

Non-invasive MR Imaging Diagnosis of Transplant Rejection

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1. PURPOSE OF THE STUDY

a. Brief Summary

The purpose of this study is to develop a non-invasive imaging test for in vivo detection of kidney transplant rejection. We propose to use an FDA-approved iron supplement "off label" as a marker for macrophages. After intravenous injection, the iron compound is taken up by macrophages and causes a detectable signal on magnetic resonance (MR) images. Since kidney transplants that undergo a rejection contain macrophages, but not unimpaired transplants, this approach should enable us to detect kidney transplant rejection with a simple imaging test.

b. Objectives

If successful, this ferumoxytol-based MR imaging test could reduce the number of renal allograft biopsies and associated anesthesia. If at least some of these biopsies could be replaced by a non-invasive imaging test, this would have immense impact on patient management and patient quality of life, and reduce health care costs. Exclusion of renal allograft rejection based on ferumoxytol-enhanced MRI would help avoid unnecessary biopsies, accelerate diagnostic workup of other reasons for an impaired renal function, and ultimately, improve patient outcomes. The proposed non-invasive imaging test could be in principle also applied to evaluation of other solid organ or stem cell transplants. Data derived from this project will be used as preliminary data for a subsequent NIH grant application.

c. Rationale for Research in Humans

Initial studies have been performed in animal models. We now have to verify the animal model results can be reproduced in patients.

2. STUDY PROCEDURES

a. Procedures

The patients and their parents will be informed about the nature of the study and a written informed consent will be obtained. 12-24 h before a planned MRI study, Ferumoxytol will be administered intravenously at a dose of 5 mg Fe/kg over approximately 1-2 minutes. The blood pressure and respiratory rate will be monitored before and directly after the injection, and the patients will be observed for any potential adverse events for at least 30 minutes after the iron oxide administration. The patients will undergo MR imaging at 1-7 days after iron oxide injection. For some patients, we have the option to get a MR- exam before the injection of the contrast agent for comparison of pre and post images. This long interval between injection and imaging is needed in order to allow for sufficient time for macrophage phagocytosis to occur. Patients will be placed supine in a 3T MR scanner, the area of the transplant kidney will be covered by a dedicated surface coil, and T1- and T2-weighted MR images will be obtained of the transplant, using axial and/or coronal T1-LAVA and T2-FSE sequences as well as multi-TE SE, multi-TI IR sequences and QSM sequences for iron quantifications. All MR images will be transferred to a postprocessing workstation and T1- and T2-relaxation times will be calculated of the kidney transplant as well as adjacent muscle and bone marrow in a pelvic bone as an internal standard. The patients will undergo a routine biopsy of their transplant within 3 weeks before or (preferably) after the MRI study. Based on renal biopsies and histopathology results, patients will be divided into groups of "no rejection" and "positive rejection". Various MRI and histopathological parameters will be compared between these two groups using student's t-tests, an analysis of variance (e.g. for comparison of data from multiple time points, multiple pulse sequences, different histopathologic stains) and regression analyses. Significant differences will be assigned for a p-value of less than 0.05.

Ferumoxytol is an FDA approved iron supplement, and it is slowly metabolized and release iron in vivo. In order to determine the effect and clearance of ferumoxytol, we propose to monitor the participant's blood iron levels immediately before and up to 6 months (3-4 times in total) after ferumoxytol injection. Furthermore, we want to investigate the blood for interaction between blood plasma proteins and iron as well as related immune cell responses and potential mechanisms for hypersensitivity reactions. We will collect small blood samples (<5ml each) from the participant when we administer ferumoxytol intravenously and when the participant visits Stanford for their routine blood tests to avoid additional visits or needle sticks. The tests will be ordered through Stanford Clinical Laboratories.

b. Procedure Risks

Once MRI contraindications have been excluded (e.g. MRI incompatible metal devices), the imaging test itself does not involve any risks for the patient. Contrast agent injection will occur outside of the magnet, in order to allow for close observation of the patient for any side effects. Of note, our other clinical imaging studies with ferumoxytol did not show any subjective or objective side effects in our patient population so far.

c. Use of Deception in the Study

NA

d. Use of Audio and Video Recordings

NA

e. Alternative Procedures or Courses of Treatment

The alternative procedure to verify a suspected transplant rejection is a transplant biopsy, which is performed in children under general anesthesia. A biopsy is associated with risk of bleeding, vascular fistulas, other organ injuries and anesthesia complications.

f. Will it be possible to continue the more (most) appropriate therapy for the participant(s) after the conclusion of the study?

yes, all patients undergo a biopsy

g. Study Endpoint(s)

Endpoint: To generate a non-invasive, easily applicable and widely available MR imaging test for evaluation of kidney transplant rejection. Primary endpoint is to develop a sensitive and reliable, non-invasive and quantitative imaging test for detection of macrophages in kidney transplants as a biomarker for rejection. As a secondary endpoint, since different oxygenation states of iron have scaled but measurable changes on the inherent tissue magnetic susceptibility, we plan to also evaluate the utility of simultaneously generated 3D maps of arterial and venous supplies of kidney transplants, provided by our imaging method, in comparison with ultrasound as the current diagnostic standard. The ultimate goal is to provide a comprehensive evaluation of the morphology, vascular supply and host immune responses in kidney transplants with one single, non-invasive and comprehensive diagnostic test.

3. BACKGROUND

a. Past Experimental and/or Clinical Findings

In patients with end stage renal failure, renal transplantation is the treatment modality of choice, leading to markedly improved quality of life and survival when compared to chronic dialysis. To date, more than 1.4 million adult patients and 70.000 children have received renal allografts. However, a major complication of renal allograft transplantation in children and adolescents is an acute or chronic rejection, which causes almost half of the kidney transplant losses. Currently the diagnosis of rejection relies on allograft biopsies, which are invasive, nearly always require general anesthesia in children and are prone to sampling errors. A non-invasive diagnostic test, which could visualize and monitor allograft rejection directly and longitudinally in vivo would save invasive biopsies and anesthesia, reduce potentially associated complications and reduce health care costs.

b. Findings from Past Animal Experiments

We chose macrophages as our target for imaging transplant rejection, because macrophages play a critical role in transplant rejection and because macrophages can be imaged with immediately clinically applicable MR imaging approaches. Macrophages are key inflammatory mediators of the innate immune response that contribute to both acute and chronic allograft rejection through a variety of mechanisms. Macrophages are attracted to sites of immune complex formation by complement fragments (e.g. C5a) and specific cytokines/chemokines. In the transplant, the macrophages are activated by IFN gamma (produced by T cells or NK cells) and TNF alpha (produced by APCs) which leads to a pro inflammatory cascade with production of reactive oxygen species, progressive transplant injury, and ultimately, graft rejection. In renal allografts that undergo rejection, CD68 positive macrophages comprise approximately 50% of the infiltrating leukocyte population, they co-localize with areas of tissue-damage and fibrosis, are preponderant in more severe forms of rejection and represent an independent predictor of worse outcomes. As new immune-modulating therapeutic agents enter the clinic whose mechanism of action involves diminishing macrophage infiltration or presence in allografts, it becomes increasingly important to identify those transplants heavily infiltrated by macrophages, as well as monitoring response to these new therapies.

4. RADIOISOTOPES OR RADIATION MACHINES

a. Standard of Care (SOC) Procedures

Identify Week/Month of Study	Name of Exam	Identify if SOC or Research
NA	NA	NA

b. Radioisotopes

- i. Radionuclide(s) and chemical form(s)
NA
- ii. Total number of times the radioisotope and activity will be administered (mCi) and the route of administration for a typical study participant
NA
- iii. If not FDA approved: dosimetry information and source documents (package insert, Medical Internal Radiation Dose [MIRD] calculation, and peer reviewed literature)
NA

c. Radiation Machines – Diagnostic Procedures

- i. Examination description (well-established procedures)
NA
- ii. Total number of times each procedure will be performed (typical study participant)

NA

- iii. Setup and techniques to support dose modeling

NA

- iv. FDA status of the machine and information on dose modeling (if procedure is not well-established)

NA

d. Radiation Machines – Therapeutic Procedures

- i. Area treated, dose per fraction/number of fractions, performed as part of normal clinical management or due to research participation (well-established procedures)

NA

- ii. FDA status of the machine, basis for dosimetry, area treated, dose per fraction and number of fractions (if procedure is not well-established)

NA

5. DEVICES USED IN THE STUDY

a. Investigational Devices (Including Commercial Devices Used Off-Label)

Investigational Device 1	
Name:	NA
Description:	NA
Significant Risk? (Y/N)	NA
Rationale for Non-Significant Risk	NA
Investigational Device 2	
Name:	N/A
Description:	N/A
Significant Risk? (Y/N)	N/A
Rationale for Non-Significant Risk	N/A
Investigational Device 3	
Name:	N/A
Description:	N/A
Significant Risk? (Y/N)	N/A
Rationale for Non-Significant Risk	N/A

b. IDE-Exempt Devices

IND-Exempt Device 1	
Name:	MRI
Description:	GE Healthcare, is a designated NSR device per the published IRB guidance GUI-7m

IND-Exempt Device 2	
Name:	N/A
Description:	N/A
IND-Exempt Device 3	
Name:	N/A
Description:	N/A

6. DRUGS, BIOLOGICS, REAGENTS, OR CHEMICALS USED IN THE STUDY

a. Investigational Drugs, Biologics, Reagents, or Chemicals

Investigational Product 1	
Name:	Ferumoxytol (Feraheme, AMAG Pharmaceuticals), IND 111 154
Dosage:	5mg Fe/kg bodyweight
Administration Route:	intravenous
Investigational Product 2	
Name:	N/A
Dosage:	N/A
Administration Route:	N/A
Investigational Product 3	
Name:	N/A
Dosage:	N/A
Administration Route:	N/A

b. Commercial Drugs, Biologics, Reagents, or Chemicals

Commercial Product 1	
Name:	N/A
Dosage:	N/A
Administration Route	N/A
New and different use? (Y/N)	N/A
Commercial Product 2	
Name:	N/A
Dosage:	N/A
Administration Route	N/A
New and different use? (Y/N)	N/A
Commercial Product 3	
Name:	N/A
Dosage:	N/A
Administration Route	N/A
New and different use? (Y/N)	N/A

7. DISINFECTION PROCEDURES FOR MEDICAL EQUIPMENT USED ON BOTH HUMANS AND ANIMALS

We will use MR scanners at Lucas Center or Lucile Packard Childrens Hospital at Stanford University. The bed/table and accessories that are used for the animals is different than the table humans use. Physiologic monitoring equipment is cleaned with a commercial disinfectant such as Roccal, Conclick, Sani-Wipes, or a 10 Bleach solution. All RF coils and positioning accessories are wrapped in plastic wrap or plastic bags for use with animals. Everything, even if it is animal use only, is cleaned with the above disinfectants after every use even if they are wrapped in plastic. The Lucas Center is checked yearly by several groups at Stanford who approve animal research in human

systems: Stanford Health & Safety. The facility is reviewed by: Stanford APLAC panel; USDA; NIH; and Aaalac.

8. PARTICIPANT POPULATION

a. Planned Enrollment

30 solid organ transplant recipients, to monitor for transplant rejection and evaluate the utility of 3D maps of arterial and venous supplies to the transplant.

b. Age, Gender, and Ethnic Background

We expect all 30 participants to be pediatric kidney transplant patients, any gender, any ethnic background. Referrals only accepted from Drs. Grimm and Concepcion.

c. Vulnerable Populations

We expect all 30 participants to be pediatric kidney transplant patients. The study adds on a non-invasive imaging study to the standard of care for these patients. Informed Consent and Assent practices will be followed.

d. Rationale for Exclusion of Certain Populations

NA

e. Stanford Populations

NA

f. Healthy Volunteers

NA

g. Recruitment Details

Participants will be referred from their treating physician's office, either Dr. Grimm or Dr. Concepcion invitation only .

h. Eligibility Criteria

i. Inclusion Criteria

recipient of a solid organ transplant, scheduled for a standard of care rejection survey

ii. Exclusion Criteria

contraindication for MRI hemosiderosis/hemochromatosis, diagnosed based on routine lab values obtained within 30 days prior to MRI scan and/or precontrast MRI

i. **Screening Procedures**

Referring treating physician discusses study with potential participant. Potential participant is referred to study coordinator to schedule a screening visit. The protocol director or other IRB approved trained personnel complete consent/assent, MRI screen, and chart review lab values .

j. **Participation in Multiple Protocols**

We will ask the participant and their family if they are enrolled in any other studies. If they are enrolled in a drug study, we will consult with the other PI before proceeding to avoid any conflicts.

k. **Payments to Participants**

Reimbursement for gas/mileage to a max of \$50 per visit to the family/driver. A \$20 Amazon gift card to the participant.

l. **Costs to Participants**

No research costs will be charged to the participants.

m. **Planned Duration of the Study**

The study is expected to enroll patients for two years, with data analysis continuing for possibly another year total: three years . i screening: 20-45 minutes; ii 90-120 minutes; iii 3-5 hours per participant, including 3D reconstructions, plus another 40-100 hours of collective data analysis.

9. RISKS

a. **Potential Risks**

i. Investigational devices

NA

ii. Investigational drugs

Dr. Daldrup-Link has applied USPIO ultra small superparamagnetic iron oxides as MR contrast agents in phase II and III clinical trials in adult patients. In addition, Dr. Daldrup-Link has currently two active clinical trials on the use of ferumoxytol in children for MR imaging of bone lesions and for whole body tumor staging. The USPIO contrast agents

are very well tolerated and show excellent safety profiles. The delivered iron dose via a typical ferumoxytol administration is in the order of 150-500 mg iron oxides note that these are coated iron particles, not free iron, which is equivalent to or lower than the iron dose administered with one blood transfusion. USPIO are slowly metabolized in the liver and not excreted via the kidneys. Thus, they are safe to use in patients with renal insufficiencies and are not associated with any risk of nephrogenic sclerosis a potential adverse event after injections of certain gadolinium chelates. Expected risks include dizziness and nausea, possible localized twitching sensation, etc. Anaphylaxis or anaphylactoid reactions were reported in 0.2 of subjects, which is in the order of other MR contrast agents.

iii. Commercially available drugs, biologics, reagents or chemicals

NA

iv. Procedures

MRI w/ contrast Magnetic fields do not cause harmful effects at the levels used in the MRI machine. However, the MRI scanner uses a very strong magnet that will attract some metals and affect some electronic devices. In some cases, having those devices means the participant should be excluded. Additionally, when contrast is injected, as with any intravenous injection, there are risks of bruising, bleeding, or infection from the venipuncture; and allergic reaction to the injected contrast.

v. Radioisotopes/radiation-producing machines

NA

vi. Physical well-being

NA

vii. Psychological well-being

Some small risk of a patient experiencing claustrophobia. This is an infrequent but regular occurrence in any MRI facility. Every effort is made to minimize this risk and the research and clinical staff are well trained to act appropriately.

viii. Economic well-being

NA

ix. Social well-being

NA

x. Overall evaluation of risk

Low - innocuous procedures such as phlebotomy, urine or stool collection, no therapeutic

agent, or safe therapeutic agent such as the use of an FDA approved drug or device.

b. International Research Risk Procedures

NA

c. Procedures to Minimize Risk

An MRI screening form will be completed prior to participation. Any potential contraindication to MRI revealed by the comprehensive screening form will result in participant exclusion. The research team will use the clinical systems and the RedCap database for data management. RedCap is maintained by Clinical Informatics at Stanford University as a HIPAA compliant, secure, encrypted database for research purposes. Data exported by the researchers from these secure systems will only be exported in a deidentified form to minimize risks to confidentiality.

d. Study Conclusion

Endpoint: To generate a non-invasive, easily applicable and widely available MR imaging test for evaluation of kidney transplant rejection. Primary endpoint is to develop a sensitive and reliable, non-invasive and quantitative imaging test for detection of macrophages in kidney transplants as a biomarker for rejection. As a secondary endpoint, since different oxygenation states of iron have scaled but measurable changes on the inherent tissue magnetic susceptibility, we plan to also evaluate the utility of simultaneously generated 3D maps of arterial and venous supplies of kidney transplants, provided by our imaging method, in comparison with ultrasound as the current diagnostic standard. The ultimate goal is to provide a comprehensive evaluation of the morphology, vascular supply and host immune responses in kidney transplants with one single, non-invasive and comprehensive diagnostic test.

In terms of individual participation, each patient's experimental participation is over when their individual MRI is collected, analyzed, and reported. If a patient experiences an anaphylactoid reaction to the study agent during initial administration, participation would be terminated - administration of the agent would be discontinued, counteracting medication would be given, and the patient would not undergo MRI. Such reactions are very rare reported in 0.2 of subjects but anticipated, and as such, the contrast agent is only administered in a clinical setting under the direct supervision of an experienced MD, with access to a crash cart and appropriate anti-anaphylactoid medication.

e. Data Safety Monitoring Plan (DSMC)

- i. Data and/or events subject to review

Of note, Dr. Daldrup-Link has applied iron oxide nanoparticles as MR contrast agents in phase II and III clinical trials in adult patients. These contrast agents are very well tolerated and show excellent safety profiles. The delivered iron dose via a typical

ferumoxytol administration is in the order of 150-500 mg iron oxides note that these are coated iron particles, not free iron, which is equivalent to or lower than the iron dose administered with one blood transfusion. Iron oxide nanoparticles are slowly metabolized in the liver and not excreted via the kidneys. Thus, they are safe to use in patients with renal insufficiencies and are not associated with any risk of nephrogenic sclerosis a potential adverse event after injections of certain gadolinium chelates. Anaphylaxis or anaphylactoid reactions were reported in 0.2 of subjects, which is in the order of or lower compared to other MR contrast agents see FDA report: Lu M, Cohen MH, Rieves D, Pazdur R. FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease. Am J Hematol. 2010;85 5 :315-9. In the unlikely event of an anaphylactoid reaction, appropriate actions will be taken as with any other contrast agent reaction and the event will be reported to the IRB.

- ii. Person(s) responsible for Data and Safety Monitoring

Dr. Daldrup-Link will serve as the ME. In an unanticipated and unlikely event of any adverse event or anaphylactoid reaction to this contrast agent, appropriate actions will be taken to treat the reaction and the event will be reported to the IRB.

- iii. Frequency of DSMB meetings
NA
- iv. Specific triggers or stopping rules

Any signs of an anaphylactoid reaction, such as rash, urticaria, nausea, cough, breathing difficulty will lead to discontinuation of contrast administration and symptomatic treatment.

- v. DSMB Reporting

The ME will forward written reports to the appropriate entities via email

- vi. Will the Protocol Director be the only monitoring entity? (Y/N)
y
- vii. Will a board, committee, or safety monitor be responsible for study monitoring? (Y/N)
y

f. Risks to Special Populations

The study involves a non-invasive imaging study and the off-label use of an FDA approved drug, with an FDA IND to do so. Side effects for this drug have been observed in less than 0.2 of the population. Informed Consent and Assent are acquired appropriately and all data and safety precautions are observed. Risks are thus assessed as minimal.

10. BENEFITS

Participants may immediately benefit from more accurate assessment of arterial and venous supply to their allograft. In the long term, validating a method for accurately assessing rejection status/health of an allograft without general anesthesia or invasive biopsy reduces risk and cost to the patient.

11. PRIVACY AND CONFIDENTIALITY

All participant information and specimens are handled in compliance with the Health Insurance Portability and Accountability Act (HIPAA) and privacy policies of Stanford University, Stanford Health Care, and Stanford Children's Health.