Evaluation of plasma chitotriosidase, plasma cytokines and cystine crystal scoring in skin and hair for the non-invasive therapeutic monitoring of nephropathic cystinosis.

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Ethical review	Approved WMO
Status	Will not start
Health condition type	Metabolic and nutritional disorders congenital
Study type	Observational invasive

Summary

ID

NL-OMON42771

Source
ToetsingOnline

Brief title
Non-invasive markers for monitoring of nephropathic cystinosis.

Condition

- Metabolic and nutritional disorders congenital
- Protein and amino acid metabolism disorders NEC
- Renal disorders (excl nephropathies)

Synonym
Cystinosis

Research involving

Human

Sponsors and support

Primary sponsor: University Hospitals Leuven, Belgium
Source(s) of monetary or material Support: KU Leuven,Raptor Pharmaceuticals INC

Intervention

Keyword: Chitotriosidase, Cystine crystals, Cytokines, Nephropathic cystinosis

Outcome measures

Primary outcome

A. cut-off values of plasma Chitotriosidase activity representing good

therapeutic control of cystinosis (WBC cystine levels < 1 nmol/mg protein)

B. Cut-off values of plasma Chitotriosidase activity representing bad

therapeutic control of cystinosis (WBC cystine levels > 2 nmol/mg protein)

C. Development of strategy for skin & cystine crystals scoring to correlate

with good and bad therapeutic control of cystinosis.

Secondary outcome

A. Correlation between WBC cystine levels and markers of macrophage activation

in cystinosis

B. Correlation between WBC cystine levels and skin & hair cystine crystals

C. Correlation between markers of macrophage activation and skin & cystine

crystals

D. Correlation between alternative markers mentioned above and eGFR in patients

without renal graft.

Study description

Background summary

General background on cystinosis

Cystinosis is an autosomal recessive disease caused by bi-allelic mutations in the CTNS gene encoding lysosomal cystine transporter cystinosin [Town et al. 1998]. The disease is characterized by lysosomal cystine tissue accumulation in all body tissues. Kidneys are first affected with renal Fanconi syndrome being the initial symptom in the majority of patients. When left untreated renal disease invariably progresses towards end stage renal failure during the first decade of life [Gahl et al. 2002]. Extra-renal organs (eyes, endocrine organs, muscles, gastro-intestinal tract, central and peripheral nervous system) are also affected by cystinosis, generally later in life [Wilmer et al. 2010]. The amino thiol cysteamine is the only available drug for decreasing lysosomal cystine accumulation that delays the progression of renal and extra-renal organ damage in cystinosis [Markello et al. 1993; Nesterova et al. 2008]. Cysteamine bitartrate salt is the most widely used cysteamine formulation and is marketed as an immediate-release preparation (Cystagon*) that has to be administered every 6 hours and a delayed-release preparation (Procysbi*) that has to be administered every 12 hours.
Currently, WBC cystine assay is the gold standard for the diagnosis of cystinosis and therapeutic monitoring of cysteamine therapy; however, it is neither ideal nor practical monitoring tool.
It was introduced by Oshima et al. who used [14C] cystine and E.coli cystine-binding protein [Oshima et al. 1974]. Currently most laboratories switched to HPLC [de Graaf-Hess et al. 1999] or LC-MS/MS [Chabli et al. 2007] methods to avoid radioactive exposure. Regardless of the large sample needed to separate WBC for HPLC or LC-MS/MS cystine assays (*10ml), both methods are tedious and are not widely available except in very few highly specialized laboratories worldwide.
While, biochemical cystine determination can be well-standardized, the major source of imprecision is attributed to WBC isolation and storage [Levtchenko et al. 2004]. Furthermore, blood for cystine assay must be taken 6 hours after the last cysteamine dose in case of Cystagon* or 12.5 hours in case of Procysbi* which is not always clinically feasible.
Cystine preferentially accumulates in polymorphonuclear leucocytes (PMN), thus, in patients with lymphocyte predominance (due to young age or viral infection); PMN cells should be used for cystine detection which poses additional technical problems [Levtchenko et al. 2004]. Furthermore, PMN cells harboring cystine are very short living cells (*12hours); therefore WBC cystine represents relatively a short time of therapeutic control. Hypothetically, if a patient complies with cysteamine treatment strictly few days before the assay, he may appear properly controlled regardless of previous compliance status.
Therefore, in this research proposal we intend to develop and to evaluate

alternative strategies for monitoring the response to cysteamine therapy in cystinosis patients.

Macrophage activation by cystine crystals

Recent evidence suggests that the immune system might play a role in the pathogenesis of nephropathic cystinosis and its rapid progression to ESRD unlike other types of Fanconi syndromes [Prencipe et al. 2014]. Prencipe et al. detected the stimulation of inflammasome related cytokines as IL-1β and IL-18 in human peripheral mononuclear cells when exposed to cystine crystals, in the plasma of cystinotic patients and in the serum and tissues of Ctns knocked-out mice.
Chitotriosidase is a fully active chitinase produced by activated macrophages. Its elevation is documented in several lysosomal storage disorders. [Hollak et al. 1994, Guo et al. 1995]. Our preliminary data demonstrate that plasma chitotriosidase activity is significantly elevated in cystinotic patients over both normal and renal controls. Chitotriosidase activities also correlated with WBC cystine contents in cysteamine treated patients above 2 years of age. Control human macrophages were potently activated in-vitro when exposed to different concentrations of cystine crystals through the significant elevations of chitotriosidase activity in both supernatant and cell lysate. Furthermore, chitotriosidase activity was significantly increased in the plasma of cystinotic knocked-out versus wild-type mice. [Elmonem et al, 2014]

When compared to WBC cystine assay, plasma chitotriosidase is much simpler, faster, more economic, stable and needs a much smaller sample (1ml or less) which is more convenient, especially in young children. On the other hand, chitotriosidase is produced by macrophages which have a very long life-span (months to years), and therefore should provide a better notion about therapeutic response over a longer time period.

Confocal microscopy of skin and hair for detection of cystine crystal:

Recently, Chiaverini et al., have shown that reflectance confocal microscopy (RCM) is able to detect dermal cystine deposition in young patients with cystinosis. Cystine deposits were visualized as bright, round, or oval-shaped dermal particles of variable size. These particles appear to be speci*c to cystinosis, as no particles were identi*ed in control patients. The nature of the deposits was further confirmed by electron microscopy analysis that showed that the particles correspond to crystalline cystine deposits within fibroblasts in the reticular dermis (Chiaverini et al 2013). The examination was quick (5 minutes), painless, and well tolerated even by the youngest patients.
RCM scoring of dermal cystine deposition could be also used as a non-invasive

marker of tissue cystine load and hence to cysteamine treatment response.

Study objective

Based on this background information and our preliminary data in this project we aim to perform longitudinal study in cystinosis patients to correlate clinical course under cysteamine therapy and

1) Markers of macrophage activation in patients plasma: chitotriosidase activity, Interleukin-18 (IL-18) ,interleukin-1beta (IL-1*) and Interleukin-6.

2) Quantitative analysis of cystine crystals in patients* skin and hair by RCM.

Study design

This study is has an open label desing.

Fifty cystinotic patients either on cysteamine treatment or at start of cysteamine treatment will be prospectively recruited and followed up for a period of 2 years.
Being an orphan disease, patients will be recruited from four different centers caring for nephropathic cystinosis patients, three in Europe at: UZ Leuven, Leuven, Belgium; Radboud University Medical Center, Nijmegen, The Netherlands and Bambino Gesu Pediatric Hospital, Rome, Italy and one in Egypt at: Cairo University Children Hospital, Cairo, Egypt.

Every 3 months the following parameters will be evaluated:
1- Clinical and basic laboratory evaluation including: serum creatinine, urinary albumin/creatinine ratio, CRP, corneal cystine crystals (once per year).
2- Cystine assay in WBCs by liquid chromatography tandem mass spectrometry. Samples will be taken 6 hours after Cystagon* intake or 12.5 hours after Procysbi* intake.
3- Chitotriosidase activity in plasma by the fluorometric substrate 4-methylumbelliferyl-triacetylchitotrioside.
4- Plasmatic IL-1β and IL-6 levels by IL-1β/IL-1F2 and IL-6 Quantikine HS ELISA Kit (R&D Systems) and plasmatic IL-18 levels by IL-18 ELISA kit (Medical and Biologic Laboratories, Nagoya, Japan).
5- Dermal and/or hair cystine crystal score by reflectance confocal microscopy

Study burden and risks

Not applicable

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

- confirmed diagnosis of nephropathic cystinosis
- age > 6 months
- patients at diagnosis
- patients under cysteamine treatment
- patients with renal Fanconi syndrome
- patients after kidney transplantation
- women of childbearing age can be included

Exclusion criteria

- age < 6 months
- intolerance of oral cysteamine treatment
- use of other cysteamine preparations than cysteamine bitartrate (Cystagon* or Procysbi*)

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Will not start
Enrollment:	24
Type:	Anticipated

Ethics review

Approved WMO

Date:	23-03-2016
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

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	NL55449.091.15