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EDUCATIONAL REVIEW

Clinical and laboratory approaches in the diagnosis of renal tubular acidosis

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Abstract In the absence of a gastrointestinal origin, a maintained hyperchloremic metabolic acidosis must raise the diagnostic suspicion of renal tubular acidosis (RTA). Unlike adults, in whom RTA is usually secondary to acquired causes, children most often have primary forms of RTA resulting from an inherited genetic defect in the tubular proteins involved in the renal regulation of acid–base homeostasis. According to their pathophysiological basis, four types of RTA are distinguished. Distal type 1 RTA, proximal type 2 RTA, mixed-type 3 RTA, and type 4 RTA can be differentiated based on the family history, the presenting manifestations, the biochemical profile, and the radiological findings. Functional tests to explore the proximal wasting of bicarbonate and the urinary acidification capacity are also useful diagnostic tools. Although currently the molecular basis of the disease can frequently be discovered by gene analysis, patients with RTA must undergo a detailed clinical study and laboratory work-up in order to understand the pathophysiology of the disease and to warrant a correct and accurate diagnosis.

Keywords Renal tubular acidosis · Metabolic acidosis · Inherited diseases · Diagnosis · Functional tests

Introduction

The term renal tubular acidosis (RTA) refers to a group of chronic diseases characterized by hyperchloremic metabolic acidosis caused by the inability of the renal tubule to retain bicarbonate (HCO_3^-) or to secrete hydrogen ions (H^+) in the presence of normal or mildly impaired glomerular filtration rate. Although in adults RTA is frequently diagnosed in the context of systemic diseases or exposure to drugs or toxins, most pediatric cases correspond to primary disorders resulting from specific genetic defects in a protein involved in the processes of HCO_3^- reabsorption, HCO_3^- regeneration and H^+ secretion [1].

This review will focus on the clinical and biochemical findings that will lead to the diagnosis of RTA, with special emphasis on the basis and the practical aspects of functional tests useful for an accurate diagnosis. The underlying molecular defect should also be identified for a complete characterization of primary RTA [2]. However, it is of note that conventional sequencing of genes so far known to cause primary distal RTA (see below) does not disclose mutations in up to 20–25 % of patients. It is also worth noting that nowadays a growing number of children with clinical suspicion of RTA are probably insufficiently studied from a pathophysiological point of view as a result of the broader availability of genetic analysis.

According to their pathophysiological basis, the following types of RTA are distinguished: type 1 RTA is caused by the inability of the distal convoluted tubule and the collecting tubule to maximally increase the urinary elimination of H^+ in the presence of metabolic acidosis; type 2 RTA results from impaired HCO_3^- reabsorption in the proximal tubule; type 3 RTA is a mixed form of type 1 and type 2 RTA; type 4 RTA is caused mainly by defective production of ammonium (NH_4^+) resulting from either aldosterone deficiency or aldosterone resistance [3].

Clinical approach to diagnosis

Table 1 summarizes the genetic and molecular basis, as well as the clinical, biochemical, and radiological findings useful to identify the subtype of RTA [1–12]. Information on acquired forms of RTA secondary to drugs and toxins or associated to systemic diseases is not included because this review mostly

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Table 1 Clinical and genetic characteristics of the four types of primary renal tubular acidosis (RTA). All forms of primary RTA are characterized by a genetic basis and, biochemically, normal serum anion gap hyperchloremic metabolic acidosis

RTA type	Defective gene	Involved protein	Inheritance	Clinical, biochemical, and radiological features	Associated extrarenal findings
1	*ATP6V1B1*	ATP6V1B1	AR	Initial presentation is typically in infants or early childhood with failure to thrive and episodes of vomiting, dehydration, and polyuria; serum potassium low or in the lower limit of normality; calciuria normal or high; hypocitraturia; development of nephrocalcinosis detected by ultrasounds at few weeks of age is the rule; some patients develop urolithiasis	Severe early deafness
	ATP6V0A4	ATP6V0A4	AR		Late deafness
	SLC4A1	AE1	AR	Variable age of presentation in infancy or childhood; failure to thrive; muscle weakness; serum potassium low or in the lower limit of normality; no reported data on citrate excretion and scarce information on calciuria which has been found normal in some patients; medullary nephrocalcinosis in the majority; frequent rickets and bone deformities	Hemolytic anemia, mainly SAO in Thai population
	SLC4A1	AE1	AD	Presentation in childhood or adulthood; milder manifestations; serum potassium low or normal; no reported data on calcium and citrate excretions; nephrocalcinosis in about half of the patients	
2	*SLC4A4*	NBCe1	AR	Very rare disorder, reported in few families; presentation in infancy or early childhood; growth failure; serum potassium low or normal; calciuria normal; urine citrate not reported (presumably normal); no nephrocalcinosis or urolithiasis	Ocular (cataracts, glaucoma and band keratopathy) Neurological (mental retardation, familial migraine)
	Unknown	Unknown	AD	Two families reported; presentation in infancy or early childhood; growth failure; serum potassium low or normal; normocalciuria; urine citrate not reported (presumably normal); no nephrocalcinosis or urolithiasis	
3	*CA2*	Carbonic anhydrase 2	AR	Presentation in infancy or early childhood; growth failure; serum potassium low or normal; levels of calcium and citrate excretions likely depending on the variable degree of proximal and distal components of RTA; nephrocalcinosis in a minority of patients	Osteopetrosis Cerebral calcification after the 2nd year of life and mental retardation High prevalence in Arab population
4	*NR3C2*	MC receptor	AD	Renal form of primary PHA type 1: presentation in infants, variable severity, with failure to thrive, vomiting and dehydration; hyponatremia, hyperkalemia and high concentrations of serum aldosterone; normal urine citrate andcalcium excretion; no nephrocalcinosis	
	SSCNN1A	α subunit of ENaC	AR	Systemic form of primary PHA type I: neonatal presentation severe renal salt wasting, life-threatening hypovolemia, extreme hyperkalemia, metabolic acidosis, and markedly elevated plasma renin activity and aldosterone levels	Pulmonary infections
	SCNN1B	B subunit of ENaC	AR		
	SCNN1G	γ subunit of ENaC	AR		
	WNK 4	WNK 4	AD	Forms of primary PHA type II*: presentation in childhood and adulthood; hyperkalemia and hypertension with normal glomerular filtration rate, mild acidosis, suppressed plasma renin levels and circulating aldosterone relatively low for the degree of hyperkalemia; hypercalciuria in some patients. More severe phenotype and growth impairment in patients with *KLHL3* or *CUL3* mutations	Myalgias, periodic paralysis, and dental abnormalities in a subset of patients
	WNK1	WNK1	AD		
	KLHL3	KLHL3	AR or AD		
	CUL3	CUL3	AD		

AR autosomal recessive; *AD* autosomal dominant; *N* normal; ↓ low; ↑ high; *NR* not reported in the vast majority of published cases; *SAO* Southeast Asian ovalocytosis; *MC* mineralocorticoid; *PHA* pseudohypoaldosteronism; *ENaC* epithelial sodium channel; *WNK* with-no-lysine kinase; *KLHL* Kelch-like; *CUL* cullin; * PHA type II has also been named as Gordon's syndrome, familial hyperkalemic hypertension, "chloride-shunt" syndrome and, in children without arterial hypertension, Spitzer–Weinstein syndrome

deals with congenital primary types of RTA, which are more frequently found in pediatric patients.

Most types of primary RTA present early, within the first weeks or months of life. The study of the family history may facilitate diagnosis because of the disease's hereditary transmission and the higher occurrence of some forms of RTA in particular population groups. It should be pointed out that in the forms of RTA that follow an autosomal recessive pattern of transmission, the parents are carriers and the patient being evaluated may be the first in the family to have the disease.

Type 1 distal RTA is the most common form of primary RTA in Western countries. It is characterized by the inability to maximally decrease urine pH and enhance urinary NH_4^+ excretion in the presence of sustained metabolic acidosis, hypokalemia, early development of nephrocalcinosis, and frequent association with nerve deafness.

Isolated type 2 proximal RTA, caused by a decrease in the renal threshold for HCO_3^- reabsorption in the absence of alterations in the transport of other solutes, is extremely rare. The vast majority of genetic forms of type 2 proximal RTA are found as a component of Fanconi syndrome caused by inborn metabolic diseases (e.g., cystinosis) rather than isolated proximal RTA. The distinctive feature of proximal RTA is the massive waste of HCO_3^- that makes it difficult to achieve and maintain normal bicarbonatemia values in spite of high doses of alkali. When the serum HCO_3^- concentration falls below the renal threshold, bicarbonaturia ceases and the urine pH becomes acidic.

Type 3 RTA has proximal (type 2 RTA) and distal (type 1 RTA) components. In addition to type 3 RTA caused by loss of function of carbonic anhydrase (CA) 2, as mentioned in Table 1, cases of permanent distal type 1 RTA with transiently impaired proximal reabsorption of HCO_3^- can be found in infants; this form of type 3 RTA should not be considered as a separate entity from distal type 1 RTA.

Type 4 hyperkalemic RTA of hereditary origin is most frequently observed in children with resistance to the action of aldosterone, mainly primary pseudohypoaldosteronism (PHA) type 1. As shown in Table 1, recent findings have shed light on the molecular basis of type 2 PHA, a rare entity.

Type 4 RTA diagnosed in patients with renal chronic interstitial nephropathies (aldosterone resistance) associated with some degree of renal failure, as well as a form of hyperkalemic distal type 1 RTA described in pediatric patients with hydronephrosis, have not been included in Table 1 because they are not considered as inherited or primary.

Laboratory approach to diagnosis

Basal studies

Plasma anion gap (AG) Plasma or serum AG must be the first biochemical work-up in the diagnosis of a child with chronic metabolic acidosis. All types of RTA are characterized by hyperchloremic metabolic acidosis, i.e., normal AG. For the reliable interpretation of plasma AG, calculated as $(Na^+ + K^+) - (Cl^- + HCO_3^-)$, the range of normal values of AG should be determined for each laboratory and even for each individual compared with the baseline values, although this is difficult to accomplish in the clinical setting. The AG value represents the difference between unmeasured anions and unmeasured cations, and is affected by variations in the plasma concentrations of albumin, phosphate, calcium, and magnesium [13].

Urinary ammonium and pH In the study of metabolic acidosis, NH_4^+ and pH should be measured in conjunction in the same urine sample and when the patient is acidotic. A normal response of kidneys to metabolic acidosis involves the lowering of urine pH and the stimulus of production and urinary elimination of NH_4^+. A normal adult under a common Western diet eliminates about 40 mEq/day of NH_4^+. This figure is greater in children when estimated on a per-kilogram basis because of the production of H^+ that results from the formation of new bone. It is worth noting that chronic metabolic acidosis gives rise to a marked increase of urinary NH_4^+, even up to 5–8 times the normal value, which may preclude the maximum decrease of urine pH that reflects free H^+ concentrations, whereas short-term metabolic acidosis results in a minimum urine pH <5.5, but urinary NH_4^+ concentrations do not increase maximally. For an accurate interpretation of urine pH and NH_4^+ values, it should also be kept in mind that highly diluted urine, a very low concentration of urinary sodium, and bacterial growth may all interfere with the normal achievement of a minimum pH without intrinsic defects of renal acidification [14, 15].

Urinary AG The majority of clinical laboratories do not measure NH_4^+ in urine because it is cumbersome. The urinary AG $(Na^+ + K^+ - Cl^-)$ may be considered an indirect index of urinary NH_4^+ excretion in the presence of hyperchloremic metabolic acidosis [16]. High concentrations of NH_4^+ are associated with high concentrations of Cl^- and the urine AG becomes negative. Positive values $(Na^+ + K^+ > Cl^-)$ indicate inappropriately low NH_4^+ excretion. However, some limitations must be kept in mind, for example, the correlation between urinary AG and NH_4^+ has been shown to be weak in neonates and young infants [17].

Urinary osmolal gap Likewise, urinary AG does not correlate with NH_4^+ when this is excreted in the company of anions other than Cl^-. In this case, urinary NH_4^+ can be roughly estimated by calculating the urine osmolal gap, i.e., urine osmolality – $(2Na^+ + 2\ K^+ + \text{urea} + \text{glucose})$ with urea and

glucose concentrations expressed in mmol/l (to convert from mg/dl to mmol/l, divide by 2.8 and 18, respectively). A value >100 mOsm/kg H_2O suggests high urinary NH_4^+ [18, 19]. This method is valuable for bedside screening for gross changes in urinary NH_4^+ concentration and is used in the diagnostic study of patients with diabetic ketoacidosis or D-lactic acidosis [13].

Functional tests

Ammonium chloride load The medical literature describes the use of several acidifying agents, such as ammonium chloride (NH_4Cl), calcium chloride and arginine hydrochloride, to explore the renal response to metabolic acidosis that consists of stimulation of distal excretion of H^+, increased proximal ammoniagenesis and trapping of NH_4^+ in the collecting duct lumen.

Administration of NH_4Cl has been the most often utilized and it has classically been considered a crucial test in the diagnosis of distal RTA [20]. However, nowadays its clinical application is quite restricted because patients with RTA are spontaneously acidotic. The NH_4Cl test is poorly tolerated since it induces nausea and vomiting, and the ability to acidify the urine may be assessed with less aggressive explorations (see below). A single dose of 75 mEq/m^2 of NH_4Cl in infants administered diluted via nasogastric tube or 150 mEq/m^2 in children over 1 h in gelatin-coated capsules has usually been given, collecting subsequent urine samples over approximately a 6–8-h period. Relatively lower doses of 100 mg/kg (53.5 mg= 1 mEq) have been used in adults [21]. The test must be validated by confirming that the NH_4Cl dose induces metabolic acidosis: tCO_2 in blood at least <18 mmol/l in infants and <21 mmol/l in older children. In normal individuals urine pH drops below 5.5 and urinary NH_4^+ increases up to 57±14 (mean±SD) μEq/min/1.73 m^2 in infants aged 1–16 months and 80±12 μEq/min/1.73 m^2 in children aged 7–12 years [22]. The capacity of urinary acidification is blunted in patients with distal RTA and preserved in patients with proximal RTA, when the plasma HCO_3^- is below the renal threshold, and patients with type 4 RTA. As for NH_4^+ excretion, it is low in children with type 1 and type 3 RTA (defective secretion of H^+) as well as in type 4 RTA (resistance to aldosterone) and it is expected to be normal in children with pure type 2 RTA.

Administration of NH_4Cl for a 3-day period represents a more potent stimulus for NH_4^+ excretion. Adults with isolated proximal RTA retain their ability to acidify urine normally in response to 3-day NH_4Cl loading (2 mEq/kg/day by oral route), but their elimination of urinary NH_4^+ is inappropriately low in comparison with healthy controls, likely reflecting the impairment of proximal ammoniagenesis [23].

Bicarbonate load This test allows the calculation of the fractional excretion (FE) of HCO_3^- when the plasma HCO_3^- concentration is normal and the urine-to-blood (U-B) pCO_2 difference when the urine becomes more alkaline than the blood. FE of HCO_3^- is determined by collecting the urine sample under mineral oil and using the formula (Urine HCO_3^- × Plasma creatinine × 100) / (Plasma HCO_3^- × Urine creatinine). It should be remembered that introduction of oil into the gas analyzer may damage the equipment, so special care must be taken in the analysis of these oil-protected urine samples.

In proximal RTA, large amounts of HCO_3^- are excreted when plasma HCO_3^- is above the renal threshold, whereas in patients with distal RTA, the bicarbonaturia is normal unless there is a transient proximal HCO_3^- wasting, as found in some infants in whom the FE of HCO_3^- has been reported to range from 6 to 15 % in the presence of normal plasma HCO_3^- achieved during intravenous infusion of sodium HCO_3^- [24, 25]. In patients diagnosed with CA 2 deficiency and primary type 3 RTA, the values of FE of HCO_3^- with normal bicarbonatemia depend on the severity of the impairment of proximal HCO_3^- reabsorption, but the amounts of oral alkali needed to correct the acidosis are much lower than in patients with pure type 2 proximal RTA [26], indicating that the loss of urinary HCO_3^- is not that high [26–28]. In type 4 RTA, FE of HCO_3^- in the setting of normal plasma HCO_3^- is usually considered to be between 5 and 10 %, although very little data based on clinical studies are available [29].

This test is usually performed by administering 4 mEq/kg of oral sodium bicarbonate (1 g=12 mEq) [30]. However, this dose does not normalize plasma HCO_3^- in a large proportion of patients with marked HCO_3^- wasting, such as those with proximal RTA. These patients require larger doses of oral bicarbonate [10] or the intravenous infusion of a 3.75 % solution of sodium HCO_3^- at rates varying from 0.3 to 0.8 ml/min to cause an increment of 2–3 mEq/l/h of plasma HCO_3^- and to minimize extracellular volume expansion, as classically reported [24]. However, even if a normal plasma HCO_3^- concentration is not achieved, the verification of massive bicarbonaturia when plasma HCO_3^- is below normal levels is likely better evidence of defective proximal reabsorption [3, 31].

The measurement of urine pCO_2 when the urinary pH is higher than that of blood is a sensitive index of distal nephron H^+ secretion. A favorable chemical gradient facilitates H+ secretion by the collecting duct. Within the tubular lumen, H^+ ions combine with HCO_3^- to form H_2CO_3, which as a result of the lack of CA in the luminal side of this segment of the nephron, dehydrates slowly into CO_2 and water. The unfavorable surface-to-volume relationship limits CO_2 diffusion out of the lumen and generates a high pCO_2 in the renal

medulla and final urine. Provided that urine pH and HCO_3^- concentration increase above 7.6 and 80 mEq/l, respectively, the U-B pCO_2 gradient should be greater than 20 mmHg in normal individuals. In patients with primary type 1 distal RTA the U-B pCO_2 value is around 0 or may even be negative, whereas it is expected to be normal in type 2 RTA and type 4 RTA. In patients with osteopetrosis and CA deficiency (type 3 RTA), acidification is impaired and U-B pCO_2 in alkaline urine has been reported to be low [28]. However, two children reported by Ohlsson et al. [26] had normal values of 64 and 40 mmHg of U-B pCO_2 in the setting of alkaline urine induced by sodium bicarbonate treatment (5–6 mEq/kg/day).

Acetazolamide administration Acetazolamide inhibits CA 2, thus decreasing HCO_3^- reabsorption at the proximal tubule and causing enhanced bicarbonaturia. Alon et al. [32] reported that the difference in U-B pCO_2 in alkaline urine induced by oral acetazolamide was comparable to that obtained after a dose of 2.5 mEq/kg of sodium bicarbonate in children and adolescents with normal and disturbed distal acidification capacity. The maximum urinary pH after administration of the alkalinizing agent was achieved more rapidly with acetazolamide than with sodium bicarbonate (160 vs. 116 min) and acetazolamide was more palatable and better tolerated. On the basis of these results, the authors concluded that oral acetazolamide, at a dose of 15–20 mg/kg, can replace sodium bicarbonate in the assessment of U-B pCO_2. Interestingly, assessment of urine pH and HCO_3^- excretion following intravenous acetazolamide has been found to be no different in patients with CA deficiency and healthy controls [33].

Furosemide test Furosemide blocks the Na^+-K^+-$2Cl^-$ cotransporter in the thick ascending limb of the loop of Henle, thus increasing the delivery of sodium chloride to the distal segments of the nephron. This stimulates sodium ion reabsorption by the cortical collecting duct and generates a favorable lumen-negative transtubular voltage that, in turn, stimulates secretion of H^+ and potassium to urine. Thus, administration of furosemide to normal individuals results in acidification of urine in association with a kaliuretic response and an increase of NH_4 excretion [34]. Furosemide causes a sharp decrease of urinary pH by a mechanism completely different to NH_4Cl and without inducing metabolic acidosis. Furosemide is usually given at a dose of 1 mg/kg either by intravenous or oral route and urine samples are collected for a period of 4 h. Urine pH drops below 5.3 whereas urine NH_4^+ and potassium excretion increase 2–3 times, approximately, with respect to baseline values [34, 35]. Intravenous administration of furosemide causes marked increases in plasma renin activity and aldosterone concentration, whereas the activities these hormones do not increase significantly when the diuretic is given by mouth. This difference may be important when evaluating patients with hyperkalemic RTA. Patients with reduced distal H^+ secretion as a result of either low distal delivery of sodium (e.g., nephrotic syndrome), or impaired, but reversible, sodium distal reabsorption (e.g., sickle cell anemia or lithium administration), will respond normally to the furosemide test, whereas patients with primary distal type 1 RTA do not correct the acidification defect or the low NH_4^+ excretion.

Furosemide+fludrocortisone test The simultaneous administration of furosemide and fludrocortisone has been proposed to replace administration of NH_4Cl in the diagnosis of distal RTA [21]. The advantage of adding fludrocortisone to furosemide is that the mineralocorticoid action stimulates reabsorption of sodium by the principal cells of the collecting duct a few minutes after its administration, thus facilitating the secretion of H^+ and the decrease of urinary pH. The test is better tolerated that the NH_4Cl load and causes a decrease of urinary pH and an increase of urinary NH_4^+ in a shorter period of time. Oral administration of 40 mg of furosemide and 1 mg of fludrocortisone to healthy adults acidifies the urine to a pH <5.3 and increases NH_4+ excretion up to 85±23 μEq/min (mean±SE), whereas patients with distal RTA fail to acidify their urine to pH <5.3 and do not significantly increase NH_4^+ excretion over the basal values.

Table 2 proposes summarized practical protocols for the clinical application of the above-described functional tests in the diagnosis of primary RTA in infants and children. Other tests, such as phosphate or sulfate loads, are not indicated in the study of pediatric patients with primary types of RTA, but can be of some usefulness to explore the origin of renal acidification defects (back flux of secreted H^+, voltage-, gradient-defects) mainly in adults with secondary forms of RTA.

Summary of integrated diagnostic approach

Metabolic acidosis in children is usually found in the setting of acute diseases, such as systemic infections, dehydration, etc. In the presence of maintained or frequently recurrent metabolic acidosis, the following diagnostic work-up is proposed:

- High plasma AG→look for inherited metabolic diseases, ingestion of toxins, or advanced chronic renal failure.
- Normal plasma AG→diarrhea causing fecal loss of HCO_3^- as the first diagnostic option; in acidosis, patients have normal ability to decrease urine pH and negative urinary AG.
- Normal plasma AG+absence of gastrointestinal disorder+normal ability to maximally acidify urine+negative

Table 2 Functional tests in the clinical study of patients with primary forms of renal tubular acidosis (RTA)

Drug or agent	Protocol				Comments
	Dose	Samples collection		Calculations	
		Blood	Urine		
Ammonium chloride	Infants: 75 mEq/m^2, ng Children: 150 mEq/m^2, oral	T_0,T_3 and T_6	Spontaneous minuted voiding; from T_0 to T_6		Minuted collection of urines is not needed if NH_4^+ values are expressed as μEq/dl GF
Sodium bicarbonate	4 mEq/kg, oral	T_0 and when urine pH>blood pH	Spontaneous voiding, under mineral oil; from T_0 toT_4 or before if two consecutive urines become alkaline	$FEHCO_3^-$ U-B pCO_2	In forms of RTA with high wasting of HCO_3^- iv infusion of $NaHCO_3$ may be necessary to normalize bicarbonatemia
Acetazolamide	15–20 mg/kg, oral	T_0 and when urine pH>blood pH	Spontaneous voiding, under mineral oil; from T_0 toT_4 or before if two consecutive urines become alkaline	$FEHCO_3^-$ U-B pCO_2	
Furosemide	1 mg/kg, oral or iv	T_0 and T_4	Spontaneous voiding; from T_0 toT_4		Oral potassium 3 days before the test if marked hypokalemia
Furosemide+ fludrocortisone	1 mg/kg+1 mg/1.73 m^2, respectively, oral	T_0 and T_4	Spontaneous voiding; from T_0 toT_4		Oral potassium 3 days before the test if marked hypokalemia

ng nasogastric; *iv* intravenous; *T* timing of sample collection in hours, i.e., T_0 means basal (immediately before the drug administration), T_n means "n" hours after the drug administration; *GF* glomerular filtrate. $FEHCO_3^-$: fractional excretion of HCO_3^-; *U-B* urine – blood. Measurements in blood samples: pH, pCO_2, HCO_3^-, electrolytes, creatinine, and osmolality; in urines: pH, electrolytes, creatinine, osmolality and HCO_3^- and NH_4^+ when appropriate. Although blood samples are not strictly required for the furosemide and furosemide+fludrocortisone tests, but will facilitate a more complete interpretation of the results

Table 3 Differential diagnosis of primary types of renal tubular acidosis (RTA) based on laboratory work-up findings in conditions of spontaneous metabolic acidosis or in response to functional tests if applicable, as explained in the manuscript's text

	Type 1 distal RTA	Type 2 proximal RTA	Type 3 RTA	Type 4 RTA
In the presence of acidosis				
- Plasma anion gap	Normal	Normal	Normal	Normal
- Urinary NH_4^+ *	Low	Normal	Low	Low
- Plasma potassium	Low/normal**	Low/normal	Low/normal	High
- Minimal urinary pH	>5.5	<5.5	>5.5	<5.5
With normal bicarbonatemia				
- Fractional excretion of bicarbonate	<5 %	>10–15 %	>5 %	>5–10 %
- Urine – blood pCO_2 (mm Hg) in alkaline urine	<20	>20	<20	>20

*Directly measured and/or indirectly estimated by urinary anion gap. The urinary osmolal gap can be used as a rough indirect index of ammonium excretion in selected cases (see text)

**There are forms of hyperkalemic distal RTA in children with obstructive uropathy

urinary AG or high NH_4^+ elimination in the presence of acidosis→think of proximal RTA; to confirm it, urine pH below 5.5 when the child is acidotic and massive bicarbonaturia following bicarbonate load should be demonstrated. Primary pure RTA is exceptional. Investigate signs of proximal tubular dysfunction (low molecular weight proteinuria, hyperaminoaciduria, glucosuria, hypophosphatemia with relative hyperphosphaturia, hypouricemia with relative hyperuricosuria) since the majority of proximal RTAs form part of Fanconi syndrome, idiopathic or secondary to toxics or metabolic diseases (e.g., cystinosis).

- Normal plasma AG+low NH_4^+ excretion in urine, assessed by indirect indexes or, preferably, by direct determination→look at plasma potassium concentration.
- Normal plasma AG+low NH_4^+ excretion in urine+hyperkalemia+normal capacity to lower urine pH→type 4 RTA→look for obstructive uropathy and renal failure.
- Type 4 RTA+normal glomerular filtration rate+absence of structural abnormalities of the kidneys and urinary tract→study sodium and potassium metabolism, including plasma renin activity and aldosterone, to confirm hypoaldosteronism or pseudohypoaldosteronism.
- Normal plasma AG+low NH_4^+ excretion in urine+hyperkalemia+decreased capacity to lower urine pH→type 1 RTA→look for obstructive uropathy.
- Normal plasma AG+low NH_4^+ excretion in urine+normal/low plasma potassium→confirm type 1 RTA by demonstrating inability to maximally acidify urine in response to spontaneous metabolic acidosis, to acidosis induced by NH_4Cl or to the furosemide+fludrocortisone test. Once type 1 RTA is confirmed→calculate FE of HCO_3^- in the presence of normal bicarbonatemia to assess associated proximal wasting of HCO_3^- (type 3 RTA) and confirm that U-B pCO_2 in alkaline urine is low to demonstrate the primary origin of the acidification defect. If primary type 1 distal RTA is diagnosed, look for nephrocalcinosis and hypocitraturia and study hearing at diagnosis and in the follow-up.

The above schematic diagnostic approach must be preceded and completed by a detailed anamnesis and family tree, as well as a physical examination particularly focused on the assessment of bone growth and distinctive phenotypic features. Nowadays, the final diagnosis of any type of primary RTA should also lead to the search for mutations in the involved genes [36].

The distinctive biochemical characteristics of each type of primary RTA useful for differential diagnosis are schematically shown in Table 3.

Incomplete distal RTA

The term incomplete distal RTA refers to an entity of questionable clinical meaning defined by normal acid–base equilibrium in blood, inability to maximally acidify the urine, and normal preservation of NH_4^+ excretion. This disorder has been reported in asymptomatic children with hypocitraturia [37], in children with posterior urethral valves [38], in individuals with osteoporosis [39], and associated with urolithiasis and nephrocalcinosis. Recently, a congenital primary form has also been found in a kindred harboring a heterozygous truncation mutation in the *ATP6V1B1* gene and having hypocitraturia, hypercalciuria, inappropriate urinary acidification after acute NH_4Cl load, and impaired U-P pCO_2 gradient in alkaline urine [40].

Key Points

• RTA is characterized by normal anion gap hyperchloremic metabolic acidosis.

• The four primary types of RTA can be distinguished on the basis of their clinical manifestations, the presenting biochemical profile and, if needed, the response to functional tests. Genetic studies should be performed to identify the involved pathogenic gene but are not strictly necessary for the diagnosis of RTA.

• A correct assessment of urinary acidification capability requires the simultaneous measurement of pH and ammonium in the same urine sample.

Conflict of interest The authors declare no conflicts of interest.

Questions (answers are provided following the reference list)

1. All types of RTA are characterized by:
   a. Hypokalemic metabolic alkalosis
   b. Hyperchloremic metabolic acidosis
   c. Hypercalciuria
   d. Hyperkalemic metabolic acidosis
2. Nephrocalcinosis is a characteristic radiological finding of:
   a. Type 1 RTA
   b. Type 2 RTA
   c. Type 1 and 2 RTA
   d. Type 4 RTA
3. Which of the following statements is true?
   a. Type 2 RTA results from impaired HCO_3^- reabsorption in the proximal tubule
   b. Type 4 RTA results from defective production of NH_4^+ by the proximal tubule
   c. Type 3 RTA is a mixed form of type 2 and type 4 RTA
   d. None of the above
4. All of the following are typical features of primary distal type 1 RTA except
   a. Recessive autosomal inheritance in the majority of cases
   b. Nephrocalcinosis in at least 50 % of patients
   c. Negative urinary anion gap in the presence of acidosis
   d. Low or negative urinary-to-blood pCO_2 gradient in alkaline urine
5. Regarding the normal response of kidneys to metabolic acidosis, one of the following statements is false
   a. Urinary pH is acid (<5–5.5) and NH_4^+ concentration is elevated
   b. The reduced ability to lower urine pH may not be caused by intrinsic defects of renal acidification
   c. Elimination of NH_4^+ in urine is higher in acute than chronic metabolic acidosis
   d. Bicarbonaturia drops to 0

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Answers

1. B
2. A
3. A
4. C
5. C