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Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

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Abstract

Introduction

In the past decades, short-term results after solid organ transplantation have markedly improved. Disappointingly, this has not been accompanied by parallel improvements in long-term outcomes after transplantation. To improve graft and recipient outcomes, identification of potentially modifiable risk factors and development of biomarkers is required. We provide the rationale and design of a large prospective cohort study of solid organ transplant recipients (TransplantLines).

Methods and analysis

TransplantLines is designed as a single center prospective cohort study and biobank including all different types of solid organ transplant recipients, as well as living organ donors. Data will be collected from transplant candidates before transplantation, during transplantation, at 3 months, 6 months, 1 year, 2 years, 5 years, and subsequently every 5 years after transplantation. Data from living organ donors will be collected before donation, during donation, at 3 months, 1 year, and 5 years after donation and subsequently every 5 years. Primary outcomes are mortality and graft failure. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, i.e. physical and psychological functioning, including quality of sleep, and neurological problems such as tremor and polyneuropathy.

Ethics and dissemination

Ethical approval has been obtained from the relevant local ethics committee. The TransplantLines cohort study is designed to deliver pioneering insights in transplantation and donation outcomes. The study design allows comprehensive data collection on perioperative

care, nutrition, social- and psychological functioning and biochemical parameters. This may provide a rationale for future intervention strategies to more individualized, patient-centered transplant care and individualization of treatment.

Strength and limitations

- Large biobank and cohort study with extensive data collection on a myriad topics related to transplantation and/or donation
- Inclusion of all types of solid organ transplant recipients
- Long follow-up to assess many relevant clinical outcomes
- Single center study
- Residual confounding cannot be excluded due to observational design

Background

Solid organ transplantation is the preferred treatment for end-stage organ failure. During the past decades, advances in immunosuppressant medications, treatment of infections, perioperative medical care, and surgical techniques (including living donation) have led to important improvements in early post-transplant graft and patient survival.[1] However, on the long-term, graft failure is a major cause of patient mortality and morbidity in all types of transplantation.[2-4] For example, in renal transplant recipients, half of the cadaveric renal allografts fail within a timeframe of 10 years.[5] Apart from reduced survival, transplant recipients often develop health problems that greatly reduce their perceived quality of life (Figure 1).[6,7,8]

The multitude of health problems that recipients experience after transplantation include amongst others obesity, diabetes, hypertension, heart failure, and malignancies.[9-11] These are likely the consequence of a combination of factors, including (1) continuous exposure to treatment with immunosuppressive drugs necessary for prevention of rejection of the transplanted organ, (2) damage induced by pre-existing exposure to end-stage organ failure, and (3) adverse life-style and environmental factors, all potentially expressed against (4) a background of increased (epi)genetic susceptibility. Among these, immunosuppressive treatment, adverse life-style, and environmental factors are good candidates for modification to decrease the load of post-transplant health problems. It should be realized that immunosuppressive treatment is currently mainly “one-size fits all”. Hence, improvement can be achieved by development of biomarkers that can allow for recognition of transplant recipients in which immunosuppressive load can be safely reduced or in which certain drugs can better be avoided, and for biomarkers which can guide such individualized immunosuppressive treatment. To improve long-term transplant outcomes, it is imperative to identify modifiable risk factors, especially among those recipients who are at increased risk.

To date, it is largely unknown in which transplant recipients immunosuppressive medication can be safely reduced to prevent the development of health problems. Furthermore, in terms of healthcare costs, it is important to prevent recurrent hospital admissions, re-transplantations or – in case of kidney transplantation – return to dialysis, which are all associated with very high expenses.[12] To effectively develop interventions to reduce mortality and morbidity after transplantation, more research is necessary on clinical and biochemical risk factors present in transplant recipients. Also, the use of living donors for kidney and liver transplantation requires a living donor program with good long-term outcomes for the donor and recipient. Living kidney donors, for example, have an increased risk for end-stage renal disease (ESRD)[13,14], while only registry data exist on the effect of living donor characteristics on recipient outcomes.[15 16]

Until now, many registries and large cohort studies focus on one type of solid organ transplantation, limiting comparability between different transplant populations. As a result, studies investigating biomarkers, quality of life, and development of health problems and adverse outcomes across different solid organ transplant populations are scarce. Despite the differences which exists in patient characteristics and treatment after different solid organ transplantations, there are many similarities in health problems that occur among subtypes of transplantation. The objective of TransplantLines study is to identify risk factors for development of long-term health problems after transplantation and to develop new interventions to improve outcome, both combined for all solid organ transplant recipients as well as specific for each subtype of transplantation.

Methods/Design

Study design and setting

The TransplantLines study is a unique, novel prospective biobank and cohort study, which aims to provide a better understanding of causes of disease- and ageing-related outcomes and health problems, both physical and psychological, in solid organ transplant recipients and donors (ClinicalTrials.gov Identifier: NCT03272841). The University Medical Center Groningen (UMCG) is the largest transplantation center in the Netherlands, and the only Dutch center that covers all types of solid organ transplantation, as well as living kidney and liver donation programs. The study protocol has been approved by the Institutional Review Board (METc 2014/077), adheres to the UMCG Biobank Regulation, and is in accordance with the WMA declaration of Helsinki and the declaration of Istanbul. All participants will give written informed consent upon enrollment. Follow-up and prospective events will be recorded over time. An overall participation rate of 85% is expected across the different transplant populations and a total number of 3000 participants is aimed.

Transplant patients

The study population comprises all solid organ transplant recipients, i.e. heart-, lung-, kidney-, liver-, and small bowel transplant recipients. Both new transplant candidates as well as transplant recipients are eligible to participate in the study. Participants of all ages will be included in TransplantLines. Children (age <18 years) will be eligible for participation upon consent by a legal representative (<12 years) or a shared consent of both the child and legal representatives (≥12 years). The study will also include candidates for re-transplantation. Exclusion criteria for participation in the TransplantLines study will be no mastery of the Dutch language or no capability to intellectually comprehend questionnaires or physical tests.

Living donors

Living kidney and liver donors will also be included in the study. The goal of including donors is to study the effects of donation, improve living donor safety and donors will serve as controls for their recipients, allowing for matched longitudinal analyses. Prospective living kidney and liver donor candidates (≥18 years old) will be eligible to participate in the study, as well as living organ donors who have donated an organ prior to the start of the TransplantLines study. Exclusion criteria will be no mastery of the Dutch language or no capability to intellectually comprehend questionnaires or physical tests.

Transplant recipients timeline

All participants of the TransplantLines study will be examined at fixed time points as shown in **Figure 2**. Transplant candidates will be first seen at pre-transplant screening. Prior to transplantation, all transplant candidates undergo a routine clinical screening. Generally, transplant candidates will be transplanted if surgery risks and transplant benefit are optimized, based on an individualized multidisciplinary clinical decision. Further study visits will be performed at time of transplantation, at 3 months, 6 months, 12 months, and 2 years after transplantation, and hereafter follow-up will be performed every 5 years.

Transplant recipients with a functional graft for at least 1 year post-transplantation and who received a solid organ transplant prior to the start of the TransplantLines study will be included at the next outpatient clinic visit. Henceforth, patients will be examined every five years and follow-up samples will be collected. Aside from the fixed time points, biobank samples of transplant recipients will be collected if a biopsy of the transplanted solid organ is necessary, on clinical indication.

Living donors timeline

All donors of the TransplantLines study will be examined at fixed time points as shown in **Figure 2**. The first study visit of donor candidates will occur at pre-donation screening. Prior to donation, all candidates undergo a routine clinical screening. Generally, donors will be accepted if surgery risks and transplant benefit are optimized, based on an individualized multidisciplinary clinical decision taking national and international guidelines into account.[17,18] Subsequently, study visits will be performed at time of nephrectomy, and at 3 months post-donation. At 12 months post-donation, donors will fill in a questionnaire and at 5 and 10 years post-donation there will be another study visit. Hereafter follow-up will be performed every five years. Living organ donors who have donated an organ prior to the start of the TransplantLines study, will be included at their next donor follow-up visit to their outpatient clinic.

Data collection

Biobank

Blood, 24-hour urine, feces, nails, and hair will be collected of participants at each TransplantLines visit. Participants will be instructed to collect a 24-hour urine sample according to strict protocol at the day before their visit to the outpatient clinic, i.e. discard their morning urine specimen, collect all subsequent urine throughout the next 24 hour and include the next morning's first specimen of the day of the visit to the outpatient clinic. Blood will be drawn after an 8-12 hour overnight fasting period in the morning after completion of the 24-hour urine collection.

As blood samples, 1 serum tube of 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube of 6 mL, 1 lithium-heparin tube of 10 mL, and 1 PAXgene tube of 10 mL will be collected of each participant at each TransplantLines visit. Subsequently, tubes will be centrifuged at 1300g for 10 minutes, except the citrate tube which is centrifuged at 2500g for 10 minutes. Of

the 24-hour urine collection, three urine tubes will be collected of which one tube will be partially acidified. All blood- and urine samples will be subsequently aliquoted and shipped to the core laboratory for storage in -80°C (-112 °F) freezers (Panasonic, 's-Hertogenbosch, the Netherlands) (**Table 1**). Blood- and urine samples will be analyzed in the following years for multiple research questions that will arise.

Participants will be asked to collect a feces sample the day prior to the TransplantLines visit. A FecesCatcher (TAG Hemi VOF, Zeijen, the Netherlands) will be sent at the patients' home, and feces sample will be collected in appropriate tubes and frozen immediately after collection. The participant will transport the feces sample in cold storage (with ice cubes or in a cooler) to the TransplantLines visit the following day. Subsequently, the feces sample will be immediately stored at -80°C (-112 °F). Feces samples will be primarily used for microbiome analyses. Solid organ transplant recipients have a shift in the gut microbiome with a decrease in predominant organisms, a loss of bacterial diversity, and emergence of new dominant population. This may result in increased risk of infection, rejection, and mortality. Therefore, we would like to examine the gut microbiome in relation to the development of health problems after transplantation.

Clinical and laboratory characteristics

Clinical laboratory measurements requested by the physician will be included in the study database upon patient consent. Demographic characteristics along with data on medication use will be provided by the participants and will be verified using the electronic hospital records. Medical information including donor and recipient information at time of transplantation, underlying disease, hospital admissions, complications after transplantation, co-morbidities, graft failure, and mortality will be extracted from the electronic hospital records.

Questionnaires

Biobank data will be expanded with an extensive set of questionnaires to collect data on physical, psychological, and social impact of undergoing a transplantation (**Figure 3**). Transplant candidates will be asked to fill out a comprehensive questionnaire during screening prior to transplantation and at 1 year post-transplantation. Transplant recipients with a functional graft for more than 1 year post-transplantation and who received the solid organ prior to start of the TransplantLines study will be asked to complete the same questionnaire. A subset of questionnaires will be provided at the other predefined time-points, i.e. at 3, at 6 months, and at 2 years after transplantation. Topics addressed by questionnaires include among others nutritional intake and diet, health-related quality of life, life-style factors such as physical activity, sleep quality, and smoking behavior, psychological impact such as anxiety, depression, coping, and well-being, and social impact such as employment and family relationships. Specification of all the different questionnaires with related subject is shown in **Table 2**. Questionnaires will be send digitally or by mail, as requested. During study visits, all questionnaires will be checked by a trained investigator for completeness and validity.

Standard assessments

Blood pressure (mmHg) will be measured according to a standard clinical protocol using an automatic device (Philips Suresign VS2^{+}, Andover, MA, USA). To prevent a white-coat effect, participants will be seated during which blood pressure and heart rate will be measured four times, with an interval of three minutes between measurements. Hereafter, participants will be asked to stand up straight for one minute, after which blood pressure and heart rate measurements will be repeated once in standing position. Measurements will be performed

with participants being on their regular medication, including anti-hypertensive drugs at trough.

Anthropometry measurements will include body weight, body length, and waist- and hip circumference. Body weight (kg) will be measured in light weight clothing without shoes using a calibrated digital measuring scale (SECA 877, Seca GMBH, Hamburg, Germany). Height (cm) will be measured using a wall-secured stadiometer (SECA 222). Waist- and hip circumference (cm) will be calculated using a measuring tape roll with standardized retraction mechanism (SECA 201). Waist circumference will be measured midway between the lowest rib and the iliac crest with the participant in standing position. Hip circumference will be determined at the maximum circumference over the trochanter major. All anthropometry measurements will be assessed twice, with inclusion of a third measurement contingent upon a difference of more than half a kilogram in weight or more than one centimeter in length.

Handgrip strength will be assessed with the Jamar Hydrolic Hand Dynamometer (Patterson Medical JAMAR 5030J1, Warrenville, Canada).[19] Participants will be instructed to sit in a chair with their shoulders in adduction, their arms rotated into neutral position, their elbows flexed to 90º, and forearms and wrists held in neutral position. Hereafter, participants will be instructed to perform a maximal isometric contraction. Handgrip strength will be tested three times with an interval of 30 seconds rest for recovery between each attempt. The dominant hand will be stated in all measurements. Furthermore, to create uniformity among assessments, the second handle position of the hand dynamometer will be utilized which has been shown to be the most accurate position.[20]

Lung function will be measured by means of an Asma-1 handheld spirometer (Vitalograph, Buckingham, United Kingdom).[21] Of all participants, the Forced Expiratory Volume (FEV1), as marker of lung function, will be recorded.

Body composition will be determined using a multifrequency bio-electrical impedance device (BIA, Quadscan 4000, Bodystat Ltd, Douglas, British Isles) at 5, 50, 100, and 200 Hz, which allows to distinct between lean body mass and fat body mass taking into account differences in volume status.[22] Main outcome variables from the BIA are estimated fat mass, fat free mass, and body fat percentage. In brief, the BIA measurement will be performed with the participant in supine position with arms and legs abducted from the body. Sensor electrodes will be placed on the dorsum of the right hand and feet, with a minimal distance of five centimeters between the electrodes. Measurement will not be executed if the participant has a temperature exceeding 37.9°C/100.2°F or has a functioning ICD/pacemaker.

Advanced glycation endproducts (AGEs) will be determined using an AGE reader SU (DiagnOptics Technologies, Groningen, The Netherlands).[23] The AGE reader SU measures skin autofluorescence (AF) by using the characteristic fluorescent properties of certain AGEs to estimate the level of AGEs accumulation in the skin. AGEs have been implicated in the pathogenesis of vascular damage and cardiovascular disorders and aid to characterize the cardiovascular risk profile of transplant recipients.[24]

Transplant recipients are known to be at increased risk for cutaneous malignancies, mainly related to long-term use of immunosuppressive medication.[25] To identify which transplant recipients are especially prone to develop dermatological health problems, a detailed dermatological history with emphasis on malignancies and subsequent treatment will be obtained. Next, a standardized dermatological examination will be performed by the trained investigator. The dermatological examination includes the determination of eye color, natural hair color at adolescence and skin type according to the classification of Fitzpatrick.[26] In addition, the presence and quantity of lentigines, moles, freckles, and warts are examined.

To assess frailty, the Clinical Frailty Scale (CFS) will be scored at study visits by the trained investigator. The CFS is a validated frailty measurement and frailty is scored based on

clinical judgment on a continuous scale from 1 (very fit) to 9 (terminally ill). A CFS-score of ≥ 5 is generally considered to be frail.[27]

To assess nutritional status, a Patient-Generated Subjective Global Assessment (PG-SGA, PT-Global, Philadelphia, USA) will be scored.[27,28] The PG-SGA is an patient-centered adaptation of the original Subjective Global Assessment (SGA). The different domains assessed by the PG-SGA are: 1) changes in body weight, 2) changes in nutritional intake, 3) symptoms which negatively influence intake, absorption, and utilization of nutrients, 4) level of activities and function, 5) conditions that increase nutritional risk or requirements, 6) metabolic stress, and 7) physical examination. Based on the PG-SGA score, subjects can be classified as well-nourished, moderately malnourished or severely malnourished.

Physical protocol

In addition to standard assessments, participants in the "physical" arm of the protocol will be asked to accomplish a standing balance test, a 2-Minute Walk Test (2MWT), a 4-Meter Walk Test (4MWT), a dexterity test, a Five Time Sit To Stand test (FTSTS), Timed Up and Go test (TUG), a rigorous neurological examination, and a breath analysis. With inclusion of the first four tests together with the handgrip strength, the five physical components of the National Health Institute Toolbox for motor assessment are being assessed.[29]

The *standing balance test* will be performed with an accelerometer (Axivity, Newcastle, United Kingdom), attached to the lower back. The standing balance test has been described in detail previously.[29] Balance will be evaluated in 5 different positions, i.e. 1) feet together on hard surface, eyes open; 2) feet together on hard surface, eyes closed; 3) feet together on foam surface (Balance Pad Elite; Airex Specialty Foams, Aargau, Switzerland), eyes open; 4) feet together on foam surface, eyes closed, and 5) feet in tandem stance, eyes open. Participants will be asked to have arms crossed on their chest and each position will be

tested for 50 seconds. Upon failure, with recording of time to failure, a second attempt will be performed. In case of non-success at the second attempt, the test will be discontinued.

Endurance will be tested with a *2MWT*.[30] The 2MWT has been shown to be highly correlated, without compromising validity and reliability, with the 6-minute walking test, an important submaximal exercise test.[31,32] To calculate distance covered by subjects on the 2MWT, two pylons are set apart 15 meters and subjects are instructed to walk as fast as possible without running, until the investigator commands to stop. Participants are updated on the remaining time after 1.00 and 1.45 minutes, and the final five seconds are indicated by a countdown. The total walking distance in 2 minutes is recorded in total meters covered with remaining scored in centimeters.

Locomotion, measured as gait speed, will be tested with a *4MWT*. Gait speed is a simple measure to summarize the overall disease burden and disability.[33,34] In brief, two pylons will be set apart 4 meters and instructed to walk at usual pace. Seconds from start to end of the 4 meters will be recorded. The 4MWT is measured twice after first a trial round.

Manual dexterity will be measured in all transplant recipients using the *9-Hole Peg Test* (9-HPT, Sammons Preston Rolyan, Chicago, IL). The 9-HPT requires participants to repeatedly place and remove nine pegs into nine holes, one at a time, as quickly as possible, and is considered to be the gold standard metric for manual dexterity.

Functional mobility will be tested in participants using the *FTSTS* and *TUG*. The FTSTS is a functional performance measure of leg strength or the force-generating capacity of muscle by using the body's weight for resistance during functional activities.[35] The FTSTS will be executed three times after a first trial round. Participants will be instructed to stand up five times as fast as possible, from sitting position with their feet flat on the floor and arms folded across the chest. Measurements start upon command, and subsequently the time

required to stand up and return sitting is recorded. Time is measured in seconds and this task is repeated five times.[36]

The TUG is a basic test for functional mobility and is based on strength, coordination, and balance.[37] For the test, a pylon and chair will be put apart 3 meters. The test will be performed four times, with the first round being a trial. Participants are instructed to stand up from the chair without support of the arms, subsequently walk with their normal gait speed around the pylon, and go back to the chair to sit down again. In case, participants use a walking aid in normal day life, the test will be performed with the use of a walking aid. The TUG is measured in seconds, from the moment the participant is instructed to get up until the moment the participant sits down again.

Transplant recipients have an increased susceptibility to develop peripheral neuropathy and tremor, mainly due to the continuous use of immunosuppressive medication, especially calcineurin inhibitors.[38,39] Therefore, an extensive neurological examination will be performed and will consist of strength testing, classifying polyneuropathy, and tremor quantification. Detailed strength testing of different muscle groups (feet flexion/extension, hip flexion, biceps flexion and wrist extension) will be performed with a digital dynamometer (C.I.T. Technics, Haren, the Netherlands).[40] Hereafter, sensibility tests will be performed using a pin-prick and monofilament pen (Novo Norisk BV, Alphen aan de Rijn, the Netherlands) on bare skin five times per measurement at the dorsal side of the 1st phalange of both feet with the subject closing their eyes. Upon failure of sensibility, the dorsal side of the foot and lower limb will be tested. Proprioception will be measured by moving the 1st phalange of both feet in dorsal flexion and plantar flexion five times with the participants closing their eyes. Upon failure, the dorsal side of the foot and index finger will be measured.

Vibration sense will be measured using a handheld biothesiometer (Bio Medical Instrument Co, Ohio, USA).[41] The biothesiometer has a rubber tractor that vibrates at 100 Hz

when operating from 50 Hz mains. In brief, participants will be measured in a supine position on a bed barefooted. The vibrating tractor will be applied bi-laterally to four different measurement points of the participants: top of the hallux, forefoot, lateral malleoli, and wrist. Before applying the vibrating tractor to the points to be tested, the amplitude of the vibrating tractor is increased from zero to the point where the vibration is perceptible and beyond the threshold to the highest amplitude possible to familiarize participants with the sensation. For the measurement, the participants will be asked to concentrate on the test and report the first sensation of the vibration by saying "Stop". Each measurement point is tested twice. If the difference between the first two measurements is greater than 20%, the measurement point is tested a third time.

Prior to tremor quantification, participants will be asked to complete part C of the Fahn-Tolosa-Marin tremor rating scale that involves tremor-related functional disability.[42] The questionnaire involves speaking, bringing liquids to the mouth, eating, hygienic care, dressing, writing, work and household related tasks. The questionnaire uses a 5-point scale, with '0= no functional ability', and 4= 'severe disability, the task cannot be executed'.

To quantify tremor, two accelerometers (University Medical Center Groningen, Groningen, the Netherlands) will be attached to the dorsal side of both hands. The accelerometers will record movement in the coronal, transversal and sagittal planes as well as linear acceleration and deceleration in both hands continuously during the measurements. Amplitudes and frequency of these measurements will be recorded on a stand-alone computer. Participants will be asked to assume seven different positions while seated, which are measured for 30 seconds each: arms down, wrists extended; arms forward, wrists and fingers relaxed; arms forward, wrists and fingers in 0 position; index fingers pointed towards each other; bilateral finger-nose task; weighted arms down with wrists expanded; weighted arms forward with wrists and fingers extended.

Finally, participants will be asked for collection of a breath sample in which hydrogen and methane will be measured with the Quintron BreathTracker (Milwaukee, Wisconsin, USA).[43] Both hydrogen and methane are exclusively formed by anaerobic fermentation in the gut, and therefore can be utilized as markers for metanogenic microflora in transplant recipients.[44]

Cognitive protocol

Participants randomized into the "cognitive" arm of the study protocol undergo a series of neuropsychological tests performed by a trained neuropsychologist or masterstudent neuropsychology under supervision of a trained neuropsychologist. The tests are administered in a quiet room with no disturbances. For timed tests, a digital clock is used. The tests are performed in a fixed order and no feedback regarding the results is given to the participant during administration. An overview of the neuropsychological tests is specified in **Table 3**.

Neuropsychological tests

The *Cognitive Screening Test (CST)* is a Dutch screening test for dementia, measuring orientation in time and place, and memory for common facts.[45] The questionnaire consists of 20 items (e.g. date of birth, name of the reigning monarch, season) and the score is calculated as the total of questions answered correctly with a maximum of 20.

Nederlandse Leestest voor Volwassenen (NLV), Dutch version of the National Adult Reading Test. The participant has to read aloud a list of 50 irregularly spelled words. The total score on the test is converted into an estimation of the premorbid intelligence quotient.[46]

The *Clock Drawing Test (CDT)* is a cognitive screening instrument.[47] Participants are asked to draw a clock and set the time to 'a quarter to two'. A maximum total score of 14 can be achieved.

The *15 Words Test* (Dutch version of the Rey Auditory Verbal Learning Test (RAVLT), measures verbal memory.[48] In this task a set of 15 unrelated words is presented to the participant, consecutively over five trials. Participants are asked to recall as many words as possible immediately after each trial (Immediate Recall). The score is the total words recalled in 5 trials, with a maximum of 75. After 20 minutes, participants are asked to recall as many of the 15 words as possible (Delayed Recall). Additionally, a recognition task will be performed. Participants are presented with a list of 30 words and are asked which words they recognize from the list they have been presented before.

Digit Span, subtest of the Wechsler Adult Intelligence Scale (WAIS-IV).[49] This subtest consists of two tasks, the Digit Span Forward and the Digit Span Backward. The Digit Span Forward is a task for immediate auditory memory span. In this task, participants are asked to repeat a series of numbers in the same order as the examiner did. The Digit Span Backward measures working memory. Participants have to repeat the presented numbers in reversed order. The score is the total strings repeated, with a maximum of 32.

The *Word Fluency*, subtest of the Groninger Intelligentie Test (GIT-2), is a verbal task measuring semantic memory.[50] Participants are asked to name as many words within a certain category within one minute. Total score per category (respectively animals and professions) were calculated.

The *Controlled Oral Word Association Test (COWAT)* is a verbal task measuring executive control.[51] Participants have to name as many words as possible that start with a specific letter within one minute. In the meantime, participants have to comply to several rules that are given on beforehand. Total scores from three different starting letters (D-A-T) were calculated.

The *Symbol Digit Modalities Test (SDMT)* measures psychomotor speed.[52] The test consists of matching symbols and numbers as fast as possible in 90 seconds. The total score of correct matches is calculated.

Trail Making Test (TMT). This test consists of two parts: Trail Making Test – A (TMT-A) and Trail Making Test – B (TMT-B). Part A is a measure of attention and information processing speed. This task involves connecting 25 numbers in ascending order, as quickly as possible. The TMT-B is a measure of divided attention and cognitive flexibility. In this condition, numbers as well as letters have to be connected in ascending order, alternating between numbers and letters (1-A-2-B- etc.). Both parts of the test are timed to completion (number of seconds).

The *Key Search Test* is a subtest of the Behavioral Assessment of the Dysexecutive Syndrome (BADS) and assesses the ability to plan and monitor progress. Participants are presented with a square which represents a field in which 'keys have been lost' . Participants must show how they would search the field to find the keys. Searching strategy is scored by means of functionality and maximum total score of 16 can be achieved.

Outcomes

The primary outcomes of the TransplantLines study are all-cause mortality and graft failure, which is defined as death due to failure of the transplanted organ, return to organ replacement therapy or re-transplantation. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, e.g. physical and psychological functioning, quality of sleep, and neurological problems such as tremor and polyneuropathy.

The TransplantLines biobank study aims to identify risk factors for health problems and patient-centered outcomes (e.g. adverse drug events, lifestyle, quality of life, social participation, physical and cognitive functioning). Due to the nature of the biobank, not all research questions are predefined and will arise during the course of inclusion. In contrast to many other studies, TransplantLines also aims to identify and ameliorate complaints experienced by transplant recipients, such as tremors and diarrhea, which to date have largely been overlooked by clinicians.

Data management and analysis

Data will be recorded digitally in an electronic case report form (eCRF) in a certified Electronic Data Capture and Clinical Data Management System (Utopia Data Management System version 1.13.6, Research Data Support, University Medical Center Groningen). All data are checked by trained researchers and are subsequently stored anonymously in a secured electronic environment. The TransplantLines database will be linked to registries and databases of the Dutch Health Database (DHD), Netherlands Comprehensive Cancer Organisation (IKNL), Central Bureau of Statistics (CBS), InterAction Database (IADB), Dutch Nephrology Registration/Registration Renal Replacement Therapy (Nefrovisie, Renine), Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA), National Organ Transplant Registry (NOTR), PHARMO Institute for Drug Outcome Research (PHARMO), Routine Outcome Monitoring (RoQua) and the Dutch Institute of Clinical Auditing database (DICA) through a generic layer. A data management board will be formed to maintain data infrastructure, construct Material Transfer Agreements (MTA) and to govern use of the TransplantLines biobank and database. Extractions from TransplantLines database will be performed using a retrieval suite in Utopia software package only after approval of the data management board. Data will always be extracted

anonymously. SPSS statistics version 23 (IBM, Armonk, NY), R version 3.2.3 (CRAN, Vienna, Austria), STATA 14.1 (STATA Corp., College Station, TX) or a similar statistical package will be used for analysis. Data collection and management is performed in accordance with the Handbook for Adequate Natural Data Stewardship (Netherlands Federation of University Medical Centers, 2017).

Discussion

The TransplantLines prospective cohort study seeks to identify risk factors for the development of long-term health problems after transplantation and ultimately to develop new and innovative interventions to improve graft survival, patient survival, and quality of life after transplantation. The TransplantLines biobank will encompass all solid organ transplantations and living organ donors. It will consist of follow-up data from all fields that are involved in organ transplantation; internal medicine, surgery, gastroenterology, hepatology, pulmonology, cardiology, dermatology, neurology, occupational medicine, children's medicine, (neuro)psychology, physiotherapy, and social work.

Although short-term transplant outcomes have improved in the last decades, graft and recipient life expectancy remains limited. In the TransplantLines study, data and samples will be collected before, during, and after transplantation to gather further insight in the impact of transplantation on transplant recipients. In addition, we aim to preemptively detect those transplant recipients who are at increased risk to develop graft failure or health problems. By investigating a wide range of clinical, social/psychological and biochemical parameters, this study aims to contribute to increased transplant survival, patient survival, but also to an increased quality of life and a more patient-centered approach to transplant care.

Our study has strengths and limitations. The major strengths of this study are the collection of extensive data on a myriad topics related to transplantation, the inclusion of all

types of solid organ transplant recipients and living organ donors, and a study with a long follow-up to assess many relevant clinical outcomes. Limitations of the current study are that it comprises a single center study and that residual confounding cannot be excluded in analyses in the TransplantLines study due to its observational design.

TransplantLines may serve as a basis for hypothesis-generating studies that yield insights in a wide range of clinical, social/psychological and biochemical parameters in solid organ transplant recipients, as well as living donors. Biomarkers may be identified to develop more individualized immunosuppressive treatment. This will lead to novel clinical trials in transplantation and patient-tailored approaches for new treatment options. Furthermore, the results of TransplantLines may serve to identify new modifiable risk factors and lifestyle factors in transplantation. Ultimately, this information will likely contribute to a more individualized treatment for transplant patients and improved living donor screening and follow-up. Thereby we aim to qualitatively and quantitatively improve outcomes after transplantation.

Study status

Data collection is in progress.

List of abbreviations used

METc – Medical Ethical Committee

UMCG – University Medical Center Groningen

EDTA - Ethylenediaminetetraacetic acid

SOP – Standard Operating Procedure

BIA – Bio-Impedance Analysis

ICD – Implantable Cardioverter-Defibrillator

SAF – Skin Auto-Fluorescence

AGE – Advanced Glycation End-product

PG-SGA - Scored Patient-Generated Subjective Global Assessment

2MWT – 2-Minute Walk Test

FTSTS – Five Time Sit To Stand Test

TUG – Timed Up and Go

CRF – Clinical Research Form

PI – Principal Investigator

Author contributions

M.F.E. designed the study, conducts the study for transplant recipients, wrote the manuscript, and is responsible for final content of the manuscript; A.W.G.N. conducts the study for transplant recipients and revised the manuscript; M.vL. conducts the study for living donors and wrote the part of living donors in the manuscript; A.L.Z. conducts the study for transplant recipients, living donors and wrote the cognitive protocol; R.M.D., S.P.S, T.J.K., and M.C.J.O. conduct the study for transplant recipients; N.R.H. conducts the study for living donors; M.J.S., C.A., E.R., J.M.S., R.A.P., G.D., R.J.P., H.G.D.L., K.D., E.A.M.V., V.E.dM gave input from their field of knowledge when designing the study and revised the manuscript; R.A.P. is responsible for kidney biobank sampling and logistics; H.B. conducts the study and revised the manuscript; S.J.L.B. designed the study, is principal investigator and project leader, and takes full responsibility for the whole study. All collaborators have revised the manuscript and approved the final version of the manuscript.

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Competing interests

None

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Table 1. Overview stored samples per participant in the TransplantLines Biobank

Sample	Color code	Tube size	Number	Temperature
Serum	Red	1500 µL	4	-80°C /-112°F
EDTA plasma	Purple	1500 µL	6	-80°C /-112°F
Buffy coat	Purple	N/A	1	-80°C /-112°F
Blood with RBC	Purple	1500 µL	2	-80°C /-112°F
Lithium-heparin	Green	1500 µL	4	-80°C /-112°F
Citrate	Blue	500 µL	4	-80°C /-112°F
PAXgene	Transparent	2.5 mL	1	-80°C /-112°F
24 hour urine	Yellow	1500 µL	6	-80°C /-112°F
Acidified 24 hour urine	Yellow	2000 µL	2	-80°C /-112°F
Feces	Black	20 mL	1	-80°C /-112°F
Nails	Purple	0.5 µL	1	-80°C /-112°F
Hair	Purple	2000 µL	1	-80°C /-112°F

Table 2. List of questionnaires in the TransplantLines study

Questionnaires	Related subject
EQ6D[53]	EuroQoL 6 dimensions
VAS scale[54]	Visual Analogue Scale
SF36[55]	Short Form-36 Health Survey
SQUASH[56]	Short Questionnaire to Assess Health - Enhancing Physical Activity
BAASIS[57]	Adherence to Immunosuppressive Drugs
MTSOSDS-R59[58]	Modified Transplant Symptom Occurrence and Symptom Distress scale
CIS[59]	Checklist Individual Strength (Fatigue)
PSQI[60]	Pittsburgh Sleep Quality Index
STAI6[61]	Short form State Trait Anxiety Inventory
PHQ9[62]	Patient Health Questionnaire (Depression)
CFQ[63]	Cognitive Functioning Questionnaire
WHO-5[64]	World Health Organization-5 (Well-Being Index)
TxEQ[65]	Transplant Effects Questionnaire
Mastery scale[66]	Pearlin Mastery Scale
UCL-47	Utrecht Coping List-47
USER-P	Utrecht Scale for Evaluation of Revalidation - Participation
Work	Participation in Labor
WRFQ[67]	Work Role Functioning Questionnaire
FAD	Family Assessment Device
ABO	Active Engagement, Protective Buffering and Overprotection Questionnaire
Social support	Social Support Questionnaire
DAG/BHQ[68]	Bowel Health Questionnaire
FFQ[69]	Food Frequency Questionnaire
Self-efficacy movement	LIVAS-scale for Physical Self-Efficacy
Sedentary behavior	OBiN Sedentary Behavior Questionnaire
Smoking behavior	Smoking behavior questionnaire
Alcohol use[a]	The Alcohol Use Disorders Identification Test

[a]The AUDIT questionnaire will only be gathered from liver transplant recipients

Table 3. Overview of the different tests performed in TransplantLines study per study protocol

General (all protocols)

Parameter/test	**Details**
General parameters	Collection of Biobank material and evaluation of questionnaires, check quality of data
Blood Pressure	Using an automatic or semi-automatic device
Weight	Using digital measuring scale
Length	Using measuring tape fixed to wall
Waist- and hip size	Using measuring tape roll
BIA	Bio-Impedance Analysis (Quadscan 4000)
SAF	Skin Auto-Fluorescence (AGE reader SU)
Dermatological questionnaire	After physical examination by student researcher
Clinical Frailty Scale	After physical examination by student researcher
PG-SGA	Scored Patient-Generated Subjective Global Assessment
Long function	Using spirometry (Vitalograph Asma 1)
Breath analysis	Using Quintron Breath Tracker
Physical protocol	
Parameter/test	**Details**
Balance Test	Using Axivity Accelerometer
Hand grip	Using Hydrolic Hand-held Dynamometer
Physical Strength	Multiple muscle groups, using Digital Dynamometer
Sensibility Tests	Using pin-prick, monofilament and biothesiometer
Tremor analysis	Using Tetras scale and Axivity Accelerometers
Manual dexterity	Using Dexterity PEG-Board
Cognitive protocol	
Nederlandse Leestest voor Volwassenen	Dutch version of the National Adult Reading Test (NART)
Digit Span	Subtest of theWechsler Adult Intelligence Scale (WAIS-IV)
15 Wordstest	Dutch version of Ray Auditory Verbal Learning Test (RAVLT)

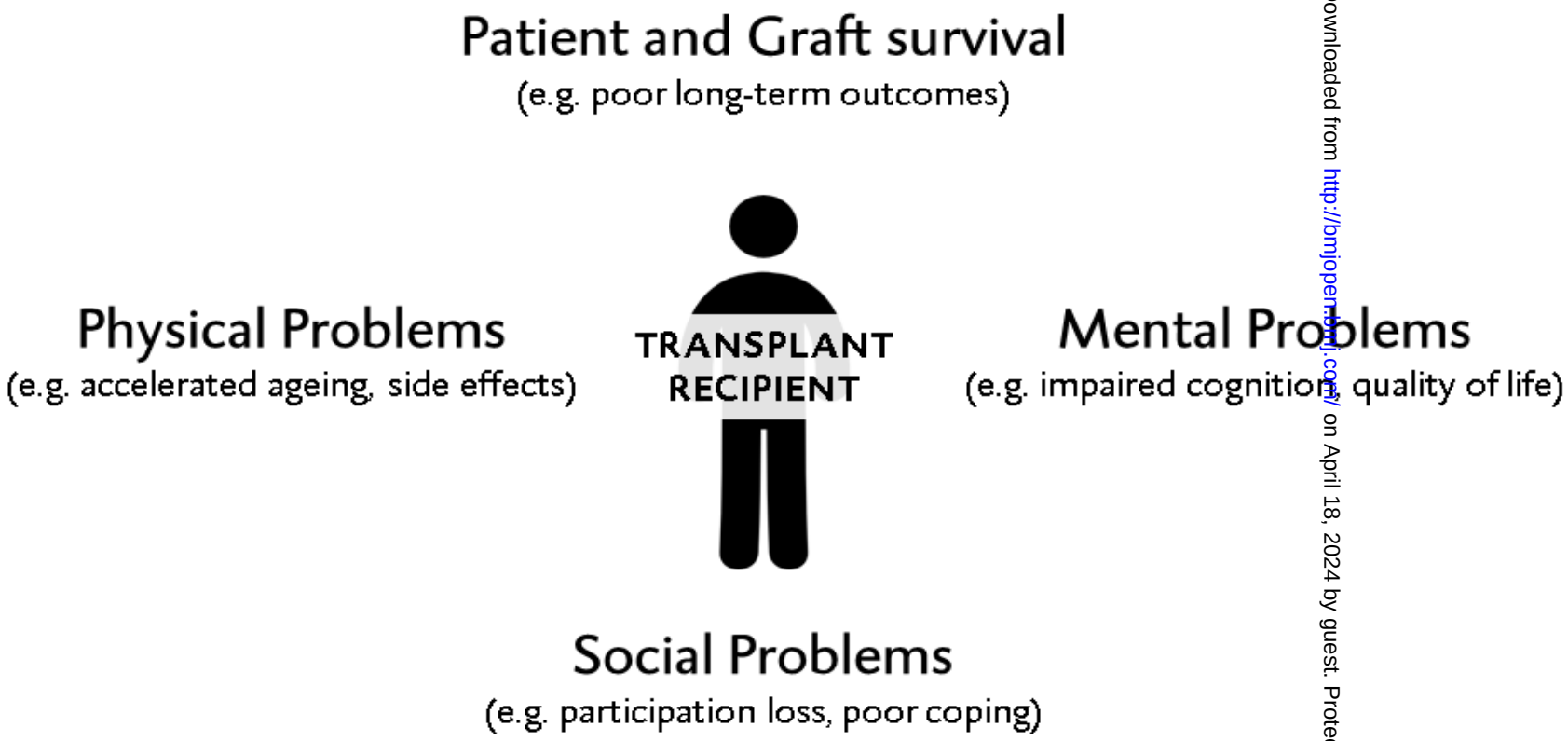
Cognitive Screening Test	Cognitive Screening Test (20)
Trail Making Test	
Clock-drawing Test	
Symbol Digit Modalities Test	
Letter Fluency Test	Dutch version of the Controlled Word Association Test (COWAT)
Word Fluency Test	Subtest of the Groningen Intelligence Test (GIT)
Key Search Test	Subtest of the Behavioral Assessment of the Dysexecutive Syndrome (BADS)
Nederlandse Leestest voor Volwassenen	Dutch version of the National Adult Reading Test (NART)

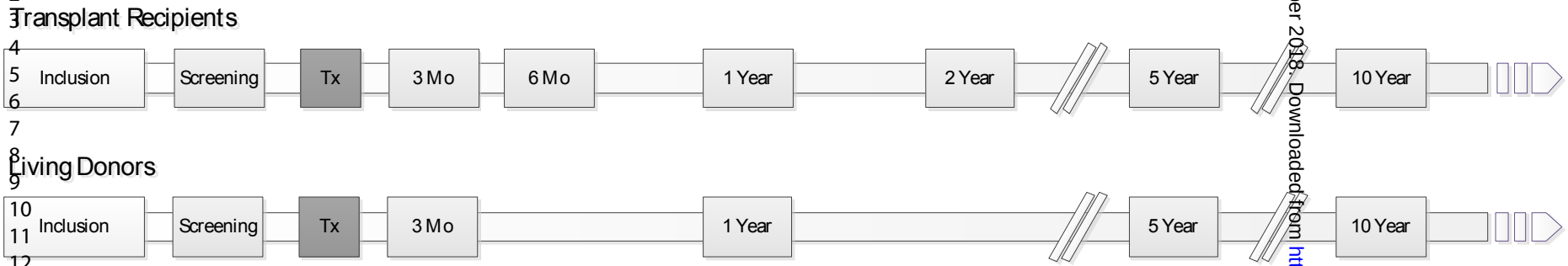
Figure legends

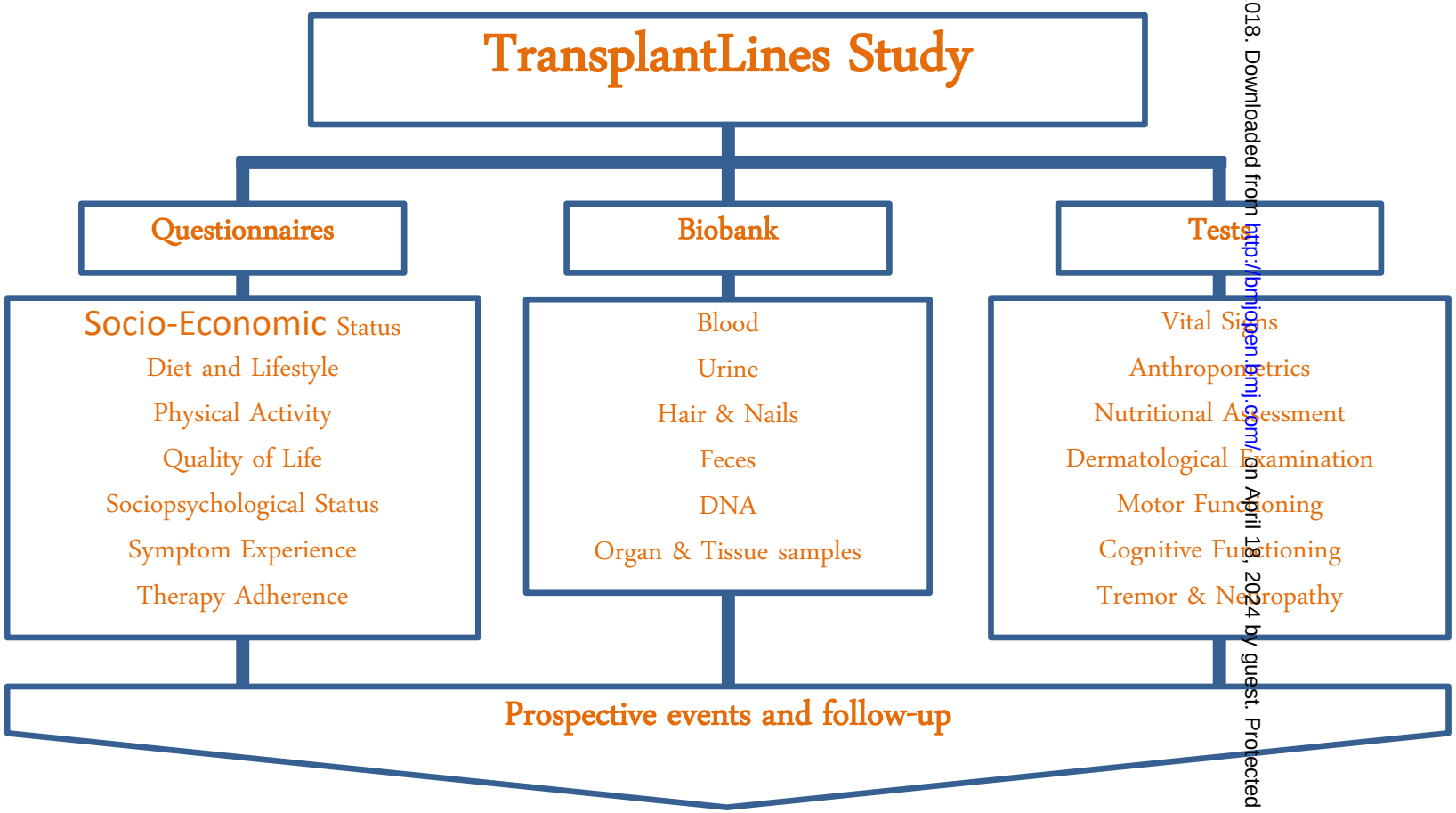
Figure 1. Overview of different health problems that arise on the long term after transplantation, both physical, psychological, and social.

Figure 2. Flowchart of the different visits in the TransplantLines study. At every study visit biobank, general tests, and questionnaires will be performed. Specifically addition at each timepoints; at transplantation perioperative residual material will be collected. At 3 months after transplantation cognitive protocol will be performed. At 6 months physical protocol will be carried out. At 12 months randomization to physical or cognitive protocol will occur. At 2 years after transplantation, a limited set of tests will be executed. Follow-up will be performed each 5 years.

Figure 3. Overview of the three main pillars of the TransplantLines study, i.e. questionnaires, biobank, and tests. The collection of data in these pillars at multiple time points will allow to investigate whether biomarkers at baseline can better predict occurrence of adverse outcomes and whether correction could possibly result in an improved survival.







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Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

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Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

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Abstract

Introduction

In the past decades, short-term results after solid organ transplantation have markedly improved. Disappointingly, this has not been accompanied by parallel improvements in long-term outcomes after transplantation. To improve graft and recipient outcomes, identification of potentially modifiable risk factors and development of biomarkers is required. We provide the rationale and design of a large prospective cohort study of solid organ transplant recipients (TransplantLines).

Methods and analysis

TransplantLines is designed as a single center prospective cohort study and biobank including all different types of solid organ transplant recipients, as well as living organ donors. Data will be collected from transplant candidates before transplantation, during transplantation, at 3 months, 6 months, 1 year, 2 years, 5 years, and subsequently every 5 years after transplantation. Data from living organ donors will be collected before donation, during donation, at 3 months, 1 year, and 5 years after donation and subsequently every 5 years. Primary outcomes are mortality and graft failure. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, i.e. physical and psychological functioning, including quality of sleep, and neurological problems such as tremor and polyneuropathy.

Ethics and dissemination

Ethical approval has been obtained from the relevant local ethics committee. The TransplantLines cohort study is designed to deliver pioneering insights in transplantation and donation outcomes. The study design allows comprehensive data collection on perioperative

care, nutrition, social- and psychological functioning and biochemical parameters. This may provide a rationale for future intervention strategies to more individualized, patient-centered transplant care and individualization of treatment.

Strength and limitations

- Large biobank and cohort study with extensive data collection on a myriad topics related to transplantation and/or donation
- Inclusion of all types of solid organ transplant recipients
- Long follow-up to assess many relevant clinical outcomes
- Single center study
- Residual confounding cannot be excluded due to observational design

Background

Solid organ transplantation is the preferred treatment for end-stage organ failure. During the past decades, advances in immunosuppressant medications, treatment of infections, perioperative medical care, and surgical techniques (including living donation) have led to important improvements in early post-transplant graft and patient survival.[1] However, on the long-term, graft failure is a major cause of patient mortality and morbidity in all types of transplantation.[2-4] For example, in renal transplant recipients, half of the cadaveric renal allografts fail within a timeframe of 10 years.[5] Apart from reduced survival, transplant recipients often develop health problems that greatly reduce their perceived quality of life (Figure 1).[6-8]

The multitude of health problems that recipients experience after transplantation include amongst others obesity, diabetes, hypertension, heart failure, and malignancies.[9-11] These are likely the consequence of a combination of factors, including (1) continuous exposure to treatment with immunosuppressive drugs necessary for prevention of rejection of the transplanted organ, (2) damage induced by pre-existing exposure to end-stage organ failure, and (3) adverse life-style and environmental factors, all potentially expressed against (4) a background of increased (epi)genetic susceptibility. Among these, immunosuppressive treatment, adverse life-style, and environmental factors are good candidates for modification to decrease the load of post-transplant health problems. It should be realized that immunosuppressive treatment is currently mainly “one-size fits all”. Hence, improvement can be achieved by development of biomarkers that can allow for recognition of transplant recipients in which immunosuppressive load can be safely reduced or in which certain drugs can better be avoided, and for biomarkers which can guide such individualized immunosuppressive treatment. To improve long-term transplant outcomes, it is imperative to identify modifiable risk factors, especially among those recipients who are at increased risk.

To date, it is largely unknown in which transplant recipients immunosuppressive medication can be safely reduced to prevent the development of health problems. Furthermore, in terms of healthcare costs, it is important to prevent recurrent hospital admissions, re-transplantations or – in case of kidney transplantation – return to dialysis, which are all associated with very high expenses.[12] To effectively develop interventions to reduce mortality and morbidity after transplantation, more research is necessary on clinical and biochemical risk factors present in transplant recipients. Also, the use of living donors for kidney and liver transplantation requires a living donor program with good long-term outcomes for the donor and recipient. Living kidney donors, for example, have an increased risk for end-stage renal disease (ESRD),[13, 14] while only registry data exist on the effect of living donor characteristics on recipient outcomes.[15, 16]

Until now, many registries and large cohort studies focus on one type of solid organ transplantation, limiting comparability between different transplant populations. As a result, studies investigating biomarkers, quality of life, and development of health problems and adverse outcomes across different solid organ transplant populations are scarce. Despite the differences which exists in patient characteristics and treatment after different solid organ transplantations, there are many similarities in health problems that occur among subtypes of transplantation. The objective of TransplantLines study is to identify risk factors for development of long-term health problems after transplantation and to develop new interventions to improve outcome, both combined for all solid organ transplant recipients as well as specific for each subtype of transplantation.

Methods/Design

Study design and setting

The TransplantLines study is a unique, novel prospective biobank and cohort study, which aims to provide a better understanding of causes of disease- and ageing-related outcomes and health problems, both physical and psychological, in solid organ transplant recipients and donors (ClinicalTrials.gov Identifier: NCT03272841). The University Medical Center Groningen (UMCG) is the largest transplantation center in the Netherlands, and the only Dutch center that covers all types of solid organ transplantation, as well as living kidney and liver donation programs. The study protocol has been approved by the Institutional Review Board (METc 2014/077), adheres to the UMCG Biobank Regulation, and is in accordance with the WMA declaration of Helsinki and the declaration of Istanbul. All participants will give written informed consent upon enrollment. Follow-up and prospective events will be recorded over time. An overall participation rate of 85% is expected across the different transplant populations and a total number of 3000 participants is aimed.

Transplant patients

The study population comprises all solid organ transplant recipients, i.e. heart-, lung-, kidney-, liver-, and small bowel transplant recipients. Both new transplant candidates as well as transplant recipients are eligible to participate in the study. Participants of all ages will be included in TransplantLines. Children (age <18 years) will be eligible for participation upon consent by a legal representative (<12 years) or a shared consent of both the child and legal representatives (≥12 years). The study will also include candidates for re-transplantation. Exclusion criteria for participation in the TransplantLines study will be no mastery of the Dutch language or no capability to intellectually comprehend questionnaires or physical tests.

Living donors

Living kidney and liver donors will also be included in the study. The goal of including donors is to study the effects of donation, improve living donor safety and donors will serve as controls for their recipients, allowing for matched longitudinal analyses. Prospective living kidney and liver donor candidates (≥18 years old) will be eligible to participate in the study, as well as living organ donors who have donated an organ prior to the start of the TransplantLines study. Exclusion criteria will be no mastery of the Dutch language or no capability to intellectually comprehend questionnaires or physical tests.

Transplant recipients timeline

All participants of the TransplantLines study will be examined at fixed time points as shown in **Figure 2**. Transplant candidates will be first seen at pre-transplant screening. Prior to transplantation, all transplant candidates undergo a routine clinical screening. Generally, transplant candidates will be transplanted if surgery risks and transplant benefit are optimized, based on an individualized multidisciplinary clinical decision. Further study visits will be performed at time of transplantation, at 3 months, 6 months, 12 months, and 2 years after transplantation, and hereafter follow-up will be performed at 5 years after transplantation and every consecutive 5 years. At time of transplantation means during operation prior to incision, and at that timepoint the blood samples are being drawn by the anesthesiologists taking care of the patient. The difference with this sample compared to other previous samples is that this sample is taken during operation, whereas the other samples are not. For example, a kidney transplant candidate can be screened and included in the TransplantLines study, but may need to wait two years on the waiting list prior to receiving the actual transplantation. Since we realize that a study of this size and duration combined with the frequency of study visits will result in lower subject adherence, we estimate a 10% dropout overall in follow-up in this transplant candidates group.

Transplant recipients with a functional graft for at least 1 year post-transplantation and who received a solid organ transplant prior to the start of the TransplantLines study will be included at the next outpatient clinic visit. Henceforth, patients will be examined every five years and follow-up samples will be collected. Aside from the fixed time points, biobank samples of transplant recipients will be collected at times of protocol biopsies that are performed in the kidney transplant program (6 months after transplantation) and the heart transplant program (repeatedly during the first year after transplantation) and if a biopsy is taken, on clinical indication, usually because of worsening of transplant function, with suspicion of acute or chronic rejection.

If a subject gets retransplanted with the same kind of organ, this will be classified as graft failure and end of follow-up. Subjects will not be included in the primary database twice. Yet, we will allow for inclusion of subjects retransplanted with the same kind of organ with a new ID in the transplant candidate group, but this will be with the intention to build over time a separate cohort with data and a biobank on retransplantations. When a transplant recipient is later on transplanted with another kind of organ, follow-up will be for the initially transplanted organ. Transplant recipients receiving a combined transplantation, e.g. kidney-pancreas and kidney-liver, will be treated as separate groups, not to be included in overall analyses for the much larger groups of subjects with single transplanted organs.

In case that a transplant recipient moves to another region of the Netherlands or abroad, the transplant recipient will always require continued medical care and follow-up by a medical specialist, who will require thorough medical information on the patient and the transplanted organ, to allow for continued dedicated care. Therefore, the medical specialist who will continue care will seek contact for information and it will usually be possible to continue follow-up on long-term outcome and events via this medical specialist. So, follow-up is usually assured and loss to follow-up will be rare. Since study visits are combined with a

routine clinical visit, subjects who move out of our region will be excluded from further study visits for the TransplantLines study.

Living donors timeline

All donors of the TransplantLines study will be examined at fixed time points as shown in **Figure 2**. The first study visit of donor candidates will occur at pre-donation screening. Prior to donation, all candidates undergo a routine clinical screening. Generally, donors will be accepted if surgery risks and transplant benefit are optimized, based on an individualized multidisciplinary clinical decision taking national and international guidelines into account.[17, 18] Subsequently, study visits will be performed at time of nephrectomy, and at 3 months post-donation. At 12 months post-donation, donors will fill in a questionnaire and at 5 and 10 years post-donation there will be another study visit. Hereafter follow-up will be performed every five years. Living organ donors who have donated an organ prior to the start of the TransplantLines study, will be included at their next donor follow-up visit to their outpatient clinic.

Patient and Public Involvement

The aim of the TransplantLines study is to provide a better understanding of the causes of disease- and ageing-related outcomes and health problems. This aim was derived from patient surveys and parts of the collected data are co-designed by patients and healthcare professionals. Because of its scope, patients will play a role in the organization of the study, helping with recruitment and conduct of the study. Also, students from a broad range of studies will play a role in the organization of the study, e.g. master students from medicine, biomedical sciences, neuropsychology, psychology, physical therapy, communication sciences, dietician students, and laboratory technician students. Patients and collaborators will

be informed of major study results by press releases from the University Medical Center Groningen.

Data collection

Biobank

Blood, 24-hour urine, feces, nails, and hair will be collected of participants at each TransplantLines visit. Participants will be instructed to collect a 24-hour urine sample according to strict protocol at the day before their visit to the outpatient clinic, i.e. discard their morning urine specimen, collect all subsequent urine throughout the next 24 hour and include the next morning's first specimen of the day of the visit to the outpatient clinic. Blood will be drawn after an 8-12 hour overnight fasting period in the morning after completion of the 24-hour urine collection. Blood drawing and receipt of the collected 24 hour urine samples is performed by experienced nurses at our outpatient clinic.

As blood samples, 1 serum tube of 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube of 6 mL, 1 lithium-heparin tube of 10 mL, and 1 PAXgene tube of 10 mL will be collected of each participant at each TransplantLines visit. Subsequently, tubes will be centrifuged by technicians at 1300g for 10 minutes, except the citrate tube which is centrifuged at 2500g for 10 minutes. Of the 24-hour urine collection, three urine tubes will be collected of which one tube will be partially acidified. All blood- and urine samples will be subsequently aliquoted by technicians and shipped to the core laboratory for storage in -80°C (-112 °F) freezers (Panasonic, 's-Hertogenbosch, the Netherlands) (**Table 1**). Blood- and urine samples will be analyzed in the following years for multiple research questions that will arise.

Participants will be asked to collect a feces sample the day prior to the TransplantLines visit. A FecesCatcher (TAG Hemi VOF, Zeijen, the Netherlands) will be sent at the patients' home, and feces sample will be collected in appropriate tubes and frozen

immediately after collection. The participant will transport the feces sample in cold storage (with ice cubes or in a cooler) to the TransplantLines visit the following day. Subsequently, the feces sample will be immediately stored at -80°C (-112 °F). Feces samples will be primarily used for microbiome analyses. Solid organ transplant recipients have a shift in the gut microbiome with a decrease in predominant organisms, a loss of bacterial diversity, and emergence of new dominant population. This may result in increased risk of infection, rejection, and mortality. Therefore, we would like to examine the gut microbiome in relation to the development of health problems after transplantation.

Additional blood and urine samples will also be collected in the event of worsening graft function and an organ-transplant biopsy is indicated. Prior to the biopsy, 1 serum tube of 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube of 6 mL, 1 lithium-heparin tube of 10 mL, and 1 PAXgene tube of 10 mL will be collected. At the same time, 1 serum tube of 10 mL and 1 EDTA sample of 10 mL and 1 spot urine sample of 10 mL will be collected and directly stored on ice to prevent (ongoing) in vitro complement activation.

Furthermore, during transplant surgery and transplant biopsies, tissue samples will be collected of the transplanted organ and surrounding tissues, including fat-, skin-, ureter-, tracheal-, biliary-, arterial and venous tissue, that have been discarded as pathological waste.

Table 1. Overview stored samples per participant in the TransplantLines Biobank

Sample	Color code	Tube size	Number	Temperature
Serum	Red	1500 µL	4	-80°C /-112°F
EDTA plasma	Purple	1500 µL	6	-80°C /-112°F
Buffy coat	Purple	N/A	1	-80°C /-112°F
Blood with RBC	Purple	1500 µL	2	-80°C /-112°F
Lithium-heparin	Green	1500 µL	4	-80°C /-112°F

Citrate	Blue	500 µL	4	-80°C /-112°F
PAXgene	Transparent	2.5 mL	1	-80°C /-112°F
24 hour urine	Yellow	1500 µL	6	-80°C /-112°F
Acidified 24 hour urine	Yellow	2000 µL	2	-80°C /-112°F
Feces	Black	20 mL	1	-80°C /-112°F
Nails	Purple	0.5 µL	1	-80°C /-112°F
Hair	Purple	2000 µL	1	-80°C /-112°F

Clinical and laboratory characteristics

Clinical laboratory measurements requested by the physician will be included in the study database upon patient consent. Most study visits are at the outpatient clinic, and for these visits blood samples will be taken fasting in the morning. It is unlikely that at these study visits multiple labs will be obtained at the same day, but if they are taken, only the lab obtained at the time of the study visit will be included in the database. In the likely rare case that multiple labs are taken at a day of a study visit, this will likely be a sign of an acute event that occurred after the study visit and it will then later on be linked to the database as the event that occurred. At the visit for transplant surgery, multiple labs will be obtained at the same day. At that day, only the lab results coming available from the samples which are taken at the same time of sampling during surgery, to provide for samples that will be included in the biobank will be linked to the database. These lab results are recognizable by the routine assays that are performed, because they are more extensive and include other routine lab results than the routine lab results coming available from samples taken at other times at the same day. Demographic characteristics along with data on medication use will be provided by the participants and will be verified using the electronic hospital records. Medical information including donor and recipient information at time of transplantation, underlying disease,

hospital admissions, complications after transplantation, further surgical or other interventional treatments, co-morbidities, graft failure, and mortality will be extracted from the electronic hospital records.

Questionnaires

Biobank data will be expanded with an extensive set of questionnaires to collect data on physical, psychological, and social impact of undergoing a transplantation (**Figure 3**). Transplant candidates will be asked to fill out a comprehensive questionnaire during screening prior to transplantation and at 1 year post-transplantation. Transplant recipients with a functional graft for more than 1 year post-transplantation and who received the solid organ prior to start of the TransplantLines study will be asked to complete the same questionnaire. A subset of questionnaires will be provided at the other predefined time-points, i.e. at 3, at 6 months, and at 2 years after transplantation. Topics addressed by questionnaires include among others nutritional intake and diet, health-related quality of life, life-style factors such as physical activity, sleep quality, and smoking behavior, psychological impact such as anxiety, depression, coping, and well-being, and social impact such as employment and family relationships. Specification of all the different questionnaires with related subject is shown in **Table 2**. Questionnaires will be send digitally or by mail, as requested. During study visits, all questionnaires will be checked by a trained investigator for completeness and validity.

Table 2. List of questionnaires in the TransplantLines study

Questionnaires	Related subject
EQ6D[19]	EuroQoL 6 dimensions
VAS scale[20]	Visual Analogue Scale
SF36[21]	Short Form-36 Health Survey

SQUASH[22]	Short Questionnaire to Assess Health - Enhancing Physical Activity
BAASIS[23]	Adherence to Immunosuppressive Drugs
MTSOSDS-R59[24]	Modified Transplant Symptom Occurrence and Symptom Distress scale
CIS[25]	Checklist Individual Strength (Fatigue)
PSQI[26]	Pittsburgh Sleep Quality Index
STAI6[27]	Short form State Trait Anxiety Inventory
PHQ9[28]	Patient Health Questionnaire (Depression)
CFQ[29]	Cognitive Functioning Questionnaire
WHO-5[30]	World Health Organization-5 (Well-Being Index)
TxEQ[31]	Transplant Effects Questionnaire
Mastery scale[32]	Pearlin Mastery Scale
UCL-47	Utrecht Coping List-47
USER-P	Utrecht Scale for Evaluation of Revalidation - Participation
Work	Participation in Labor
WRFQ[33]	Work Role Functioning Questionnaire
FAD	Family Assessment Device
ABO	Active Engagement, Protective Buffering and Overprotection Questionnaire
Social support	Social Support Questionnaire
DAG/BHQ[34]	Bowel Health Questionnaire
FFQ[35]	Food Frequency Questionnaire
Self-efficacy movement	LIVAS-scale for Physical Self-Efficacy
Sedentary behavior	OBiN Sedentary Behavior Questionnaire
Smoking behavior	Smoking behavior questionnaire
Alcohol use[a]	The Alcohol Use Disorders Identification Test

[a]The AUDIT questionnaire will only be gathered from liver transplant recipients

Standard assessments

Blood pressure (mmHg) will be measured according to a standard clinical protocol using an automatic device (Philips Suresign VS2^{+}, Andover, MA, USA). To prevent a white-coat effect, participants will be seated during which blood pressure and heart rate will be measured

four times, with an interval of three minutes between measurements. Hereafter, participants will be asked to stand up straight for one minute, after which blood pressure and heart rate measurements will be repeated once in standing position. Measurements will be performed with participants being on their regular medication, including anti-hypertensive drugs at trough.

Anthropometry measurements will include body weight, body length, and waist- and hip circumference. Body weight (kg) will be measured in light weight clothing without shoes using a calibrated digital measuring scale (SECA 877, Seca GMBH, Hamburg, Germany). Height (cm) will be measured using a wall-secured stadiometer (SECA 222). Waist- and hip circumference (cm) will be calculated using a measuring tape roll with standardized retraction mechanism (SECA 201). Waist circumference will be measured midway between the lowest rib and the iliac crest with the participant in standing position. Hip circumference will be determined at the maximum circumference over the trochanter major. All anthropometry measurements will be assessed twice, with inclusion of a third measurement contingent upon a difference of more than half a kilogram in weight or more than one centimeter in length.

Handgrip strength will be assessed with the Jamar Hydrolic Hand Dynamometer (Patterson Medical JAMAR 5030J1, Warrenville, Canada).[36] Participants will be instructed to sit in a chair with their shoulders in adduction, their arms rotated into neutral position, their elbows flexed to 90º, and forearms and wrists held in neutral position. Hereafter, participants will be instructed to perform a maximal isometric contraction. Handgrip strength will be tested three times with an interval of 30 seconds rest for recovery between each attempt. The dominant hand will be stated in all measurements. Furthermore, to create uniformity among assessments, the second handle position of the hand dynamometer will be utilized which has been shown to be the most accurate position.[37]

Lung function will be measured by means of an Asma-1 handheld spirometer (Vitalograph, Buckingham, United Kingdom).[38] Of all participants, the Forced Expiratory Volume (FEV1), as marker of lung function, will be recorded.

Body composition will be determined using a multifrequency bio-electrical impedance device (BIA, Quadscan 4000, Bodystat Ltd, Douglas, British Isles) at 5, 50, 100, and 200 Hz, which allows to distinct between lean body mass and fat body mass taking into account differences in volume status.[39] Main outcome variables from the BIA are estimated fat mass, fat free mass, and body fat percentage. In brief, the BIA measurement will be performed with the participant in supine position with arms and legs abducted from the body. Sensor electrodes will be placed on the dorsum of the right hand and feet, with a minimal distance of five centimeters between the electrodes. Measurement will not be executed if the participant has a temperature exceeding 37.9°C/100.2°F or has a functioning ICD/pacemaker.

Advanced glycation endproducts (AGEs) will be determined using an AGE reader SU (DiagnOptics Technologies, Groningen, The Netherlands).[40] The AGE reader SU measures skin autofluorescence (AF) by using the characteristic fluorescent properties of certain AGEs to estimate the level of AGEs accumulation in the skin. AGEs have been implicated in the pathogenesis of vascular damage and cardiovascular disorders and aid to characterize the cardiovascular risk profile of transplant recipients.[41]

Transplant recipients are known to be at increased risk for cutaneous malignancies, mainly related to long-term use of immunosuppressive medication.[42] To identify which transplant recipients are especially prone to develop dermatological health problems, a detailed dermatological history with emphasis on malignancies and subsequent treatment will be obtained. Next, a standardized dermatological examination will be performed by the trained investigator. The dermatological examination includes the determination of eye color, natural hair color at adolescence and skin type according to the classification of

Fitzpatrick.[43] In addition, the presence and quantity of lentigines, moles, freckles, and warts are examined.

To assess frailty, the Clinical Frailty Scale (CFS) will be scored at study visits by the trained investigator. The CFS is a validated frailty measurement and frailty is scored based on clinical judgment on a continuous scale from 1 (very fit) to 9 (terminally ill). A CFS-score of ≥ 5 is generally considered to be frail.[44]

To assess nutritional status, a Patient-Generated Subjective Global Assessment (PG-SGA, PT-Global, Philadelphia, USA) will be scored.[44, 45] The PG-SGA is an patient-centered adaptation of the original Subjective Global Assessment (SGA). The different domains assessed by the PG-SGA are: 1) changes in body weight, 2) changes in nutritional intake, 3) symptoms which negatively influence intake, absorption, and utilization of nutrients, 4) level of activities and function, 5) conditions that increase nutritional risk or requirements, 6) metabolic stress, and 7) physical examination. Based on the PG-SGA score, subjects can be classified as well-nourished, moderately malnourished or severely malnourished.

Randomization and additional physical and cognitive tests

In addition to standard assessments, participants will receive additional physical tests or cognitive tests at their study visit at 12 months post-transplantation or at the first study visit if it concerns transplant recipients with a functioning graft for more than 1 year who were transplanted before the start of TransplantLines. Participants will be randomized (1:1 ratio) into either the “physical” arm or the “cognitive” arm of the study. Randomization will be performed for each transplant-program separately to ensure balanced randomization of subjects for each type of solid-organ transplant. Participants randomized into the “physical” arm of the study protocol will be asked to accomplish a standing balance test, a 2-Minute

Walk Test (2MWT), a 4-Meter Walk Test (4MWT), a dexterity test, a Five Time Sit To Stand test (FTSTS), Timed Up and Go test (TUG), a rigorous neurological examination, and a breath analysis. With inclusion of the first four tests together with the handgrip strength, the five physical components of the National Health Institute Toolbox for motor assessment are being assessed.[46] A subset of these tests will also be performed at the 6 months post-transplantation study visit in all solid-organ transplant recipients. Participants randomized into the "cognitive" arm of the study protocol undergo a series of neuropsychological tests performed by a trained neuropsychologist or master student neuropsychology under supervision of a trained neuropsychologist. The tests are administered in a quiet room with no disturbances. For timed tests, a digital clock is used. The tests are performed in a fixed order and no feedback regarding the results is given to the participant during administration. An overview of the neuropsychological tests is specified in **Table 3**. A subset of these test will also be performed at 3 months post-transplantation in all solid-organ transplant recipients.

Table 3. Overview of the different tests performed in TransplantLines study per study protocol

General (all protocols)

Parameter/test	**Details**
General parameters	Collection of Biobank material and evaluation of questionnaires, check quality of data
Blood Pressure	Using an automatic or semi-automatic device
Weight	Using digital measuring scale
Length	Using measuring tape fixed to wall
Waist- and hip size	Using measuring tape roll
BIA	Bio-Impedance Analysis (Quadscan 4000)
SAF	Skin Auto-Fluorescence (AGE reader SU)
Dermatological questionnaire	After physical examination by student researcher
Clinical Frailty Scale	After physical examination by student researcher

PG-SGA	Scored Patient-Generated Subjective Global Assessment
Long function	Using spirometry (Vitalograph Asma 1)
Breath analysis	Using Quintron Breath Tracker
Physical protocol	
Parameter/test	**Details**
Balance Test	Using Axivity Accelerometer
Hand grip	Using Hydrolic Hand-held Dynamometer
Physical Strength	Multiple muscle groups, using Digital Dynamometer
Sensibility Tests	Using pin-prick, monofilament and biothesiometer
Tremor analysis	Using Tetras scale and Axivity Accelerometers
Manual dexterity	Using Dexterity PEG-Board
Cognitive protocol	
Nederlandse Leestest voor Volwassenen	Dutch version of the National Adult Reading Test (NART)
Digit Span	Subtest of theWechsler Adult Intelligence Scale (WAIS-IV)
15 Wordstest	Dutch version of Ray Auditory Verbal Learning Test (RAVLT)
Cognitive Screening Test	Cognitive Screening Test (20)
Trail Making Test	
Clock-drawing Test	
Symbol Digit Modalities Test	
Letter Fluency Test	Dutch version of the Controlled Word Association Test (COWAT)
Word Fluency Test	Subtest of the Groningen Intelligence Test (GIT)
Key Search Test	Subtest of the Behavioral Assessment of the Dysexecutive Syndrome (BADS)

Physical Protocol Measurements and Tests

The *standing balance test* will be performed with an accelerometer (Axivity, Newcastle, United Kingdom), attached to the lower back. The standing balance test has been described in

detail previously.[46] Balance will be evaluated in 5 different positions, i.e. 1) feet together on hard surface, eyes open; 2) feet together on hard surface, eyes closed; 3) feet together on foam surface (Balance Pad Elite; Airex Specialty Foams, Aargau, Switzerland), eyes open; 4) feet together on foam surface, eyes closed, and 5) feet in tandem stance, eyes open. Participants will be asked to have arms crossed on their chest and each position will be tested for 50 seconds. Upon failure, with recording of time to failure, a second attempt will be performed. In case of non-success at the second attempt, the test will be discontinued.

Endurance will be tested with a *2MWT*.[47] The 2MWT has been shown to be highly correlated, without compromising validity and reliability, with the 6-minute walking test, an important submaximal exercise test.[48, 49] To calculate distance covered by subjects on the 2MWT, two pylons are set apart 15 meters and subjects are instructed to walk as fast as possible without running, until the investigator commands to stop. Participants are updated on the remaining time after 1.00 and 1.45 minutes, and the final five seconds are indicated by a countdown. The total walking distance in 2 minutes is recorded in total meters covered with remaining scored in centimeters.

Locomotion, measured as gait speed, will be tested with a *4MWT*. Gait speed is a simple measure to summarize the overall disease burden and disability.[50, 51] In brief, two pylons will be set apart 4 meters and instructed to walk at usual pace. Seconds from start to end of the 4 meters will be recorded. The 4MWT is measured twice after first a trial round.

Manual dexterity will be measured in all transplant recipients using the *9-Hole Peg Test* (9-HPT, Sammons Preston Rolyan, Chicago, IL). The 9-HPT requires participants to repeatedly place and remove nine pegs into nine holes, one at a time, as quickly as possible, and is considered to be the gold standard metric for manual dexterity.

Functional mobility will be tested in participants using the *FTSTS* and *TUG*. The FTSTS is a functional performance measure of leg strength or the force-generating capacity of

muscle by using the body's weight for resistance during functional activities.[52] The FTSTS will be executed three times after a first trial round. Participants will be instructed to stand up five times as fast as possible, from sitting position with their feet flat on the floor and arms folded across the chest. Measurements start upon command, and subsequently the time required to stand up and return sitting is recorded. Time is measured in seconds and this task is repeated five times.[53]

The TUG is a basic test for functional mobility and is based on strength, coordination, and balance.[54] For the test, a pylon and chair will be put apart 3 meters. The test will be performed four times, with the first round being a trial. Participants are instructed to stand up from the chair without support of the arms, subsequently walk with their normal gait speed around the pylon, and go back to the chair to sit down again. In case, participants use a walking aid in normal day life, the test will be performed with the use of a walking aid. The TUG is measured in seconds, from the moment the participant is instructed to get up until the moment the participant sits down again.

Transplant recipients have an increased susceptibility to develop peripheral neuropathy and tremor, mainly due to the continuous use of immunosuppressive medication, especially calcineurin inhibitors.[55, 56] Therefore, an extensive neurological examination will be performed and will consist of strength testing, classifying polyneuropathy, and tremor quantification. Detailed strength testing of different muscle groups (feet flexion/extension, hip flexion, biceps flexion and wrist extension) will be performed with a digital dynamometer (C.I.T. Technics, Haren, the Netherlands).[57] Hereafter, sensibility tests will be performed using a pin-prick and monofilament pen (Novo Norisk BV, Alphen aan de Rijn, the Netherlands) on bare skin five times per measurement at the dorsal side of the 1st phalange of both feet with the subject closing their eyes. Upon failure of sensibility, the dorsal side of the foot and lower limb will be tested. Proprioception will be measured by moving the 1st

phalange of both feet in dorsal flexion and plantar flexion five times with the participants closing their eyes. Upon failure, the dorsal side of the foot and index finger will be measured.

Vibration sense will be measured using a handheld biothesiometer (Bio Medical Instrument Co, Ohio, USA).[58] The biothesiometer has a rubber tractor that vibrates at 100 Hz when operating from 50 Hz mains. In brief, participants will be measured in a supine position on a bed barefooted. The vibrating tractor will be applied bi-laterally to four different measurement points of the participants: top of the hallux, forefoot, lateral malleoli, and wrist. Before applying the vibrating tractor to the points to be tested, the amplitude of the vibrating tractor is increased from zero to the point where the vibration is perceptible and beyond the threshold to the highest amplitude possible to familiarize participants with the sensation. For the measurement, the participants will be asked to concentrate on the test and report the first sensation of the vibration by saying "Stop". Each measurement point is tested twice. If the difference between the first two measurements is greater than 20%, the measurement point is tested a third time.

Prior to tremor quantification, participants will be asked to complete part C of the Fahn-Tolosa-Marin tremor rating scale that involves tremor-related functional disability.[59] The questionnaire involves speaking, bringing liquids to the mouth, eating, hygienic care, dressing, writing, work and household related tasks. The questionnaire uses a 5-point scale, with '0= no functional ability', and 4= 'severe disability, the task cannot be executed'.

To quantify tremor, two accelerometers (University Medical Center Groningen, Groningen, the Netherlands) will be attached to the dorsal side of both hands. The accelerometers will record movement in the coronal, transversal and sagittal planes as well as linear acceleration and deceleration in both hands continuously during the measurements. Amplitudes and frequency of these measurements will be recorded on a stand-alone computer. Participants will be asked to assume seven different positions while seated, which are

measured for 30 seconds each: arms down, wrists extended; arms forward, wrists and fingers relaxed; arms forward, wrists and fingers in 0 position; index fingers pointed towards each other; bilateral finger-nose task; weighted arms down with wrists expanded; weighted arms forward with wrists and fingers extended.

Finally, participants will be asked for collection of a breath sample in which hydrogen and methane will be measured with the Quintron BreathTracker (Milwaukee, Wisconsin, USA).[60] Both hydrogen and methane are exclusively formed by anaerobic fermentation in the gut, and therefore can be utilized as markers for metanogenic microflora in transplant recipients.[61]

Cognitive Protocol Measurements and Tests

The *Cognitive Screening Test (CST)* is a Dutch screening test for dementia, measuring orientation in time and place, and memory for common facts.[62] The questionnaire consists of 20 items (e.g. date of birth, name of the reigning monarch, season) and the score is calculated as the total of questions answered correctly with a maximum of 20.

Nederlandse Leestest voor Volwassenen (NLV), Dutch version of the National Adult Reading Test. The participant has to read aloud a list of 50 irregularly spelled words. The total score on the test is converted into an estimation of the premorbid intelligence quotient.[63]

The *Clock Drawing Test (CDT)* is a cognitive screening instrument.[64] Participants are asked to draw a clock and set the time to 'a quarter to two'. A maximum total score of 14 can be achieved.

The *15 Words Test* (Dutch version of the Rey Auditory Verbal Learning Test (RAVLT), measures verbal memory.[65] In this task a set of 15 unrelated words is presented to the participant, consecutively over five trials. Participants are asked to recall as many words as possible immediately after each trial (Immediate Recall). The score is the total

words recalled in 5 trials, with a maximum of 75. After 20 minutes, participants are asked to recall as many of the 15 words as possible (Delayed Recall). Additionally, a recognition task will be performed. Participants are presented with a list of 30 words and are asked which words they recognize from the list they have been presented before.

Digit Span, subtest of the Wechsler Adult Intelligence Scale (WAIS-IV).[66] This subtest consists of two tasks, the Digit Span Forward and the Digit Span Backward. The Digit Span Forward is a task for immediate auditory memory span. In this task, participants are asked to repeat a series of numbers in the same order as the examiner did. The Digit Span Backward measures working memory. Participants have to repeat the presented numbers in reversed order. The score is the total strings repeated, with a maximum of 32.

The *Word Fluency*, subtest of the Groninger Intelligentie Test (GIT-2), is a verbal task measuring semantic memory.[67] Participants are asked to name as many words within a certain category within one minute. Total score per category (respectively animals and professions) were calculated.

The *Controlled Oral Word Association Test (COWAT)* is a verbal task measuring executive control.[68] Participants have to name as many words as possible that start with a specific letter within one minute. In the meantime, participants have to comply to several rules that are given on beforehand. Total scores from three different starting letters (D-A-T) were calculated.

The *Symbol Digit Modalities Test (SDMT)* measures psychomotor speed.[69] The test consists of matching symbols and numbers as fast as possible in 90 seconds. The total score of correct matches is calculated.

Trail Making Test (TMT). This test consists of two parts: Trail Making Test – A (TMT-A) and Trail Making Test – B (TMT-B). Part A is a measure of attention and information processing speed. This task involves connecting 25 numbers in ascending order,

as quickly as possible. The TMT-B is a measure of divided attention and cognitive flexibility. In this condition, numbers as well as letters have to be connected in ascending order, alternating between numbers and letters (1-A-2-B- etc.). Both parts of the test are timed to completion (number of seconds).

The *Key Search Test* is a subtest of the Behavioral Assessment of the Dysexecutive Syndrome (BADS) and assesses the ability to plan and monitor progress. Participants are presented with a square which represents a field in which 'keys have been lost'. Participants must show how they would search the field to find the keys. Searching strategy is scored by means of functionality and maximum total score of 16 can be achieved.

Outcomes

The primary outcomes of the TransplantLines study are all-cause mortality and graft failure, which is defined as death due to failure of the transplanted organ, return to organ replacement therapy or re-transplantation. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, e.g. physical and psychological functioning, quality of sleep, and neurological problems such as tremor and polyneuropathy.

The TransplantLines biobank study aims to identify risk factors for health problems and patient-centered outcomes (e.g. adverse drug events, lifestyle, quality of life, social participation, physical and cognitive functioning). Due to the nature of the biobank, not all research questions are predefined and will arise during the course of inclusion. In contrast to many other studies, TransplantLines also aims to identify and ameliorate complaints experienced by transplant recipients, such as tremors and diarrhea, which to date have largely been overlooked by clinicians.

Data management, analysis, and access to data and samples

Data will be recorded digitally in an electronic case report form (eCRF) in a certified Electronic Data Capture and Clinical Data Management System (Utopia Data Management System version 1.13.6, Research Data Support, University Medical Center Groningen). Data entry is performed by the trained investigators. The trained investigator who performed assessments at the study visit of a participant is responsible for data entry of that participant. All data are later checked again by the trained investigators and are subsequently stored anonymously in a secured electronic environment. The TransplantLines database will be linked to registries and databases of the Dutch Health Database (DHD), Netherlands Comprehensive Cancer Organisation (IKNL), Central Bureau of Statistics (CBS), InterAction Database (IADB), Dutch Nephrology Registration/Registration Renal Replacement Therapy (Nefrovisie, Renine), Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA), National Organ Transplant Registry (NOTR), PHARMO Institute for Drug Outcome Research (PHARMO), Routine Outcome Monitoring (RoQua) and the Dutch Institute of Clinical Auditing database (DICA) through a generic layer. A data management board will be formed to maintain data infrastructure, construct Material Transfer Agreements (MTA) and to govern use of the TransplantLines biobank and database. Extractions from TransplantLines database will be performed using a retrieval suite in Utopia software package only after approval of the data management board. Data will always be extracted anonymously. SPSS statistics version 23 (IBM, Armonk, NY), R version 3.2.3 (CRAN, Vienna, Austria), STATA 14.1 (STATA Corp., College Station, TX) or a similar statistical package will be used for analysis. Data collection and management is performed in accordance with the Handbook for Adequate Natural Data Stewardship (Netherlands Federation of University Medical Centers, 2017). A team consisting of medical doctors of the

different fields involved, called Research Team TransplantLines, is installed to decide and prioritize who will get access to the samples and data of the TransplantLines biobank and cohort study. Use of samples and data can be requested by internal and external researchers against a reasonable fee. All samples are stored at -80°C and access is logged in a linked database. The logging system also provides for registration of multiple access and the number of freeze-thaw cycles that samples have undergone. Multiple access to samples is possible, but for each specific project a new request needs to performed and approved by the Research Team TransplantLines. The data coming available from the assays performed at the provided samples will be linked to the TransplantLines database and be made available to researchers in the certified Electronic Data Capture and Clinical Data Management System which will allow for evaluation and statistical analyses. This environment will also monitor and log data handling and store results of analyses.

Missing data handling

Concerning treatment of missing data and inability to generate data from missing samples, we will apply statistical methods using maximum likelihood and multiple imputation, which are now standard for dealing with participant loss and missing data.[70] These methods provide more consistent and efficient estimates of population parameters than methods relying on complete cases, mean imputation, last observation carried forward or single-imputation regression methods.[70-73] As advised in authoritative reports, these analyses will be complemented with sensitivity analyses to assess robustness of findings.[74-76]

Discussion

The TransplantLines prospective cohort study seeks to identify risk factors for the development of long-term health problems after transplantation and ultimately to develop new

and innovative interventions to improve graft survival, patient survival, and quality of life after transplantation. The TransplantLines biobank will encompass all solid organ transplantations and living organ donors. It will consist of follow-up data from all fields that are involved in organ transplantation; internal medicine, surgery, gastroenterology, hepatology, pulmonology, cardiology, dermatology, neurology, occupational medicine, children's medicine, (neuro)psychology, physiotherapy, and social work.

Although short-term transplant outcomes have improved in the last decades, graft and recipient life expectancy remains limited. In the TransplantLines study, data and samples will be collected before, during, and after transplantation to gather further insight in the impact of transplantation on transplant recipients. In addition, we aim to preemptively detect those transplant recipients who are at increased risk to develop graft failure or health problems. By investigating a wide range of clinical, social/psychological and biochemical parameters, this study aims to contribute to increased transplant survival, patient survival, but also to an increased quality of life and a more patient-centered approach to transplant care.

Our study has strengths and limitations. The major strengths of this study are the collection of extensive data on a myriad topics related to transplantation, the inclusion of all types of solid organ transplant recipients and living organ donors, and a study with a long follow-up to assess many relevant clinical outcomes. Limitations of the current study are that it comprises a single center study and that residual confounding cannot be excluded in analyses in the TransplantLines study due to its observational design. It is also a limitation that transplant recipients with limited language skills and/or poor comprehension are excluded, because these patients are likely those who are at higher risk of poor compliance and high risk social behavior, which would possibly have worse outcomes. A further limitation is that our infrequent collection of biobank samples may limit utility in detecting biomarkers for routine monitoring of transplant health and detection of suitable biomarkers

may only be possible if sampling happens to fall near the time of a clinical event. It may be considered a strength that in addition to taking samples at fixed time points, we also take samples when biopsies are performed, both at times of protocol biopsies and at times of biopsies taken at clinical indication, usually because of worsening of transplant function, with suspicion of acute or chronic rejection.

TransplantLines may serve as a basis for hypothesis-generating studies that yield insights in a wide range of clinical, social/psychological and biochemical parameters in solid organ transplant recipients, as well as living donors. Biomarkers may be identified to develop more individualized immunosuppressive treatment. This will lead to novel clinical trials in transplantation and patient-tailored approaches for new treatment options. Furthermore, the results of TransplantLines may serve to identify new modifiable risk factors and lifestyle factors in transplantation. Ultimately, this information will likely contribute to a more individualized treatment for transplant patients and improved living donor screening and follow-up. Thereby we aim to qualitatively and quantitatively improve outcomes after transplantation.

Study status

Data collection is in progress.

List of abbreviations used

METc – Medical Ethical Committee

UMCG – University Medical Center Groningen

EDTA - Ethylenediaminetetraacetic acid

SOP – Standard Operating Procedure

BIA – Bio-Impedance Analysis

ICD – Implantable Cardioverter-Defibrillator

SAF – Skin Auto-Fluorescence

AGE – Advanced Glycation End-product

PG-SGA - Scored Patient-Generated Subjective Global Assessment

2MWT – 2-Minute Walk Test

FTSTS – Five Time Sit To Stand Test

TUG – Timed Up and Go

CRF – Clinical Research Form

PI – Principal Investigator

Author contributions

M.F.E. designed the study, conducts the study for transplant recipients, wrote the manuscript, and is responsible for final content of the manuscript; A.W.G.N. conducts the study for transplant recipients and revised the manuscript; M.vL. conducts the study for living donors and wrote the part of living donors in the manuscript; A.L.Z. conducts the study for transplant recipients, living donors and wrote the cognitive protocol; R.M.D., S.P.S, T.J.K., and M.C.J.O. conduct the study for transplant recipients; N.R.H. conducts the study for living donors; A.M.B., M.J.S., C.A., E.R., J.M.S., F.A.J.A.B, R.A.P., S.P.B., G.D., R.J.P., H.G.D.L., K.D., E.A.M.V., V.E.dM gave substantial input from their field of knowledge when designing the study and revised the manuscript; R.A.P. is responsible for kidney biobank sampling and logistics; H.B. conducts the study and revised the manuscript; S.J.L.B. designed the study, is principal investigator and project leader, and takes full responsibility for the whole study. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the submitted work. All collaborators have revised the manuscript and approved the final version of the manuscript.

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Competing interests

None

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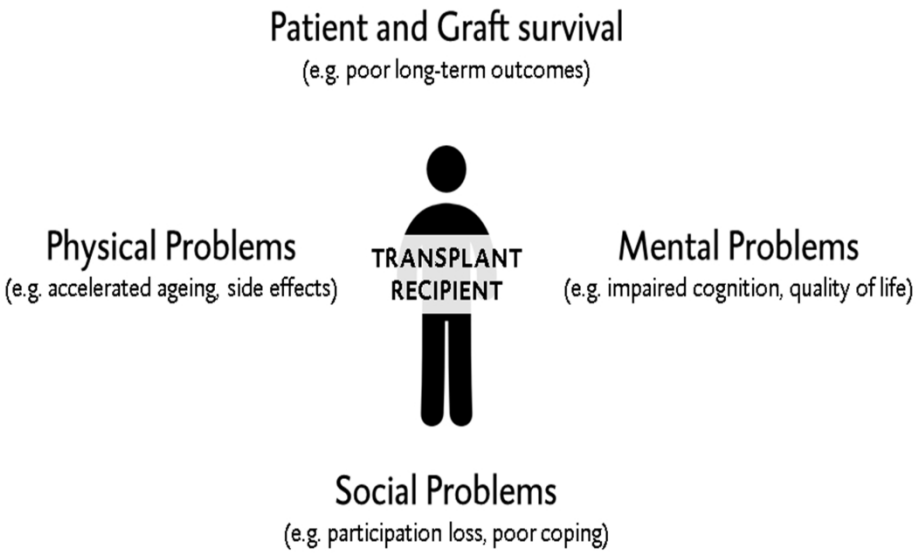
Figure legends

Figure 1. Overview of different health problems that arise on the long term after transplantation, both physical, psychological, and social.

Figure 2. Flowchart of the different visits in the TransplantLines study. At every study visit biobank, general tests, and questionnaires will be performed. Specifically addition at each timepoints; at transplantation perioperative residual material will be collected. At 3 months after transplantation cognitive protocol will be performed. At 6 months physical protocol will be carried out. At 12 months randomization to physical or cognitive protocol will occur. At 2 years after transplantation, a limited set of tests will be executed. Follow-up will be performed each 5 years.

Figure 3. Overview of the three main pillars of the TransplantLines study, i.e. questionnaires, biobank, and tests. The collection of data in these pillars at multiple time points will allow to investigate whether biomarkers at baseline can better predict occurrence of adverse outcomes and whether correction could possibly result in an improved survival.



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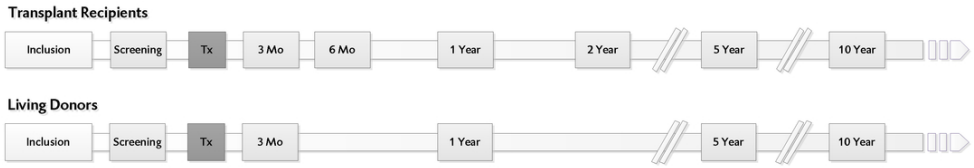

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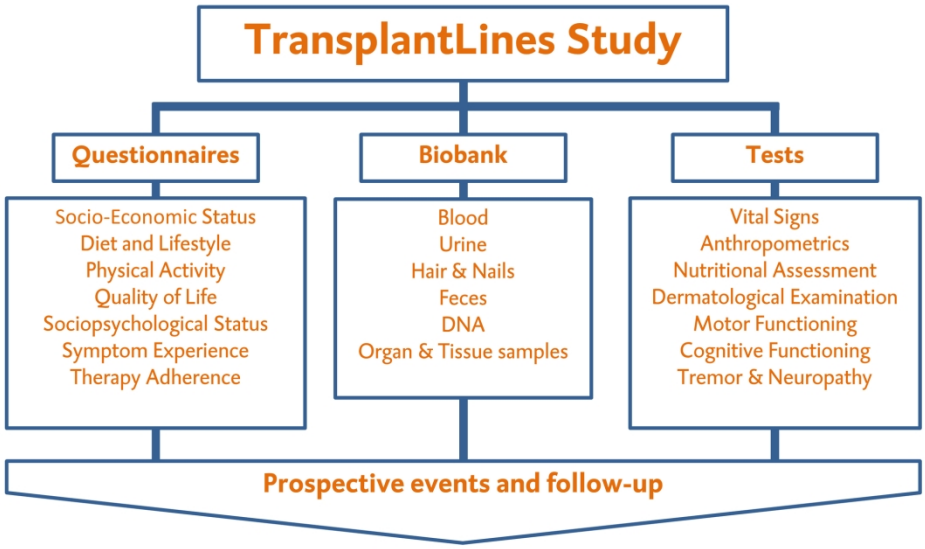

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