ADOLESCE-NT: More rapid deterioration of renal function after renal transplantation in adolescence: effect of the endocrine system on immunological and pharmacokinetic factors

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Answering the question if there are any associations between hormonal status in adolescence on the one hand, and immunological reactivity and pharmacodynamics on the other hand; and if such associations can explain the more rapid deterioration of...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Nephropathies
Study type	Observational invasive

Summary

ID

NL-OMON43848

Source
ToetsingOnline

Brief title
ADOLESCE-NT

Condition

- Nephropathies

Synonym
kidney disease, nephropathy

Research involving
Human

Sponsors and support

Primary sponsor: Erasmus MC, Universitair Medisch Centrum Rotterdam
Source(s) of monetary or material Support: Stichting Sporten voor Sophia

Intervention

Keyword: adolescence, kidney transplant, pharmacokinetics, T-lymphocytes

Outcome measures

Primary outcome

Primary objective: the relationship of the endocrine and immunological status,

measured as:

1 T-cell flow cytometry

2 Cytokine production capacity in Tnaive, Tmem and Treg subsets

a Analysis of the cytokine production capacity of CD4 and CD8 naïve,

and memory T cell subsets

b Establishing the number and the origin of the CD4+CD25+CD127-FoxP3+

T cells

c STAT5 activation by GH and IL-2

3 TREC levels, including normalization for DNA input

Secondary outcome

The secondary objectives are the relationship of the endocrine status and

pharmacokinetic parameters:

1. Trough levels of CNI and MMF at every outdepartment consultation, and

calculation of variability

2. Ratio trough level / relative daily dose of CNI and MMF

both in relation to polymorphisms of Cyp3a4 and 5, and ABCB1

Study description

Background summary

Regarding renal transplantations at childhood age, transplant survival for transplantation at ages 14-24 years is inferior to that at other ages, as appears from data of transplantations performed between 1990 and 2000 in the Eurotransplant region (unpublished data) and other studies. Evaluation of more recent transplantations in the Collaborative Transplant Study has confirmed this (http:/www.ctstransplant.org).

In the first months after transplantation the incidence of acute rejections in the age group 12-16 years is higher than that in the younger recipients. Furthermore, the percentage of patients with complete reversal of acute rejection episodes is less in adolescents than in younger age groups. Transplant survival in the first phase after transplantation does not differ between age groups; differences develop over the course of the years. Still, acute rejections in the early phase after transplantation may affect survival of the transplant kidney on the long term, which suggests there may be a relationship between the two phenomena.

The psychological changes in adolescence, manifested as desire for liberty and independence, may well result in poorer therapy compliance, which no doubt may play a role in the poorer transplant survival. A review on compliance showed a mean prevalence of non-adherence of 43% in adolescents, a significantly higher proportion than in younger patients and to mixed pediatric/adolescent populations (22%). Twenty-three percent of late acute rejection episodes in pediatric kidney transplants and 14.4% of all graft losses have been reported to be associated with non-adherence. However, one must take into account that quantification of non-adherence is difficult since no accepted and reliable method for assessment is currently available. Assessment of utilization of medication such as pill counts and assessment of prescription refill rates are often used in clinical drug trials. MEMS (medication event monitoring system), another, quite expensive method used, works via electronic monitoring devices attached to medication container caps and records the time and day the bottle is opened and closed. All mentioned techniques have in common that they do not measure the actual drug ingestion.
Despite the shortcomings of subjective assessment, self-reporting represents the most utilized and probably the most cost-effective way to monitor adherence in clinical settings. Self-report at a confidential interview has been best measured for detection of both missed doses and erratic timing of medication. Unfortunately, for obvious reasons patients may be reluctant to disclose non-adherence to their physician. Disclosure to an independent researcher seems to be more accurate than to clinical staff.
Finally, blood drug level monitoring may be helpful when a patient*s *through*

level is either inexplicably low or high or variable.

Nevertheless the higher incidence of acute rejections in the first months after transplantation can most likely not be ascribed to the poorer therapy compliance, as this is expected to exert effects on the longer term. There are no reports describing whether the increased incidence of acute rejections and the more rapid deterioration of graft function at these ages can be explained by other factors. The enormous changes in hormonal status in adolescence could affect the immunological reactivity or the pharmacokinetics of immunosuppressants. Activation of the immune system occurs in many other diseases in this period: autoimmune diseases such as SLE and Wegener*s granulomatosis often start to flare up in this period; the minimal changes nephrotic syndrome often exacerbates in this period before abating in adulthood. The starting point of this study is therefore the hypothesis that the inferior transplant survival in adolescence is caused by altered immunological reactivity.

Development of the immune system
T-cells play an important role in the acceptation and rejection of a transplanted kidney. T-cells mature in the thymus, an organ that grows until pubertal age and then gradually involutes, during which process epithelium is replaced with fatty tissue. T-cells are released from the thymus as naive T-cells that have not yet been in contact with alloantigens. In the circulation they slowly proliferate in the absence of antigenic stimuli, the so-called homeostatic proliferation. In the presence of antigenic stimuli a proliferation burst may occur.

Reference values of the most important lymphocyte subpopulations that circulate in the blood (CD3/CD4/CD8 positive T-cells, B-cells, and NK cells) show only slight changes after the 10th year of life. Also the levels of circulating immunoglobulines A, G and M show little variation after this age.3Still there are indications that also at this stage of life an important development takes place in the lymphocytic system, notably within the T-cell compartment: the number of naive T-cells goes down, whereas the number of memory-T-cells goes up.

The naive T-cells / memory T-cells ratio can be estimated by measuring the concentration of T-cells that contain TRECs. TRECs, T-cell receptor excision circles, are DNA molecules generated as a by-product during recombination of the TCR genes, and which are not duplicated during cell division. As a result, at mitosis the TREC is passed on to one of the two daughter cells. The TREC level decreases with ageing, as a result both of the reduced production of T-cells and the many cell divisions which T-cells in the immune system undergo during an individual*s life. There is a very good correlation between age and TREC levels in adults, which is being used in forensic medicine. In certain circumstances (diseases, persistent viral infections) the immune system will age (more rapidly). On the basis of the TREC levels an individual*s

immunological age can then be estimated and plotted against the chronological age. In puberty the TREC level might occasionally be extremely high, among other things due to the effect of growth hormone on the thymus (see below). The larger number of naive T-cells could result in a stronger immunological reactivity to a transplanted kidney.

In the present study we will investigate, apart from the TREC levels, the distribution and activity of the different T-cell subsets in the circulation. Activity will be investigated of the memory T cells in particular, i.e. the CD4 T effector memory cells (Tem) and the central memory T cells (Tcm), and of regulatory T-cells (Tregs). Once Tems have been in contact with a certain antigen, they will proliferate and produce cytokines at renewed contact. In this way they activate the immune response. In addition we will study the role of regulatory T-cells (Tregs). Tregs, CD4+CD25+CD127-FoxP3+, play an important role in maintaining the equilibrium in the immune response. These cells are capable of controlling unwanted immune responses, for example in autoimmune processes, and in rejection of transplanted organ. The question is how the ratio of memory and regulatory T-cells changes during the different developmental stages in childhood. It may well be that in adolescence, under the influence of hormonal changes, the Tem and the Tcm are activated while the Treg are still in a resting phase, which may result in a fiercer immune response against the transplanted kidney. We hypothesize that this is the most active period, compared to the pre-adolescence and the young adult phases.

Hormonal changes in children with chronic kidney disease and after renal transplantation.
It is well known that adolescence is characterized by major hormonal changes, regarding both the hypothalamus-gonadal axis and the growth hormone axis.

Puberty of children with chronic kidney disease (CKD) is delayed by about 2 years. Boys reach their final height at a median age of about 20 years, girls at 18 years, versus median 18 and 16 years, respectively, in healthy children. The growth spurt is shorter and less pronounced, so that the final height is clearly shorter than in healthy young people. In 35-60% of the children, final height is less than 2 standard deviations below average. The delayed puberty development manifests itself in delayed bone maturation, which after transplantation is even more delayed with corticosteroids use. This is why most children after a successful renal transplantation will continue to grow for a longer period. The hypothalamus-gonadal axis is suppressed notably in the pre-transplantation phase, and is in the subnormal range in the transplanted adolescent, especially in the first months after transplantation. This leads to the delayed sexual maturation in children with CKD. The average age of menarche in these girls mainly depends on the age at which transplantation is performed during pubertal development , but is later in comparison with the 13.3 years in healthy girls. High to very high growth hormone levels are also found during the pubertal growth spurt in children after transplantation, comparable with the levels in healthy children during puberty.

Relationship endocrinology - immunology in adolescence
If the sex hormone development should be the cause of the difference in transplant survival, it is to be expected that a difference between boys and girls would occur in puberty, and which thereafter would persist. 7-year transplant survival grouped by age and sex, is in the 12-14 years age group already decreased in girls, who reach puberty earlier than do boys. In boys the decline does not occur until age 14-16 years. In this age category there is no longer a difference between boys and girls. Also in adults there is no difference in transplant survival between men and women, so we may assume that sex hormones do not play an important role in the puberty effect.

There are, however, clear indications that the growth hormone axis affects the immune system, and notably the thymus. Growth hormone (GH) acts through the GH receptor, which is in the cytokine receptor family. Theoretically it is possible that the high GH levels during and after the maximum growth spurt influence the other cytokine receptors and consequently exert a disturbing effect. To the best of our knowledge this has not been investigated in healthy adolescents nor adolescents with kidney transplants so far. The substantial levels of GH and growth factors such as IGF-I and the binding proteins during puberty possibly influence graft acceptation at pubertal age.

Associations between GH, IGF-1 and the immune system have been reviewed extensively. GH is produced in the pituitary gland. The major role of GH obviously is promoting growth, which is achieved by stimulation of the production of IGF-1 in the liver, which through exerting action on the growth plates results in increased body height. In addition, however, GH and IGF-1 are also involved in the immune system. Here, too, GH acts through the GH receptors, which are present on cells of many types, including lymphocytes in the thymus and thymus epithelium. Receptor cleavage leads to signal transduction via JAK2 and subsequently via a number of members of the STAT family, i.e. 1, 3, 5a and 5b. Activated STAT5 may tone down a large part of the immunosuppressive effect of corticosteroids on lymphocytes 22. JAK2 is also stimulated by cytokines such as interleukin (IL)-3, -5, -6, -12, -13, prolactin, erythropoetin and interferon -γ. If the extracellular domain of the GH receptor is cleaved, it can circulate in the blood as IGF binding protein (IGF-BP 1 t/m 6), which competes with the receptors. In humans and experimental animals with GH-deficiency, GH therapy leads to growth of the thymus and the cell populations therein. GH stimulates the repopulation of the thymus also after damage to the thymus by radiation or treatment with cytostatics. The total number of lymphocytes increases, but the ratio of CD4+ and CD8+ cells does not alter. Also the number of circulating T-cells which just have been released from the thymus, assessed from the TREC level, increases.
These considerations support our hypothesis that a strong activity of GH as occurs in adolescence may increase the risk of acute and chronic transplant rejection by generalized activation of the T-cell system, although a specific effect is not recognized.

On the other hand, however, an in vitro study has shown that purified T-cells under the influence of IGF-1 produced the Th2 cytokines IL10 and IL4, not the inflammatory cytokines IL-2, IL-5, IL-6, interferon -γ and IL-1β, IL-8, tumor necrosis factor alpha. This would, contradictory to our hypothesis, precisely point at an anti-inflammatory role of IGF-1. T-cell subsets are established and undergo dramatic changes during childhood. Reference values are determined in children aged 0-18 years, but have not been compared with hormonal status. Some parameters are highly variable in adolescents (Langerak, pers comm.). A study in healthy adolescents might provide the answers whether or not there is a relation between the immune system, specifically T-cells, and hormonal status of hormones such as GH and sex hormones, independent of factors connected to kidney disease, that might be of influence on this relation.

Also multicenter clinical research on GH treatment for small body height in children after renal transplantation has not evidently documented an increase in the incidence of acute rejections. However, the following prerequisites for treatment applied: proper suppression of the immune system with daily doses of prednisolone, rather than every other day; and not any rejection episode in the year before start of treatment. For that matter, GH treatment is rarely started earlier than after one year after transplantation.
Of the lymphocyte subsets, only B-cell and CD25+ T-cell levels are lower during treatment with GH as compared with the preceding period. A mixed lymphocyte culture (MLC) of pediatric recipients and their living kidney donors showed hyporesponsivity after transplantation; addition of GH to the medium resulted in an MLC of healthy adults in strong stimulation of the proliferative and cytotoxic response and the production of interferon -γ, whereas this effect was seen in only 3 of the 20 pediatric recipients.

In summary, in vitro and in vivo studies provide arguments pro and con about an immuno-activating effect of GH. The present study will investigate whether there is a relationship between hormone development, particularly that of the GH axis, and the immunological activity in children and young adults.

Pharmacokinetics and -genetics
The concentration-time curves that are yearly prepared for all transplanted children in Erasmus MC-Sophia, at first sight show with regard to cyclosporine and MMF no differences with age. Regarding tacrolimus, the eldest children (15 until 18 years) show a higher trough level and a higher curve at a similar relative starting dose, although the same trough level is aimed at (4-8 mcg/l).

In spite of comparable or even higher blood concentrations, the pharmacokinetics could be different, e.g. as a result of the effect of the drug on and in the cells.
Most studies of the age effect on pharmacokinetics and -dynamics describe a special position of only the very young children, while the adolescents show no peculiarities in metabolism or bioavailability. One study, in children treated with TCL and MMF, without steroids, reports that children older than 12 years

require less tacrolimus than do the younger children. This is confirmed by our own findings. Several factors have been proposed to explain this age effect, such as difference in distribution volume, in relative liver size and thus rate of conversion, and in glomerular filtration rate.

Regarding the individual enzymes involved in absorption and metabolisation of the immunosuppressives: CYP 3A4 reaches adult levels around the end of the 1st year of life, the glucuronidating enzymes UGT1A9 and UGT2B4 shortly after the 2nd year of life. Contrasting findings have been reported for ABCB1, which is negatively involved in the absorption of calcineurin inhibitors in the bowel: on the one hand a persistently low level until the 10th year and next increasing levels until the 55th year of life; on the other hand a maximum level at birth and next a decrease until a stable level at the age of 0.5 to 2 years. An age-dependent effect of ABCB1 polymorphisms (ABCB1 c.2677GG genotype, c.2677GT genotype c.2677TT genotype and ABCB1 c.1236C>T genotype) on the bioavailability of cyclosporine has been described, with the largest effect in adolescence. Also the CYP3a4 intron 6 C>T polymorphism and CYP 3a5*3 are associated with altered tacrolimus and cyclosporine A metabolism. The literature does not contain reports of evident effects of sex hormone or GH on bioavailability or metabolism of calcineurin inhibitors or MMF. Our own data show a slight difference in trough levels versus relative dose of tacrolimus between adolescents and younger children, and boys and girls in puberty. The numbers of patients are too small, however, to draw conclusions.

In conclusion, the data collected in our own clinical experience and from literature indicate that the hormonal changes in adolescence affect immunological reactivity and pharmacokinetics. Nevertheless, the data are not unanimous concerning the direction and the size of these effects. Therefore a study is justified to evaluate the relation of hormonal, immunological and pharmacokinetic parameters in a group of kidney transplant recipients in adolescence or the adjacent age groups, with comparison to healthy controls.

Study objective

Answering the question if there are any associations between hormonal status in adolescence on the one hand, and immunological reactivity and pharmacodynamics on the other hand; and if such associations can explain the more rapid deterioration of renal function and loss of the graft.

Study design

Prospective cross-sectional (single center) and longitudinal observational (multicenter) study.

Study burden and risks

Burden for the patientpopulation is very low and consist of:
1. a maximum of 3 venipunctures, which are combined with the regular controls.
2. a maximun of 3 X-rays of the hand, of which 2 are usually made within the regular controls. Therefore at least 1 additional x-ray will be made.

Burden for the controlgroup consists of 1 venapuncture and 1 X-ray of the hand.

Time patients have to spent for participation is little.

The risks of participation are negligible.
Risks of venipuncture are hematoma, pain, bleeding, and in immune compromised patients there is a very small risk of infection.
Risk of hand X-rays, in particular due to the radiation exposure. This exposure is very small.

The burden and risks of participation therefore are very small

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adolescents (12-15 years)
Adolescents (16-17 years)
Adults (18-64 years)
Children (2-11 years)
Elderly (65 years and older)

Inclusion criteria

planned renal transplantation, or status after renal transplantation
age 8 - 30 years

Exclusion criteria

No informed consent
treatment with rituximab or chemotherapy
pubertas precox

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	12-04-2012
Enrollment:	190
Type:	Actual

Ethics review

Approved WMO Date:	15-03-2012
Application type:	First submission
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)
Approved WMO Date:	22-05-2014
Application type:	Amendment
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)
Approved WMO Date:	19-10-2016
Application type:	Amendment
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL38406.078.11