Is This Elderly Patient Dehydrated? Diagnostic Accuracy of Hydration Assessment Using Physical Signs, Urine, and Saliva Markers

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Abstract: Objectives: Dehydration in older adults contributes to increased morbidity and mortality during hospitalization. As such, early diagnosis of dehydration may improve patient outcome and reduce the burden on healthcare. This prospective study investigated the diagnostic accuracy of routinely used physical signs, and non-invasive markers of hydration in urine and saliva. Design: Prospective diagnostic accuracy study. Setting: Hospital acute medical care unit and emergency department. Participants: One hundred and thirty older adults (59 males, 71 females, mean (SD) age = 78 (9) y). Measurements: Participants with any primary diagnosis underwent a hydration assessment within 30min of admittance to hospital. Hydration assessment comprised seven physical signs of dehydration (tachycardia (>100bpm), low systolic blood pressure (<100mmHg), dry mucous membrane, dry axilla, poor skin turgor, sunken eyes, and long capillary refill time (>2s)), urine color, urine specific gravity (USG), saliva flow rate (SFR) and saliva osmolality. Plasma osmolality (Posm) and the blood urea nitrogen to creatinine ratio (BUN:Cr) were assessed as reference standards of hydration, with 21% of participants classified with water-loss dehydration (Posm >295mOsm/kg), 19% classified with water-and-solute-loss dehydration (BUN:Cr >20) and 60% classified as euhydrated. Results: All physical signs showed poor sensitivity (0-44%) for detecting either form of dehydration, with only low systolic blood pressure demonstrating potential utility for aiding the diagnosis of water-and-solute-loss dehydration (diagnostic OR = 14.7). Neither urine color, USG, nor SFR could discriminate hydration status (area under the receiver operating characteristic curve, AUCROC = 0.49-0.57, P>0.05). In contrast, saliva osmolality demonstrated moderate diagnostic accuracy (AUCROC = 0.76, P<0.001) to distinguish both dehydration types (70% sensitivity, 68% specificity, OR =5.0 (95%CI 1.7-15.1) for water-loss dehydration, and 78% sensitivity, 72% specificity, OR =8.9 (95%CI 2.5-30.7) for water-and-solute-loss dehydration). Conclusions: With the exception of low systolic blood pressure, which could aid in the specific diagnosis of water-and-solute-loss dehydration, physical signs and urine markers show little utility to determine if an elderly patient is dehydrated. Saliva osmolality demonstrated superior diagnostic accuracy compared with physical signs and urine markers, and may have utility for the assessment of both water-loss and water-and-solute-loss dehydration in older individuals. It is particularly noteworthy that saliva osmolality was able to detect water-and-solute-loss dehydration, for which a measurement of plasma osmolality would have no diagnostic utility.

Letter

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11th September 2014

To the editor in chief of *Journal of the American Medical Directors Association*,

Re: Manuscript # JAMDA-D-14-00269, "Is this elderly patient dehydrated? Diagnostic accuracy of hydration assessment using physical signs, urine and saliva markers"

Thankyou for allowing us to resubmit the above manuscript to your journal. We have responded to the reviewers comments (see below), with changes in the manuscript highlighted in red text. We hope you feel that these changes have improved the manuscript.

Please don't hesitate to contact me if you require further information. I look forward to hearing from you.

Kind regards,

Prof. Neil Walsh.

Response to reviewers:

Reviewer #1:

Many thanks for your very insightful and constructive comments. Below are our responses to your comments, and changes within the manuscript are highlighted in red text. We hope you agree that these small changes have improved the manuscript.

1) Only 42% of screened individuals entered the study, and 31% had sufficient parameters to be analyzed. Although you show numbers in Figure 1, please comment on the large number of excluded subjects. Doesn't this affect the usefulness of the screening in clinical practice?

Thanks. As you have noted, of those that were screened (n =420), a large number of participants were excluded (58%). However, it should be noted that these were excluded due to ethical reasons of conducting the research as stipulated to us by the ethics committee (e.g. patients unable to provide consent (incapacity) for the research study (n=98), or that the research should not interfere with routine care of the patient and in those who had already began treatment (n = 88)), or because participants declined to take part (n = 54). We have now included this information at the start of the results sections, along with percentages of those excluded (lines 197-199). In terms of the application of the usefulness in clinical practice, the reasons outlined above do not preclude the usefulness of the measures in the current study being used in clinical practice (i.e. in all patients admitted to hospital).

In light of this being a proof of concept study for saliva indices, we did in this instance exclude participants who had potential confounding effects on saliva (e.g. oral trauma, recent dental surgery, swallowing problems etc), although it should be noted that only 2 participants were excluded for this (both had swallowing problems), and in light of your excellent point, we have now added this information to the results (lines 198-199,) and Figure 1, and have also now acknowledged in the

discussion that future studies should investigate whether saliva indices have utility, in patients with oral related problems (please see lines 341-344). Thanks.

2) "and allowing for an approximate one-third exclusion rate from data analysis (due to missing reference tests, and co-morbidities that preclude the use of the reference standards), a total of 178 participants were recruited into the study." It appears that the exclusion rate was higher than anticipated? Please comment.

Please note that the allowance for the one third exclusion rate (for missing reference tests and co-morbidities that affected the reference standards), was for those who might be excluded from the data analysis after they were already recruited into the study (i.e. n = 178 recruited). The N for which we analyzed data was n = 130, with 48 excluded from the data analysis. The proportion excluded from analysis of those recruited (48/178, 27%) is therefore actually lower, not higher than the anticipated 1/3 exclusion rate. Thanks.

3) "participants with a history of renal disease (n = 24), or who were in cardiac failure (n = 1) were excluded from data analysis." Please specify the criteria for renal disease and cardiac failure. What level of renal disease (stage?) or creatinine or other. For CHF, only "history" or other criteria? As you point out, the presence of renal disease, starvation, malnutrition (among others) limit the usefulness of the BUN/Cr ratio. It would be useful to discuss the level of renal disease that you excluded.

Thanks. For this study, we excluded from data analysis, all participants who had any known previous history of renal disease (CKD stage 1-5) or if they were in cardiac failure as diagnosed by the clinician. We have now clarified this and added this information to the methods section (Line 165). In line with comment 1 above, we have also now added a sentence to the discussion where we discuss how future studies should investigate the utility of these indices in these relatively small populations (lines 318-320). Thanks.

4) Please discuss relationship between saliva and blood osmolality. If the values are generally highly correlated, is there any benefit in using saliva rather than blood. Is it quicker, cheaper, easier to use saliva. Given a paucity of saliva in 25% of subjects, should blood be favored?

This is a very helpful observation and the changes we have made (described below) in response have improved the take home message of the manuscript. Many thanks.

As we have addressed in the manuscript (lines 55-61, 169-171 and in Figure 1), plasma osmolality is elevated in, and will only detect water-loss dehydration. In water-and-solute loss dehydration, plasma osmolality is either normal or low, and thus has no diagnostic utility for this type of dehydration. Given the differential response of plasma osmolality to these two types of dehydration, we feel it would be inappropriate to report, or rely on the correlation between saliva and plasma osmolality to determine saliva's utility as a diagnostic method. In the current study, saliva osmolality was able to detect a proportion of patients with water-and-solute dehydration (sensitivity 78%), and is an easier to perform and non-invasive so has advantages over blood sampling.

With this in mind, based on your excellent point , as this limitation of plasma (blood) osmolality for detecting water-and-solute-loss only dehydration was not as prominent as it should be in the manuscript, we have now added a sentence to the end of the abstract (lines 29-31) and to the discussion and conclusion where we address this (lines 312-314, 351-352).

We were able to collect a quantity of saliva in 126/130 patients (97%)- reported on lines 210 and 328, although as we have stated (lines 213, 327) we only had adequate saliva (at least 25ul) to assess osmolality using our osmometer in 75% of samples. However, we have addressed this limitation in the discussion, (line 327-333) where we say that micro osmometers are in development that can assess

osmolality on nano-gram quantities. We hope you feel that this is adequately addressed in the manuscript. Thanks.

Reviewer #2:
Many thanks for reviewing our manuscript.

*Title Page (Containing author details)

Is this elderly patient dehydrated? Diagnostic accuracy of hydration assessment using physical signs, urine and saliva markers

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Keywords: Dehydration, diagnosis, older, hypovolemia, osmolality, clinical

Running header: Dehydration assessment of older adults.

Is this elderly patient dehydrated? Diagnostic accuracy of hydration assessment using physical signs, urine and saliva markers

ABSTRACT

Objectives: Dehydration in older adults contributes to increased morbidity and mortality during hospitalization. As such, early diagnosis of dehydration may improve patient outcome and reduce the burden on healthcare. This prospective study investigated the diagnostic accuracy of routinely used physical signs, and non-invasive markers of hydration in urine and saliva. **Design:** Prospective diagnostic accuracy study. **Setting:** Hospital acute medical care unit and emergency department. **Participants**: One hundred and thirty older adults (59 males, 71 females, mean (SD) age = 78 (9) y). **Measurements:** Participants with any primary diagnosis underwent a hydration assessment within 30min of admittance to hospital. Hydration assessment comprised seven physical signs of dehydration (tachycardia (>100bpm), low systolic blood pressure (<100mmHg), dry mucous membrane, dry axilla, poor skin turgor, sunken eyes, and long capillary refill time (>2s)), urine color, urine specific gravity (USG), saliva flow rate (SFR) and saliva osmolality. Plasma osmolality (Posm) and the blood urea nitrogen to creatinine ratio (BUN:Cr) were assessed as reference standards of hydration, with 21% of participants classified with water-loss dehydration (Posm >295mOsm/kg), 19% classified with water-and-solute-loss dehydration (BUN:Cr >20) and 60% classified as euhydrated. **Results:** All physical signs showed poor sensitivity (0-44%) for detecting either form of dehydration, with only low systolic blood pressure demonstrating potential utility for aiding the diagnosis of water-and-solute-loss dehydration (diagnostic OR = 14.7). Neither urine color, USG, nor SFR could discriminate hydration status (area under the receiver operating characteristic curve, AUC_{ROC} = 0.49-0.57, P>0.05). In contrast, saliva osmolality demonstrated moderate diagnostic accuracy (AUC_{ROC} = 0.76, P<0.001) to distinguish both dehydration types (70% sensitivity, 68% specificity, OR =5.0 (95%CI 1.7-15.1) for water-loss dehydration, and 78% sensitivity, 72% specificity, OR =8.9 (95%CI 2.5-30.7) for water-and-solute-loss dehydration). **Conclusions:** With the exception of low systolic blood pressure, which could aid in the specific diagnosis of water-and-solute-loss dehydration, physical signs and urine markers show little utility to determine if an elderly patient is dehydrated. Saliva osmolality demonstrated superior diagnostic accuracy compared with physical signs and urine markers, and may have utility for the assessment of both water-loss and water-and-solute-loss dehydration in older individuals. It is particularly noteworthy that saliva

osmolality was able to detect water-and-solute-loss dehydration, for which a measurement of plasma osmolality would have no diagnostic utility.

INTRODUCTION

Dehydration in older adults is a significant clinical problem. A diagnosis of dehydration is associated with the presence of co-morbidities, longer hospital stay, additional future hospitalization and higher mortality rates [1-5]. The point-prevalence of dehydration in community-dwelling older adults in the USA was reported as 17-28% [6;7]. In many cases, simple and inexpensive oral rehydration is sufficient to treat dehydration and halt the progress of more serious fluid-deficit related illnesses such as acute kidney injury. However, upon hospitalization, many patients may be denied the correct course of treatment due to physician misdiagnosis of dehydration [7]. Therefore, accurate and early identification of dehydration in older adults admitted to hospital is vital to alleviate ill-health and the significant economic burden of treating dehydration on healthcare [1;2].

No single 'gold-standard' marker of hydration status exists [8], although blood biochemistry including plasma osmolality, electrolytes and blood urea nitrogen to creatinine ratio (BUN:Cr) represent criterion methods of identifying dehydration in a clinical setting [9-12]. However, blood sample collection is invasive and laboratory analysis is time-consuming, often delaying the course of treatment by hours. To aid an initial diagnosis of dehydration before requesting blood biochemistry confirmation, clinicians may use a variety of simple screening measures, albeit in a non-systematic way, that may include; presenting signs and symptoms of dehydration [11;13;14], patient history [13], orthostatic blood pressure change [15], and/or urinary parameters [16]. Nevertheless, these screening methods are often characterized by poor diagnostic performance [11;17-21]. To confound hydration assessment further, the term 'dehydration' is poorly defined and is used to characterize many water and solute deficits relating to whole body fluid deficits [7]. In order to simplify clinical practice researchers have suggested the classification of clinical dehydration into two distinct types. Firstly, water-loss dehydration (also termed hypertonic hypovolemia, or intracellular dehydration), which is hypertonic in nature and occurs when water loss proportionally exceeds solute loss. Water loss dehydration is typically defined as a plasma osmolality ≥295mOsm/kg [12;22]. Secondly, water-and-solute-loss dehydration (also

termed intravascular volume depletion or extracellular dehydration), which may be isotonic or hypotonic due to equal, or greater proportional loss of solutes than water [10;12;23], and typically defined as a BUN:Cr ≥20 in the absence of hypertonicity [22]. To the best of our knowledge, there are few [18;19], rigorous studies that have investigated the diagnostic accuracy of clinical physical signs and/or urine indices to detect dehydration in hospitalized older adults using a criterion reference method, and none which have simultaneously assessed the utility of any hydration marker to assess both types of dehydration.

In a series of studies (in young healthy adults) we have shown that rapid measurements made from non-invasive collection of saliva fluid can be used to identify water-loss dehydration [24-26]. For example, decreases in whole saliva flow rate and increases in whole saliva osmolality were shown to track progressive modest dehydration (equivalent to 1-3% body mass loss). The utility of these novel saliva markers of dehydration has not yet been examined in a clinical, older adult population, although encouragingly, the presence of a dry tongue was identified as the clinical sign most strongly associated with dehydration in an elderly cohort [14]. To this end, the purpose of this prospective study was to determine, and compare, the diagnostic accuracy of clinical physical signs routinely used in hospital settings [11;13;14], along with saliva (flow rate and osmolality) and urine indices (color and specific gravity) [27], to detect static (one-point in time) water-loss, and water-and-solute-loss dehydration in a hospitalized, older adult cohort using primary reference standards; plasma osmolality and BUN:Cr [10;12;22;28].

METHODS

Experimental design and procedures

The study was conducted as a prospective, hospital-based cross-sectional study. All measures of hydration status were performed within 30 minutes of admission, with no disruption to routine care in the following order; examination of physical signs of dehydration, collection of saliva, blood and urine. For the reference standards of whole body hydration assessment, a blood sample was collected by the clinical research fellow or a specialist phlebotomist and analyzed for plasma osmolality (within 15min) and BUN:Cr (within 2h). For consistency, all physical examinations and assessment of confidential medical information was carried out by the same clinical research fellow (a junior doctor with five years clinical experience), who was

blinded to the results of the reference standards and the saliva and urine index test results when conducting the physical examination. Saliva and urine samples were collected, and analyzed by an independent research assistant who had been trained in the handling and assessment of saliva and urine samples by a postdoctoral researcher, and who was blinded to the physical examination results. All osmolality analyses were made by a trained research assistant. Details of the patients' medical condition, history and medication were recorded retrospectively after the reference and index test results had been established.

Participants

A convenience sample of adults over 60 years of age admitted consecutively to the acute medical care unit or emergency department of Gwynedd Hospital, Bangor, UK, with any primary diagnosis and capacity to consent were enrolled between May and November 2011 during the times the investigators were available (09:00h – 17:00h, Monday-Friday). Participant exclusion criteria included: oral trauma or dental surgery within 14 days, swallowing problems, salivary gland tumors, if they were deemed too unwell by the medical staff to participate in the study, if they were assessed as not having capacity to consent, or if they had already begun any form of medical treatment or rehydration therapy (oral or intravenous). Participant flow through the study is depicted in **Figure 1.** All participants recruited provided fully informed written consent, and the study adhered to the Declaration of Helsinki and was approved by the North West Wales Research Ethics Committee (Ref: 11/WA/0023).

Assessment of hydration status

Reference standards

Blood sample collection and analysis

Blood samples were collected from an antecubital or dorsal metacarpal vein without venestasis into one serum separation vacutainer, and one lithium heparin coated vacutainer (Becton Dickinson, Oxford, UK). Serum blood urea nitrogen and serum creatinine were assessed at the hospital clinical biochemistry department using an automated biochemistry analyzer (Olympus AU 2700 chemistry immuno analyzer, Beckman Coulter, USA). The lithium heparin treated blood was centrifuged immediately upon collection at 1500 *g* for 10 minutes at 4 °C. The plasma was aspirated and triplicate measurements of osmolality were

made immediately using a freezing point depression osmometer (Model 330 MO, Advanced Instruments, Massachusetts, USA). Standard control solutions (290 mOsm/kg) were run through the osmometer and checked daily to ensure acceptable limits of precision (±2 mOsm/kg). The analytical coefficient of variation for repeated sample plasma osmolality measurements was 0.7% (1.9 mOsm/kg).

Index tests

Clinical assessment of physical signs of dehydration

The clinical assessment consisted of seven physical signs of dehydration that are routinely used in Gwynedd Hospital; tachycardia (resting heart rate >100 beats per minute), low resting systolic blood pressure (<100mmHg), dry mucous membrane (inside of the cheek, dry *v*s. wet), axillary dryness (assessed by palpating the armpit, dry *v*s. moist), poor skin turgor (measured by pinching the skin on the dorsum of the hand and observing if the tissue fold returned to normal immediately), presence of sunken eyes as assessed by the clinical research fellow, and long capillary refill time (> 2s, assessed by holding the patients hand at heart level and blanching the participant's right index finger using moderate pressure and assessing the length of time for the return of normal color). Each physical sign was assessed with the participant rested and seated upright and assessed dichotomously.

Saliva sample collection and analysis

Unstimulated whole saliva samples were collected using a pre-weighed Versi-sal® collection device (Oasis Technology, USA) as previously described [29]. Participants firstly swallowed in order to empty the mouth of residual saliva, before saliva was collected by placing the Versi-sal® collection device under the tongue. Saliva collection was performed with minimal orofacial movements and accurately timed. After 4 min, the collection device was inspected for volume of saliva by weighing it immediately (to the nearest milligram) and subtracting the pre-weight. If the volume was insufficient for osmolality analysis (< 25µl), the swab was replaced under the tongue for a further 4 min. By assuming the density of saliva to be 1.00g/ml, saliva flow rate (SFR) was calculated by dividing the volume collected by the time of collection [24]. Saliva was recovered from the collection device by centrifugation at 1500 *g* for 10 min, and assessed immediately in duplicate for saliva osmolality using a freezing point depression osmometer (Model 330 MO, Advanced

Instruments, Massachusetts, USA). The analytical coefficient of variation for repeated sample saliva osmolality measurements was 0.8% (0.9 mOsm/kg).

Urine sample collection and analysis

A mid-flow urine sample was collected and immediately analyzed for urine color [27] and urine specific gravity (USG) using a handheld refractometer (Atago URC-Osmo refractometer, Japan).

Sample size calculation and data analysis

The desired sample size for dehydrated participants (n = 20 water-loss only) was calculated using the following equation:

$$n \geq \frac{(1.96)^2\, p(1 - p)}{x^2}$$

Where p = desired sensitivity (70%) as a proportion, and x = desired confidence interval (20%) as a proportion [30]. Assuming a prevalence of impending water-loss dehydration (plasma osmolality ≥ 295mOsm/kg) of 17% [7], and allowing for an approximate one-third exclusion rate from data analysis (due to missing reference tests, and co-morbidities that preclude the use of the reference standards), a total of 178 participants were recruited into the study. Medical records for participants were accessed after enrolment, and due to potential influencing effects on the reference standards assessed in this study, participants with a history of renal disease (CKD stage 1-5, n = 24), or who were in cardiac failure as diagnosed by a clinician (n = 1) were excluded from data analysis. Participants were also excluded from data analysis if the reference tests were not available (n = 11), if they had an abnormally low (<10) BUN:Cr which may be indicative of renal disease or the syndrome of inappropriate antidiuretic hormone (n = 8), or if they were taking glucocorticoid medication (n = 4) which affects the validity of the BUN:Cr [10]. Based on the reference standards, participants with a presenting plasma osmolality ≥295mOsm/kg were classified as having impending water-loss dehydration [12;22]. Of the remaining participants, those with a BUN:Cr ≥20 in the absence of hypertonicity [22] were classified as having water-and-solute-loss dehydration, and the remaining participants formed the euhydrated control group (normal plasma osmolality and BUN:Cr).

To assess the diagnostic accuracy of saliva and urine indices, and clinical physical signs for assessment of hydration status, both water-loss, and water-and-solute-loss dehydration groups were separately compared with the euhydrated control group. Both dehydration groups were also combined to form a generic dehydration group for comparison with euhydration. For all dichotomized clinical physical sign data, the following were calculated; area under the receiver operating characteristic curve (AUC_{ROC}) as a measure of global diagnostic accuracy, sensitivity, specificity, positive and negative likelihood ratios (LR), and the diagnostic odds ratio (OR) generated by logistical regression. For continuous variable data (urine color, USG, SFR and saliva osmolality), the degree to which each variable could discriminate between dehydration and euhydration was assessed using AUC_{ROC}. For variables that could distinguish hydration status, the single cut-off value that provided the optimal discrimination was identified as the point on the curve with the largest vertical displacement from the reference line, and sensitivity, specificity, overall diagnostic accuracy, positive and negative LR, and the diagnostic OR were calculated. For all diagnostic analyses 95% confidence intervals were constructed. To compare AUC_{ROC}, a method was adopted that accounts for the correlation between samples from the same individual [31]. Group data were analyzed using one-way ANOVA. Data were analyzed using Microsoft excel (Microsoft, USA), SigmaPlot version 12.0 (Systat Software, Inc. USA) and SPSS version 20 (IBM Corporation, USA) software. Significance was accepted as $P < 0.05$ for all ANOVA, logistic regression and AUC_{ROC} analyses.

*****Insert Figure 1 about here*****

RESULTS

Participant characteristics

A total of 420 participants were screened for inclusion, with 242 excluded, largely due to ethical considerations of conducting the research, or declining to take part (n = 240, 57%), or due to swallowing problems (n = 2, 1%). Therefore, 178 participants were enrolled into the study (n = 85 males, n = 93 females) with mean age (SD) 78 (9) y. After further exclusions for data analysis, data were analyzed for n = 130 participants (n = 59 males, n = 71 females; mean age 78 (9), range 60-101y), of which n = 27 (21%) were classified as water-loss dehydrated, n = 25 (19%) were classified as water-and-solute-loss dehydrated, and n = 78 (60%) were classified as euhydrated. Of the 27 participants in the water-loss only dehydration

group, 10 also had an elevated BUN:Cr (≥20). There were no differences between the groups for age (**Table 1**). By design, participants with water-loss dehydration had elevated plasma osmolality, and participants with water-and-solute-loss dehydration had elevated BUN:Cr compared with euhydrated control (**Table 1**).

Insert Table 1 about here

Feasibility of collecting index tests

All clinical physical sign assessments were conducted in all 130 participants. Saliva was collected in all but four participants (1 water-loss dehydrated, 2 water-and-solute-loss dehydrated, and 1 euhydrated control). For these four participants SFR was recorded as zero, and SFR data was therefore analyzed for $n = 130$. There was adequate saliva (> 25µl) to assess saliva osmolality in 98 participants (75%). In comparison urine samples could not be collected in 45 participants, who were unable to urinate within 30 min of the blood collection. One participant provided a urine sample containing blood, confounding interpretation. Urine color and specific gravity were therefore analyzed in 84 participants (65%).

Diagnostic accuracy of clinical physical signs

Diagnostic data for all seven clinical physical signs for both types of dehydration are shown in **Table 2** and **Figure 2**. No clinical physical sign in isolation could discriminate between euhydration and either form of dehydration (AUC_{ROC} range 0.44-0.57). Individually, all clinical physical signs performed poorly in terms of detecting dehydration with sensitivity ranging from 0–44%. They did however generally perform better at detecting euhydration, with specificity ranging from 60-99%. For detecting water-and-solute-loss dehydration, a low resting systolic blood pressure (<100mmHg) demonstrated high diagnostic odds and likelihood ratios (14.7 (95% CI 1.6-138.3) and 12.5 (95%CI 1.5-107 respectively)), suggesting potential utility in aiding the diagnosis of this specific type of dehydration.

Insert Table 2 about here

Diagnostic accuracy of urine and saliva indices

There were no differences between any of the three groups for urine color, USG or SFR (**Table 1**). Furthermore, when assessed using ROC analyses, neither urine color, USG or SFR were able to discriminate between dehydration and euhydration (AUC_{ROC} range 0.49-0.57, all $P > 0.05$, **Table 3**). Saliva osmolality

was greater in participants with both forms of dehydration than euhydrated control ($P < 0.001$, **Table 1**), but more importantly, was able to distinguish both types of dehydration separately from euhydration (AUC_{ROC} = 0.76, $P < 0.01$ for both types of dehydration individually and combined, **Table 3**). Based on the ROC analysis, the saliva osmolality cut-off that provided the optimum balance between sensitivity and specificity was calculated as: 95, 97, and 94 mOsm/kg for water-loss only, water-and-solute-loss only, and both forms of dehydration combined, respectively. The diagnostic accuracy of saliva osmolality to detect all dehydration types is displayed in **Table 4**. Saliva osmolality was able to identify water-loss dehydration, water-and-solute-loss dehydration, and both forms of dehydration combined with a sensitivity of 70, 78 and 76%, and specificity of 68, 72, and 68%, respectively. Importantly, when AUC_{ROC} curves were compared, the ability of saliva osmolality to discriminate hydration status was superior ($P < 0.05$) to all clinical physical signs and urine indices for both types of dehydration in older adults (**Figure 2**).

*****Insert Table 3 and Table 4 about here*** ***Insert Figure 2 about here*****

DISCUSSION

Dehydration in older adults is a leading cause of hospitalizations, contributing to increased morbidity and mortality during clinical care, and poorer functional status of the individual [1-5;32]. As such, early identification of hydration status is paramount to prevent the development of further co-morbidities, and to reduce the burden on healthcare [1;2]. This prospective study sought to investigate the diagnostic accuracy of routinely used clinical physical signs and urine indices, and novel, simple, non-invasive saliva indices. The main finding was that currently used clinical physical signs were not able to discriminate between dehydration and euhydration, and thus provide little help to the physician making an initial hydration assessment. The exception was a low systolic blood pressure which could aid in the specific diagnosis of water-and-solute-loss dehydration. Whilst showing promise in young healthy cohorts [27], urine analysis demonstrated no utility in identifying dehydration in an older adult cohort admitted to hospital. However, the novel finding from the study was that saliva osmolality could discriminate between dehydration and euhydration, and importantly, was sensitive to both water-loss, and water-and-solute-loss forms of dehydration, demonstrating superior diagnostic accuracy than urinary parameters and currently used clinical physical signs. Saliva collection is non-invasive, and easy to collect, and therefore, may have practical utility as an initial screening method for impending dehydration in older adults.

Despite a relative paucity of clear supporting evidence, clinicians may rely on an array of simple physical screening tests to aid the hydration assessment of patients admitted to hospital. Whilst showing some clinical promise in young children [33;34], clinical physical signs often demonstrate poor diagnostic performance when applied to older adults, likely due to; a loss of skin elasticity with advancing age affecting skin turgor, smoking and cold environmental temperatures causing peripheral vasoconstriction which may result in false positives for capillary refill time, and anticholinergic medications and a reliance on mouth breathing in the elderly which can result in a dry oral mucosa [11;17;35]. Findings from previous studies investigating the utility of clinical physical signs should also be viewed with caution where they have adopted a non-criterion reference standard, e.g. difference in weight gain after rehydration [13], urinary measures [16], or relied on a clinicians overall diagnosis [14] as opposed to a more, objective biochemical criterion measure such as plasma osmolality [7;10-12;17;22;36]. Furthermore, previous studies have been limited by failing to characterize the diagnostic accuracy of clinical physical signs in assessing both forms of dehydration commonly encountered in a clinical setting, i.e. water-loss, and water-and-solute-loss dehydration [10].

A particular strength of the current study was that both forms of dehydration were characterized simultaneously using valid biochemical assessments as reference standards, including the preferred direct measurement of plasma osmolality as opposed to calculated osmolality [12;37]. We observed that no clinical physical sign could discriminate between either type of dehydration and euhydration when assessed using AUC_{ROC}, and thus, should not be used in isolation to diagnose hydration status in older adults admitted to hospital. However, although not sensitive (16%), a low (<100mmHg) sitting systolic blood pressure, may aid the physician in making a diagnosis of water-and-solute-loss dehydration owing to its very high specificity (i.e. low false positive rate), high diagnostic odds ratio (OR =14.7), and high positive likelihood ratio (OR =12.5). This finding is in line with the well-known effects of a loss of extracellular fluid (intravascular volume depletion) on blood pressure responses. Although researchers have previously focused on orthostatic blood pressure responses to assess hydration [13;15], altering posture may be impractical in a clinical setting, particularly in bed-ridden patients. Therefore, a sitting blood pressure assessment may have practical value for the clinician.

Urinary markers have been reported as valid methods to assess acute changes in hydration status in young healthy people [27]. In the current study, neither USG nor urine color were able to discriminate between dehydration and euhydration. This is likely due in part, to the decreased renal function that is characteristic of older age, and to a potential confounding effect on urine of the many types of medications that an older adult cohort are likely to be prescribed. In support, previous studies have also shown that urine indices are poor markers of hydration status in elderly patients [13;19], in critically ill patients [20] and in young children with gastroenteritis [38]. Urine collection is not always possible when required, and was only able to be collected in 65% of participants in the current study, and in only 79% of elderly patients in a recent clinical study [13]. Taken together, we do not recommend the use of USG or urine color as screening tools for dehydration in older adults.

To the best of our knowledge, this is the first study that has investigated the diagnostic accuracy of saliva indices to assess dehydration in older adults admitted to hospital. Saliva sample collection is simple and non-invasive and has previously been shown to track modest water-loss dehydration in young healthy males [24-26]. Saliva flow rate was not associated with either form of dehydration, but the novel finding of the current study was that saliva osmolality was able to detect both forms of dehydration with sensitivity >70% and diagnostic OR >5. Although a sensitivity to detect dehydration of 72-78% may only be described as "fair to moderate" [39], it is important to stress that any novel diagnostic marker should be compared against what is currently used in clinical practice, and in the case of the present study, a high saliva osmolality (>94mOsm/kg) was able to detect more cases of both types of dehydration than any single clinical physical sign or urinary marker without compromising specificity (**Figure 2**). It is also worth re-iterating that saliva osmolality was able to detect water-and-solute-loss dehydration, for which a measurement of plasma osmolality would have no diagnostic utility. Furthermore, the cohort in the current study reflects a representative, older adult clinical population, admitted with any primary diagnosis, and we did not remove participants taking medications (except for 4 patients taking glucocorticoid medications). Thus, the fact that a single marker is able to achieve a sensitivity > 70% for both types of dehydration at one-point in time regardless of medication is promising. It remains unknown whether saliva osmolality can also identify both types of dehydration in the relatively small proportion of patients in this study taking glucocorticoid

medication, in patients with heart failure, and in those with various stages of kidney disease. Finally, since we set our reference standard cut-off at the lower end of the dehydration continuum to reflect impending, or pre-clinical dehydration [10;12;22], the measurement of saliva osmolality may have practical utility in identifying those individuals with modest dehydration, so that further biochemistry analysis can confirm the presence of, and type of dehydration, in order that specific, tailored rehydration is commenced to prevent the patient developing more severe dehydration along with its associated co-morbidities and poorer outcome.

There are a few limitations of saliva that we must acknowledge. Firstly, in the current study, the requirement of 25 µl of saliva sample for analysis meant that only 75% of the samples could be analyzed (although a measurable quantity of saliva was collected from 97% of participants compared with only 65% of participants able to provide a urine sample). However, point of care devices that utilize nano-technology for the assessment of saliva osmolality are under development [40;41]. For example, the osmolarity of tears can now be assessed using the principle of impedance on as little as 50nl [42;43]. Thus, this limitation should not be seen to detract from the future application of saliva osmolality to assess hydration status in clinical care. Secondly, with saliva sampling in a clinical population, there may be a potential confounding effect of anything which can affect saliva flow rate, e.g. anticholinergic medications, or recent food/fluid consumption [44;45]. This is potentially important since a decrease in saliva flow explained in part, the increase in saliva osmolality observed during acute dehydration in young healthy males [24-26]. However, we observed only a small association between saliva flow rate and osmolality (r = -0.40), suggesting that in the current study, saliva osmolality was largely independent of saliva flow rate. The physiological mechanisms responsible for an increase in saliva osmolality during dehydration are unclear, but may be due to an increase in water absorption in the saliva gland and/or neural factors [24-26]. Finally, although we excluded only 2 participants with swallowing problems, further research should investigate the diagnostic utility of saliva indices in patients with this, and other oral-related problems (e.g. oral trauma, recent dental surgery, salivary gland tumors etc).

CONCLUSIONS

In conclusion, with the exception of low systolic blood pressure, which could aid in the specific diagnosis of water-and-solute-loss dehydration, physical signs and urine markers show little utility to determine if an

elderly patient is dehydrated. Saliva osmolality demonstrated superior diagnostic accuracy compared with physical signs and urine markers for the assessment of both water-loss and water-and-solute-loss dehydration. It is particularly noteworthy that saliva osmolality was able to detect water-and-solute-loss dehydration, for which a measurement of plasma osmolality would have no diagnostic utility. The measurement of saliva osmolality has potential utility as a screening method to aid the diagnosis of impending dehydration in older adults.

COMPETING INTERESTS DECLARATION:

The study was funded by HydraDx Inc, who were interested in identifying if saliva indices other than those presented herein had utility for hydration assessment. MBF and PRB were employed as research assistants by HydraDx on this study.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS:

Figure 1. Participant flow through the study. BUN, blood urea nitrogen.

Figure 2. ROC curve comparison between clinical physical signs, saliva and urine indices for the assessment of dehydration. Data are shown for both forms of dehydration combined (A), water-loss dehydration only (B) and water-and-solute-loss dehydration (C). The cut-off that provides the optimum discrimination between sensitivity (true positive rate) and specificity (false positive rate) is plotted. BP, low systolic blood pressure; SE, sunken eyes; CR, capillary refill time; Tc, Tachycardia; AD, axillary dryness, ST, skin turgor, DM, dry mucous membrane; Sosm, saliva osmolality; SF, saliva flow rate; UrC, urine color; USG, urine specific gravity. Vertical error lines represent sensitivity 95% CI, horizontal error lines represent specificity 95% CI.

Table 1

Table 1. Group data for age, blood reference tests and urine and saliva index tests of hydration.					
		Water-loss only dehydrated (*n* = 27)	**Water-and-solute-loss only dehydrated (*n* = 25)**	**Euhydrated controls (*n* = 78)**	***P* value (one-way ANOVA)**
	Age (Yr)	78.3 (9.6)	80.1 (9.6)	76.3 (7.7)	0.14
Reference tests	Plasma osmolality (mOsm/kg)	299 (6)†	283 (6)	283 (9)	<0.001
	BUN:Cr	18.8 (5.5)	24.3 (4.7)‡	15.7 (2.6)	<0.001
Index tests	Urine specific gravity	1.017 (0.006)	1.016 (0.007)	1.016 (0.006)	0.77
	Urine color	4.1 (1.6)	3.9 (1.8)	3.9 (1.7)	0.87
	Saliva flow rate (µl/min)	56 (55)	86 (183)	77 (90)	0.57
	Saliva osmolality (mOsm/kg)	136 (58)*	140 (66)*	92 (45)	<0.001
Values represent mean (standard deviation). BUN:Cr; blood urea nitrogen to creatinine ratio.					
† Significantly greater than water-and-solute-loss only dehydrated and euhydrated control groups ($P < 0.001$).					
‡ Significantly greater than water-loss only dehydrated and euhydrated control groups ($P < 0.001$).					
* Significantly greater than euhydrated control group ($P < 0.01$).					

Table 2

Table 2. Diagnostic accuracy of clinical signs to determine both forms of dehydration in combination, and separately (water-loss only, and water-and-solute loss dehydration) in older adults >60yr. Values in parentheses represent 95% Confidence intervals.

	All dehydration					**Water-loss only dehydration**					**Water-and-solute-loss only dehydration**			
Clinical assessment	**AUC_{ROC}**	**Positive LR**	**Negative LR**	**Diagnostic OR**		**AUC_{ROC}**	**Positive LR**	**Negative LR**	**Diagnostic OR**		**AUC_{ROC}**	**Positive LR**	**Negative LR**	**Diagnostic OR**
Low systolic BP (< 100 mmHg)	0.53	6.0	0.9	6.4		0.49	N/A	1	N/A		0.57	**12.5**	**0.9**	**14.7***
	(0.43-0.64)	(0.7-54.2)	(0.8-1.0)	(0.7-59.1)		(0.37-0.62)	N/A	(1)	N/A		(0.44-0.71)	(1.5-107.6)	(0.7-1.0)	(1.6-138.3)
Tachycardia (HR > 100 bpm)	0.50	1.0	1.0	1.0		0.44	0.5	1.2	0.4		0.56	1.6	0.8	1.9
	(0.40-0.60)	(0.5-1.9)	(0.8-1.2)	(0.4-2.3)		(0.32-0.56)	(0.2-1.5)	(1.0-1.4)	(0.1-1.5)		(0.43-0.70)	(0.8-3.0)	(0.6-1.1)	(0.7-5.0)
Dry mucous membrane	0.51	1.1	1.0	1.1		0.51	1.0	1.0	1.0		0.52	1.1	0.9	1.2
	(0.41-0.62)	(0.7-1.6)	(0.7-1.3)	(0.5-2.3)		(0.38-0.63)	(0.6-1.7)	(0.7-1.4)	(0.4-2.5)		(0.39-0.65)	(0.7-1.9)	(0.6-1.4)	(0.5-3.0)
Axillary dryness	0.54	1.3	0.9	1.4		0.53	1.2	0.9	1.3		0.56	1.4	0.8	1.7
	(0.44-0.64)	(0.8-2.0)	(0.7-1.2)	(0.7-3.0)		(0.40-0.65)	(0.6-2.1)	(0.7-1.3)	(0.5-3.1)		(0.43-0.70)	(0.8-2.4)	(0.6-1.2)	(0.7-4.2)
Poor skin turgor	0.55	1.3	0.9	1.5		0.53	1.2	0.9	1.3		0.57	1.4	0.8	1.7
	(0.45-0.65)	(0.8-2.0)	(0.6-1.1)	(0.7-3.1)		(0.40-0.66)	(0.7-2.0)	(0.6-1.3)	(0.5-3.2)		(0.44-0.70)	(0.8-2.3)	(0.5-1.2)	(0.7-4.3)
Sunken eyes	0.51	1.2	1.0	1.2		0.43	0.6	1.1	0.5		0.56	1.9	0.9	2.2
	(0.41-0.62)	(0.5-2.8)	(0.8-1.1)	(0.5-3.4)		(0.35-0.60)	(0.1-2.5)	(0.9-1.2)	(0.1-2.7)		(0.42-0.69)	(0.8-4.6)	(0.7-1.1)	(0.7-6.7)
Capillary refill > 2 S	0.50	1.0	1.0	1.0		0.52	1.2	1.0	1.2		0.48	0.8	1.0	0.8
	(0.40-0.60)	(0.5-2.0)	(0.8-1.2)	(0.4-2.4)		(0.39-0.64)	(0.5-2.7)	(0.8-1.2)	(0.5-3.5)		(0.36-0.61)	(0.3-2.3)	(0.9-1.3)	(0.2-2.7)

AUR_{ROC}, area under the receiver operating characteristic curve; LR, likelihood ratio; OR, odds ratio; BP, blood pressure; HR, heart rate; ***** $P < 0.05$ significantly associated with hydration status by logistic regression analysis. N/A, not assessed as sensitivity was 0%.

Table 3

Table 3. Receiver operating characteristic (ROC) area under the curve (AUC) analysis for urine and saliva indices for the detection of dehydration in older adults (>60yr).

	ROC analysis	
	AUC	***P* value**
All dehydration		
USG	0.53	0.67
	(0.39-0.66)	
Urine color	0.52	0.79
	(0.39-0.65)	
Saliva flow rate	0.56	0.25
	(0.46-0.66)	
Saliva osmolality	**0.76**	**<0.001**
	(0.66-0.86)	
Water loss only dehydration		
USG	0.55	0.53
	(0.39-0.72)	
Urine color	0.54	0.61
	(0.38-0.70)	
Saliva flow rate	0.55	0.46
	(0.43-0.67)	
Saliva osmolality	**0.76**	**<0.001**
	(0.66-0.87)	
Water and solute loss dehydration		
USG	0.50	0.98
	(0.32-0.69)	
Urine color	0.49	0.91
	(0.31-0.67)	
Saliva flow rate	0.57	0.28
	(0.44-0.71)	
Saliva osmolality	**0.76**	**0.001**
	(0.62-0.89)	

Values in parentheses represent 95% confidence intervals. USG, urine specific gravity.

Table 4

Table 4. Diagnostic accuracy of saliva osmolality to determine both forms of dehydration in combination, and separately (water-loss only, and water-and-solute loss dehydration) in older adults >60yr.

	Diagnostic accuracy	Positive LR	Negative LR	Diagnostic OR
Both forms of dehydration combined	71%	2.4	0.4	6.9
	(63-80%)	(1.6-3.6)	(0.2-0.6)	(2.8-17.5)
Water-loss dehydration	69%	2.2	0.4	5.0
	(59-79%)	(1.4-3.5)	(0.2-0.9)	(1.7-15.1)
Water-and-solute-loss dehydration	73%	2.8	0.3	8.9
	(63-83%)	(1.7-4.4)	(0.1-0.8)	(2.5-30.7)

Values in parentheses represent 95% Confidence intervals. LR, likelihood ratio; OR, odds ratio.

Figure 1

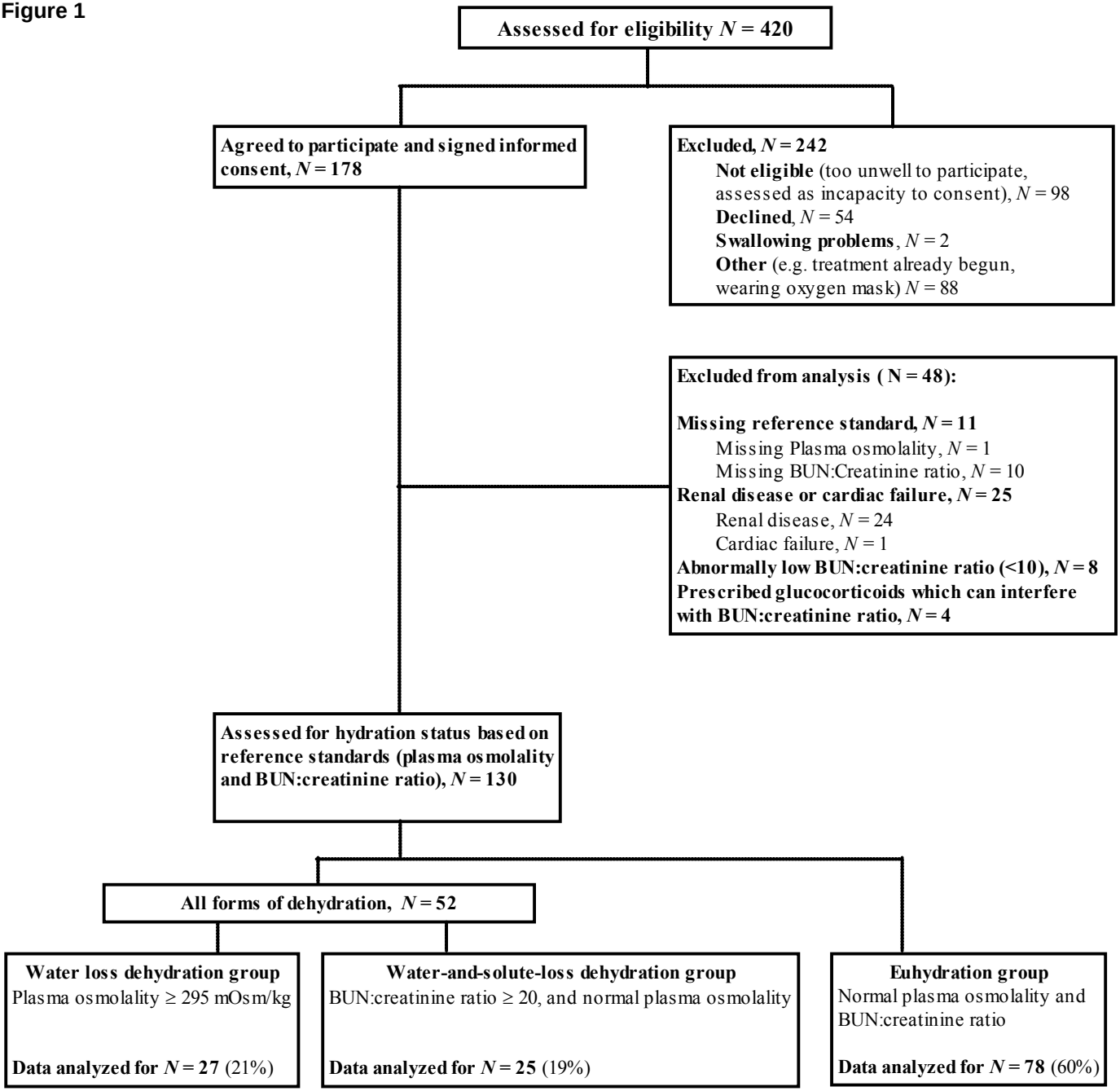
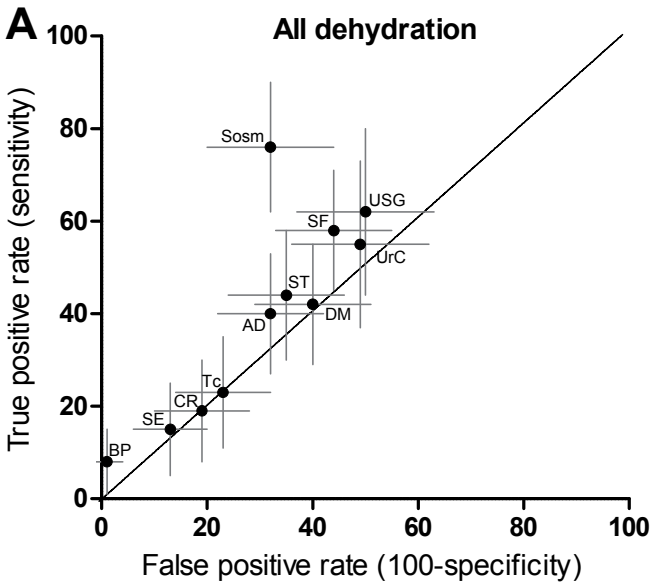
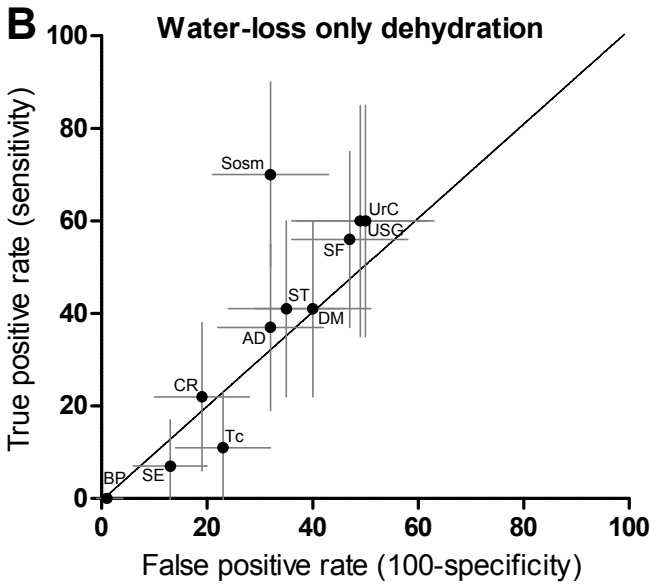

Assessed for eligibility N = 420
Agreed to participate and signed informed consent, N = 178
Excluded, N = 242
Not eligible (too unwell to participate, assessed as incapacity to consent), N = 98
Declined, N = 54
Swallowing problems, N = 2
Other (e.g. treatment already begun, wearing oxygen mask) N = 88
Excluded from analysis (N = 48):
Missing reference standard, N = 11
Missing Plasma osmolality, N = 1
Missing BUN:Creatinine ratio, N = 10
Renal disease or cardiac failure, N = 25
Renal disease, N = 24
Cardiac failure, N = 1
Abnormally low BUN:creatinine ratio (<10), N = 8
Prescribed glucocorticoids which can interfere with BUN:creatinine ratio, N = 4
Assessed for hydration status based on reference standards (plasma osmolality and BUN:creatinine ratio), N = 130
All forms of dehydration, N = 52
Water loss dehydration group
Plasma osmolality ≥ 295 mOsm/kg
Data analyzed for N = 27 (21%)
Water-and-solute-loss dehydration group
BUN:creatinine ratio ≥ 20, and normal plasma osmolality
Data analyzed for N = 25 (19%)
Euhydration group
Normal plasma osmolality and BUN:creatinine ratio
Data analyzed for N = 78 (60%)

Figure 2


A
All dehydration
True positive rate (sensitivity)
False positive rate (100-specificity)
0
20
40
60
80
100
Sosm
USG
SF
UrC
ST
DM
AD
Tc
CR
SE
BP


B
Water-loss only dehydration
True positive rate (sensitivity)
False positive rate (100-specificity)
0
20
40
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80
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Sosm
UrC
USG
SF
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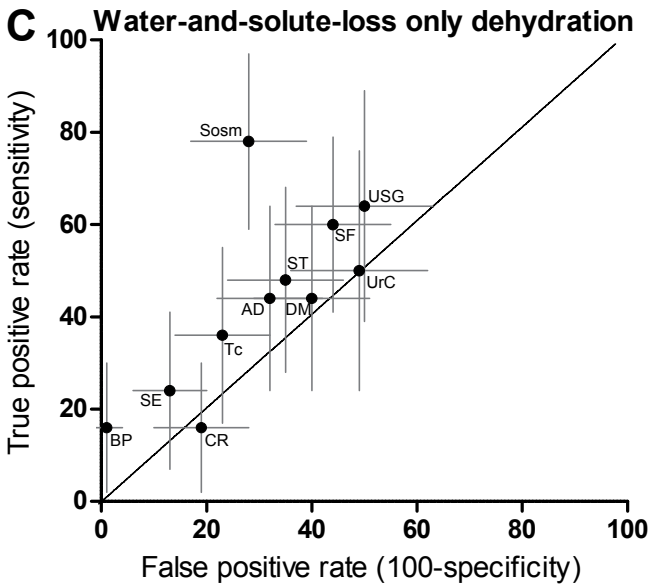
DM
AD
CR
Tc
SE
BP


C
Water-and-solute-loss only dehydration
True positive rate (sensitivity)
False positive rate (100-specificity)
0
20
40
60
80
100
Sosm
USG
SF
ST
UrC
AD
DM
Tc
SE
BP
CR

Manuscript # JAMDA-D-14-00269, "Is this elderly patient dehydrated? Diagnostic accuracy of hydration assessment using physical signs, urine and saliva markers" – Response to reviewers

Reviewer #1:

Many thanks for your very insightful and constructive comments. Below are our responses to your comments, and changes within the manuscript are highlighted in red text. We hope you agree that these small changes have improved the manuscript.

1) Only 42% of screened individuals entered the study, and 31% had sufficient parameters to be analyzed. Although you show numbers in Figure 1, please comment on the large number of excluded subjects. Doesn't this affect the usefulness of the screening in clinical practice?

Thanks. As you have noted, of those that were screened (n =420), a large number of participants were excluded (58%). However, it should be noted that these were excluded due to ethical reasons of conducting the research as stipulated to us by the ethics committee (e.g. patients unable to provide consent (incapacity) for the research study (n=98), or that the research should not interfere with routine care of the patient and in those who had already began treatment (n = 88)), or because participants declined to take part (n = 54). We have now included this information at the start of the results sections, along with percentages of those excluded (lines 197-199). In terms of the application of the usefulness in clinical practice, the reasons outlined above do not preclude the usefulness of the measures in the current study being used in clinical practice (i.e. in all patients admitted to hospital).

In light of this being a proof of concept study for saliva indices, we did in this instance exclude participants who had potential confounding effects on saliva (e.g. oral trauma, recent dental surgery, swallowing problems etc), although it should be noted that only 2 participants were excluded for this (both had swallowing problems), and in light of your excellent point, we have now added this information to the results (lines 198-199,) and Figure 1, and have also now acknowledged in the discussion that future studies should investigate whether saliva indices have utility, in patients with oral related problems (please see lines 341-344). Thanks.

2) "and allowing for an approximate one-third exclusion rate from data analysis (due to missing reference tests, and co-morbidities that preclude the use of the reference standards), a total of 178 participants were recruited into the study." It appears that the exclusion rate was higher than anticipated? Please comment.

Please note that the allowance for the one third exclusion rate (for missing reference tests and co-morbidities that affected the reference standards), was for those who might be excluded from the data analysis after they were already recruited into the study (i.e. n = 178 recruited). The N for which we analyzed data was n = 130, with 48 excluded from the data analysis. The proportion excluded from analysis of those recruited (48/178, 27%) is therefore actually lower, not higher than the anticipated 1/3 exclusion rate. Thanks.

3) "participants with a history of renal disease (n = 24), or who were in cardiac failure (n = 1) were excluded from data analysis." Please specify the criteria for renal disease and cardiac failure. What level of renal disease (stage?) or creatinine or other. For CHF, only "history"

or other criteria? As you point out, the presence of renal disease, starvation, malnutrition (among others) limit the usefulness of the BUN/Cr ratio. It would be useful to discuss the level of renal disease that you excluded.

Thanks. For this study, we excluded from data analysis, all participants who had any known previous history of renal disease (CKD stage 1-5) or if they were in cardiac failure as diagnosed by the clinician. We have now clarified this and added this information to the methods section (Line 165). In line with comment 1 above, we have also now added a sentence to the discussion where we discuss how future studies should investigate the utility of these indices in these relatively small populations (lines 318-320). Thanks.

4) Please discuss relationship between saliva and blood osmolality. If the values are generally highly correlated, is there any benefit in using saliva rather than blood. Is it quicker, cheaper, easier to use saliva. Given a paucity of saliva in 25% of subjects, should blood be favored?

This is a very helpful observation and the changes we have made (described below) in response have improved the take home message of the manuscript. Many thanks.

As we have addressed in the manuscript (lines 55-61, 169-171 and in Figure 1), plasma osmolality is elevated in, and will only detect water-loss dehydration. In water-and-solute loss dehydration, plasma osmolality is either normal or low, and thus has no diagnostic utility for this type of dehydration. Given the differential response of plasma osmolality to these two types of dehydration, we feel it would be inappropriate to report, or rely on the correlation between saliva and plasma osmolality to determine saliva's utility as a diagnostic method. In the current study, saliva osmolality was able to detect a proportion of patients with water-and-solute dehydration (sensitivity 78%), and is an easier to perform and non-invasive so has advantages over blood sampling.

With this in mind, based on your excellent point , as this limitation of plasma (blood) osmolality for detecting water-and-solute-loss only dehydration was not as prominent as it should be in the manuscript, we have now added a sentence to the end of the abstract (lines 29-31) and to the discussion and conclusion where we address this (lines 312-314, 351-352).

We were able to collect a quantity of saliva in 126/130 patients (97%)- reported on lines 210 and 328, although as we have stated (lines 213, 327) we only had adequate saliva (at least 25ul) to assess osmolality using our osmometer in 75% of samples. However, we have addressed this limitation in the discussion, (line 327-333) where we say that micro osmometers are in development that can assess osmolality on nano-gram quantities. We hope you feel that this is adequately addressed in the manuscript. Thanks.

Reviewer #2:
Many thanks for reviewing our manuscript.