Prevalence of distal renal tubular acidosis in primary Sjögren’s syndrome

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Abstract

Objectives. Our objectives were to analyse the prevalence of distal renal tubular acidosis (dRTA) in primary SS (pSS) and to compare a novel urinary acidification test with furosemide and fludrocortisone (FF) with the gold standard ammonium chloride (NH₄Cl) to detect dRTA.

Methods. Urinary acidification was assessed in 57 pSS patients using NH₄Cl and FF. A urinary acidification defect was defined as an inability to reach a urinary pH of <5.3 after NH₄Cl.

Results. The prevalence of complete dRTA (urinary acidification defect with acidosis) was 5% (3/57). All three patients had positive SSA/Ro and SSB/La autoantibodies and impaired kidney function. The prevalence of incomplete dRTA (urinary acidification defect without acidosis) was 25% (14/57). Compared with patients without dRTA, patients with incomplete dRTA had significantly lower venous pH and serum bicarbonate and higher urinary pH. SSB/La antibodies were more prevalent in the dRTA groups (P < 0.05). Compared with NH₄Cl, the positive and negative predictive values of FF were 46% and 82%, respectively. Vomiting occurred more often during the urinary acidification test with NH₄Cl than with FF (9 vs 0, P < 0.05).

Conclusion. Incomplete dRTA is common in pSS and causes mild acidaemia and higher urinary pH, which may contribute to bone demineralization and kidney stone formation. FF cannot replace NH₄Cl in testing urinary acidification in pSS, but may be considered as a screening tool, given its reasonable negative predictive value and better tolerability.

Key words: ammonium chloride, fludrocortisone, furosemide, pH, urinary acidification.

Introduction

Distal renal tubular acidosis (dRTA) is a well-known complication of primary SS (pSS) [1]. dRTA is classified as complete or incomplete [1]. Complete dRTA is defined as a non-anion gap metabolic acidosis with a urinary pH >5.3. Patients with incomplete dRTA maintain a serum bicarbonate level within the normal range, but are unable to acidify their urine after an acid load [2]. dRTA indicates a failure of the intercalated cells in the kidney collecting duct to secrete hydrogen ions [3, 4]. If the secretion of protons is severely impaired, the secretion of other cations, including potassium, is increased to maintain electroneutrality. This explains why complete dRTA is often accompanied by hypokalaemia due to renal potassium loss, which may even result in hypokalaemic paralysis [5-9]. Other, more long-term complications of dRTA include osteomalacia [10] and kidney stones [11]. Therefore the detection of dRTA is clinically relevant because treatment with potassium citrate may prevent these complications [12].

In addition to dRTA, other renal manifestations of pSS may include tubulointerstitial nephritis, proximal RTA and nephrogenic diabetes insipidus [13, 14]. Proximal RTA is characterized by impaired reabsorption of bicarbonate rather than a failure to secrete protons. Proximal RTA can be differentiated from dRTA by analysing if other
functions of the proximal tubule are perturbed (the presence of hypophosphataemia, hypouricaemia, glucosuria or tubular proteinuria) or by performing a bicarbonate infusion test [15].

Most of the literature on dRTA in pSS concerns case reports or small case series and therefore the true prevalence of dRTA in pSS remains unclear [6, 9]. The prevalence of pSS in patients with new-onset dRTA is reported to be 5% [16]. Although Bossini et al. [13] analysed complete and incomplete dRTA more systematically in 60 patients with pSS, the ammonium chloride (NH4Cl) test was only performed in 12 patients. Recently Walsh et al. [17] proposed an alternative urinary acidification test using a single administration of furosemide and fludrocortisone (FF). The FF combination FF maximally stimulates urinary acidification because of an increased distal delivery of sodium to the collecting duct by furosemide and direct stimulation of hydrogen secretion by fludrocortisone [17]. In this study, FF was shown to be as effective as NH4Cl in testing urinary acidification, and was also quicker and better tolerated [17].

In the present study we determined the prevalence of both complete and incomplete dRTA using the NH4Cl test. Furthermore, we compared the diagnostic performance of the urinary acidification test with FF with the urinary acidification test with NH4Cl. To do so, we performed both the NH4Cl and FF urinary acidification tests in a large cohort of patients with pSS.

Methods

Study cohort

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center (MEC-2013-075). pSS was defined according to the revised American–European Consensus Group classification criteria [18]. Additional inclusion criteria for this study included age >18 years, no other underlying autoimmune disease and an estimated glomerular filtration rate of >30 ml/min. Patients with pSS were recruited from our outpatient clinic and through the advertisement of this study on the website of the Dutch SS patient society (Fig. 1). ANA, SSA/RO52, SSA/RO60, SSB/La autoantibodies and RF were measured in all patients using previously reported methods [19]. The results of salivary gland biopsy were retrieved when available. Finally, the European League Against Rheumatism (EULAR) SS Disease Activity Index (ESSDAI) was calculated for all of the patients.

Urinary acidification tests

All subjects underwent both urinary acidification tests using NH4Cl and FF on separate days with a minimum of 1 week between the tests. Patients were allowed to continue their medication except for medication that would interfere with the urinary acidification tests (mineralocorticoid receptor blockers, loop diuretics, fludrocortisone). Patients were instructed to fast prior to the NH4Cl test to prevent vomiting; fasting was not necessary prior to the FF test. At baseline, serum and urine were collected followed by administration of the test medication. NH4Cl was given at a dose of 1 ml/kg body weight accompanied by water and ingested over a period of 30 min to prevent gastric irritation. The FF test included a single oral administration of 40 mg of furosemide and 1 mg of fludrocortisone, as described previously [17]. In both tests, hourly urine samples were collected for 6 h to measure urinary pH. Urinary pH was measured immediately by one of the investigators (T.B.) using an electrode pH meter (HI 991001, Hanna Instruments, IJsselstein, The Netherlands). During both tests, patients were monitored for nausea and vomiting.

dRTA

Complete dRTA was defined as serum bicarbonate <21 mmol/l, normal anion gap, positive urine anion gap, impaired urinary acidification and the absence of any other known causes for dRTA (e.g. medication, hypercalciuria) [1]. Incomplete dRTA was defined as an abnormal NH4Cl test accompanied by serum bicarbonate in the normal range [1]. In both the NH4Cl and FF tests, a urinary acidification defect was defined as a failure to achieve a urinary pH <5.3 within 4 h after intake of the study drugs [17].

Statistics

All results are expressed as mean (s.d.). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by comparing the FF test results with the NH4Cl test results, considering an abnormal response to the NH4Cl test as definition of disease. Comparisons of continuous variables between the three groups (no dRTA, incomplete dRTA, complete dRTA) were performed using one-way analysis of variance with the least significant difference post hoc test. Categorical data (presence or absence of autoantibodies) were analysed using the Fisher’s exact test. A P-value <0.05 was considered significant. All analyses were performed using SPSS version 21 (IBM, Armonk, NY, USA).

Results

Study cohort and baseline characteristics

The study cohort included 57 patients with pSS (Fig. 1). Although 62 patients were invited to participate in the study, 5 patients were excluded because they were unable to complete both urinary acidification tests due to repeated vomiting or because of withdrawal of consent. The baseline characteristics of the study cohort are shown in Table 1. Of the 30 patients with a retrievable salivary gland biopsy, the mean focus score was 3.1 (s.d. 2.5). In addition to medication for pSS, other commonly used drugs in this cohort were renin-angiotensin inhibitors (nine patients) and NSAIDs (seven patients).

Prevalence of dRTA

Seventeen patients were unable to acidify their urine to a pH <5.3 after NH4Cl (Fig. 2B). None of these patients used NSAIDs, which can also cause dRTA [20]. Among these
17 patients, 3 had a baseline serum bicarbonate level of <21 mmol/l [16.9 mmol/l (s.d. 0.58)] (Table 1). They were diagnosed as complete dRTA secondary to pSS, because they also had a normal serum anion gap [10.3 mEq/l (s.d. 0.5)], a positive urine anion gap [+33 mEq/l (s.d. 9)] and no other explanations for dRTA, including medication or hypercalciuria. They also showed no signs of proximal renal tubular acidosis, as indicated by the absence of hypophosphataemia, hypouricaemia and glucosuria. Although all three patients had prior episodes of hypokalaemia, they were normokalaemic at baseline in this study (Table 1); two patients were receiving potassium supplementation. The remaining 14 patients were diagnosed as incomplete dRTA since they had normal baseline serum bicarbonate levels.
bicarbonate but were unable to acidify their urine to pH <5.3. In summary, the prevalence of complete dRTA was 5% (3/57) and the prevalence of incomplete dRTA was 25% (14/57).

The FF test is less sensitive but better tolerated

In order to compare the results of the NH$_4$Cl test with the novel FF test, we performed both tests in the same cohort of patients. Twenty-four patients were unable to acidify their urine to pH <5.3 with the FF test (Fig. 2D). A comparison between the NH$_4$Cl and FF tests showed not only that more patients were unable to acidify their urine to a pH <5.3 (24 vs 17 patients), but also that six patients did acidify their urine with FF but not with NH$_4$Cl (Table 2). Considering the NH$_4$Cl test as gold standard, the sensitivity and specificity of the FF test were 65% and 68% and the PPV and NPV were 46% and 82%, respectively.

Comparison of the side effects during both tests showed that vomiting was significantly more common in patients undergoing the NH$_4$Cl test than in patients undergoing the FF test (9 vs 0, $P < 0.05$). The three patients diagnosed with complete dRTA also failed to reach a urine pH of <5.3 with the FF test. None of the patient characteristics reported in Table 1 were different in the six patients with a false-negative FF test ($P > 0.05$ for all). The six patients used the following drugs: HCQ ($n = 4$), renin-angiotensin inhibitors ($n = 1$) and NSAIDs ($n = 1$); none of these patients used immunosuppressive drugs.

Correlation of dRTA with disease parameters

We also analysed whether acid-base-related parameters and kidney function differed between patients without dRTA, with incomplete dRTA and with complete dRTA (Fig. 3). Patients with incomplete dRTA had significantly

**TABLE 2** Comparison of urinary acidification in patients with primary SS after NH$_4$Cl or FF

<table>
<thead>
<tr>
<th></th>
<th>NH$_4$Cl</th>
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<tr>
<td>$U_{\text{pH}} &gt; 5.3$</td>
<td>11</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>$U_{\text{pH}} &lt; 5.3$</td>
<td>6</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>40</td>
<td>57</td>
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FF: furosemide and fludrocortisone; NH$_4$Cl: ammonium chloride; $U_{\text{pH}}$: urine pH.
lower values of serum bicarbonate and venous pH and higher values of urinary pH than patients without dRTA (Fig. 3). The three patients with complete dRTA had the lowest venous pH, lowest serum bicarbonate and lowest urinary pH, although the latter was not different from the patients with incomplete dRTA. The patients with complete dRTA also had a higher serum creatinine than the other two groups. None of these patients used NSAIDs.

When we analysed the autoantibody prevalence in the three groups, SSB/La autoantibodies were more prevalent in patients with incomplete dRTA (79%) and complete dRTA (100%) than in patients without dRTA (45%) (supplementary Fig. S1, available at Rheumatology Online). These prevalences showed a statistically significant difference only between the patients without dRTA and those with incomplete dRTA, probably due to the low number of patients with complete dRTA. Finally, we analysed whether the ESSDAI or disease duration were different in patients with dRTA (complete and incomplete combined) compared to with patients without dRTA. The ESSDAI was 3.0 (s.d. 1.9) in dRTA vs 2.4 (s.d. 1.9) in controls and the disease duration was 13.0 years (s.d. 5.0) in dRTA vs 11.4 years (s.d. 8.0) in controls (\( P > 0.05 \) for both).

**Discussion**

Our objective was to analyse the prevalence of dRTA in pSS and to compare the diagnostic performance of FF with NH4Cl in assessing urinary acidification. The prevalence of complete dRTA was 5% and that of incomplete dRTA was 25%. The prevalence of complete dRTA in this study was the same as in the cohort of 60 patients with pSS reported by Bossini et al. [13]. All three patients with complete dRTA also had reduced kidney function. This may be due to tubulointerstitial nephritis, which is a common renal manifestation of pSS, although this was not confirmed with a kidney biopsy.

The prevalence of incomplete dRTA in our study was much higher than in the cohort reported by Bossini et al. (25% vs 0%), although they performed the NH4Cl test in only 12 patients [13]. Although patients with incomplete

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**Fig. 3** Comparison of acid-base-related parameters and kidney function

The four panels show the distribution of (A) serum bicarbonate, (B) serum creatinine, (C) venous pH and (D) urine pH under baseline conditions in the three groups. The three groups are classified as no dRTA \( (n = 40) \), incomplete dRTA \( (n = 14) \) and complete dRTA \( (n = 3) \). Statistical analysis was performed by one-way analysis of variance followed by a post hoc test. dRTA: distal renal tubular acidosis; NS: not significant.
dRTA by definition do not have metabolic acidosis, the patients in our cohort did have mild acidaemia and a higher urinary pH at baseline. This is a novel finding with potential clinical implications, because even mild acidaemia may contribute to bone demineralization [21], and higher urinary pH may predispose to kidney stone formation [11]. Indeed, Arampatzis et al. [22] diagnosed incomplete dRTA in 1 of 15 males with recurrent calcium stone formation. Eriksson et al. [11] reported 10 patients who presented with dRTA and urolithiasis who went on to develop pSS in subsequent years.

The higher autoantibody prevalence in patients with complete and incomplete dRTA may have pathophysiological significance. Although autoantibodies causing dRTA have not been identified, several reports suggest that autoantibodies against carbonic anhydrase [23] or acid-base transporters [24] are involved in the pathogenesis of dRTA in pSS. If the presence of autoantibodies causing dRTA is confirmed, screening for these antibodies would facilitate early identification and treatment of dRTA.

The second focus of this study was to analyse whether the recently reported urinary acidification test with FF could replace NH₄Cl [17]. We confirmed better tolerability of the FF test, but unfortunately the overall diagnostic performance of FF was poor. One exception was the reasonable NPV. Therefore we believe FF could be considered as a first screening test and NH₄Cl could be reserved for those patients who fail to acidify their urine to pH <5.3 with FF. One caveat with this approach is the possibility of a false-negative test result. For example, if the FF test had been used as initial screening test for dRTA in this cohort, dRTA would have been missed in 6 of 17 patients (35%). Why these six patients had a false-negative test result remains unclear, but it is probably due to the different mechanisms by which urinary acidification is tested (directly giving an acid load vs indirectly stimulating H⁺ secretion).

Because it remains unclear whether incomplete dRTA always results in complications, it may be acceptable to miss these false negatives initially. Conversely, early identification of patients with a urinary acidification defect could provide a rationale for treatment with potassium citrate, which was recently shown to improve BMD even in healthy older adults [21].

Why did the urinary acidification test with FF in the present study perform worse than in the initial report by Walsh et al. [17]? One important difference is that half of the patients in the study by Walsh et al. had complete dRTA, which is more likely to result in a positive FF test. In addition, FF was only tested in patients with previously confirmed dRTA and not in patients in whom dRTA may be absent, incomplete or complete. Indeed, in response to Walsh et al.’s report, Viljoen et al. [25] reported the results of performing both urinary acidification tests in 10 patients with recurrent nephrolithiasis and/or nephrocalcinosis. They also identified three patients who were able to acidify their urine to a pH <5.3 with NH₄Cl but not with FF. In agreement with our recommendation, Viljoen et al. [25] proposed that the urinary acidification test with FF should be used as an initial screening test to be followed up by NH₄Cl if urine pH remains ≥5.3. It is unclear whether the FF test should be repeated after a certain period of time in the case of a normal test result. There are no data to indicate what the disease-free period is after a negative test result. Therefore, at present, we recommend it be left to the discretion of the treating physician to repeat the FF test after a few years.

The strength of our study is the large cohort of patients with pSS in whom urinary acidification was tested functionally, which allowed us to establish the true prevalence of incomplete dRTA. A limitation of our study was that we do not know whether the presence of incomplete dRTA leads to poorer outcomes during follow-up, as our study was cross-sectional. A prospective cohort study aiming to determine the clinical significance of incomplete dRTA is currently ongoing.

In conclusion, incomplete dRTA is common in pSS and causes mild acidaemia, which may potentially contribute to organ damage. FF cannot replace NH₄Cl for testing urinary acidification in pSS, but it may be considered as a screening test, given its reasonable negative predictive value and better tolerability.

**Rheumatology key messages**

- In this SS cohort, the prevalence of incomplete distal renal tubular acidosis was high (25%).
- Furosemide with fludrocortisone cannot replace ammonium chloride in testing for distal renal tubular acidosis in pSS.
- Fludrocortisone may be considered as a screening tool, given its reasonable negative predictive value and better tolerability.

**Acknowledgements**

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**Supplementary data**

Supplementary data are available at *Rheumatology* Online.

**References**