Simplifying the Evaluation of Children With New Onset Diabetes: Utility of Pancreatic Autoantibodies for Diabetes Type Classification and Use of Serum Bicarbonate to Diagnose and Classify Diabetic Ketoacidosis.

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Introduction

Diabetes mellitus in youth is common, with an estimated worldwide annual incidence of 80,000 children under age 15 years\(^1\) and the incidence is rising\(^2\). Diabetic ketoacidosis (DKA) at presentation of new onset diabetes mellitus (NODM) occurs in approximately 15 to 70% of patients in Europe and North America\(^3\). The incidence of DKA at presentation is higher in younger children, ethnic minorities, families of lower socioeconomic status, and regions with a lower prevalence of diabetes\(^3\)–\(^7\), which includes many resource limited countries. In an era of rising health care cost and increasing availability of sophisticated diagnostic technologies, evaluating the evidence for the utility of testing procedures has become paramount.

In the evaluation of pediatric patients with new onset diabetes, routine measurement of pancreatic autoantibodies at diagnosis is a frequent practice, although there is currently no universal agreement regarding whether this is necessary. Further, the standard initial diagnostic evaluation for children with new onset diabetes and especially those suspected to have diabetic ketoacidosis incorporates a panel of laboratory tests, including a venous blood gases (VBG). Acidemia is defined as arterial pH <7.35 or venous pH <7.3, and traditionally, a venous pH <7.3 or a serum HCO3 <15 mmol/L is used to confirm the diagnosis of DKA, with lower values of both indicating greater severity of the condition. Venous pH <7.1 or serum HCO3 <5 mmol/L has been used to define severe DKA\(^3\)–\(^8\). These definitions are incorporated into hospital practice guidelines, and emergency room physicians and pediatricians often base management decisions, for example, admission to hospital and use of intravenous versus subcutaneous insulin therapy on the venous pH value. However, the VBG can be costly, needs to be drawn in a separate collection tube, and in remote or resource limited settings is frequently not readily available.
The main objective of this research was to evaluate the usefulness of the pancreatic autoantibody panel in clinical practice, and to evaluate the ability of serum HCO3, measured at presentation as part of a routine basic metabolic panel, to accurately predict DKA and classify its severity in a large population of youth with new onset diabetes. Our specific aims were:

1. To determine the number of patients whose diabetes type was reclassified after obtaining the results of the pancreatic autoantibody panel, to describe their clinical characteristics, and to develop and test the utility of a clinical scoring system to assist in the selection of patients for whom pancreatic autoantibody panel measurement is warranted.

   This work was submitted as an original article to Diabetes Care.

2. To investigate whether serum HCO3 levels can be used to accurately diagnose DKA and classify its severity in children with new onset diabetes mellitus.

   This work was submitted as an original article to Pediatrics.
References


Title: Utility of Pancreatic Autoantibodies for Classification of New Onset Diabetes in Children and Adolescents

Running Title: Pancreatic Autoantibodies for Classifying Diabetes

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STRUCTURED ABSTRACT

**Objective:** To determine whether measuring pancreatic autoantibodies (PAA) in pediatric new onset diabetes can be restricted to patients with equivocal diabetes type.

**Research Design and Methods:** Retrospective analysis of all patients with new onset diabetes admitted to Boston Children's Hospital from 10/1/07-7/1/13 who had measurement of PAA (glutamic acid decarboxylase, insulin, IA-2). Data collection included initial diagnosis of diabetes type before PAA results and at follow up. We used logistic regression to predict type 1 diabetes and developed a clinical score to classify diabetes type.

**Results:** Of 1089 patients (45.1% female, 76.7% white, age 10.6±4.5 years), initial diagnosis was 1021 (93.8%) type 1 diabetes, 42 (3.9%) type 2 diabetes, and 26 (2.4%) other. Seventy-eight of 993 (7.9%) patients with clinical type 1 diabetes were PAA- and 12 of 42 (28%) patients with clinical type 2 diabetes were PAA+. Less than 6% of patients had a change in diagnosis of diabetes type at follow up. Data from a subset of 736 subjects were used to develop a scoring system to predict type 1 diabetes. Using weight z-score, age and race the scoring system had 91.7% sensitivity, 82% specificity and a positive predictive value of 98.6%, and suggested PAA measurement was unnecessary in 85.3% of subjects. Findings were similar in a validation cohort of 234 subjects.

**Conclusions:** Application of a simple scoring system may reduce to ~15% the number of PAA measurements needed to classify diabetes type, resulting in substantial cost savings. Clinical judgment should guide the decision to measure PAA.
**Key words:**

pancreatic autoantibodies; insulin antibody; IA2 antibody; GAD antibody; type 1 diabetes mellitus; type 2 diabetes mellitus; scoring system; child

**Abbreviations:**

BMI body mass index, DKA diabetic ketoacidosis, GAD glutamic acid decarboxylase, HbA1c Hemoglobin A1c, IAA insulin autoantibody, IA-2 insulinoma antigen 2, MODY maturity onset diabetes of the young, PAA pancreatic autoantibodies, VBG venous blood gas
INTRODUCTION.

Diabetes is classified into the following general categories: Type 1 diabetes, Type 2 diabetes, gestational diabetes mellitus, and specific types of diabetes due to other causes (e.g., monogenic diabetes, diseases of the exocrine pancreas, drug- or chemical-induced) (1). Any classification system likely represents an artificial division of a spectrum of beta-cell function and insulin resistance, with autoimmunity influencing the rate of beta-cell decline (2,3). The utility of classifying diabetes type has been debated (4,5); however, it is important to characterize subjects for research studies, and in clinical practice classification influences management decisions.

In children and adolescents with new onset diabetes, the clinical presentation, including age, ethnicity, pubertal status, body habitus, signs of acanthosis nigricans, presence of diabetic ketoacidosis, and family history inform the designation of diabetes type (6,7). Specific types of diabetes are usually easily recognized based on the history, exposures, and co-morbid conditions. However, monogenic forms of diabetes may be difficult to distinguish clinically from type 1 or type 2 diabetes, and although a careful history can heighten suspicion, misdiagnosis and under-recognition are common (8–10). At many institutions pediatric endocrinologists routinely measure pancreatic autoantibodies (PAA) in all children with new onset diabetes; however, whether this should be part of a patient’s evaluation at the time of diagnosis is controversial. The SEARCH for Diabetes in Youth study group, for example, base the etiological classification of diabetes type on determination of autoimmunity and insulin sensitivity and have suggested this strategy be used both for research purposes and clinical practice (11,12). Others have proposed using a combination of autoimmune status and C-peptide levels as a proxy for beta cell function to define pediatric diabetes phenotypes (13). On the other hand, the most recent ISPAD
The guidelines state that PAA measurement “may assist in confirming diabetes type” (6); the recommendation to measure PAA is restricted to children suspected of having type 2 diabetes and to obese patients with a clinical picture suggestive of type 1 diabetes (7). There is not universal agreement regarding whether PAA should be measured in all patients with new onset diabetes, regardless of clinical presentation, or, alternatively, whether PAA measurement should be performed in selected patients in whom the type of diabetes is uncertain.

The main objective of this study was to evaluate the usefulness of a PAA panel in clinical practice. Specifically, we aimed to determine the number of patients whose diabetes type was reclassified after obtaining the results of the PAA panel, and to describe their clinical characteristics. We hypothesized that measurement of the PAA panel can be omitted in the majority of patients with a classic clinical presentation of type 1 diabetes, and can be restricted to patients suspected of having type 2 or equivocal diabetes type. We also aimed to develop and test the utility of a clinical scoring system to assist in the selection of patients for whom PAA measurement is warranted.
RESEARCH DESIGN AND METHODS

Study Design and Study Population

This was a cross-sectional retrospective review of all patients with new onset diabetes presenting to Boston Children’s Hospital (BCH) between October 1, 2007 and July 1, 2013. Subjects were identified using BCH’s Diabetes Inpatient Quality Improvement Database, which contains a register of all patients with newly diagnosed diabetes mellitus, including their presumed diabetes type assigned by the inpatient diabetes care team before obtaining the results of the PAA panel. Subjects met inclusion criteria if they had new onset diabetes and had PAA measured within 5 days of initial diagnosis. Exclusion criteria were a previous diagnosis of diabetes mellitus and neonatal diabetes. New onset diabetes was defined as random plasma glucose ≥ 200 mg/dL with classic symptoms of diabetes mellitus (polyuria, polydipsia, weight loss), or a fasting plasma glucose ≥ 126 mg/dL (14). Diabetic ketoacidosis (DKA) was defined as venous pH < 7.3. The study was approved by the Institutional Review Board at BCH.

Data Collection

We used Childrens360, a data extraction tool linked to the electronic medical record, to obtain subject data. We collected information on age at diagnosis, race, anthropometric measures, and diabetes type as described above. Diabetes types were designated as type 1 diabetes, type 2 diabetes or other. For subjects whose height was not recorded at the time of diagnosis, heights from outpatient visits within 60 days of diagnosis were extracted, and the height measurement that was closest to the date of diagnosis was selected to calculate the patient’s body mass index (BMI) at diagnosis. We collected laboratory data including concentrations of plasma glucose, serum electrolytes, serum and/or urine ketones, hemoglobin A1c (HbA1c), venous blood gas
(VBG), and PAA, which included autoantibodies to glutamic acid decarboxylase (GAD), insulin (IAA), and insulinoma antigen 2 (IA-2).

**Outcomes**

Our main outcome was the proportion of patients for whom the PAA profile changed the clinically assigned of diabetes type at the time of diagnosis (hereafter referred to as initial diagnosis). Our secondary outcome was the performance of a clinical scoring system to identify patients in whom the measurement of PAA can be omitted with minimal likelihood of an incorrect diagnosis.

**Diabetes Group Definitions**

Subjects were classified into six groups based on their initial diagnosis and PAA status: type 1 diabetes/PAA+, type 1 diabetes/PAA-, type 2 diabetes/PAA+, type 2 diabetes/PAA-, other type/PAA+ and other type/PAA-. We assumed that type 1 diabetes/PAA+ subjects had autoimmune type 1 diabetes and that type 2 diabetes/PAA- subjects had classic type 2 diabetes. For all other groups, the first author carefully reviewed the clinic notes after diagnosis to determine the final assessment of diabetes type by the patient’s pediatric endocrinologist (hereafter referred to as final diagnosis).

A final diagnosis of type 1 diabetes was defined as either type 1 diabetes/PAA+ or any initial diagnosis (type 1 diabetes, type 2 diabetes or other type) with follow-up assignment of type 1 diabetes. Similarly, a final diagnosis of type 2 diabetes was either type 2 diabetes/PAA- or any initial diagnosis (type 1 diabetes, type 2 diabetes or other type) with follow-up assignment of type 2 diabetes. For subjects whose physician’s records documented uncertainty in the assignment of diabetes type (variously referred to as hybrid, type 1.5, type 3, double diabetes,
mixed type, etc.), final diagnosis was designated as equivocal. All other types of diabetes distinct
from type 1 and type 2, such as iatrogenic, medication-induced, and monogenic diabetes, were
designated as other.

Laboratory Analysis

Serum bicarbonate (HCO3) concentration was measured using quantitative enzyme-based
determination on the Roche/Hitachi cobas c systems platform. The VBG, plasma glucose and
serum electrolytes were determined using the Radiometer ABL 825 instrument. Urine ketone
concentrations were measured using Acetest tablets. Hemoglobin A1c (HbA1c) was measured by
immunoassay using the Roche Integra 800 platform. GAD was measured by quantitative ELISA,
IA-2 and IAA by quantitative radioimmunoassay (all at ARUP, Salt Lake City, UT, USA).
Before January 1, 2009, IAA was measured in the Clinical Immunology Laboratory at the Joslin
Diabetes Center using a competitive insulin antibody assay (15); GAD and IA2 were measured
using quantitative radioimmunoassay (16). All other measurements were performed in the BCH
Clinical Chemistry Laboratory.

Statistical Analysis

We used mean and standard deviation or median and interquartile range, as appropriate, for
continuous variables and proportions for categorical variables to describe subject characteristics.
Analyses of continuous data were performed with Student’s t-test and Wilcoxon rank sum test
for normally and non-normally distributed data, respectively. Chi square test was used to
compare categorical variables. Subjects (n=32) were excluded from the primary analysis if no
data for a final diagnosis of diabetes type were available.
For the development of the PAA clinical scoring system, subjects were divided into a study cohort (January 1, 2009 – July 1, 2013) and a validation cohort (October 1, 2007 – December 31, 2008). The study cohort was used to build a logistic regression model to predict a final diagnosis of type 1 diabetes. We first performed a univariate screen to assess the variables age, race (white vs. other), weight z-score, BMI z-score, DKA status, ketonuria, pH, serum HCO3 concentration, plasma glucose concentration, and anion gap as predictors of type 1 diabetes. We then used forward selection in a multivariable model to identify variables that remained predictors of type 1 diabetes with a significance level of at least 0.1. C-statistics and receiver operator characteristics (ROC) were used to assess the goodness of fit of models including all significant predictors as well as simplified models including only a subset of significant clinical predictors. An area under the curve (AUC) >0.90 was defined a priori as the minimum requirement for the final model. Beta estimates of the selected predictors of type 1 diabetes were used to weigh their importance and assign, accordingly, points for the scoring system. The ROC curve was used to determine a cut-off for classifying a subject as likely to have type 1 diabetes and, therefore, not requiring PAA measurement. Results of each ROC curve were presented as mean AUC with 95% CI.

Performance of the scoring system was assessed by separately calculating sensitivity, specificity and positive predictive value in the study cohort and in the validation cohort.

For all analyses, p-value <0.05 was considered significant. Analyses were performed using SAS Software Version 9.4 (SAS Institute Inc., Cary, NC, USA).
RESULTS

Subjects

The cohort consisted of 1263 subjects, of whom 1089 (86.2%) met eligibility criteria. The initial diagnosis was type 1 diabetes in 1021 (93.8%), type 2 diabetes in 42 (3.9%) and other type of diabetes in 26 (2.4%) subjects. Baseline characteristics of subjects are shown in Table 1.

Subjects with an initial diagnosis of type 1 diabetes were more likely to be white, younger, have lower weight z-score and BMI z-score, ketonuria, and present with DKA. Of all eligible subjects, 86.5% were positive for one or more PAA. The majority of subjects tested positive for GAD (70.4%) and IA-2 (70.4%), while less than half tested positive for IAA (41.4%). Results of PAA status by diabetes type assigned at diagnosis are shown in Table 2.

Final Diagnosis

Data to assign a final diagnosis of diabetes type were available for 1057 subjects (Table 3). Median time from initial diagnosis to most recent follow up assessment was 922 days (IQR 50, 1794). Sixty subjects (5.7%) had a change in their diagnosis following PAA panel results. Of 992 subjects who received an initial diagnosis of type 1 diabetes, 77 (7.8%) had a negative PAA panel. Of these, the majority (66.2%) retained the diagnosis of type 1 diabetes, whereas 26 (2.6%) received an alternate final diagnosis, including type 2 diabetes (n=8), equivocal type (n=12), monogenic diabetes (n=3), diabetes related to propionic acidemia (n=1), abnormal insulin secretion related to Alagille syndrome (n=1), and steroid induced diabetes (n=1). Of the 42 subjects with an initial diagnosis of type 2 diabetes, 30 (71.4%) and 6 (14.3%), retained a final diagnosis of PAA- and PAA+ type 2 diabetes, respectively; whereas, in 6 (14.3%) subjects the final diagnosis was changed to PAA+ type 1 diabetes. Of the 22 subjects who had an initial diagnosis of other type of diabetes, 13 (59.1%) received a final diagnosis of PAA+ type 1 diabetes.
diabetes. All other subjects were PAA- and were assigned a final diagnosis of equivocal type (n=3), type 2 diabetes (n=3), monogenic diabetes (n=1), steroid-induced diabetes (n=1) and diabetes associated with cancer therapy (high dose dexamethasone, L-asparaginase, total body irradiation) (n=1).

**Development of PAA Clinical Scoring System**

The scoring system was developed based on the data from 809 subjects in the study cohort (Figure 1). The univariate screen revealed age, weight z-score, BMI z-score, race, ketonuria, serum HCO3 concentration, anion gap, plasma glucose, BUN and BUN/creatinine ratio as significant predictors of type 1 diabetes. Given expected co-linearity of BMI z-score and weight z-score, only the latter was introduced into the multivariable selection process. Age, weight z-score, race and ketonuria remained significant in the multivariable model. Comparison of a model including all four variables vs. only including the three variables age, weight z-score and race, revealed minimally different ROC characteristics with a small reduction in AUC (0.94 [95% CI 0.90-0.97] vs. 0.92 [95% CI 0.87-0.96]), but a value that remained >0.9. Therefore, for ease of implementation, the three variable model was selected (Figure 2a). Points for each of the 3 variables were assigned as shown in Table 4. Based on the ROC characteristics of the total score as a predictor of type 1 diabetes, a cut-off of less than 3 points was chosen to predict type 1 diabetes (Figure 2b). Conversely, a score of 3 or above suggests obtaining a PAA panel to determine definitively the diabetes type.

**Evaluation of PAA Clinical Scoring System**
The scoring system was evaluated in 736 study cohort subjects for whom weight z-score, race and age data were available. The scoring system had a sensitivity of 91.6%, specificity of 82% and PPV of 98.6% (Table 5).

Of 248 subjects in the validation cohort, 234 had no missing data for the variables weight z-score, race and age. Subjects in the validation cohort had, on average, a 0.4% (4 mmol/mol) lower HbA1c level at diagnosis (10.9 ± 2.0 % [95 ± 22 mmol/mol] vs. 11.3 ± 2.3 % [99 ± 25 mmol/mol], p= 0.01). They were more likely than subjects in the study cohort to have positive IAA antibodies (71.3% vs. 33.8%, p<0.0001) and had a lower likelihood of having positive GAD antibodies (53.3% vs. 78.0%, p<0.0001). However, there was no difference in the percentage of subjects who had a positive PAA panel (91.1% vs. 88.2%, p=0.14). The scoring system had a sensitivity of 93.3%, specificity of 90.9% and PPV of 99.5% in the validation cohort (Table 6).

When assessing the ability of the scoring system to correctly identify subjects most likely to have a change of diabetes type following PAA results (i.e. subjects with type 1 diabetes/PAA-, type 2 diabetes/PAA+, or initial diagnosis of other type, n=99), we found that 58 of 67 subjects (86.6%) were correctly assigned a score of less than 3 points (i.e. type 1 diabetes), and 21 of 32 (65.6%) were correctly assigned a score of 3 or greater (i.e. type 2 or other diabetes type). Overall, 80 of 99 subjects (80.8%) with the greatest likelihood of receiving a different final diagnosis after obtaining the PAA results were correctly classified using the scoring system.
CONCLUSIONS

These results provide compelling evidence that in a predominantly white population, a clinical diagnosis of diabetes type could be made correctly in a majority of patients with new onset diabetes. A different diabetes type was assigned after obtaining the results of a PAA panel in only a small number of patients. Further, we showed that a simple clinical scoring system has high sensitivity and specificity to identify patients with classic type 1 diabetes, thereby limiting the need to measure PAA to only about 10 to 15% of patients.

In our large population of pediatric and young adult patients between 6 months and 25 years of age with newly diagnosed diabetes mellitus, we found that less than 6% of patients were reclassified after the results of the PAA panel were available. Slightly higher rates of reclassification (based on C-peptide levels, pancreatic autoantibody results, or clinical course) have been described in previous studies. In a group of ethnically diverse patients, Lipton et al. found that as many as 19.8% (22 out of 111) of their patients’ diabetes type was reclassified over an average of 7.8 years of follow-up (17). In a cohort of 580 German and Austrian patients with type 2 diabetes, 10.3% were reclassified on average 2.4 years after follow up (18). Rates of reclassification might be expected be higher in ethnically diverse populations with more type 2 diabetes, and in patients initially diagnosed with type 2 diabetes. In our relatively small subset of 42 patients with an initial diagnosis of type 2 diabetes, 6 (14.3%) were reclassified as type 1 diabetes (n=6).

Consistent with other reports, based on the measurements of insulin, GAD and IA-2 antibodies, 86.5% of our total population and 89.6% of patients with a clinical diagnosis of type 1 diabetes were PAA positive (19,20). Our scoring system showed high sensitivity and specificity to
identify children with a final diagnosis of type 1 diabetes, potentially allowing PAA measurement to be omitted in a majority of these patients and restricting their measurement to patients with an initial diagnosis of type 2, equivocal or unclear types of diabetes. The high overall prediction accuracy of the scoring system is not surprising. In the SEARCH cohort (2), all three predictors used in our scoring system are strongly associated with lower fasting C-peptide levels at diagnosis, a strong predictor of beta cell function decline (21), which occurs fastest in youth with autoimmune type 1 diabetes (2).

The aim of our scoring system was to identify and recommend PAA testing only for those at greatest risk for having a diabetes type other than classic type 1 diabetes. This strategy is consistent with the most recent ISPAD guidelines that suggest PAA measurement be used to assist in confirming diabetes type (6). Owing to the increased prevalence of overweight and obesity in youth in the general population, clinical features of type 1 and type 2 diabetes have, increasingly, become less distinct, representing different points on the spectra of insulin sensitivity, beta cell autoimmunity and beta cell loss (22). Between 10% and 40% of youth diagnosed with type 2 diabetes have positive PAA (23–25). In our study, 12 of 42 (28.6%) of patients initially diagnosed with type 2 diabetes had a positive PAA panel; however, 6 (50%) patients were later classified as having type 1 diabetes. Several studies, including the TODAY study, have shown significant clinical and biochemical differences between PAA positive and negative youth with clinical type 2 diabetes (12,26–28). Dabelea at al. further showed that the main predictor of beta cell decline in all forms of diabetes is autoimmunity, regardless of BMI status (2). Our scoring system is consistent with the notion that recognition of autoimmunity is particularly important in overweight and obese youth with suspected type 2 diabetes because it may impact clinical management, and especially the decision to initiate insulin therapy. For
example, the scoring system always recommends screening for any obese child with a BMI z-score >2 if they are also over 10 years old and/or of non-white race. Further, the scoring system performed well in recommending PAA screening for subjects with type 2 diabetes, with 37 (94.9%) out of 39 patients with a final diagnosis of type 2 diabetes receiving the appropriate recommendation (score ≥3) to perform a PAA screen.

Our data also suggest that clinicians need to maintain a high level of suspicion for monogenic diabetes in children with a clinical phenotype suggestive of type 1 diabetes. Patients with maturity onset diabetes of the young (MODY) may be the hardest to recognize (29). For example, in our cohort, one patient with MODY3 and one with MODY4 had a score ≥3, suggesting a PAA panel measurement, whereas 2 other patients with MODY had a score of <3. However, both patients were ultimately identified on clinical grounds. A detailed family history and monitoring of the clinical course frequently raises the suspicion of monogenic diabetes (29).

Several limitations of the study merit comment. First, its retrospective nature did not allow for rigorous phenotyping of our subjects. Further, details regarding the rationale for the clinician’s initial and final diagnosis were limited, including the impact of the PAA results on the final diagnosis. However, we believe that availability of these details would not have changed the primary findings. Second, we excluded patients who did not have a PAA panel measured within 5 days of diagnosis. However, our analysis comparing patients who did and did not have PAA measured revealed that the two groups did not differ significantly, except for a higher rate of severe DKA and higher frequency of associated electrolyte abnormalities in patients who did not have a PAA panel measured. Third, the PAA panel obtained at BCH included GAD, IA-2 and insulin antibodies, but not islet cell or zinc transporter 8 antibodies. Adding measurement of the
latter antibody to the three autoantibody panel might have increased the antibody positive patients by to 2-3 percent (30,31). Most likely, however, this would mainly have affected the children with PAA- type 1 diabetes, a majority of whom are classified as type 1 diabetes by the scoring system. Lastly, the racial/ethnic composition of our diabetes population may differ compared to other locations in the United States (32) and elsewhere in the world. These results, therefore, may only be generalizable to populations with a similar racial/ethnic composition; i.e., predominantly White.

In summary, our study provides data regarding the utility of determining the autoimmune status in a large cohort of pediatric patients with new onset diabetes. We conclude that a simple scoring system can screen patients who are most likely to benefit from PAA measurement. We believe that PAA determination can be restricted to a relatively small number of selected patients with new onset diabetes. Careful evaluation of the clinical phenotype at presentation and over time is crucial in determining appropriate diabetes management, regardless of diabetes type classification. Further prospective studies should evaluate how clinical presentation and determination of autoimmune status impact clinical management, in particular, for overweight and obese youth with diabetes.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 2: ROC curves of multivariable model and of scoring system to predict type 1 diabetes

Figures represent ROC curves of the multivariable model including age, weight z-score and race as predictor of type 1 diabetes (panel A), and of the total assigned score as predictor of type 1 diabetes (panel B). Based on the ROC curve in panel B, sensitivity and specificity maximized at 3 points. A cut-off of <3 points was thus chosen to predict type 1 diabetes, conversely suggesting PAA measurements for individuals whose score is ≥3.
REFERENCES


**Table 1: Characteristics of Subjects**

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<thead>
<tr>
<th></th>
<th>All (n=1089)</th>
<th>Type 1 (n=1021)</th>
<th>Type 2 (n=42)</th>
<th>Other (n=26)</th>
<th>p-value*</th>
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<td>Age (y)</td>
<td>10.6 ± 4.5</td>
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<td>15.3 ± 2.8</td>
<td>13.1 ± 3.5</td>
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<td>Sex (% Female)</td>
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<td>467 (45.8)</td>
<td>18 (42.9)</td>
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<td>Weight z-score</td>
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<td>BMI z-score</td>
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<td>-0.16 ± 1.48</td>
<td>2.34 ± 0.72</td>
<td>1.17 ± 1.30</td>
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<td>Race (%)</td>
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<td></td>
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<td>(93 ± 30)</td>
<td>(92 ± 24)</td>
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<td>DKA (%)</td>
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<td>7.9</td>
<td>22.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Severe†</td>
<td>3.6</td>
<td>3.7</td>
<td>2.6</td>
<td>4.6</td>
<td>0.74</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 (7.29, 7.43)</td>
<td>7.36 (7.28, 7.43)</td>
<td>7.37 (7.33, 7.41)</td>
<td>7.36 (7.30, 7.41)</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum Bicarbonate (mmol/L) ‡</td>
<td>21 (14, 28)</td>
<td>21 (13, 29)</td>
<td>22 (18, 26)</td>
<td>22 (14, 29)</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>409 ± 178</td>
<td>414 ± 176</td>
<td>351 ± 198</td>
<td>305 ± 142</td>
<td>0.03</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>14 ± 6</td>
<td>15 ± 6</td>
<td>11 ± 5</td>
<td>12 ± 4</td>
<td>0.0004</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.47 ± 0.19</td>
<td>0.46 ± 0.19</td>
<td>0.59 ± 0.17</td>
<td>0.56 ± 0.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BUN/Creatinine Ratio</td>
<td>34 ± 18</td>
<td>35 ± 18</td>
<td>20 ± 7</td>
<td>24 ± 9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>132 ± 5</td>
<td>132 ± 5</td>
<td>134 ± 4</td>
<td>133 ± 3</td>
<td>0.08</td>
</tr>
<tr>
<td>Anion Gap</td>
<td>17 ± 6</td>
<td>18 ± 6</td>
<td>15 ± 6</td>
<td>16 ± 6</td>
<td>0.03</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation, unless otherwise indicated.

*Comparison of type 1 vs. type 2 diabetes. Comparison for “other” diabetes type (n=26) not shown

†pH <7.1; ‡median (interquartile range)


<table>
<thead>
<tr>
<th></th>
<th>Initial Diagnosis</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Type 1</td>
<td>Type 2</td>
<td>Other</td>
<td></td>
<td></td>
<td>p-value*</td>
</tr>
<tr>
<td>PAA+ N(%)</td>
<td>942 (86.5)</td>
<td>915 (89.6)</td>
<td>12 (28.6)</td>
<td>16 (6.5)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 antibody+ N(%)</td>
<td>216 (19.9)</td>
<td>203 (19.9)</td>
<td>8 (19.1)</td>
<td>6 (23.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 antibodies+ N(%)</td>
<td>449 (41.3)</td>
<td>443 (43.4)</td>
<td>1 (2.4)</td>
<td>5 (19.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 antibodies+ N(%)</td>
<td>277 (25.5)</td>
<td>269 (26.4)</td>
<td>3 (7.1)</td>
<td>5 (19.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAD+ N(%)</td>
<td>751† (70.4)</td>
<td>734 (73.4)</td>
<td>7 (16.7)</td>
<td>10 (38.5)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA-2+ N(%)</td>
<td>752‡ (70.4)</td>
<td>732 (73.1)</td>
<td>8 (19.1)</td>
<td>13 (50.0)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAA+ N(%)</td>
<td>442§ (41.4)</td>
<td>430 (42.9)</td>
<td>4 (9.8)</td>
<td>8 (30.8)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Comparison of type 1 vs. type 2 diabetes. Comparison for “other” diabetes type (n=26) not shown

† N missing = 21
‡ N missing = 19
§ N missing = 20
Table 3: Categorization of Subjects by Initial Diagnosis and Final Diagnosis

<table>
<thead>
<tr>
<th>Initial Diagnosis / PAA status</th>
<th>Type 1 (n=986)</th>
<th>Type 2 (n=47)</th>
<th>Equivocal (n=15)</th>
<th>Other (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA+</td>
<td>915 (86.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAA-</td>
<td>52 (4.8)</td>
<td>8 (0.8)</td>
<td>12 (1.1)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA+</td>
<td>6 (0.6)</td>
<td>6 (0.6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAA-</td>
<td>0</td>
<td>30 (2.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA+</td>
<td>13 (1.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAA-</td>
<td>0</td>
<td>3 (0.3)</td>
<td>3 (0.3)</td>
<td>3 (0.3)</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to the percentage of the total population (n=1057; 32 missing due to lack of follow up).
### Table 4: PAA Clinical Scoring System

<table>
<thead>
<tr>
<th>Variable</th>
<th>Points</th>
<th>Beta Estimate (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Z-score</td>
<td></td>
<td>1.34 (0.19)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>0.31 (0.06)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10-15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;15</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td>0.58 (0.15)</td>
</tr>
<tr>
<td>White</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL SCORE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If &lt; 3</td>
<td>→</td>
<td>assume type 1 diabetes</td>
</tr>
<tr>
<td>If ≥3</td>
<td>→</td>
<td>obtain PAA panel</td>
</tr>
</tbody>
</table>

Variables included in the scoring system, their categorization, and points assigned for each subcategory are shown. Beta estimates are shown for each variable and indicate the weight of one measurement unit of this variable in relation to the other two. A total score of <3 is suggestive of type 1 diabetes, whereas if the score is ≥3, obtaining a PAA panel is recommended.
Table 5: Performance of Scoring System using Weight z-score, Age and Race

<table>
<thead>
<tr>
<th>Score</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1</td>
</tr>
<tr>
<td>Study cohort</td>
<td>(n=736)</td>
</tr>
<tr>
<td>Score &lt;3</td>
<td>n=686</td>
</tr>
<tr>
<td>Likely Type 1, omit PAA</td>
<td>(85.3)</td>
</tr>
<tr>
<td>Score ≥3</td>
<td>58</td>
</tr>
<tr>
<td>Type 2 or other, obtain PAA</td>
<td>(7.9)</td>
</tr>
<tr>
<td>Validation cohort</td>
<td>(n=234)</td>
</tr>
<tr>
<td>Score &lt;3</td>
<td>n=223</td>
</tr>
<tr>
<td>Likely Type 1, omit PAA</td>
<td>(88.9)</td>
</tr>
<tr>
<td>Score ≥3</td>
<td>15 / 223</td>
</tr>
<tr>
<td>Type 2 or other, obtain PAA</td>
<td>(6.4)</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent percentages of all patients in their respective cohort (study or validation cohort).
Figure 1: Study Algorithm

1089 subjects met eligibility criteria

32 had no follow-up data

1057 were included in analysis of the change of diabetes type after PAA

Study cohort: 809 subjects

No weight z-score available: 15 subjects

No race data available: 58 subjects

Included in scoring system analysis: 736 subjects

Validation cohort: 248 subjects

No weight z-score available: 0 subjects

No race data available: 14 subjects

Included in scoring system validation: 234 subjects
Figure 2: ROC curves of multivariable model and of scoring system to predict type 1 diabetes

Figures represent ROC curves of the multivariable model including age, weight z-score and race as predictor of type 1 diabetes (panel A), and of the total assigned score as predictor of type 1 diabetes (panel B). Based on the ROC curve in panel B, sensitivity and specificity maximized at 3 points. A cut-off of <3 points was thus chosen to predict type 1 diabetes, conversely suggesting PAA measurements for individuals whose score is ≥3.
Serum bicarbonate can substitute for venous pH in evaluating children with new onset diabetes

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Corresponding Author: Julia von Oettingen, MD, Dr. med., Boston Children’s Hospital, 300 Longwood Avenue, Boston, MA, 02115, julia.vonoettingen@childrens.harvard.edu, 617-355-7476

Short Title: Serum bicarbonate for evaluating diabetic ketoacidosis

Abbreviations: AUC area under the curve, BCH Boston Children’s Hospital, BHOB beta-hydroxybutyrate, DKA diabetic ketoacidosis, DM diabetes mellitus, HCO₃⁻ serum bicarbonate, NODM new onset diabetes mellitus, ROC receiver operator characteristic, VBG venous blood gas, vpH venous pH measurement

Key Words: diabetes, diabetic ketoacidosis, bicarbonate, blood gas, child

Funding Source: There was no funding support for this study.

Financial Disclosure Statement: Dr. Rhodes receives research funding from Merck, and her spouse owned stock in Bristol-Myers Squibb and owns stock in Pfizer, GlaxoSmithKline, Sanofi and Merck. Drs. von Oettingen, Wolfsdorf, and Feldman have no financial relationships relevant to this article to disclose.

Conflict of Interest: The authors have no conflict of interest to disclose.

What’s Known on This Subject:
Diabetic ketoacidosis (DKA) is a common and serious first manifestation of diabetes mellitus in children. During initial evaluation, the venous blood pH is frequently used to make the diagnosis and classify the severity of DKA.

What This Study Adds:
This study demonstrates that the serum bicarbonate concentration is a simple and accurate predictor of the presence and severity of DKA and can be used in lieu of vpH, especially in resource poor settings where access to vpH measurement is limited.
Contributor’s Statement:

**Julia von Oettingen:** Dr. von Oettingen conceptualized and designed the study, and carried out data collection and data analysis. She drafted the initial manuscript.

**Joseph Wolfsdorf and Erinn Rhodes:** Drs. Wolfsdorf and Rhodes conceptualized and designed the study, and supervised data collection and analysis. They critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

**Henry Feldman:** Dr. Feldman supervised data analysis. He critically reviewed and revised the manuscript, and approved the final manuscript as submitted.
Structured Abstract

Objective: To investigate whether serum bicarbonate (HCO3) levels can be used to accurately diagnose diabetic ketoacidosis (DKA) and classify its severity in children with new onset diabetes mellitus (NODM).

Methods: Retrospective study of all patients with NODM requiring treatment with insulin presenting to Boston Children’s Hospital from 10/1/07 – 7/1/13. DKA was defined as blood glucose ≥200 mg/dL, vpH <7.3 and urine ketones ≥2+. Linear regression was used to assess serum HCO3 as a predictor of vpH, and logistic regression to evaluate serum HCO3 as a predictor of DKA and severe DKA.

Results: 690 patients (47% female, mean age 10.8±4.3 years, 76.7% white, 19.4% DKA) were included in the study cohort. The relationship between serum HCO3 and vpH was log-linear (r=0.87, 95% CI 0.85-0.89, p<0.001). HCO3 predicted vpH using the formula vpH=6.81301+(0.17823*ln[HCO3]); R² 0.75 (p<0.001). The receiver operator characteristics curve showed an area under the curve of 0.97 (95% CI 0.96-0.99, p<0.001). HCO3 cut-offs of <15 and <18 mmol/L had sensitivities of 82.1% and 91.8%, and specificities of 97.7% and 91.7%, respectively, to diagnose DKA. Cut-offs of <5 and <8 mmol/L had sensitivities of 52.4% and 95.2%, and specificities of 99.9% and 96.7%, respectively, to diagnose severe DKA. Findings were similar in a validation cohort of 197 subjects.

Conclusions: Serum HCO3 concentration alone can substitute for vpH to diagnose DKA and classify severity in children with NODM. It is suggested as an alternative to reliance on vpH, especially in resource poor settings where access to vpH measurement may be limited.
Introduction

Diabetes mellitus in youth is common, with an estimated worldwide annual incidence of 80,000 children under age 15 years\(^1\) and the incidence is rising\(^2\). Diabetic ketoacidosis (DKA) at presentation of new onset diabetes mellitus (NODM) occurs in approximately 15 to 70% of patients in Europe and North America\(^3\). The incidence of DKA at presentation is higher in younger children, ethnic minorities, families of lower socioeconomic status, and regions with a lower prevalence of diabetes\(^3\)–\(^7\), which includes many resource limited countries.

In many pediatric academic medical centers in the United States, the standard initial diagnostic evaluation for children with NODM and especially those suspected to have DKA includes a panel of laboratory tests to measure venous blood gases (VBG) and the concentrations of plasma glucose, serum electrolytes, bicarbonate (HCO\(_3\)), blood urea nitrogen (BUN), creatinine, hemoglobin A1c (HbA1c), and serum or urinary ketones. Acidemia is defined as arterial pH <7.35 or venous pH <7.3, and traditionally, a vpH <7.3 or a serum HCO\(_3\) <15 mmol/L is used to confirm the diagnosis of DKA, with lower values of both indicating greater severity of the condition. Venous pH <7.1 or serum HCO\(_3\) <5 mmol/L has been used to define severe DKA\(^3\)–\(^8\). These definitions are incorporated into hospital practice guidelines, and emergency room physicians and pediatricians often base management decisions, for example, admission to hospital and use of intravenous versus subcutaneous insulin therapy on the vpH value. However, in remote or limited resource settings, VBGs are not readily available.

A recent study of pediatric patients seen in an emergency room suggested that the venous HCO\(_3\) concentration accurately predicts abnormal vpH in children with DKA\(^9\). Similarly, in adults, venous HCO\(_3\) concentrations predicted arterial pH with a high degree of sensitivity and
specificity\textsuperscript{10}. The objective of our study was to evaluate the ability of serum HCO3, measured at presentation as part of a routine basic metabolic panel, to accurately predict DKA and classify its severity in a large population of youth with NODM.
Patients and Methods

Study Design and Study Population

This was a cross-sectional retrospective chart review of all patients with NODM presenting to Boston Children’s Hospital (BCH) between October 1, 2007 and July 1, 2013. Inclusion criteria were new onset diabetes, serum HCO3 concentration measured within 15 minutes of an initial vpH measurement, and urinary ketone measurement within 4 hours of vpH measurement. These cut-offs were chosen a priori to avoid introduction of effects related to response to therapy. Exclusion criteria were a previous diagnosis of diabetes and neonatal diabetes. DKA was defined as blood glucose $\geq 200$ mg/dL, vpH $<7.3$ and urine ketones $\geq 2+$. Mild, moderate, and severe DKA were defined as vpH 7.2 - 7.29, 7.1 - 7.19 and $< 7.1$, respectively. Subjects were divided into a study cohort (January 1, 2009 – July 1, 2013) and a validation cohort (October 1, 2007 – December 31, 2008). The study was approved by the Institutional Review Board at BCH.

Data Collection

We used the BCH inpatient Diabetes Quality Improvement Database to identify eligible subjects. This database includes all patients with diabetes admitted to BCH. Virtually all patients with NODM referred to BCH who require treatment with insulin are admitted to hospital. We used Patient360, a data extraction tool linked to the electronic medical record, to extract subject data. We collected information on patient age at diagnosis, race/ethnicity, anthropometric measures, date and time of laboratory analyses, and laboratory data including concentrations of plasma glucose, serum electrolytes and HCO3, serum and/or urine ketones, HbA1c and VBG.

Outcome Measures
Our main outcome measure was the correlation between serum HCO3 concentration and vpH in a population of children and adolescents at the time of their initial diagnosis of diabetes mellitus. Secondary outcomes were the accuracy of serum HCO3 concentration to diagnose DKA and classify its severity, including a determination of serum HCO3 cut-offs that would best correlate with the traditionally used vpH cut-offs of <7.3 to define presence of DKA and <7.1 to define severe DKA.

**Laboratory Analysis**

Serum HCO3 concentration was measured using quantitative enzyme-based determination on the Roche/Hitachi cobas c systems platform. The VBG, plasma glucose and serum electrolytes were determined using the Radiometer ABL 825. Urine ketone concentrations were measured using Acetest tablets. HbA1c was measured by immunoassay using the Roche Integra 800 platform. All measurements were performed in the BCH Clinical Chemistry Laboratory.

**Statistical Analysis**

Standard descriptive statistics were used to describe subject characteristics. These included percentages for categorical variables, and mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate, for continuous variables. Analyses of continuous data utilized Student’s t-test and Wilcoxon rank sum test for normal and non-normal data, respectively, and chi-square tests to compare categorical variables. The relationship between serum HCO3 and vpH was assessed by Pearson’s correlation. Given that the relationship appeared log-linear rather than linear, and because vpH is a log of hydrogen ion content, serum HCO3 was log-transformed for further analyses. Linear regression was used to assess serum
HCO3 as a predictor of pH, and to create a formula to calculate the v pH as a function of serum HCO3. A priori, a strong correlation was defined as a Pearson correlation coefficient $\geq 0.7$ and a very strong correlation as a coefficient $\geq 0.85$.

For our secondary outcomes, logistic regression and receiver operator characteristic (ROC) curves were used to evaluate serum HCO3 concentration as a predictor of DKA or severe DKA. An area under the curve (AUC) of greater than 0.9 was chosen a priori as the minimum acceptable goodness-of-fit. The Hosmer and Lemeshow test was used to assess how well the model was calibrated; i.e. how well predicted DKA event rates matched expected event rates. Other variables included in the model using a purposeful selection approach were age, race/ethnicity, serum osmolality, concentrations of plasma glucose, serum sodium, and BUN, BUN/creatinine ratio and anion gap. Variables were retained if the c-statistic improved by more than 10% or if there was a change in the beta coefficient of more than 10%. Characteristics for different serum HCO3 concentration cut-offs to predict presence of DKA and severe DKA from the model included sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The final analysis was then replicated in the validation cohort.

For all analyses, p-value $<0.05$ was considered significant. Analyses were performed using SAS Software Version 9.4 (SAS Institute Inc., Cary, NC, USA).
Results

Subjects

The NODM cohort consisted of a total of 987 subjects who had laboratory measurements performed at BCH at the time of diagnosis. Sixty two subjects were excluded because of missing laboratory data: vpH (n=61), serum HCO3 (n=26), urine ketones (n=8). Of the remaining 925 subjects, 235 were excluded because VBG and serum HCO3 were not measured within 15 minutes of each other (n=154), and/or urinary ketones were not measured within 4 hours of the initial VBG measurement (n=119). Thirty-eight subjects did not meet the criteria for either time frame. Overall, 690 subjects met inclusion criteria and were included in the primary study analysis.

For the validation cohort, we identified 284 subjects of whom 18, 11 and 8 did not have vpH, serum HCO3 and/or urine ketone measurements, respectively. Of the remaining 263 subjects, 66 were excluded because VBG and serum HCO3 measurements were not obtained within 15 minutes of each other (n=44), and/or absence of urine ketones measured within 4 hours of the VBG measurement (n=26). Five subjects met neither of the two criteria. A total of 197 subjects (48% female, 85% white, 16.8% DKA) were included in the analyses performed on the validation cohort.

Baseline characteristics for all subjects are shown in Table 1. There were no significant differences between the study and validation cohort with respect to demographic characteristics, diabetes type or severity of presentation. Laboratory characteristics for the study cohort are shown in Table 2.

Relationship between serum HCO3 concentration and venous pH
Serum HCO3 concentration correlated with v pH, \( r = 0.79 \) (95% CI 0.76 – 0.82). The relationship appeared to be log-linear, and log transformation of serum HCO3 values showed a strong correlation between the natural log of HCO3 and v pH (Figure 1), \( r = 0.87 \) (95% CI 0.85 – 0.89). A linear regression model with serum HCO3 as the sole predictor of v pH yielded the following formula: Venous pH = 6.81301 + (0.17823*ln[HCO3]), \( R^2 0.75, p<0.001 \). The range of serum HCO3 concentrations with their corresponding v pH values is shown in the supplementary Table.

**Serum HCO3 concentration as a predictor of DKA**

The ROC curve derived from the logistic regression model assessing serum HCO3 concentration as a predictor of DKA showed an AUC of 0.97 (95% CI 0.958-0.987, p<0.001) (Figure 2a), with a maximum rescaled \( R^2 \) of 0.79. The association of predicted probabilities and observed responses showed a concordance of 96.8%. No additional variables met criteria to be included as predictors or confounders. For the prediction of severe DKA, the AUC was 0.99 (95% CI 0.991-0.999, p<0.001) (Figure 2b), with a maximum rescaled \( R^2 \) of 0.82 and an association of predicted probabilities and observed responses showed a concordance of 99.4%. Sensitivity, specificity, NPV and PPV of different serum HCO3 concentration cut-off values for diagnosis of DKA and for classification of severe DKA are shown in Table 3.

**Validation**

Using the validation cohort, application of a serum HCO3 cut-off value of <15 and <18 mmol/L for the diagnosis of DKA yielded a sensitivity of 84.9% and 100%, specificity of 97% and 89.6%, PPV of 84.9% and 66%, and NPV of 97% and 100%, respectively. For the classification of severe DKA, a cut-off of <5 and <8 mmol/L yielded a sensitivity of 87.5% and
100%, specificity of 98.9% and 96.8%, PPV of 77.8% and 57.1%, and NPV of 99.5% and 100%, respectively.
Discussion

The results of our study confirm a very strong log-linear relationship between serum HCO3 concentration and vpH in patients with NODM. The findings suggest that serum HCO3 levels can reliably be used to diagnose and classify DKA in pediatric patients with new onset diabetes, and that VBG analysis is not required for the initial evaluation of these patients. Based on the formula derived to calculate the vpH based on the serum HCO3 level, the clinician can choose a serum HCO3 cut-off level for the diagnosis and classification of DKA based on their intent to maximize either the sensitivity or the specificity of the test.

Several other studies have been performed in an attempt to simplify and render more efficient the initial diagnosis and subsequent management of DKA. Sheikh-Ali et al. evaluated quantitative serum beta-hydroxybutyrate (BOHB) as a diagnostic test for DKA on the basis that pH, HCO3 and anion gap are relatively non-specific for the diagnosis of DKA. They suggested a BOHB level >3 mmol/L as a cut-off to diagnose DKA, but found a 20% discordance between BOHB and the conventional criteria using pH < 7.3, HCO3 <18 mmol/L and glucose ≥200 mg/dL\textsuperscript{11}. At least three groups have suggested end-tidal CO2 as a simpler and less invasive diagnostic or monitoring tool for DKA\textsuperscript{12–14}. Although end-tidal CO2 had a strong linear correlation with vpH and HCO3 concentration, the agreement was not narrow enough to confirm DKA, especially in cases of mild acidosis\textsuperscript{13,14}. Measurement of end-tidal CO2 has not been widely adopted and may be more suitable for monitoring rather than for initial diagnosis of DKA and classification of its severity. Another attempt to simplify the initial evaluation of patients with DKA has been with the measurement of electrolytes using a VBG analyzer rather than serum, which entails the use of a VBG alone\textsuperscript{19}. While this approach may be feasible in some
settings, many remote and resource limited settings are not equipped with VBG analyzers and may only have the capacity to measure serum electrolytes.

Only one previous study has assessed the utility of venous HCO3 for prediction of pH in children. Similar to our results, this study demonstrated a strong correlation between serum HCO3 concentrations and vpH, and concluded with a recommendation to validate their findings in additional patient cohorts. While a relatively large percentage of their population (~39% of 300 patients) presented with DKA, only 8 subjects had severe DKA – possibly limiting the interpretation for the classification of mild to moderate versus severe DKA. The authors suggested the best statistical cut-off of 18.5 mmol/L for serum HCO3 to define the presence of DKA, and 10.5 mmol/L to define severe DKA. These cut-offs, particularly the latter, are higher than suggested in most of the pediatric literature, possibly a result of the small number of subjects with severe DKA. We are providing a formula to calculate vpH from HCO3, and are suggesting multiple different cut-offs of serum HCO3 levels to predict and classify DKA. This allows clinicians to opt for erring on the side of caution and minimize the number of false negative cases by choosing a higher HCO3 cut-off, or minimize false positive cases and overtreatment by choosing a lower HCO3 cut-off. This is in line with the most recent adult guidelines by the American Diabetes Association that suggest a bicarbonate of 15-18 mmol/L in addition to an arterial pH <7.25-7.3 and anion gap >10 mmol/L for the diagnosis of DKA. For clinicians more used to using pH, we provide a conversion formula that aligns with traditionally used of vpH values of 7.3 and 7.1 to align with HCO3 values of 15 and 5 mmol/L, respectively.

Although minor differences exist for the criteria to diagnose DKA, the most commonly used definition in pediatrics is a combination of pH <7.3, HCO3 <15-18 mmol/L, ketonuria or ketonemia and hyperglycemia. Some definitions add the anion gap as a measure of
metabolic acidosis. In our study, serum HCO3 was the strongest predictor of the presence of DKA as defined by $\text{pH} < 7.3$ and presence of hyperglycemia and ketosis. Whereas the anion gap was also a significant predictor of DKA, it did not perform nearly as well as the serum HCO3 concentration (data not shown) and did not meet the a priori criteria for inclusion in the final statistical model. This is not surprising given that the anion gap, while indicative of acidosis, is affected by several anions that are present in DKA, including lactate, BOHB, acetoacetate, phosphate and sulfate. Finally, no other laboratory test added to the value of the HCO3 to predict DKA. This is likely a simple reflection of the natural correlation between pH and HCO3, but supports the use of HCO3 as the sole predictor of DKA.

Patients had on average four VBGs measured, adding significant cost not only to the initial evaluation but to the entire DKA management process. In an era of increasing financial constraints, there is a potential not only for simplification but also for cost-saving in the evaluation of patients with DKA.

Certain limitations of the study merit comment. First, this study was retrospective, and limited to a single, large academic referral center. While results may, as a result, be specific to the population studied, our demographics are quite representative of typical pediatric diabetes populations in large regions of the United States and Europe with regards to race/ethnicity, diabetes type and age distribution. Although the inpatient database used to identify our subjects represents a comprehensive list of all inpatients presenting with new onset diabetes requiring insulin therapy, some patients with type 2 diabetes treated with oral antihyperglycemic agents may be treated as outpatients and would not be represented. However, as the focus of the analysis is on the diagnosis and classification of DKA, the composition of the sample as insulin-
requiring patients supports the internal validity, and the large sample size and highly significant outcomes supports the generalizability to demographically similar populations.

Second, we could not differentiate between children who had been pre-treated with intravenous fluids or insulin and those who had not received any treatment before arriving at BCH. However, a previous study that was able to compare subjects who had received insulin prior to hospital admission to insulin-naïve subjects did not find a difference with regards to the ability of serum HCO3 concentration to accurately predict the presence of DKA. We also could not exclude patients with health conditions that may influence their acid-base status independent of their diabetes. While this patient population would, if anything, have biased the results towards a worse correlation of serum HCO3 level and vpH, clinicians must take into account the effect of underlying renal and pulmonary disorders on evaluation of acidosis, including DKA.

In addition, similar to other studies evaluating the relationship between parameters measured on the VBG and serum, we had to exclude a significant number of patients without near-simultaneous measurements of VBG and serum biochemistries, and ketone measurement within a few hours of diagnosis and treatment initiation. The population of excluded patients differed from the population analyzed in that they had a significantly higher prevalence of DKA (34.7 vs. 19.4%, p <0.001) and severe DKA (8.9% vs. 3%, p <0.001) and, not surprisingly, an overall higher frequency of electrolyte derangement and signs of dehydration (data not shown). These patients may have been sicker, and thus priority was not put on obtaining timely urine ketones, which accounted for the largest number of patients excluded. However, when we included all patients in the analysis, regardless of the timing of their ketone measurement, none of our outcomes changed significantly (data not shown).
Conclusion

In summary, serum HCO3 concentration is a simple, reliable and effective predictor of the presence and severity of DKA and can be used without the addition of the VBG to diagnose DKA and assess its severity. A range of HCO3 cut-off values based on desired maximal sensitivity or specificity of the test may be preferable to a single cut-off for the determination of DKA in the clinical setting allowing management decisions based on the clinical scenario. Such an approach should be considered in DKA management guidelines to facilitate management for clinicians and patients in both resource-limited and resource-rich settings. Future studies should prospectively evaluate the use of these recommendations and it may be of interest to examine the utility of HCO3 concentrations for the monitoring and resolution of DKA.
References


Acknowledgements:

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Table and Figure Legends:

Table 1: Characteristics of Subjects

Values are presented as mean (standard deviation), or as N (%) where indicated.

*Comparison of study vs. validation cohort

Table 2: Biochemical Characteristics of Subjects

All values are presented as mean (standard deviation), except for those marked by (†) which are represented as median (interquartile range), and by (††) which are presented as N (%).

*Comparison of DKA vs. no DKA.

Figure 1 Correlation between serum HCO3 and venous pH

The log-linear correlation between serum HCO3 and venous pH, including 95% confidence intervals, is shown. The X-axis displays the serum HCO3 concentration on a natural log scale.

Figure 2 Receiver Operator Characteristics Curve

ROC curves are shown for a) Serum HCO3 concentration as a predictor of DKA and b) Serum HCO3 concentration as a predictor of severe DKA. Each point on the curve represents a different HCO3 value, such that sensitivity and specificity vary with the HCO3 cut-off chosen.
### Table 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Cohort (n=690)</th>
<th>Validation Cohort (n=197)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female N (%)</td>
<td>324 (47.0)</td>
<td>95 (48.2)</td>
<td>0.75</td>
</tr>
<tr>
<td>Race/Ethnicity N (%)</td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>White</td>
<td>529 (76.7)</td>
<td>163 (82.7)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>12 (1.7)</td>
<td>2 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>49 (7.1)</td>
<td>11 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>55 (8.0)</td>
<td>10 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>45 (6.5)</td>
<td>11 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8 (4.3)</td>
<td>10.3 (4.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI (z-score)</td>
<td>-0.01 (1.32)</td>
<td>0.02 (1.43)</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes type N (%)</td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>655 (94.9)</td>
<td>186 (94.4)</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>24 (3.5)</td>
<td>6 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11 (1.6)</td>
<td>5 (2.5)</td>
<td></td>
</tr>
<tr>
<td>DKA severity N (%)</td>
<td></td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>None</td>
<td>556 (80.6)</td>
<td>164 (83.5)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>80 (11.6)</td>
<td>19 (9.6)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>33 (4.8)</td>
<td>6 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>21 (3.0)</td>
<td>8 (4.1)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Biochemical Characteristics of Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=690)</th>
<th>DKA (n=134)</th>
<th>No DKA (n=556)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous pH†</td>
<td>7.36 (7.32, 7.38)</td>
<td>7.23 (7.15, 7.27)</td>
<td>7.37 (7.35, 7.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum HCO3† (mmol/L)</td>
<td>23 (19, 26)</td>
<td>11 (8, 15)</td>
<td>24 (22, 26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum osmolality (mOsm/kg)</td>
<td>309 (13)</td>
<td>321 (17)</td>
<td>306 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>425 (167)</td>
<td>470 (172)</td>
<td>414 (168)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>15 (5)</td>
<td>15 (7)</td>
<td>14 (5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.48 (0.19)</td>
<td>0.53 (0.24)</td>
<td>0.46 (0.17)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Serum BUN/Creat</td>
<td>34 (17)</td>
<td>31 (15)</td>
<td>35 (17)</td>
<td>0.04</td>
</tr>
<tr>
<td>Anion gap (mmol/L)</td>
<td>17 (6)</td>
<td>26 (6)</td>
<td>15 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>11.3 (2.3)</td>
<td>12.1 (1.8)</td>
<td>11.1 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary ketones &gt;2+ ††</td>
<td>464 (67.2)</td>
<td>134 (100)</td>
<td>330 (59.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3: Evaluation of Serum HCO3 Concentration Cut-off Values

a) Diagnosis of DKA

<table>
<thead>
<tr>
<th>HCO3 [mmol/L]</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>82.1 %</td>
<td>97.7 %</td>
<td>89.4 %</td>
<td>95.8 %</td>
</tr>
<tr>
<td>&lt;16</td>
<td>84.3 %</td>
<td>96.2 %</td>
<td>84.3 %</td>
<td>96.2 %</td>
</tr>
<tr>
<td>&lt;17</td>
<td>88.1 %</td>
<td>94.2 %</td>
<td>78.7 %</td>
<td>97.0 %</td>
</tr>
<tr>
<td>&lt;18</td>
<td>91.8 %</td>
<td>91.7 %</td>
<td>72.8 %</td>
<td>97.9 %</td>
</tr>
</tbody>
</table>

b) Classification of Severe DKA

<table>
<thead>
<tr>
<th>HCO3 [mmol/L]</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>52.4 %</td>
<td>99.9 %</td>
<td>91.7 %</td>
<td>98.5 %</td>
</tr>
<tr>
<td>&lt;6</td>
<td>85.7 %</td>
<td>99.4 %</td>
<td>81.8 %</td>
<td>99.6 %</td>
</tr>
<tr>
<td>&lt;7</td>
<td>90.5 %</td>
<td>98.8 %</td>
<td>70.1 %</td>
<td>99.7 %</td>
</tr>
<tr>
<td>&lt;8</td>
<td>95.2 %</td>
<td>96.7 %</td>
<td>47.6 %</td>
<td>99.9 %</td>
</tr>
</tbody>
</table>
Figure 1. Log-linear correlation between serum HCO3 and venous pH, including 95% confidence intervals. The X-axis displays both HCO3 and
Figure 2. Receiver Operator Characteristics Curve

Figure 2. ROC curves are shown for a) Serum HCO3 concentration as a predictor of DKA and b) Serum HCO3 concentration as a predictor of severe DKA. Each point on the curve represents a different HCO3 value, such that sensitivity and specificity vary with the HCO3 cut-off chosen.
Main Conclusion

In conclusion, both research studies provide evidence to support simplifying the evaluation of children with new onset diabetes. The first study emphasizes the usefulness of clinical data to guide ancillary diagnostic testing, while the second study underlines that testing parsimony should be considered when evaluating children with new onset diabetes for diabetic ketoacidosis.

Specifically, our first study provides data regarding the utility of determining the autoimmune status in a large cohort of pediatric patients with new onset diabetes, and leads to the conclusion that in a predominantly white population, a clinical diagnosis of diabetes type could be made correctly in a majority of patients with new onset diabetes. A different diabetes type was assigned after obtaining the results of a pancreatic autoantibody panel in only a small number of patients. We showed that a simple clinical scoring system has high sensitivity and specificity to identify patients with classic type 1 diabetes, thereby limiting the need to measure a pancreatic autoantibody panel to only about 10 to 15% of patients. Careful evaluation of the clinical phenotype at presentation and over time is crucial in determining appropriate diabetes management, regardless of diabetes type classification.

Our second study provides evidence that serum HCO3 concentration is a simple, reliable and effective predictor of the presence and severity of DKA and can be used without the addition of the VBG to diagnose DKA and assess its severity. A range of HCO3 cut-off values based on desired maximal sensitivity or specificity of the test may be preferable to a single cut-off for the determination of DKA in the clinical setting allowing management decisions based on the clinical scenario. Such an approach should be considered in DKA management guidelines to
facilitate management for clinicians and patients in both resource-limited and resource-rich settings.
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Julia von Oettingen