

21.1 Introduction

The clinical diagnosis of infantile, nephropathic cystinosis should be considered in any child presenting with failure to thrive, growth retardation and hypophosphatemic rickets. Most patients show also polyuria, salt craving and excessive drinking. At an early stage of the disease the pathognomonic corneal crystals of cystine (slit lamp examination) might still be absent but they develop in all patients in the course of the disease. Photophobia developing over the first years of life is the result of crystalline deposits in the cornea and the characteristic retinopathy. Without treatment the patients might suffer from life-threatening dehydration episodes. The natural course of the disease is limited by progressive glomerular damage leading to endstage renal failure at school age. At age 12 years 90% of patients with infantile nephropathic cystinosis have completely lost their glomerular function.

Three subtypes of the disease have been differentiated; (1) infantile, nephropathic cystinosis; (2) late onset or adolescent type cystinosis; and (3) adult or benign, non-nephropathic cystinosis. However only adult or benign cystinosis is clearly distinct from the nephropathic forms by the absence of nephropathy. The clinical appearance of nephropathic forms of cystinosis is not uniform but varies among all patients especially in the extent of tubular dysfunction.

All types of cystinosis are inherited in an autosomal recessive manner. The chromosomal localization was assigned to chromosome 17p. The gene identified encodes a lysosomal membrane protein called cystinosin which is responsible for the transport of the disulfide cystine out of the lysosomal space into the cytosol. A defect of this lysosomal membrane transporter causes cystinosis. The 3 subtypes have been found to be allelic.

In nephropathic cystinosis clinical chemistry of blood and urine reveals Fanconi syndrome with glucosuria, generalized hyperaminoaciduria and hyperphosphaturia. Most patients show polyuria and loose potassium and bicarbonate resulting in hypokalemia and renal acidosis. Additional tubular losses of calcium, magnesium and carnitine might also occur. The degree of tubular dysfunction is variable in any patient, but also dependent upon

glomerular filtration. There is no clear distinction between infantile and late-onset type of the disease. In late-onset nephropathic cystinosis (adolescent cystinosis) the first sign of tubular dysfunction might be tubular proteinuria.

Every case of Fanconi syndrome needs to be checked for cystinosis. The biochemical diagnosis of cystinosis is verified by an increased amount of intracellular free non-protein cystine in isolated leukocytes or cultivated fibroblasts.

Symptomatic therapy corrects the renal tubular losses, especially water and salt (e.g. phosphorus, potassium and bicarbonate). Frequent vomiting and anorexia interfere with appropriate symptomatic therapy and high calorie nutrition. Nasogastral or percutaneous gastroenterotomy (PEG) tube feeding becomes necessary in many patients for the first years of life.

Cysteamine has been shown to be effective in removing the lysosomal cystine in cystinosis. The weak base cysteamine tends to distribute within the acidic lysosomal space. A mixed disulfide of cysteamine and cysteine is formed by disulfide interchange and is transported out of the lysosomal space by the carrier for lysine. In cytosol the mixed disulfide is cleaved by reduced glutathione. Early introduction of cysteamine therapy can protect the kidneys from further progression of glomerular destruction. In end-stage renal failure replacement therapy (dialysis, transplantation) becomes necessary. Longterm survival of cystinotic patients is followed by additional late sequelae e.g. distal myopathy, loss of retinal function (blindness), disturbances of memory and other cerebral functions (for review see [3]).

21.2 Nomenclature

No.	Disorder – affected component	Tissue distribution	Chromosomal localisation	MIM
21.1	Infantile nephropathic cystinosis (lysosomal membrane cystine transporter)	generalized	17p	219800
21.2	Adolescent nephropathic cystinosis (lysosomal membrane cystine transporter)	generalized	17p	219900
21.3	Benign non-nephropathic cystinosis (lysosomal membrane cystine transporter)	generalized	17p	219750

21.3 Metabolic Pathway

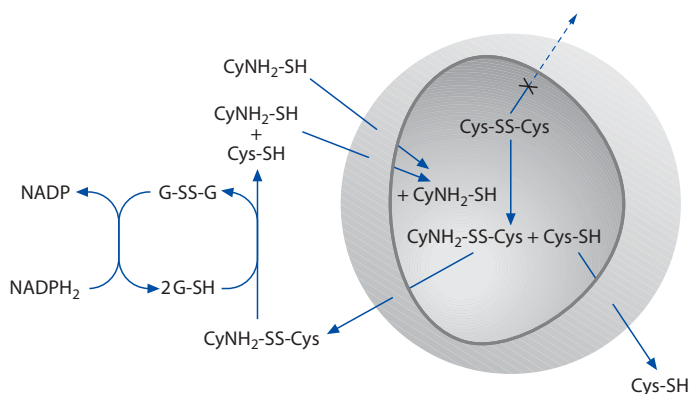


Fig. 21.1. Lysosomal handling of cystine (*Cys-SS-Cys*) in cystinosis and effect of cysteamine (*CyNH₂-SH*). *Cys-SH*, cysteine; *CyNH₂-SS-Cys*, mixed disulfide; *GSH*, reduced glutathione; *G-SS-G*, oxidized glutathione

21.4 Signs and Symptoms

Table 21.1. Infantile nephropathic cystinosis

System	Symptoms/markers	Neonate	Infant	Child	Adolescent
Unique clinical findings	Retinopathy	+	+	+	+
	Corneal crystals		±	+	+
	Photophobia		±	±	+
	Failure to thrive		±	+	+
	Polyuria/polydipsia		±	+	+
	Rickets		±	+	+
	End-stage renal failure			+	+
	Muscle weakness				±
Routine laboratory	Protein (U)	n-↑	↑	↑	↑
	PO ₄ (S)		↓	↓	↓
	K ⁺ (S)		↓	↓	↓
	Na ⁺ (S)		n-↓	n-↓	n-↓
	Creatinine (S)			↑	↑
	Urea (S)			↑	↑
	Thyroid (T ₄) (S)			n-↓	n-↓
	Metabolic acidosis		±	±	±
	PO ₄ (U)		↑	↑	↑
	K ⁺ (U)		↑	↑	↑
	Glucosuria (U)		+	+	+
	Intracellular cystine (WBC, FB)	↑	↑	↑	↑
Special laboratory	Cystine (P)	n	n	n	n
	Generalized aminoaciduria (U)		+	+	+
	Carnitine (S)		↓	↓	↓

Table 21.2. Adolescent nephropathic cystinosis (late onset)

System	Symptoms/markers	Infant	Child	Adolescent	Adult
Unique clinical findings	Retinopathy	±	+	+	+
	Corneal crystals	±	±	+	+
	Photophobia		±	+	+
	Failure to thrive		±	+	+
	Polyuria/polydipsia		±	+	+
	Rickets		±	+	+
	End-stage renal failure			+	+
	Muscle weakness			±	+
Routine laboratory	Protein (U)		n-↑	↑	↑
	PO ₄ (S)		n-↓	↓	↓
	K ⁺ (S)		n-↓	↓	↓
	Na ⁺ (S)		n-↓	n-↓	n-↓
	Creatinine (S)			n-↑	↑
	Urea (S)			n-↑	↑
	Thyroid (T ₄) (S)			n-↓	n-↓
	Metabolic acidosis			±	±
	PO ₄ (U)		n-↑	↑	↑
	K ⁺ (U)		n-↑	↑	↑
	Glucosuria (U)		±	+	+
Special laboratory	Intracellular cystine (WBC, FB)	↑	↑	↑	↑
	Cystine (P)	n	n	n	n
	Generalized aminoaciduria (U)		+	+	+

Table 21.3. Benign non-nephropathic cystinosis

System	Symptoms/markers	Neonate	Infant	Child	Adolescent	Adult
Unique clinical findings	Corneal crystals		?	±	+	+
	Photophobia			±	±	±
Routine laboratory	Normal at all ages					
Special laboratory	Intracellular cystine (WBC, FB)	↑	↑	↑	↑	↑
	Cystine (P, U)	n	n	n	n	n

21.5 Reference Values

Cystine (WBC)	<0.1–0.2 nmol cystine/mg protein ^a
Cystine (FB)	<0.1–0.2 nmol cystine/mg protein ^a
Cystine (P)	20–60 µmol/l

^a The nomenclature is not uniform. Some laboratories express their results as 1/2-cystine; 1 nmol 1/2-cystine/mg protein equals 0.5 nmol cystine/mg protein.

WBC cystine values represent mixed leukocyte preparations. These preparations are widely used but comprise an undefined variable population of cells. Intracellular cystine in cystinosis is located exclusively in lysosomes and therefore found predominantly in lysosome-rich cells like polymorphonuclear leukocytes. Therefore normal and pathological values obtained from polymorphonuclear leukocyte preparations are 2- to 3-fold higher compared to mixed leukocyte preparations [10].

Using mixed leukocyte preparations the cystine value of an individual patient might vary already by changes of the differential blood count.

Cystine concentrations are related to the amount of protein as the denominator. Contamination with non-leukocyte protein e.g. erythrocyte ghosts etc. will therefore result in falsely low values.

It is difficult to compare the results between different laboratories due to these technical problems of sample preparation.

The following methods are used for the determination of cystine:

- Ion exchange chromatography with ninhydrin detection. This time-consuming method requires only sample preparation, has high specificity but low sensitivity. An internal control of the sample is provided by other amino acids with the same analysis.
- Cystine-binding protein assay [5]. This indirect but rapid measurement method has a very high sensitivity and specificity, but disadvantages are the use of radioactive material and the questionable availability of the cystine-binding protein. It does not provide internal control of the sample tested.
- HPLC after reduction of cystine by NaBH_4 and derivatisation with bromobimane [1, 6]. This method is fast, sensitive and specific, but still used in only very few laboratories.

21.6 Pathological Values/Differential Diagnosis

Homozygote cystinosis

Cystine (WBC)	1.3–11.6 nmol cystine/mg protein ^a [3]
Cystine (FB)	3.3–7.2 nmol cystine/mg protein ^a [3]

^a The different forms of cystinosis cannot be distinguished by the cystine content of WBCs or FBs but on clinical data only.

A clear distinction between homozygote normal and heterozygote individuals is possible only when using polymorphonuclear leukocyte preparations [10].

21.7 Loading Tests

Not applicable.

21.8 Diagnostic Flow Chart

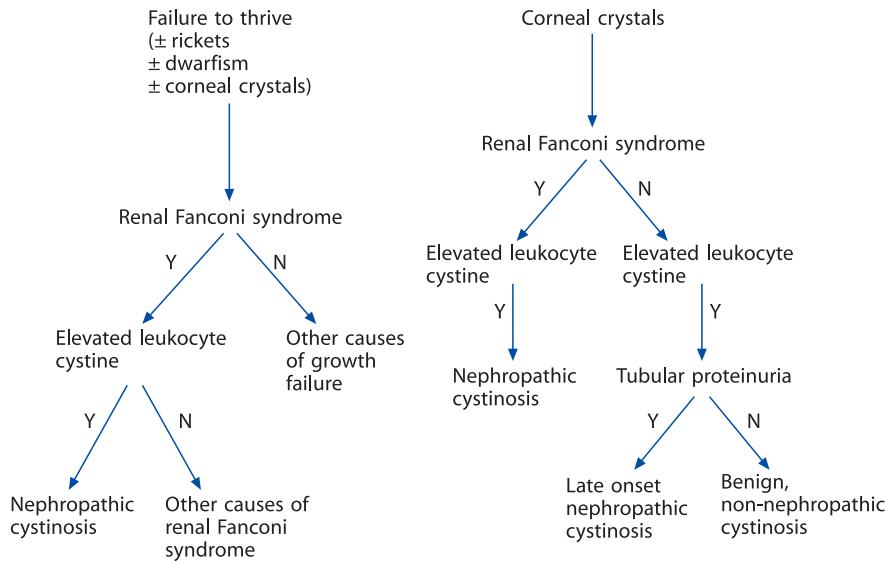


Fig. 21.2

21.9 Specimen Collection

Test	Preconditions	Material	Handling	Pitfalls
Leukocyte cystine (WBC)	None	Heparinized whole blood	Prepare mixed leukocyte fraction [4] or polymorphonuclear leukocytes [10], lyse by sonication and precipitate immediately with sulfosalicylic acid	Preparation should be performed within 24 h after blood drawing (storage of whole blood at 10–20 °C). Damage of leukocytes before final lysis results in falsely low results. Non-acidified preparations will lose cystine even under frozen conditions
Fibroblast cystine (FB)	None	Cultured fibroblasts	Wash, harvest by trypsinization or scraping, sonicate and precipitate immediately with sulfosalicylic acid	Non-acidified preparations will lose cystine even under frozen conditions

21.10 Prenatal Diagnosis

Tissue	Timing (WG)	Methodology	Pitfalls
CV	10–13	1. Direct determination of cystine (see 21.5: Reference Values) 2. Mutation analysis (see 21.11: DNA-Analysis)	Not reported
CCVS AFC	14–18	1. Direct determination of cystine (see 21.5: Reference Values) 2. Mutation analysis (see 21.11: DNA-Analysis) 3. ³⁵ S-Cystine labeling and HVE separation of acid-soluble metabolites [8]	

21.11 DNA Analysis

Disorder	Tissue	Methodology	Mutations
21.1, 21.2, 21.3	Genomic DNA	PCR SSCP Sequencing	deletions missense/nonsense (deletions → missense)

21.12 Initial Treatment

Correction of fluid and electrolyte imbalances is life saving. This includes free access to salt and water and substitution of renal losses e.g. fluid, potassium, phosphate and bicarbonate.

21.13 Summary/Comments

Nephropathic forms of cystinosis manifest as renal tubular dysfunction followed by progressive glomerular damage leading to endstage renal failure. The onset of symptoms is variable. In the most severe infantile form end-stage renal failure occurs at the end of the first decade. Cysteamine removes the intracellular cystine from lysosomes in cystinotic patients. Patients can be prevented from progression of glomerular damage if intervention begins early in life.

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