Screening for Diabetes and Prediabetes With Proposed A1c-Based Diagnostic Criteria

Running title: Screening with A1c

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**Objective:** An International Expert Committee (IEC) and the American Diabetes Association (ADA) proposed diagnostic criteria for diabetes and prediabetes based on A1c levels. We hypothesized that screening for diabetes and prediabetes with A1c measurements would differ from using oral glucose tolerance tests (OGTT).

**Research Design and Methods:** We compared prediabetes, dysglycemia (diabetes or prediabetes), and diabetes identified by proposed criteria (A1c ≥ 6.5% for diabetes, and 6.0-6.4% [IEC] or 5.7-6.4% [ADA] for High Risk/prediabetes) to standard OGTT diagnoses in three datasets. Non-Hispanic white or black adults without known diabetes who had A1c and 75 g OGTT measurements were included from the prospective Screening for Impaired Glucose Tolerance study (SIGT, n=1581), and from NHANES III (n=2014), and NHANES 2005-2006 (n=1111).

**Results:** OGTT revealed prediabetes in 35.8% and diabetes in 5.2% of combined study subjects. A1c provided ROC areas for diabetes of 0.79-0.83, but ROC areas were ≤ 0.70 for dysglycemia or prediabetes. The proposed criteria missed 70% with diabetes, 71-84% with dysglycemia, and 82-94% with prediabetes. Compared to IEC, ADA criteria for prediabetes resulted in fewer false negatives and more false positives. There were also racial differences, with false positives more common in black subjects and false negatives more common in whites. Using NHANES 2005-2006 data, approximately 5.9 million non-Hispanic U.S. adults with unrecognized diabetes and 43-52 million with prediabetes would be missed by screening with A1c.

**Conclusions:** The proposed A1c diagnostic criteria are insensitive and racially discrepant for screening, missing most undiagnosed Americans with diabetes and prediabetes.

Diabetes affects over 21 million American adults (1; 2), with a lifetime risk ranging from 20-50+% depending on sex and race (3). Identification of diabetes – and its precursor, prediabetes – can permit management to prevent complications or delay progression from prediabetes to diabetes. Because most U.S. health care systems do not have systematic screening programs, many Americans with diabetes and most with prediabetes are undiagnosed and cannot initiate programs aimed at prevention (2).

An International Expert Committee (IEC) recently proposed new diagnostic criteria based on measurement of A1c, with A1c ≥ 6.5% for diabetes and 6.0-6.4% for “High Risk” of progression to diabetes (4). The American Diabetes Association (ADA) subsequently proposed A1c ≥ 6.5% for the diagnosis of diabetes, and 5.7-6.4% for highest risk to progress to diabetes (5). Since A1c testing is readily available in the U.S., relatively well standardized, exhibits low intra-individual variation, and does not require fasting or restriction to certain times of the day (6), many clinicians might wish to use A1c measurements to screen for diabetes and prediabetes. However, the proposed diagnostic criteria were based largely on identification of diabetic retinopathy, and performance of the proposed criteria as a screening test is not understood. The IEC A1c criteria have recently been compared to testing with fasting glucose or oral glucose tolerance tests (OGTT) in various populations to diagnose diabetes (7-13) and high risk/prediabetes (10;
11; 13), but the ADA A1c criteria have not been studied.

We hypothesized that A1c diagnostic criteria would fail to identify many subjects with unrecognized diabetes or prediabetes. We evaluated the proposed criteria as screening tests in three populations, compared to the OGTT as a “gold standard” used for identification of diabetes and prediabetes around the world (14).

RESEARCH DESIGN AND METHODS

Subjects and study design. We examined three datasets where non-Hispanic white and black adult subjects without known diabetes had both an OGTT and A1c measured (15).

In the “Screening for Impaired Glucose Tolerance” study (SIGT) (16), healthcare system employees and community members in Atlanta were eligible if they had age 18 or above, non-Hispanic white or black race, no prior diagnosis of diabetes, and were not pregnant or breastfeeding, not taking glucocorticoids, and well enough to work. 1581 subjects completed the protocol.

The National Health and Nutrition Examination Surveys (NHANES) assessed adults and children across the United States. In NHANES III (17), 2057 non-Hispanic black or white subjects (to match the SIGT study population) over 40 years old with no known history of diabetes had an OGTT meeting inclusion criteria. In NHANES 2005-2006 (18), 1154 non-Hispanic adult subjects 18 years and older, met inclusion criteria. Age, body mass index, blood pressure, lipids, and family history were categorized using conventional criteria. After excluding subjects with missing data, there were 2014 subjects in NHANES III and 1111 subjects in NHANES 2005-2006, as described previously (15).

Glucose and A1c measurements. Plasma glucose and A1c measurements have been described previously (16-18). A1c measurements utilized NGSP-certified systems (see Supplemental Material in the online appendix available at http://care.diabetesjournals.org).

Classification of glucose tolerance. Glucose tolerance was classified by ADA criteria based on glucose levels in a single 75g OGTT. Subjects were grouped as: normal glucose tolerance (NGT) (FPG<100 mg/dl, with 2hrPG<140 mg/dl), prediabetes (IFG with FPG 100-125 mg/dl and 2hrPG<200 mg/dl, and/or IGT with FPG<126 mg/dl and 2hrPG 140-199 mg/dl), and diabetes (FPG≥126 mg/dl or 2hrPG≥200 mg/dl)). The additional IEC criteria identified subjects as Normal (A1c <6%), High Risk for diabetes (6.0-6.4%), and Diabetes (≥6.5%), while new ADA criteria identified subjects as Normal (A1c <5.7%), High Risk (5.7-6.4%), and Diabetes (≥6.5%). Additional evaluations utilized FPG<110 mg/dl for normal glucose tolerance. “Dysglycemia” includes prediabetes or diabetes (OGTT), or High Risk or Diabetes (A1c).

Statistical analysis. Means and frequencies were determined in aggregate and by subgroup analysis of the different glucose tolerance categories. The discriminative effectiveness of screening was evaluated by the area under receiver operating characteristic (ROC) curves using SAS 9.2 (SAS Institute, Inc. Cary, NC) for the NHANES data, and SPSS 15.0 (SPSS Inc., Chicago, IL) for SIGT data. All NHANES analyses were conducted using SAS 9.2 and SUDAAN version 10 (RTI International, Research Triangle Park, NC) to account for the complex survey design. Prevalence estimates from NHANES studies were weighted, except where noted or when small sample sizes made weighting estimates unstable. 95% confidence intervals were calculated using the Wilson Score method. Paired t-tests compared rates of apparent false positive and false negative identification of diabetes or high risk in black and white
subjects. Population estimates were derived from the results of the weighted NHANES 2005-2006 dataset and applied to nonHispanic black or white adults for which data were available, ~156 million individuals. No additional extrapolations were made.

RESULTS
As reported previously (16), the 1581 SIGT subjects had average age 48 yrs and BMI 30.3 kg/m$^2$; 42% were male, and 58% were black. The 2014 NHANES III subjects had average age 55 yrs and BMI 27.3 kg/m$^2$; 47% were male, and 10% were black. The NHANES 2005-2006 subjects had average age 46 yrs, and BMI 28.5 kg/m$^2$; 49% were male, and 13% were black (see supplemental tables 1A-1C).

Using OGTT results, 5.8% of the combined study subjects had new diabetes, and 36% had prediabetes (Table 1). Moreover, the proportions were similar in the three study populations: 4.6% and 33% in SIGT (16), 7.6% and 38% in NHANES III, and 5.2% and 36% in NHANES 2005-2006 (Supplemental Tables 1A-1C). In contrast, assessment by A1c was different from OGTT characterization. 2.3% of combined subjects were categorized as Diabetes and 6.2% were High Risk by IEC criteria, while 2.3% were categorized as diabetes and 19.5% were High Risk by ADA criteria. The three populations were categorized in similar proportions by A1c with the IEC and ADA criteria.

Thus, compared to the OGTT, A1c testing resulted in more Normal “diagnoses” (categorizations) and fewer High Risk and Diabetes “diagnoses” with both the IEC and ADA criteria. The ADA criteria resulted in a distribution of “diagnoses” more similar to the OGTT criteria by identifying more subjects as High Risk and fewer subjects as Normal. The different distribution for OGTT vs. the A1c criteria was seen in males, females, and nonHispanic whites, but not in nonHispanic blacks (supplemental Tables 1A-1C), where A1c “diagnoses” of Diabetes and High Risk using the ADA criteria were more frequent.

A1c-based categorizations were mostly incorrect for OGTT diagnoses of both prediabetes and diabetes. Both the ADA and the IEC criteria would result in more false positive (FP) and false negative (FN) categorizations compared to OGTT (Table 2) – generally more FN than FP. The IEC criteria generated more FN and fewer FP than the ADA criteria. Incorrect identifications across the three datasets averaged 90% and 71% for prediabetes (by IEC and ADA criteria, respectively, combining FP and FN), 84% and 65% for dysglycemia (all FN), and 70% for diabetes (all FN). Extrapolating the NHANES 2005-2006 findings to the nonHispanic American adult population, A1c testing would incorrectly identify diabetes in 6.5 million (FP and FN). A1c testing for prediabetes would also be incorrect for 43 million Americans using ADA criteria and 52 million by IEC criteria, with the majority of those being prediabetic by OGTT, but Normal by A1c (table 2). Importantly, the ADA criteria would additionally label nonHispanic Americans with normal results on OGTT – 5.8 million as High Risk and 75 thousand as Diabetes.

ROC analyses were performed to determine if screening categorization by A1c vs. OGTT was independent of cutoff values. ROC curve areas were lowest for prediabetes, and higher for diabetes (Figure 1). Only for diabetes were areas under the ROC curve areas $\geq 0.80$ (0.82 in SIGT, 0.80 in NHANESIII, and 0.83 in NHANES 2005-2006), while for prediabetes they were lowest (0.65 in SIGT, 0.62 in NHANESIII, and 0.68 in NHANES 2005-2006), and intermediate for dysglycemia (0.67 in SIGT, 0.66 in NHANESIII, and 0.70 in NHANES 2005-2006).

To assess alternative cutoffs, we evaluated sensitivity, specificity, PPV, and
NPV for different A1c cutoffs across the three datasets (Supplemental Tables 2A and 2B). Both the IEC and the ADA criteria are specific for diagnosing diabetes and prediabetes (>90% specificity above A1c cutoffs of 6.0%), but insensitive for both diabetes (<34% at A1c cutoff 6.5% and above) and prediabetes – lowest with IEC criteria (<31% above cutoff of 5.7%, and <13% above A1c cutoff of 6.0%). Approximately equal sensitivity and specificity would be provided by cutoffs 5.3-5.5% for prediabetes or dysglycemia, and 5.5-5.8% for diabetes. The sum of sensitivity and specificity was maximized at 5.4-5.6% for prediabetes, 5.5-5.6% for dysglycemia, and 5.7-5.9% for diabetes.

However, even cutoffs maximizing the sum of sensitivity and specificity for the NHANES 2005-2006 dataset (A1c 5.4% for High Risk and 5.7% for diabetes) would incorrectly identify diabetes in approximately 21 million (19 million FP and 2.2 million FN) and incorrectly identify High Risk in 59 million nonHispanic American adults – with fewer FN but more FP at these lower cutoffs.

Repeating the above analyses with a higher fasting plasma glucose cutoff for normal glucose tolerance (FPG<110 mg/dl) did not change the results (see Supplementary Tables 2, 3, and 4, and Supplementary Figure 1).

In addition, the impact of the A1c-based criteria differs by race. There would be more FN and fewer FP (Figure 2) in nonHispanic whites than blacks with both the IEC and ADA criteria (p<0.05 for both). Of those with diabetes by OGTT, 78% of whites vs. 51% of blacks would be FN (p<0.01), whereas 0.3% of whites vs. 1.8% of blacks without diabetes would be FP (p=0.15). With the IEC criteria for High Risk, 94% of whites vs. 77% of blacks with prediabetes by OGTT would be FN, while 1.7% of whites vs. 9.0% of blacks without prediabetes would be FP (p<0.02 that whites have more FN and less FP). At the lower ADA cutoff for High Risk, there would be fewer FN but more FP: 78% of whites vs. 56% of blacks with prediabetes by OGTT would be FN, while 8.5% of whites vs. 18% of blacks without prediabetes would be FP (p<0.05 that whites have more FN and less FP). However, the percent of incorrect High Risk subjects (FN+FP) with A1c screening for prediabetes was lower with the ADA criteria: 96% of whites and 86% of blacks with IEC criteria, vs. 87% of whites and 73% of blacks with ADA criteria.

Extrapolating from the NHANES 2005-2006 dataset, A1c screening with the IEC and ADA criteria would incorrectly identify diabetes (FP or FN) in 470,000 black and 6 million white nonHispanic Americans. The IEC criteria would incorrectly identify High Risk in 6 million black and 42 million white nonHispanic Americans. Although, there would be fewer missed subjects using the ADA criteria, potentially incorrect diagnoses would only be reduced for nonHispanic black adults, incorrectly identifying High Risk in 4 million black but 47 million white nonHispanic Americans.

**CONCLUSIONS**

Our analyses demonstrate that the IEC and ADA A1c diagnostic criteria have limitations for use in screening which include: (i) high specificity but low sensitivity, (ii) intrinsic inaccuracy even with alternative cutoffs, and (iii) discrepant application to different racial groups. The results are consistent with two different methodologies for measurement of A1c (immunoassay in SIGT and HPLC in NHANES), and with one dataset including black and white volunteers (SIGT) and two others representative of the nonHispanic American population (NHANES III and 2005-2006). While our findings support diagnosing diabetes by the proposed A1c criteria as rarely false positive, use of the proposed criteria would not be a good screening strategy to identify the many
Americans who presently have unrecognized diabetes or prediabetes as found with OGTTs – a diagnostic standard currently used around the world.

While the IEC and ADA did not recommend use of their criteria for screening, A1c testing has attractive features including simplicity, standardization, availability, low day-to-day variability, and lack of need for prior fasting or restriction in time of measurement – and A1c is widely used as an indicator of diabetes control. Additionally, the IEC stated that A1c “may be better” than glycemic criteria for diagnosing diabetes (4) and the ADA listed the A1c criteria “above” the glycemic criteria (5). However, other workers have questioned the use of A1c measurements for diagnosis (6; 19). Several groups have now applied the proposed IEC criteria to a variety of datasets. The IEC criteria would miss diagnoses of diabetes and prediabetes in an older, predominantly white American population (8), and in another NHANES population with measurement of FPG but no OGTTs (7), and ROC areas, sensitivity, and specificity were similar to those in the current study with an American population enriched in Hispanic adults (12). There was greater variability in the international studies (9-11; 13), possibly reflecting ethnic and racial differences, as well as differences in the underlying rates of undiagnosed diabetes (9; 11). None of the studies found the IEC High Risk criteria to be similar to prediabetes with OGTTs (10; 11; 13).

It is beyond our analysis to address the accuracy of OGTT vs. A1c, but they differ intrinsically. A1c within an individual fluctuates little from day to day, while an OGTT can reflect day to day differences in insulin secretion and insulin action. As such, A1c should better assess glycemic trends over several months, while OGTT should better assess immediate glucose homeostasis. Moreover, there is substantial inter-individual variation in A1c at the same levels of glycemia (20; 21) – reflecting differences in red cell penetration, glycation, hemoglobin species, red cell half-life, vitamin and medication status, etc. (22). Such variation would be expected to make it more difficult for A1c measurements to distinguish small differences in glycemia – e.g., prediabetes vs. normal.

Our findings that the proposed A1c criteria result in more FP and fewer FN in blacks compared to whites are consistent with previous reports that at similar glucose levels, A1c levels are higher in blacks than whites (23). This explains both relative under-diagnosis in whites, and over-diagnosis in blacks. The basis for such differences remains unknown.

The strengths of our study include assessments in over 4500 subjects, similarity of findings across multiple datasets with different A1c methodologies, evaluation of both IEC and ADA criteria, comparison of the general utility of A1c screening with the specific problems of the proposed criteria, and the identification of potential problems in applications to different racial/ethnic groups. One limitation is performance of only a single OGTT, but such an approach reflects clinical practice. Moreover, a single abnormal OGTT was highly predictive of subsequent diabetes in the Pimas (24), and differences found on repeat studies should be included in the variability of the ROC analyses. We also did not search for factors which can be problematic for A1c measurements, such as hemoglobinopathies and anemia. Further studies would be required before our findings with the ADA criteria could be generalized to other racial/ethnic groups, but problems with the IEC criteria have been reported with diverse populations (8; 9; 12).

While A1c screening may be of limited value, better test characteristics appear to be provided by measuring random plasma glucose (25), or glucose one hour after a 50g
Screening with A1c

oral glucose challenge (16); either could be obtained opportunistically during office visits, at any time of day and without a prior fast. Other tests or combinations of tests may also be useful, and it is possible that health economics analyses would identify one or another test as more cost-effective in different patient populations and clinical settings. We still need to identify previously unrecognized diabetes and prediabetes, in order to initiate preventive management.

Author Contributions. L.P., D.O., M.R., D.Z., and J.T. devised the research questions and formulated the research plan. D.O. wrote the manuscript and researched the data. L.P. wrote and revised the manuscript, and oversaw all research of the data. K.H. and M.R. researched the data, and edited the manuscript. D.Z researched the data. J.T. edited the manuscript.

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Table 1: Subjects identified by oral glucose tolerance test (OGTT) compared with A1c diagnoses.

<table>
<thead>
<tr>
<th>A1c categories</th>
<th>IEC</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGTT Categories</td>
<td>Normal A1c &lt; 6.0%</td>
<td>High Risk A1c = 6.0-6.4%</td>
</tr>
<tr>
<td>NGT 58% (2.3)</td>
<td>97% (0.90)</td>
<td>2.5% (0.80)</td>
</tr>
<tr>
<td>Prediabetes 36% (1.4)</td>
<td>89% (2.7)</td>
<td>10% (1.9)</td>
</tr>
<tr>
<td>Diabetes 5.8% (0.9)</td>
<td>49% (2.9)</td>
<td>21% (1.2)</td>
</tr>
</tbody>
</table>

Row-Wise %

| % of Total | NGT 57% (2.3) | 1.5% (0.46) | 0.18% (0.08) | 51% (2.8) | 7.0% (1.7) | 0.18% (0.08) |
| Prediabetes | 32% (1.7) | 3.5% (0.59) | 0.35% (0.03) | 25% (2.1) | 10% (1.2) | 0.35% (0.03) |
| Diabetes | 2.8% (0.53) | 1.2% (0.15) | 1.7% (0.28) | 1.8% (0.44) | 2.3% (0.25) | 1.7% (0.28) |

(a) - the data in A1c categories for Diabetes are the same for ADA and IEC diagnostic criteria. Every subject was mapped according to OGTT and A1c diagnostic criteria into 3x3 tables, and the percentage of the subjects within each dataset was determined. NHANES values are from weighted estimates. Values were combined across the three datasets and the mean and SEM are shown. Each value in the upper rows is the mean and SEM of row-wise percentages, and percentage of subjects in each OGTT category is also included. The lower rows show the percentage of the entire population.

Table 2: Rates of correct and incorrect classification

<table>
<thead>
<tr>
<th>OGTT Criteria</th>
<th>IEC Criteria</th>
<th>ADA Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>%Correct 97% (0.90)</td>
<td>88% (3.0)</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>%Correct 10% (1.9)</td>
<td>29% (4.0)</td>
</tr>
<tr>
<td>Dysglycemia</td>
<td>%Correct 16% (2.0)</td>
<td>35% (3.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>%Correct 30% (1.9)</td>
<td>30% (1.9)</td>
</tr>
</tbody>
</table>

Correct or incorrect classification within each diagnostic category were evaluated in each dataset, and the average and SEM of the rates across the three datasets is listed. %Correct is the proportion of subjects with a given OGTT diagnostic criteria, that were correctly identified by A1c diagnostic criteria. Incorrect diagnoses were false positives (FP) and false negatives (FN), which were further categorized as being incorrectly Normal, High Risk, or Diabetes per A1c testing. SIGT proportions were calculated from raw data; NHANES proportions were calculated from weighted estimates.
Figure 1. ROC analyses for each of the three datasets, showing A1c screening to detect prediabetes, dysglycemia, and diabetes. A. Prediabetes. B. Dysglycemia. C. Diabetes. Closed circles = 0.5% intervals of A1c, open circles = ADA and IEC cutoff for diabetes at A1c=6.5%, open squares = ADA cutoff for High Risk at A1c=5.7%, closed squares = IEC cutoff for High Risk at A1c=6.0%. Points labelled with A1c values. Dotted lines = SIGT, dashed lines = NHANES III, solid lines = NHANES 2005-2006.
**Figure 2.** Rates of false positive and false negative A1c screening compared to OGTT screening according to race across the three study populations. Open bars show nonHispanic whites and filled bars nonHispanic blacks. The rates of FP and FN subject for black and white subgroups across the populations are shown as mean ± SEM.