

Title page

Cystinuria: clinical practice recommendation

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Running headline: Cystinuria: clinical practice recommendation

Abstract

Cystinuria (OMIM 220100) is an autosomal recessive hereditary disorder in which high urinary cystine excretion leads to formation of cystine stones due to its low solubility at normal urinary pH. We developed clinical practice recommendation for diagnosis, surgical and medical treatment, and follow up of cystinuria patients. Elaboration of these Clinical Practice Recommendations spanned from June 2018 until December 2019 with a consensus conference in January 2019. Selected topic areas were chosen by the co-chairs of the conference. Working groups focusing on specific topics were formed. Group members performed systematic literature review using Medline, drafted the statements, and discussed them. They included geneticists, medical biochemists, paediatric and adult nephrologists, paediatric and adult urologists experts in cystinuria, Metabolic Nephropathy Joint Working Group of the European Reference Network for Rare Kidney Diseases (ERKNet) and eUROGEN members. Overall 20 statements were produced to provide guidance on diagnosis, genetic analysis, imaging techniques, surgical treatment (indication and modalities), conservative treatment (hydration, dietetic, alkalinisation, cystine binding drugs), follow up, self-monitoring, complications (renal failure and hypertension), and impact on quality of life. Due to the rarity of the disease and the poor level of evidence in the literature, these statements could not be graded. This clinical practice recommendation provides guidance on all aspects of the management of both adults and children with cystinuria, including diagnosis, surgery and medical treatment.

Keywords: cystine, cystinuria, D-penicillamine, potassium citrate, tiopronin and urolithiasis.

Introduction

Cystinuria (OMIM 220100) is an autosomal recessive hereditary disorder characterized by a defective reabsorption of cystine and dibasic amino acids ornithine, lysine and arginine (COLA) in the renal proximal tubule and in the epithelial cells of the gastrointestinal tract. The cystine transporter is a heterodimer comprised of two subunits joined by a disulfide bridge. The interaction of the heavy subunit (rBAT) and the light subunit ($b^{0,+AT}$) is essential for the functional expression of the whole system ($b^{0,+}$)¹. Two genes responsible for cystinuria have been identified: *SLC3A1* (2p21) which encodes the heavy subunit (rBAT) and *SLC7A9* (19q12) that encodes the light subunit ($b^{0,+AT}$)²⁻⁴. High cystine excretion leads to crystal precipitation in the distal tubule and formation of cystine stones due to its low solubility at normal urinary pH.

Worldwide, the overall prevalence of cystinuria is 1 per 7000 with high ethnogeographic variation¹. Two large studies carried out in France show that cystinuria causes 1% of all cases of urolithiasis and 4-5 % of urolithiasis in children^{5,6}.

Recommendation

1/ Classification and pathophysiology of cystinuria

a. Classification

Three phenotypes of cystinuria have traditionally been described on the basis of the urinary excretion of cystine in the obligate heterozygous parents (Table 1)^{4, 7}. More recently, a genotypic classification of cystinuria was introduced⁸ (Table 1). Most patients have genotype AA or BB which denote two mutated alleles in *SLC3A1* or *SLC7A9*, respectively. A0 and B0 denote the identification of only one mutated allele, even by extensive genotyping (see

below). Carriers of genotype A0 usually have normal cystine excretion, whereas carriers of genotype B0 have often elevated cystine excretion, however, very rarely develop stones⁹.

b. Genetic testing of cystinuria

i. Method of screening

Small variations can be detected by direct Sanger sequencing. For detection of large rearrangements (e.g. copy number variations, CNV), MLPA or real time PCR is required¹⁰.¹¹. Next generation sequencing (NGS) based panel analysis should be favoured over Sanger sequencing, as NGS-based panel approaches allow for both CNV analysis and detection of single-nucleotide changes.

Identified mutations in *SLC3A1* and *SLC7A9* are presented in Supplementary Table 1. Large scale rearrangements represent between 17% and 21% of all found variants¹⁰⁻¹². In *SLC3A1*, known deletions and insertions often involve parts of the neighbouring genes *PREPL* and *CAMKMT*¹⁰. Hypotonia–cystinuria syndrome (MIM 606407) is a rare homozygous contiguous gene deletion syndrome, removing part or all of *SLC3A1* and *PREPL* genes, associated with cystinuria type A. It is characterized by hypotonia, minor facial dysmorphism, mild to moderate intellectual disability and growth hormone deficiency¹³⁻¹⁵.

ii. Genotype – phenotype correlation

Although data are conflicting, neither the genotypic nor the previous phenotypic classification appear to be relevant with respect to the clinical course in patients with cystinuria. No difference in age of onset, number of stone emissions, or total stone events between those with type AA or BB was detected^{8, 12, 16, 17}.

iii. Role of genetic analysis in routine clinical practice

Genetic analysis is not mandatory for diagnosis, but is useful in the context of genetic counselling and in situations of clinical uncertainty. In cases with prenatal diagnosis of hyperechogenic colon, genetic testing can be recommended for the early diagnosis of cystinuria, as urinary amino acid excretion is difficult to interpret in the first months/years of life because of tubular immaturity.

Statements 1:

- Cystinuria is defined by a high urinary excretion of cystine and dibasic amino acids (ornithine, lysine and arginine) due to a defective proximal tubular reabsorption.
- We suggest performing genetic analysis in patients with cystinuria allowing to confirm the diagnosis, to classify the disease and to counsel other family members, although no strong genotype – phenotype correlations have been demonstrated.

c. Pathophysiology of cystine stones

The mechanisms involved in cystine kidney stone formation are related to the high concentration of cystine and its low solubility at normal urinary pH (Figure 1). Cystine solubility is below 250 mg/L (1.05 mmol/L) at pH <6 and reaches 500 mg/L (2.1 mmol /L) at pH > 7.5¹⁸⁻²⁰. The amount of cystine excreted by biallelic affected individuals (AA and BB) is typically greater than 400 mg/day (1.6 mmol/day) and usually reaches 600 to 1400 mg/day (2.5 to 6 mmol/day), whereas it does not exceed 50 mg/day (0.2 mmol/day) in normal subjects²¹. Cystine reabsorption in the proximal tubule is not directly sodium dependent, but high sodium intake would increase the intracellular neutral amino acids and sodium load, which might slow the apical reabsorption of cystine^{1, 22-24}.

2/ Clinical presentation and diagnosis

a. Clinical presentation

Detection of a hyperechoic colon at routine ultrasound before 36 weeks of gestation may suggest a diagnosis of cystinuria with a high positive predictive value (89%)^{25, 26}. The proposed explanation of this observation is that cystinuric foetuses have high cystine concentration in the amniotic fluid (AF). As the foetus constantly swallows AF and rBAT/b0+ transport system is exclusively expressed in the small intestine, ingesting AF with high cystine content would result in an overload of the intestinal reabsorptive capacity causing high cystine concentration and precipitation in the colon²⁷. In any case, confirmation of the diagnosis should be made after the delivery. The first renal stone detection can occur very early^{8, 28} and exceptionally at birth, suggesting the possible prenatal formation of the stones^{28, 29}.

In most of the patients, the first stone detection occurs during childhood or adolescence. Median age of first clinical event was 13 years in a European cohort⁸ and 16 years in a French study¹⁷ although the diagnosis can also be made during adulthood^{8, 12, 17, 29-31}. Type A and type B cystinuria have a similar age at diagnosis and similar clinical evolution^{8, 30}. A more severe disease with higher frequency of stone episodes has been reported in males in one study⁸, but not confirmed in other series^{12, 17}.

Cystinuria can be detected following the evaluation of a renal stone emission, hematuria or urinary tract infections or occasionally during radiologic examinations (X-Ray, renal ultrasound scan), performed for other reasons¹⁷. The siblings of cystinuric patients should be offered investigation for cystinuria as they could be asymptomatic³².

Statements 2:

- Detection of a hyperechoic colon at routine ultrasound scan before 36 weeks of gestation may suggest a diagnosis of cystinuria with a high positive predictive value.
- In patients with cystinuria having syndromic features, deletions on chromosome 2p21 including *SLC3A1* gene should be investigated.
- The first stone episode often occurs during adolescence.

b. Diagnosis of cystinuria

The diagnosis of cystinuria can be made by analysis of kidney stones, observation of cystine crystals in the urinary sediment or detection of an abnormal excretion of cystine and dibasic amino acids in the urine in adults and children.

Analysis of stone composition by infrared spectroscopy and X-ray diffraction offers the highest degree of certainty, whereas wet chemical analysis of urinary stones is considered to be obsolete due to poor results^{33, 34}. Most cystine stones may be easily recognized by their morphological aspect (Figures 2A and 2B).

Microscopic examination of the first morning urine, or even a random urine sample, can reveal the characteristic hexagonal crystals of cystine, which are highly specific for the diagnosis (Figure 2C). At least, two thirds of untreated patients will manifest these pathognomonic crystals.

The diagnosis of cystinuria can be confirmed by the measurement of elevated levels of cystine and dibasic amino acids (ornithine, lysine and arginine) in 24-hour urine specimens. For small children who cannot yet control their voiding the first or second morning urines are the most suitable and results should be reported relative to the creatinine level to compensate for urine concentration (Table 2)³⁵⁻³⁷. Although ion exchange chromatography (IEC) is

generally considered to be the reference method, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is increasingly used as a routine methodology and both methodologies could be considered as appropriate.

Genetic testing is not mandatory for diagnosis. It may be performed in specialized centers in case of atypical presentation, uncertain mode of inheritance, for genetic counseling and/or research purpose (see above Part 1). Increasingly, genetic screening of stone disease patients (children or adults) using stone-oriented NGS panels may reveal pathogenic genetic variations in *SLC3A1* and *SLC7A9*. Since not all variants are pathogenic, the interpretation of causality needs careful assessment and genetic result should be consistent with the clinical and biochemical findings.

The cyanide-nitroprusside test (CNT) is a rapid qualitative test that has been classically proposed as screening method for cystinuria ³⁸. However, due to its low reproducibility and sensitivity and the use of hazardous and unstable reagents, this test should be considered outdated.

Statements 3:

- Infrared spectroscopy and X-ray diffraction offer the highest degree of certainty for the analysis of cystine stone composition.
- Microscopic examination of the first morning urine can reveal the characteristic hexagonal crystals of cystine, which are highly specific for the diagnosis of cystinuria.
- The diagnosis of cystinuria is confirmed by the measurement of increased levels of cystine and dibasic amino acids (ornithine, lysine and arginine) in 24-hour urine specimens or in urine sample (as amino acid/creatinine ratio).

c. Imaging techniques used for diagnosis in children and adults

Diagnosis of cystinuria is not based on imaging. For diagnosis of stones in general non-contrast computed tomography scan of kidneys, ureters, and bladder (CT KUB) offers highest sensitivity and specificity ³⁹ and is often obtained prior to surgical intervention. Radiation dose can be minimised with low and ultralow dose techniques but this is dependent on body mass index ⁴⁰. The use of Hounsfield Units in cystinuric stones is of limited benefit due to the narrow range, most typically below 800 units despite generally being considered hard stones ⁴¹.

However, assessment and surveillance with ultrasound may be more appropriate in recurrent stone formers in preference to KUB X-ray or CT KUB to reduce the cumulative radiation ⁴². In children, ultrasound is often used first-line with low dose CT KUB as second line investigation.

Contrast CT intravenous pyelogram is only occasionally needed to delineate anatomy and plan surgery in selected patients ⁴³.

Statements 4:

- Non-contrast CT kidneys, ureters, and bladder offers the highest sensitivity and specificity for detecting renal and ureteric stones. Low dose and ultralow dose techniques should be employed as they have been shown to maintain sensitivity and specificity in stone detection.
- Assessment and surveillance with ultrasound may be more appropriate in recurrent cystine stone formers to minimize cumulative radiation exposure from CT KUB.
- In children, ultrasound should be used as the first-line diagnosis imaging technique.

3/ Treatment

a. Treatment of acute renal colic

The classical symptomatic treatment of a renal colic should be used with urologists involved in the management of patients from admission. Consideration should be given to analgesia requirements and signs of infection, to assess whether there is a need for urgent decompression (stent or nephrostomy) ^{44, 45}. In patients with refractory pain or those with a low probability of passing the stone with conservative management, ureteroscopic stone removal or extracorporeal shockwave lithotripsy (ESWL) should be undertaken in a timely manner. Stone size and location are used to guide discussions on the chance of spontaneous stone passage. In a recent large real-world study of all stone formers managed conservatively, the chance of spontaneous stone passage was 89%, 49% and 29% for stones <5mm, ≥5-7mm and >7mm respectively ⁴⁶. For stones in the proximal and distal ureter the passage rates were 52% and 83%. However, patients with cystinuria may be able to pass larger stones due to multiple previous stone passage and knowledge of previous ability to pass stones is important.

Statements 5:

- A non-steroidal anti-inflammatory drug should be the first drug of choice in the absence of contraindications.
- Renal decompression (with stent or nephrostomy) should be undertaken in patients with infected or obstructed kidneys.

b. Medical Expulsive Therapy

There is no specific evidence for the use of medical expulsive therapy (MET) in cystinuria patients and its use will depend on local policies.

c. Surgical treatment

i. Indications for surgical treatment.

- Ureteral stones

When observation is the strategy offered to a patient with an uncomplicated ureteral stone (i.e. absence of infection, no refractory pain, and no deterioration of renal function) that may pass spontaneously, surgical treatment should be considered if it fails to pass after a reasonable period of conservative treatment not exceeding 4 weeks from initial clinical presentation to prevent further symptoms or renal insult ^{47, 48}. Also, in case of complication during this period of time, such as persistent pain despite adequate analgesic medication, or persistent obstruction, surgical treatment should be offered ⁴⁹. In cystinuria, due to the recurrent nature of stones and higher incidence of chronic kidney disease, once a decision to treat is made, this should be expedited in a timely fashion ⁵⁰.

Statement 6:

- Cystinuria patients with ureteral stones who are well with no infection or renal impairment can be observed for a period up to 4 weeks for stone passage. Ongoing pain, renal impairment or low chance of spontaneous passage are indications for prompt treatment.

- Renal stones

Surgical management of cystine renal stones does not differ significantly from other stone compositions⁵¹⁻⁵³. However, due to frequent recurrent episodes, complete stone clearance is of importance where possible to avoid growth of residual fragments which may result in symptoms or further surgery later⁵⁴. Small renal stones may be observed with periodic evaluation as they may pass. Larger renal stones >1cm, pain, infection, hematuria or stone growth are indications that should prompt surgical intervention⁵⁵. In pediatrics, because of the fast stone growth rates and new stone formation, patients should be rendered stone-free whenever possible. However, repeated surgeries can also potentially damage the kidney and should be evaded by optimizing conservative treatment.

Statement 7:

- Due to the recurrent nature of cystine stones, complete stone clearance should be achieved whenever possible.

ii. Surgical modalities (Figures 3A & 3B)

ESWL, ureteroscopy (URS), or percutaneous nephrolithotomy (PCNL) are all options for treating cystine stones (Figure 3).

Although cystine stones are generally considered harder and more resistant to ESWL, reasonable outcomes can be achieved by well-trained urologists in adults and children⁵⁶⁻⁶⁰.

URS is the mainstay of treatment for ureteric stones and renal stones below 20 mm in children and adults^{48, 52, 61}. Although PCNL is recommended for renal stones larger than 20 mm, staged ureteroscopic procedures may be offered because cystinuric patients often have experienced multiple previous invasive procedures and high recurrence rates, thus demanding the use of the least invasive methods^{52, 62}. When PCNL is undertaken, newer miniaturized

techniques may be beneficial in reducing trauma to the renal parenchyma in recurrent stone formers ^{61, 63}.

If ureteral stent placement is considered at the end of an endourological procedure (URS or PCNL), the time of indwelling should be minimised due to the risk of encrustation which can occur within 2 weeks ⁵².

Statements 8:

- Cystinuria patients often undergo frequent procedures, therefore, ureteral stones and renal stones <20 mm should be treated with flexible ureteroscopy to avoid the insult of percutaneous nephrolithotomy.
- Whilst often considered relatively ESWL resistant, ESWL may be offered to patients with previous treatment success or to patients who wish to avoid invasive procedures, including children.
- When PCNL is considered, miniaturized techniques may offer equivalent stone-free rates to that with standard PCNL but with reduced renal insult.
- Stent dwell time should be ideally minimized to <2 weeks, due to risk of rapid encrustation.

iii. Differences in surgical treatment between children and adults

Usually the treatments strategies in children are the same than in adults. ESWL is likely to be performed under general anesthesia in children. Small recent series suggest ESWL is less frequently used today due to advances in pediatric ureteroscopy ^{61, 64}. URS in young children requires specific equipment (miniaturised ureteroscopes) and specialised surgical expertise. Besides stone-related factors, sex (in children) and age of the patient have to be considered when making a choice between retrograde and percutaneous treatment. For percutaneous

surgery, miniaturised techniques (mini and ultramini PCNL) should be used ⁶³. In young children urological procedures are associated with higher morbidity. Open surgery in paediatrics should be limited but may be required in cases of congenital anomalies of the kidney and urinary tract. Staghorn calculi may be approached with a combination of PCNL and ESWL and occasionally with open surgery.

d. Conservative treatment

i. Hydration

Fluid intake should guarantee a urine output large enough to maintain a cystine concentration below 250 mg/L (1 mmol/L) during 24 hours ^{21, 65}. In adults, over 3 liters of urine output is required for excretion of 1-1,5 g (2-3 mmol) of cystine per day ^{19, 66}. The target of urine specific gravity should be ≤ 1.005 ⁶⁷. In children these values may be adjusted to promote 24-hours urine volume of more than 2 liters /1.73 m² ⁶⁸. Patients should be strongly advised to consume fluids before bedtime and upon awakening. In some refractory paediatric cases, it could be considered to provide water during the night by mean of nasogastric tube feeding or gastrostomy.

Statements 9:

- Fluid intake should guarantee a urine output large enough to maintain a cystine concentration below 250 mg/L (1 mmol/L).
- In most adults, at least 3 liters of urine output per 24 hours are required.
- In children, urine volume should be higher than 2 liters/1.73 m².

ii. Dietetic:

The effect of a low sodium diet on reducing cystine excretion albeit modest has been demonstrated⁶⁹⁻⁷². Reducing sodium intake to 1-1.5 mEq/kg (in adults 100 mEq/day or NaCl 6 g/d) is therefore advisable even if its effect on stone activity is not supported by clinical trials²¹.

Reduced methionine intake lowers cystine production, but it should not be below the physiological requirement (i.e. 1,200–1,400 mg/day in adults)⁷³. Such reduction can be partially achieved by avoidance of foods with very high methionine and cystine content (Table 3). For adults, protein intake should be lower than 1g/kg (ideal weight) per day to reach this goal. Restriction of animal protein intake also decreases net acid excretion, increasing urine pH. However, its potential effect has not been demonstrated in clinical trials. Protein restriction is not advisable in children for concern about growth but foods with high methionine content should be reduced⁷³.

Statements 10:

- Reducing sodium intake to 1-1.5 mEq/kg (adults 100 mEq/day or NaCl 6 g/d) is recommended.
- Reduced methionine intake is recommended to lower cystine production.

iii. Alkalinisation

In order to increase cystine solubility, alkalinisation should be a cornerstone of treatment for all patients. Potassium citrate, 60–80 mEq/day (60–80 mEq/1.73 m² in children), should be preferred as alkalinizing agent, because it provides effective alkalinization without increasing sodium output^{72, 74}. In adults, the starting dose around 60 mEq/day should be carefully

adjusted until therapeutic urinary pH values are reached in each urine portion in freshly voided urine (7.5-8.0) ^{67,75, 76}. There is a risk of calcium-phosphate crystal formation at high pH value ⁶⁷. Potassium citrate should be given in three to four single doses (including large bedtime dose) or diluted in large volumes of water. Sodium bicarbonate is mainly recommended in cases with severe renal insufficiency or intolerance to potassium citrate although sodium increases urinary cystine excretion ⁷⁵. Although acetazolamide has been suggested as an additional mean to increase urine pH, it is not well tolerated and has not been shown to be superior to citrate ^{1,77}.

We recommend to reinforce medical therapies at the time of any urological intervention for stone removal ⁷⁸.

Statement 11:

- Potassium citrate, 60–80 mEq/day, is preferred as alkalinizing agent and adjusted until therapeutic pH values (7.5 to 8.0) are reached in each urine portion during 24 hours. Urinary pH should be measured in freshly voided urine.

iv. Cystine binding drugs

Tiopronin and D-penicillamine are sulfhydryls which cleave cystine into two cysteine moieties to form a mixed disulfide ⁷⁹. The solubility of the cysteine–penicillamine complex is up to 50 fold higher than that of cystine ¹. These thiol-containing agents can be used in patients who experience stone recurrence despite good adherence to the above interventions. Tiopronin and D-penicillamine are both effective treatment in reducing free urine cystine levels ^{8, 20, 80, 81}.

However, cystine binding agents carry severe side effects. It has been suggested that serious adverse event are less common with tiopronin but a large recently published study showed

that the incidence of adverse effects was similar for the two drugs ^{17, 67, 82}. Adverse effects include alterations in taste perception, muco-cutaneous lesions, proteinuria and/or nephrotic syndrome due to immune-complex membranous glomerulopathy ^{17, 83-85}, immune-mediated diseases ⁶⁸. Severe hematological reactions such as neutropenia and thrombocytopenia can also occur ⁸⁶.

Monitoring complete blood cell count and urinary protein excretion should be performed regularly. Due to potential long term effects of these agents, the duration of treatment should be evaluated case-by-case.

The optimal dosage varies from patient to patient ⁸⁰. For tiopronin, the dose is between 15 and 40 mg/kg/day in three divided doses (in adults 800-1500 mg/day divided in three doses) and around 20 mg/kg to 30 mg/kg/day in four divided doses (maximum dose 1.2 g/day) for D-penicillamine ^{21, 75, 80}. We suggest a higher dose to be given at bedtime.

Statements 12:

- Conservative treatment is based upon a stepwise strategy, using hydration, diet and alkalization as basic measures, with the addition of thiol derivatives in refractory cases.
- Monitoring of complete blood cell count and urinary protein excretion should be performed regularly in patients treated with thiol derivatives.

iv. New therapeutic options

New drugs, L-cystine diamides, have recently been proposed as L-cystine crystallization inhibitors for cystinuria ⁸⁷⁻⁹⁰, but their routine clinical use has not yet been validated. α -lipoic acid inhibits cystine stone formation in mice and its use has to be further evaluated in humans ^{91, 92}.

4/ Patients' follow up

a. Imaging techniques

Published clinical series have used combination of periodic US and non-contrast CT KUB ^{66, 78}, or US only ^{52, 80}, with non-contrast CT reserved as second line. The frequency of follow up is variable and determined on clinical review ⁵².

Statements 13:

- Offer ultrasound as first line imaging in the follow up of cystinuric patients with non-contrast CT as second line.
- The frequency of follow up varies from every 3 months to annually, depending on activity of stone formation.

b. Role of crystalluria

A positive association has been shown between stone activity and crystalluria in urine samples ⁹³⁻⁹⁵. The optimal timing of urine sample for assessing crystalluria is debated, with some authors showing positive association of stone activity with samples obtained during clinical visits ⁹³ and others with early morning urine samples ^{94, 96}. In principle, monitoring crystalluria may allow tailoring of radiological investigations, but prospective studies are needed.

Statement 14:

- Crystalluria is a useful tool in the management of patients, but assessing crystalluria requires standardized methodology that is not available in some centers.

c. Urinary cystine monitoring

Cystine co-exists in urines in soluble and non-soluble forms⁵². Their relative proportion is dependent on several factors, including urinary pH, ionic strength and urine concentration⁹⁷. Total urinary cystine is not a good predictor of clinical outcome⁹⁸. Measurement of cystine concentration in fractionated urinary collections may help monitoring the adequacy of the therapy⁶⁵.

Measuring free and bound cystine has clinical value in patients treated with cystine binding drugs to adapt treatment dose (target level of free cystine < 100 μmol/mmol creatinine), but it is not available in all centers and results of published studies need to be replicated^{80,99}.

Statement 15:

- Monitoring urinary cystine is of limited value in the management of patients with cystinuria.

d. Monitoring of other urinary parameters

Monitoring frequency depends on disease activity. In patients with rare stone events (<1/year) yearly monitoring is sufficient. In adults and in children who are able to collect 24h urines, we suggest monitoring 24h urines to assess urinary volume, sodium and creatinine excretion. Monitoring proteinuria should be recommended in three situations: 1/ when cystine binding thiols are used, 2/ in case of surgery induced nephron reduction, and 3/ in case of renal failure. Measuring calcium excretion is useful in patients with mixed stone composition^{100, 101}. If patients cannot collect 24h urines, monitoring urine density on spot samples at different times of the day helps assessing urinary dilution.

Statement 16:

In adults and in children that are able to collect 24h urines, we suggest monitoring 24h urines to assess urinary volume, sodium, creatinine excretion, and proteinuria.

e. Supersaturation tests

Measuring supersaturation may reduce the variability in cystine excretion that is related to differences in urinary cystine solubility^{102, 103}. A proprietary assay has been developed to determine cystine capacity by measuring the solubility of cystine added to urine samples. Results of this assay correlate with stone activity, although the test lacks sensitivity¹⁰⁴, limiting its clinical usefulness. Assessing urine supersaturation may also be helpful to evaluate the efficacy of cystine-binding thiol drugs, which reduce supersaturation²⁰.

Statement 17:

- Urinary supersaturation tests may be useful to evaluate the efficacy of therapy, but they are available only in a few specialized centers through a single commercial US laboratory.

f. Stone activity

Different criteria of stone activity such as changes in stone size, development of new stones, passages of stones or interventions for stones, or a combination of the above have been proposed^{8, 104, 105}. Establishing a validated methodology would be valuable for clinical research.

g. Role of self-monitoring

Although prospective studies demonstrating the benefits of self-monitoring are not available, compliance to treatment, including alkalinizing agents, has been associated with fewer urological interventions ¹⁰⁶. Density should also be measured on spot morning urine (objective <1005) ⁶⁷. Lower densities are associated with reduced risk of cystine crystalluria ^{96, 107}. Recently, smartphones applications have been developed to remind patients to drink and to monitor water intake.

Statements 18:

- All patients should self-monitor urinary pH to maintain values between 7.5 and 8.

This can be achieved with test strips, dipsticks or with electronic devices.

- Assessing urinary specific gravity may also be useful.

h. Pregnancy

The absence of stones should be assessed before pregnancy. When possible, patients should be stone free before pregnancy. There is an increased risk of stone formation in general during pregnancy due to anatomic factors and hypercalciuria. Cystine binding thiols are contra-indicated. As it is an autosomal recessive disease, the risk of transmission to children is extremely low unless the other parent is a carrier of a pathogenic variant in the same gene.

5/ Complications

a. Chronic kidney disease and high blood pressure

On average, patients with kidney stones are at higher risk of chronic kidney disease (CKD), compared to the normal population ¹⁰⁸. This risk is considerably higher in cystine stone formers ¹⁰⁹. The majority of adults with cystinuria have estimated glomerular filtration rate (eGFR) below 90 ml/min/1.73 m² ^{12, 17, 31, 50, 52}. Most often, they have eGFR ranging 60-89

ml/min/1.73 m². The incidence of end-stage kidney disease remains relatively low ^{12, 17}. However, in one study including 314 adult patients (mean age 38.7 years), 26.8% of patients had eGFR <60ml/min/1.73m² and 12% had proteinuria ¹⁷. In another recent study, 17.8% of patients had eGFR below 60ml/min/1.73m² ⁵⁰.

The risk of CKD is closely related to age and to past history of renal parenchymal damage, including previous nephrectomies, congenital abnormalities and renal hypoplasia ^{17, 50, 110}. A proteomic study suggests a potential role of inflammation in kidney damage in cystinuria ¹¹¹. There is no risk of relapse after renal transplantation ¹¹².

Hypertension is reported in 29% to 51% of adult patients with cystinuria and is associated with male gender, age and chronic kidney disease (CKD) ^{17, 50}. Another study found no difference in blood pressure between cystine vs non-cystine stone formers but cystine patients were 10 years younger ¹⁰⁹.

Statement 19:

- Patients with cystinuria are at higher risk of chronic kidney disease and early onset hypertension.

b. Adjustment of treatment as stone activity decreases with renal failure

Data on dose adaptation of chelating agents in patients with CKD are insufficient. Theoretically, patients with CKD decrease their urinary cystine excretion and consequentially their risk of forming new stones if their urine output remains elevated. Because chelating agents are predominantly eliminated by the kidneys, they tend to accumulate during renal impairment. The risk/benefits of using these drugs should be carefully evaluated in patients with CKD. Treatment should be stopped or reduced in patients with eGFR 60-89 ml/min/m² and should always be stopped in patients with lower eGFR.

b. Impact on health-related quality of life (HRQoL)

Adult patients with cystinuria have on average a lower HRQoL compared to the general population^{107, 113}. HRQoL scores are positively influenced by the duration of stone-free time since the last event¹¹³. Many patients express frustration and find that medical treatment is poorly effective or poorly tolerated¹⁰⁷. Comparisons of HRQoL between cystinuric and non-cystinuric stone formers have yielded conflicting results^{113, 114}. Different questionnaires have been used, including the SF-36 in the US population^{107, 113}. Stone specific questionnaires, including the Wisconsin Stone Quality of Life questionnaire, have also been developed¹¹⁴, but are not yet validated in different populations.

Statement 20:

- Cystinuric patients with active stone disease have lower HRQoL compared to the general population.

Conclusion

Overall 20 statements were produced to provide guidance on diagnosis, genetic analysis, imaging techniques, surgical treatment, conservative treatment, follow up, self-monitoring, and complications (renal failure and hypertension), both in adults and children.

Briefly, the diagnosis can be made by analysis of kidney stones, observation of cystine crystals in the urinary sediment or detection of an abnormal urinary excretion of cystine. Due to the recurrent nature of cystine stones, complete stone clearance should be achieved whenever possible with a patient specific surgical approach. Conservative treatment is based upon a stepwise strategy, using hydration to maintain cystine concentration below 250 mg/L, reduced sodium and methionine diet and alkalization with potassium citrate (to reach

urinary pH 7.5 to 8) as basic measures, with the addition of thiol derivatives in refractory cases.

Several aspects apparently differ between these European recommendations and a recently published United States consensus paper¹¹⁵. Strengths of the present paper are the description of detailed genetic testing, comprehensive urinary cystine values facilitating the clinical diagnosis, and description of the specificities of surgical management. The recommended urine pH target is 7.5-8.0, in accordance with previous European guidelines and a recent publication^{67, 76}. In Europe, the detection and quantification of cystine crystals is used by many centers to monitor the conservative treatment of cystinuria.

We believe that this recommendation will have a positive impact on the outcomes of patients by establishing common standards, and spreading and harmonising good practices.

Methods

Development of these Clinical Practice Recommendations is an initiative of the European Reference Network for Rare Kidney Diseases (ERKNet) in collaboration with the European Reference Network (ERN) for rare and complex urogenital diseases and conditions (eUROGEN), spanned from June 2018 until December 2019, and involved one meeting in Paris, in January 2019, to discuss selected topic areas chosen by the co-chairs of the conference. This joint recommendations development group included geneticists, medical biochemists, paediatric and adult nephrologists and paediatric and adult urologists. Working groups focusing on specific topics were formed. Group members performed systematic literature review using Medline/Pubmed® and the Cochrane Library. The following key words were used: “cystine”, “cystinuria”, “penicillamine”, “tiopronin”. A total of 1754 articles were initially identified and articles relevant to the topic of diagnosis and management of cystinuria were selected (487 papers). Case reports were excluded. A total of

148 papers were assessed, and 115 were finally selected. Statements were elaborated and discussed by experts according to their level of agreement after literature review. Due to the rarity of the disease and the poor levels of evidence in the literature, these statements could not be graded. Revisions were made by external specialists including a patient group representative.

Disclosures

B Knebelmann reports advisory board activity with Advicenne. A Bertholet Thomas is investigator of Advicenne study ADV7103. M Bultitude, K Thomas, M Daudon and E Levtchenko report consulting fees by Advicenne.

Genotype classification	Type A, <i>SLC3A1</i> pathogenic variant(s)	Type B, <i>SLC7A9</i> pathogenic variant(s)	Mixed genotype
Protein	Heavy subunit (rBAT)	Light subunit ($b^{0,+AT}$)	rBAT and $b^{0,+AT}$
Examples of genotype	AA	BB	AAB, ABB, BBB
Inheritance	Autosomal recessive (genotype AA)	Autosomal recessive (genotype BB)	Mixed
Phenotype	<p>Genotype AA individuals have elevated COLA excretion and form kidney stones.</p> <p>Carriers of genotype A0 usually have normal cystine excretion, and do not develop cystine stones</p>	<p>Genotype BB individuals have elevated COLA excretion and form kidney stones.</p> <p>Carriers of genotype B0 have often elevated cystine excretion, however, very rarely develop stones⁹</p>	<p>Genotype AAB, ABB, BBB individuals form kidney stones</p> <p>Carriers of genotype of AB have often elevated cystine excretion, very rarely develop stones¹⁰</p>

Table 1. A summary of cystinuria disease classifications. Genotype types AA and BB denote two mutated alleles in *SLC3A1* or *SLC7A9*, respectively, whereas A0 and B0 denote the identification of only one mutated allele. There may be mixed genotype groups such as AB, where individuals have one pathogenic variant in *SLC3A1* and one in *SLC7A9*, and AAB and BBA if there are more than two pathogenic variants pertaining to different genes. COLA: abbreviation for four amino acids which excretion is increased in cystinuria (cystine, ornithine, lysine, arginine).

Table 2. Urinary cystine values

	Urinary cystine excretion per day	Urinary cystine concentration
Reference values	<30 mg/day (0.13 mmol/day)	< 1 month < 39 μ mol/mmol creatinine (<80 mg/g creatinine) <1 year < 25 μ mol/mmol creatinine (< 52 mg/g creatinine) >1 year < 17 μ mol/mmol creatinine (<35 mg/g creatinine) 36
Cystinuria AA and BB genotype	>400 mg/day (1.7 mmol/day)	> 150 μ mol/mmol creatinine (> 315 mg/g creatinine)
Cystinuria A0 genotype	<100 mg/day (0.4 mmol/day)	
Cystinuria B0 genotype	40-400 mg/day (0.17-1.7 mmol/day)	

Adapted from ⁸ and ³⁶. Cystine values depend on the method of analysis and on the studied population.

Table 3. Methionine contents of several foods (mg/100 g)

(modified from reference ²¹)

Dried cod	2300
Horse meet	1300
Crayfish	1000
Sardines in oil	740
Tuna in oil	680
Other fish	600
Liver	600
Poultry meat	550-620
Other meat (pork, beef, mutton, veal)	400-550
Parmesan	930
Gruyere	900
Emmental	790
Other cheese	500-600
Eggs	390

Legends to Figures

Figure 1. Cystine solubility according to urinary pH (adapted from ²¹).

Figure 2. Cystine stones. Figure 2A. Under microscopic examination cystine stones show a yellow-brown colour and have a granular surface and crude and radial structure. Figure 2B. A different morphology is observed during alkaline therapy: these stones have a smooth or finely rough appearance, due to incorporation in their peripheral layers some proportions of calcium phosphate and the cystine crystals are often of reduced size. Figure 2C. Microscopic examination of the first morning urine can reveal the characteristic crystals of cystine: large hexagonal crystals, lamellar appearance, often stacked parallel to each other.

Figure 3. Surgical treatment algorithms. URS, ureteroscopy; fURS, flexible ureteroscopy; ESWL, extracorporeal shockwave lithotripsy; PCNL, percutaneous nephrolithotomy.

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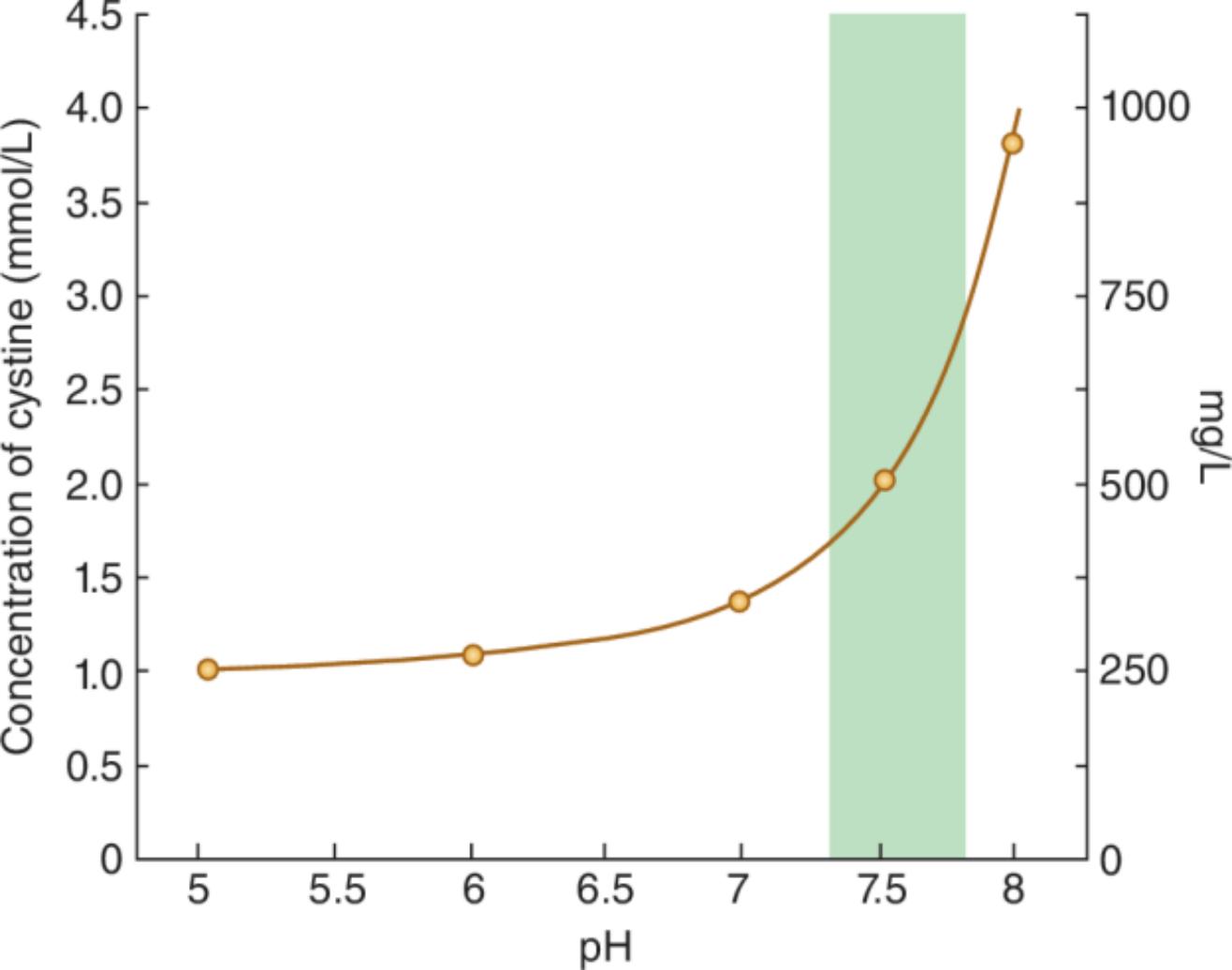
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a**Surface of cystine calculi****Section of cystine calculi****b****c**