist and graded according to Banff criteria. Samples will be classified as non-active rejection vs active rejection, by rejection type and histopathology grade, and by non-injury vs injury.
    - Levels of dd-cfDNA expressed as fraction, serum-creatinine, eGFR, proteinuria and DSA will be captured together with biopsy. Median values comparison between groups will be performed with a non-parametric approach.

Identify and define the secondary outcome and when the outcome(s) will be measured:

- At the next two follow up clinical visits planned as regular standard of care (3- and 6-months after biopsy would be ideal)
- To assess the additional benefit of dd-cfDNA measurements in predicting graft outcomes
    - dd-cfDNA measurements at diagnosis will be correlated to standard parameters collected in (post-) transplant surveillance such as demographic parameters, serum-creatinine, eGFR, proteinuria and DSA to evaluate the benefit of dd-cfDNA measurements in predicting graft loss/graft function

Short Scientific Background

Thus, different parameters such as serum-creatinine, the estimated glomerular filtration rate (eGFR), proteinuria and human leukocyte antibody profiles have been identified to suspect allograft injury. Yet so far only histology obtained via needle biopsy and classified following the BANFF classification can properly identify the cause leading to graft damage. Graft biopsy, although being a routine intervention, can cause major complications such as hemorrhage up until graft loss. That is why interest has been ignited in minimally invasive strategies to detect graft injury and/or rejection. The aim is to establish a set of different parameters including the patient's personal and demographic information, lab reports, analysis of DSA and eventually other, new biomarkers to predict graft function/failure without the need for biopsy. Ideally, these new biomarkers could predict graft damage even before already.

The quantification of donor derived cell free DNA (dd-cfDNA) has been proposed as a noninvasive marker for diagnosis of graft rejection [1]. The rationale is that rejection entails

injury which leads to increased apoptosis in the graft, accompanied by higher levels of dd-cfDNA released from these cells into the circulation. Bloom et al. showed that active graft rejection is accompanied by higher levels of dd-cfDNA [2]. Jordan et al. proved that dd-cfDNA levels are even higher in antibody-mediated rejections compared to other types of rejection [3]. Further, Bu et al. presented evidence with a large longitudinal cohort that routine monitoring of dd-cfDNA allows early identification of clinically important graft injury [4].

The inclusion of dd-cfDNA monitoring may complement well histology and traditional laboratory surveillance strategies as a prognostic marker and risk-stratification tool post-transplant.

References

1. Gielis EM, Ledeganck KJ, Winter BY de, et al. Cell-Free DNA: An Upcoming Biomarker in Transplantation. Am J Transplant 2015;15:2541–51.
2. Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. J Am Soc Nephrol 2017;28:2221–32.
3. Jordan SC, Bunnapradist S, Bromberg JS, et al. Donor-derived Cell-free DNA Identifies Antibody-mediated Rejection in Donor Specific Antibody Positive Kidney Transplant Recipients. Transplant Direct 2018;4.
4. Bu L, Gupta G, Pai A, et al. Clinical outcomes from the Assessing Donor-derived cell-free DNA Monitoring Insights of kidney Allografts with Longitudinal surveillance (ADMIRAL) study. Kidney Int. 2021 Dec 22:S0085-2538(21)01168-6.

How will answering these questions change clinical practice, change concepts about the topic or confirm the work of other investigators?

The study is intended as workshop among GETHIT Group members willing to participate sending samples. A practical exercise as training or demonstration on the novel biomarker dd-cfDNA for non-invasive solid organ transplant surveillance.

Study Methods (Observational or Experimental):

Observational, multicntre study

Patient Population (Inclusion and Exclusion Criteria):

Participants with single kidney transplant (de-novo transplant) with the indication for graft biopsy will be considered for this study

Inclusion Criteria

Participant is willing and able to give informed consent for participation in the trial.

Male or Female, aged 18 years or above (Gillick Competent).

Transplanted > 14 days and < 1 year ago.

Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

Participants not meeting the inclusion criteria

Participants not willing to comply all trial requirements

Kidney re-transplant recipients

Transplant recipients who:

- have received a transplant from a monozygotic (identical) twin
- have had allogeneic blood or bone marrow transplant
- are pregnant
- have had multiple transplanted organs
- have had a blood transfusion that contains white blood cells within the past 30 days (washed or leukocyte-depleted RBCs are acceptable)
- have had a biopsy within the past 24 hours

When will data be collected? Include timing of visits (either SOC or specifically for the study) and calendar:

Date and collected parameters (eGFR=estimated glomerular filtration rate; DSA=donor-specific antibodies; dd-cfDNA=donor derived cell free DNA; EMR=electronic medical record):

Date	Histology	eGFR	Proteinuria	DSA	dd-cfDNA	EMR
Day of biopsy (Visit 0)	X	X	X	X	X	X
Clinical follow-up on Visit 1 and 2 (ideally at days 90 and 180)		X	X			X

At Visit 1 and 2 (ideally at days 90 and 180, but may vary depending on standard of care practice

of each participant center): Clinical follow-up visits will include routine entering of demographic parameters, serum-creatinine, eGFR, DSA (if available) and proteinuria into EMR. For patients having treatment for rejection, information about drugs used will be captured too. For dd-cfDNA quantification by using AlloSeq cfDNA assay (CareDx), sample will be collected always before biopsy. 8 ml of whole blood will be drawn in Cell-Free DNA BCT (Streck), 2x tubes per patient will be taken for contingency. Plasma separation and storage will be performed within 7 days of blood draw.
Anticipated Enrollment (number of patients): 40 patients, 40 samples to be tested for quantification of dd-cfDNA levels in plasma
Study Duration (include anticipated start and end dates): March to September 2022 (Interim results) to February 2023 (Final results) Project approval in February 2022 will open the possibility to invite participant site to send out samples, call open for 4 months (March to June 2022). Reference site receiving samples will be testing them blinded in batch. There are two runs planned, each run with n=20 samples and n=4 external quality controls, which will be completed by July 2022. Participant sites will share sample information after testing, and data analysis with paired clinical variables will be executed during August 2022 to generate interim preliminary report. Final interim report as power point format will be presented to GETHIT Group participant study sites during 43 Congreso de la Sociedad Española de Inmunología (León, September 22-24, 2022). Participant sites should be gathering clinical follow up data of patients enrolled on day 90 (by October approx.) and at day 180 (by January 2023 approx.). Data analysis for assessment of clinical outcomes will be performed when all data received and will be included on the final report (February 2023). End of the study is defined when final report will be generated, minimum as power point format distributed among participant sites and funders for the study.
Publication Plan (publishing timeline and anticipated journal): Oral presentation with interim results at GETHIT Group meeting during 43 Congreso de la Sociedad Española de Inmunología (León, September 22-24, 2022). Oral presentation with final results during most appropriate GETHIT Group meeting opportunity in 2023.
Estimated Total Grant Amount and/or estimated number of free tests (for research use only) to be provided by CareDx/Longwood:

CareDx and Diagnostica Longwood will supply free of charge the necessary laboratory reagents for processing 48 AlloSeq cfDNA tests for research use only subject to agreement to:

1. Use the free of charge kits are to be used only as specified in the study plan.
2. Maintain records to show and account for the uses of reagents
3. Allow CareDx access to records to verify use of AlloSeq cfDNA testing
4. Provide written acknowledgment of receipt reagents necessary for AlloSeq cfDNA tests
5. Return or discard any kits not used for the specified purposes.
6. Refrain from use of the tests for any purpose prohibited by law.
7. Cooperate with any efforts of CareDx/Longwood to publicize the grant award.
8. Comply with reasonable requests from CareDx/Longwood for information about program activities.

The necessary laboratory reagents for processing 48 AlloSeq cfDNA (40 patient cfDNA samples + 8 cfDNA controls) research use only results are:

- 1x cfDNA control set kit
- 80x Cell-Free DNA BCT blood collection tubes (Streck)
- 1x Qiagen QIAamp Circulating Nucleic Acid Kit (Qiagen, catalog # 55114)
- 2x AllSeq cfDNA kit (CareDx, catalogue # ASCF.1(24))
- 2x MiSeq V3 (150 cycle) sequencing reagent kit (Illumina, catalog # MS-102-3001)

Other Relevant Research Information:

Internal Reference Number / Short title:

IRB Ref:
Date and Version No: 14 02 2022 v0

Principal Investigator Signature:

Firmado por FRANCISCO JOSE BOIX GINER - NIF:74239160J el día 14/02/2022 con un certificado emitido por ACCVCA-120

The approved protocol should be signed by author(s) and/or person(s) authorised to sign the protocol