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Jessica Vakili Date

Identification of an Effective Diabetes Screening Strategy:

Analysis of the Screening for Impaired Glucose Tolerance Study

By

Jessica Vakili

Master of Science in Public Health

Department of Biostatistics and Bioinformatics

Qi Long, Ph.D.

Thesis Advisor

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Jessica Vakili

B.S., University of Tennessee Knoxville, 2008

Thesis Advisor: Qi Long, Ph.D.

Committee Members: Darin Olson, MD, Ph.D.

Lawrence Phillips, MD

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A thesis submitted to the Faculty of the
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Abstract

Identification of an Effective Diabetes Screening Strategy:

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By: Jessica Vakili

Background: Establishing an effective and systematic formal screening test is ideal for managing the public health burden resulting from diabetes. The oral glucose tolerance test (OGTT) is the standard to identify diabetes with maximum sensitivity and specificity. However, this test is quite time intensive and inconvenient for patients. We sought to determine if pairing simpler tests together might be a reasonable substitute for OGTT.

Methods: The final sample of the Screening for Impaired Glucose Tolerance study involved 1,573 subjects without known diabetes. Tests collected on these subjects included random plasma glucose (RPG), hemoglobin A1c, glucose challenge test (GCT), fasting plasma glucose (FPG), and an OGTT. Four different disease statuses were defined to signify a positive diagnosis for diabetes. For each definition of diabetes, receiver operating characteristic curve were produced for individual tests as well as for combinations of two tests.

Results: Despite disease status, OGTT and FPG consistently had the two highest areas under the curve analyzing all subjects. Additionally, the majority of paired tests were significantly higher than their matching single tests proving combinations of simple tests improve screening accuracy. FPG + GCT was the combined test identified with the highest area under the curve for standard diabetes and high-risk prediabetes. Instead of using an OGTT, GCT could be performed conveniently during an outpatient visit, followed by an FPG in patients previously screened with GCT.

Discussion: Our data and results do suggest that strong consideration should be given to screening. These findings propose that opportunistic screening in a clinical setting of individuals at high-risk would be justified and thus will help delay the onset of type 2 diabetes. Further research of this screening methods' cost effectiveness would be necessary as well as future research into additional diabetes screening methods.

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Introduction

The establishment of a formal and effective screening test for diabetes is a crucial next step in the advancement in controlling the disease. This research examines if a simpler screening method, including paired tests, could be substituted for the oral glucose tolerance test (2h-OGTT). Currently, 25.8 million people or 8.3% of the population are estimated to have type 1, type 2, and gestational diabetes in the USA. [1] Even more alarming is of the 25.8 million people 7 million are undiagnosed. [1] Type 2 diabetes prevalence is rising quickly, and is expected to affect 50 million people by 2050 in the United States likely due to increasing obesity, reduced activity levels, and the aging population. [2, 3] This is the most common form of diabetes which usually occurs in people with genetic predispositions that is also compounded by other factors. These factors can raise one's risk of developing diabetes, such as high blood pressure, obesity, physical inactivity or family history. [4] Many people are unaware of their risk since diabetes often presents first as asymptomatic when blood glucose initially becomes elevated. Medical diagnosis usually occurs slowly, sometimes 8 to 12 years after glucose levels are noticed as unstable. [5] With type 2 diabetes, there is a lack of insulin, either by the body not producing enough or the body's cells ignoring it; the latter of which is known as insulin resistance. [6] Insulin is used by the body to convert glucose into energy, by transporting the sugar to cells. [6] If sugar does not reach the cells, this build-up causes complications and sugar never converts to energy.

Diabetes also disproportionally affects several race groups. According to the Centers for Disease Control and Prevention (CDC) research, when compared to white American adults, Asian Americans risk was 18% higher for a diabetes diagnosis, Hispanics risk was 66% higher, and non-Hispanic blacks had a risk 77% higher. [1] Additionally, individuals have an increased risk

with just one parent with type 2 diabetes and if both parents have diabetes, the risk approaches 40%. [3] Diabetes can lead to many adverse health outcomes for people, including increased risk of cardiovascular disease, renal disease, ophthalmologic problems, and a 10 to 14 year decrease in life expectancy. [5] Gum disease, mental health disorders, hearing loss, and nerve damage are also serious health complications many diabetics face. [7] Furthermore, diabetes is a costly disease and in 2007 expenses associated with diabetes were estimated at $174 billion. [8] These costs include direct medical costs and indirect costs, such as work loss or disability. [1] With the increasing number of diabetes cases, expenses will only continue to rise. However, by identifying people with diabetes in earlier stages through screening tests, these costs could be contained. In addition to controlling costs, finding a suitable screening test could identify individuals at high risk or at a stage where glucose is just beginning to rise, currently known as "prediabetes", and manage the progression of prediabetes into diabetes. Thus, prevention programs could be developed and enroll individuals with prediabetes or undiagnosed diabetes. Establishing a formal screening test that is simple, inexpensive, and convenient would be useful in discovering people in earlier stages of the disease and hopefully decrease or alleviate the burdens related to diabetes.

Screening for Diabetes

There is an important difference between diagnostic testing and screening for diabetes. Screening for diabetes is done to separate asymptotic high risk individuals from people at low risk for diabetes and identify people with prediabetes and to diagnose those with otherwise undetected diabetes. [9] Prediabetes is a condition where people have blood glucose or A1c levels higher than normal but not elevated enough to be considered diabetic. [1] The ADA suggests screening individuals > 45 years every 3 years and if individuals are overweight (BMI >

25 km/m^2) with one other diabetes risk factor begin screening at an earlier age. [3] A few relevant risk factors include physical inactivity, certain race/ethnicity, hypertension, and history of gestational diabetes. [3] Numerous approaches exist for screening, including risk assessment questionnaires, portable capillary blood glucose assessments, and laboratory evaluations performed at various cutoff points for blood glucose. [9] A positive screening test indicates the individual is more likely to have diabetes compared to an individual with a negative screening test and can also diagnose diabetes with methods that include blood glucose testing. [9] Questionnaires are a screening tool used for asymptomatic type 2 diabetes, requesting demographic, behavioral, and medical information about an individual. [9] Popular because of their low cost price, questionnaires often do not perform well when used as a testing method. Alternatively, random blood glucose, fasting plasma glucose, glycated hemoglobin (HbA1c), 50-g glucose challenge test, and 75-g oral glucose tolerance test are some of the available biochemical test options. [9] In one recent study, the glucose challenge test (GCT) was examined to be an accurate, convenient, and low cost screening option since gestational diabetes behaves similarly to early glucose tolerance. [10] A combination of screening tests can also be completed, either simultaneously or sequentially. For example, a questionnaire performed to reveal people at high risk for diabetes could be followed by a blood glucose test. Subsequently, a 2h-OGTT would be performed if the blood glucose levels surpassed a certain cutoff level. Additionally, a Hong Kong study evaluated combining FPG and A1c as a screening method and observed that 80% of 2h-OGTTs would be avoided by screening through this paired testing approach. [11]

Several circumstances support the relevance of screening tests including the extensive health burdens and health care costs associated with diabetes. As mentioned earlier, the number of diabetics in the US is rising rapidly and is currently considered the seventh leading cause of

death in the general population [1]. Further, this suggests that the amount of medical resources spent will only increase. Through improved screening tests, not only would people earlier in diabetes history be identified but the large number of undiagnosed individuals could also be more easily detected. In addition to alleviating burdens, the natural history of type 2 diabetes is well known, making screening tests a viable measure [9]. Prior to disease recognition, a period follows biologic onset in which diabetes is undetectable. After this period, symptoms develop and complications start and result in major disability or death [9]. Currently there is evidence if prediabetes is detected with impaired glucose tolerance in a 2h-OGTT, diabetes can be delayed or even prevented with either lifestyle changes or medications. [5] Glucose levels become difficult to lower back to normal as beta cell mass and function are lost, which may be prevented by an earlier diagnosis in the diseases natural history. [10] In addition, using exercise, diet regimen, or medical treatment earlier in the diagnosis is thought to produce better benefits compared to when treatment is delayed. Also, subjects could improve glycemic management by earlier screening test detection.

Current Screening/Testing Options

Currently, there are no standardized cutoff points chosen to determine a positive screening test, but the ADA has recommended thresholds for diagnostic testing. [9] However, such points are arbitrary but based on relationships between blood glucose and rise of developing complications of diabetes. [12] These diagnostic tests include FPG, the 2 hour value in the 75-g 2h-OGTT, and recently A1c [13]. Glucose challenge test and random plasma glucose are also available however have been mainly used for screening and not definitive diagnosis. GCT is mainly used for gestational diabetes screening in pregnant women while RPG can be used in a wide population but also requires additional symptoms to be present.

Oral glucose tolerance test (2h-OGTT) has been regarded as the "gold standard" for identifying diabetes with maximum sensitivity and specificity. [14] However, many doctors do not prefer this test, in particular in primary care, due to its inconvenience on individuals and patients aversion to schedule. [5] While it can easily be performed in a doctor's office, individuals must fast, having no caloric intake at least 8 hours before testing. An initial blood sample is drawn then the patient receives a high sugar drink and has their blood drawn at 2 hours. [15] In a diabetic, blood sugar levels will elevate more, and return to baseline much slower than a person without diabetes. [6] Diabetes is defined by 2h-OGTT as ≥199 mg/dL and pre-diabetes is identified between 140 mg/dL and 199 mg/dL for the two hour test. [16] 2h-OGTT has been criticized for its variability since accuracy of testing can be affected by patient's diet and fasting length, raising the issue of its reproducibility. [17] 2h-OGTT is also more expensive and time inefficient, especially when needed for large screening programs. [17] An additional advantage of 2h-OGTT is detecting impaired glucose tolerance levels which is helpful since it discloses that a subject is at high-risk for diabetes and cardiovascular disease. [18]

Recently, the American Diabetes Association (ADA) expanded its diagnostic recommendations for diabetes testing to include hemoglobin A1c. [16] A1c offers a non-fasting test option that can classify individuals with both high-risk prediabetes and diabetes. [19, 20] Currently, this new A1c suggestion faces some criticism and needs further evaluation. Some argue A1c has low sensitivity and is not sufficient in diagnosing the disease, as recent analysis has demonstrated. [21, 22] The hemoglobin A1c test measures individuals' average blood glucose control over a period of time, usually 3 to 4 months.[21] The percentage of glycated hemoglobin in the blood is measured and 5.7% to 6.4% has been suggested as a positive high-risk prediabetes diagnosis while 6.5% or higher indicates diabetes. [16] A1c samples possess less biologic variability than

blood glucose values, do not fluctuate from acute effects such as stress or illness, and remain stable after collection and stored at room temperature. [23] However, in certain individuals with specific types of anemia and hemoglobinopathies, care must be taken when performing these tests to not cause misleading results. [23] Additionally, using the A1c test, diabetes incidences vary among ethnicities causing some debate regarding different cutoffs for different races. [17] Further, A1c is often more expensive than other test options and is not widely available in developing countries. [23] The advantage of A1c, despite low sensitivity, is its convenience; therefore, promoting broader use of the test and potentially more diabetes diagnoses. In a study of a Dutch population, less than one-third of individuals already known to have a prediabetes diagnosis fell within the currently defined range for type 2 high-risk prediabetes of 6.0% and 6.4%. [19] However, using the ADA's recommendation of A1c diagnostic criteria which are more inclusive, people with high-risk prediabetes potentially could be missed or could be inappropriately labeled.

Another widely used test is fasting plasma glucose (FPG) performed on a patient in a fasting state. A blood sample is drawn, and if it measures greater than 125 mg/dL then a positive diabetes diagnosis is made; a measure greater than or equal to 100 mg/dL but less than 125 mg/dL constitutes prediabetes.[16] An advantage of FPG is its inexpensive cost compared to 2h-OGTT and convenience regarding time. [17] However, FPG provides information only on fasting glucose levels and is less sensitive than 2h-OGTT. [17] Prior to the ADA's recent recommendation of A1c, FPG had been a commonly administered test. [24]

RPG or random plasma glucose measures blood glucose levels without requiring the person to fast. This test is used to identify diabetes determined by a blood glucose level of 200 mg/dL or

higher plus an additional symptom including increased urination, thirst, and unexplained weight loss, but is not used to diagnose high-risk prediabetes. [5]

Study Goals

Regardless of test used, it is ideal to identify as many people with this disease as possible. Since A1c is debated as a reliable method due to poor sensitivity for diabetes and both poor sensitivity and specificity for high-risk prediabetes, it is important to determine a screening test method effective in identifying the most people with diabetes and is relatively inexpensive and time efficient. Each test discussed above, 2h-OGTT, A1c, FPG, RPG, and GCT, has been evaluated alone and this study looks further at pairing tests together. The current analysis was performed to compare single tests, identify single tests as different from the paired tests, and to evaluate the paired tests with each other. In addition, this study examined cutoff points for the best combined test resulting in maximized sensitivity and specificity. The tests cutoff points would represent a convenient and reliable screening method replacing 2h-OGTT. Our analysis of this study suggests a screening method does exist that could be beneficial in recognizing people in the early natural history of diabetes. With this information and through further research, a diabetes testing algorithm using currently available tests could be developed to achieve optimal performance in terms of identifying individuals that are at increased risk and at early stages of diabetes. This would aid in minimizing the burden on patients and associated costs. Such algorithm could be tailored to specific subpopulations, e.g., different racial groups or age groups.

Methods

Protocol and Measurements

The Screening for Impaired Glucose Tolerance Study (SIGT) enrolled patients between January 1, 2005 and March 31, 2008. Participants volunteered to participate and were recruited through posters, flyers, and other media notices. Community members, employees of the Grady Health System, Emory HealthCare, and Emory University and Morehouse School of Medicine were included. Eligible individuals had no prior diagnosis of diabetes, were not pregnant or nursing, not taking glucocorticoids and were well enough to have worked during the previous week. However, working was not an inclusion criterion. Initially, 4,024 people were interested in the study and of those 2,111 were scheduled for first visits. The study protocol was completed by 1,581 individuals and 1,573 had complete data on GCT, 2h-OGTT, A1c, and RPG.

The Emory University Institutional Review Board approved the SIGT study, which was completed during outpatient visits at Emory University and Grady Memorial Hospitals. At the participants' first visit, random plasma glucose and random capillary glucose measurements were taken, since prior fasting is not required. Next, subjects drank a 50 g oral glucose within 5 minutes and after 1 hour had measurements of plasma and capillary glucose obtained. During the patient's second visit a 2h-OGTT (75 g) was completed before 11 hours following an overnight fast. Samples were drawn at baseline, 1 and 2 hours. Additionally, plasma lipids and HbA1c were also taken from blood samples during the 2h-OGTT blood draw. The tests were completed within a maximum of 2 weeks. In addition to blood samples, health surveys were also performed for collecting general patient health information.

Sodium fluoride/oxalate preservative was used to acquire blood glucose samples and within 30 minutes of testing, samples were frozen at -80°C. [10] Hemoglobin A1c was obtained through a laboratory procedure endorsed by the National Glycohemoglobin Standardization Program. {Phillips, 2009 #1107}

Analysis

The final sample of our study included 1,573 subjects. Using ADA (American Diabetes Association) criteria, A1c ≥ 6.5%, FPG ≥ 126 mg/dl, 2-hour plasma glucose ≥ 200 mg/dl from an 2h-OGTT, or RPG ≥ 200 mg/dl signifies a positive diagnosis for diabetes. High-Risk prediabetes is diagnosed by A1c values between [5.7%, 6.4%], 2-h plasma glucose varying between 140 mg/dl and 199 mg/dl, or FPG values within the 100 mg/dl and 125 mg/dl range. Each subject had information for each of these measurements in addition to age, race (either black or white), sex, and body mass index (BMI).

Means and standard deviations for continuous variables were reported and frequencies were calculated for binary variables. Means between the false negative and true positive groups were compared by using a two sample t-test. To evaluate the effectiveness of diabetes diagnostic tests, receiver operating characteristic (ROC) curve analysis was performed. [25] The ROC curve is a graphical representation of the true positive rate (sensitivity) as a function of the false positive rate (1-specificity) at different cut-off points. [25]The most accurate prediction would result in a point in the upper left corner of the map, representing 100% sensitivity and 100% specificity. The five tests, namely, 2h-OGTT, FPG, RPG, A1c, and GCT, were compared based on the accuracy for predicting the disease status of interest by using ROC curve analysis. Four different definitions of disease status were considered: 1) FPG ≥ 110 mg/dl or 2h-OGTT ≥ 140 mg/dl

(high-risk prediabetes), 2) 2h-OGTT>199 mg/dl or FPG>125 mg/dl, 3) 2h-OGTT>199 mg/dl or A1c ≥ 6.5%or FPG>125 mg/dl and 4) 2h-OGTT>199 mg/dl or A1c ≥ 6.5%. For each definition of disease status, ROC curve analysis was performed for individual tests as well as for combinations of two tests. In the case of combinations of two tests, a best linear combination of the two diagnostic test values was constructed before standard ROC curve analysis was performed. [26] Specifically, assuming that X_1 and X_2 are the values of the two tests of interest, we first create a linear score: $U=\beta_1 X_1+ \beta_2 X_2$ where the coefficients (β) are estimated through logistic regression with the disease status of interest as the outcome variable. [27] Subsequently, the ROC curve analysis can be performed for the linear score U. [27] [4] C-statistics were reported as measures of the area under the ROC curve (AROC) for each singular and paired test and 95% confidence intervals were obtained for this value. The computed ROC curve was tested against a ROC curve due only to chance (AROC=0.5); namely, 0.5 signifies the ROC curve of a diagnostic test with no power to differentiate individuals with diabetes against those without (AROC = 0.5). C-statistics for each single test were compared against the other tests, both single and paired, to determine which tests were significantly different from one another. The paired tests were evaluated against one another also. P-values were compared for statistical significance to 0.05. All analyses were performed in SAS version 9.3 (SAS Institute, Inc., Cary, NC).

After computing and comparing AROC between all singular and paired tests, the test with the highest AROC value was chosen to represent the best test to replace 2h-OGTT. Furthermore, the sensitivity and specificity of the selected test were evaluated at a series of cut-off points, including FPG values greater than or equal to: 80, 90, 100, 110, 120 and GCT values greater than or equal to: 120, 130, 140, 150, and 160. Given sensitivity and specificity, the Youden index: $J = \max \{Sen + Spe - 1\}$ was computed to assess diagnostic accuracy for each cutoff. [28] The

Youden Index ranges between 0 and 1, and represents the maximum distance from the “chance” line to the curve. [5] The maximum value of J can be considered as the measure of highest diagnostic effectiveness. [28] Therefore, the Youden indices computed at the series of cutoffs were used to determine the optimal cutoff for the selected test, for example, FPG $\geq$ 80 and GCT $\geq$ 120.

Results

Demographics of Participants:

The SIGT study included 1,573 subjects for analysis. The study enrolled 914 (58.1%) females while 659 (41.9%) males participated (Table 1). Race was divided into two categories, 912 (58%) black individuals and 661 (42%) white subjects. Age ranged from 18 to 87 years old with a mean age of 47.9 years. The average body mass index (BMI) was 30.3 km/m^2 ranging between 15.5 to 64.9 km/m^2 (Table 1). Using the criteria established by the ADA, 336 individuals were recognized as high-risk prediabetes and 34 as positive for diabetes through A1c measures (Table 3). A1c values for the participants ranged from 4.2% to 11.03% with a mean of 5.43%. FPG values varied between 44 mg/dl and 274 mg/dl with an average of 95.3 mg/dl. According to the FPG thresholds, 434 subjects were classified as pre-diabetic and 42 were diabetic (Table 3). The 2 hour plasma glucose measure from an 2h-OGTT test varied from 30 mg/dl to 370 mg/dl with a mean at 112.5 mg/dl. Two hundred and fifty-one subjects had 2h-OGTT values indicating high-risk prediabetes while 57 would be classified as diabetic (Table 3). A diabetic diagnosis from RPG must be made with additional presence of symptoms; RPG values ranged from 56 mg/dl to 255 mg/dl and 6 individuals had RPG values indicated diabetes (Table 3). GCT measurements while mainly used to diagnose gestational diabetes were between 51 mg/dl to 241 mg/dl with an average value at 133.3 mg/dl.

Diabetes Definition as 2h-OGTT>199 or FPG>125

As an existing "gold standard", the diabetes outcome defined as 2h-OGTT > 199 mg/dL or FPG > 125 mg/dL discovered 72 individuals with a positive diabetes diagnosis. RPG and A1c were not used as part of the standard diabetes definition. ROC analysis was used to evaluate the ability

of each test to identify a positive diabetes outcome. Analyzing all subjects (n=1573), the area under the ROC curve for 2h-OGTT was 0.95 (95% CI: 0.91-0.98), FPG was 0.93 (95% CI: 0.89-0.98), GCT was 0.895 (95% CI: 0.86-0.94), RPG was 0.83 (95% CI: 0.78-0.87), and A1c was 0.82 (95% CI: 0.75-0.88) (Table 2).

We then "screened" individuals using the tests A1c, FPG and GCT first. When restricted to individuals with A1c $\geq$ 5.7%, the analysis included 370 subjects. Area under the curve for 2h-OGTT was 0.96 (95% CI: 0.92-0.997), FPG was 0.94 (95% CI: 0.89-0.98), GCT was 0.89 (95% CI: 0.84-0.93), and RPG was 0.81 (95% CI: 0.75-0.87) (Table 2). Four hundred and twenty-three individuals were included when limiting to FPG > 100 mg/dl. Listed in Table 2 for 2h-OGTT, AROC was 0.91 (95% CI: 0.86-0.96); GCT was 0.82 (95% CI: 0.76-0.88); A1c was 0.79 (95% CI: 0.72-0.86); RPG was 0.76 (95% CI: 0.69-0.82). Considering subjects with GCT > 140 mg/dl included 599 people with AROC for 2h-OGTT as 0.96 (95% CI: 0.92-0.99). FPG was 0.91 (95% CI: 0.86-0.96), A1c was 0.81 (95% CI: 0.74-0.88), and RPG – 0.78 (95% CI: 0.72-0.84) (Table 2). All AROC's in table 2 were significant when compared to chance ($p<0.0001$).

Overall when analyzing all subjects, 2h-OGTT, FPG, and GCT were each significantly different ($p<0.02$) from A1c and RPG (Table 4). A1c compared to RPG in table 4 was not significant as well as FPG compared to 2h-OGTT and GCT. However, when examining GCT and 2h-OGTT there was a statistically significant difference ($p=0.002$).

Additionally, tests were combined to consider the increased value of two tests displayed in table 2. OGTT was excluded from the paired tests since the goal is to produce OGTT like results using simpler tests. FPG + GCT produced the highest area value, .9633, followed by FPG + RPG and FPG + A1c which shared nearly identical AROC values, .9323 and .932 consecutively. The

remaining paired tests were ordered in decreasing value GCT + A1c, RPG + GCT, and finally RPG + A1c.

The majority of combined tests were significantly different from their individual matching parts (Table 5). Interestingly, FPG alone was not statistically significant when compared to either adding RPG or A1c for a paired testing option. In addition, the combination of RPG and A1c was no different from RPG alone but was statistically significant when compared to A1c alone ($p=0.001$). GCT evaluated against GCT + A1c was barely insignificant ($p=0.051$). FPG + GCT had not only the highest AUC of the combined tests, but was also found to be statistically significant from every other paired test ($p<0.03$) (Table 6). RPG + A1c appeared to be the worst choice in terms of lowest AUC and was significantly lower than all other combined options (Table 6). This testing combination would have been ideal since it is the two most inexpensive and convenient choices.

The greatest Youden index, 0.744, was provided by the cutoffs of GCT $\geq$ 120 and FPG $\geq$ 110 (sensitivity 81%, specificity 94%; Table 8). Using GCT first and subsequently FPG with these cutoffs would result in a test correctly identifying about 58 of the 72 positive individuals. To better understand what subjects potentially would be misclassified by these cutoffs, characteristics of the false positives and false negatives were compared. Fourteen people would be identified as negative when they actually were positive for diabetes. Characteristics of the 14 people identified as falsely negative had a FPG mean value of 102 mg/dl (std. dev. = 15.94), 5.45% for A1c mean (std. dev. = 0.4), and an 2h-OGTT mean value of 203 mg/dl (std. dev. = 34.1). Roughly, 92 subjects were incorrectly classified as positive. These individuals FPG mean value was 114.5 mg/dl (std. dev. = 4.5), 5.7% for A1c (std. dev. = 0.5), and 140 mg/dl for 2h-OGTT (std. dev. =35.8). Evaluating the 2-h OGTT and A1c means from the true positives

against the false negatives presented a statistically significant difference between the A1c values but not for 2h-OGTT.

Diabetes Definition: High-Risk Pre-diabetes

Being at high risk for prediabetes in this study was defined as FPG ≥ 110 mg/dl or 2h-OGTT ≥ 140 mg/dl and included 294 positive individuals. AROC analysis was used once again to determine the tests ability to predict a positive definition of high-risk prediabetes. Since this analysis eliminated subjects with FPG and 2h-OGTT values equal or above 126 or 200 respectively, 1,501 non-diabetic individuals remained to be analyzed. Similarly to the other defined outcomes, 2h-OGTT was followed by FPG, GCT, A1c, and RPG according to the area under the curve in decreasing order (Table 9). However, the difference between 2h-OGTT and the subsequent tests was much larger. 2h-OGTT was statistically different from all other single tests ($p<0.0001$; Table 10). GCT was no different from FPG but was significantly different compared to the remaining tests (Table 10). Similarly, evaluating FPG showed statistical difference compared with all other tests but GCT ($p<0.0001$; Table 10). Additionally in Table 10, A1c compared to RPG was insignificant but with all other tests was significantly less. RPG was also significantly less than the other tests ($p< 0.0001$), besides A1c (Table 10).

The highest area under the curve for paired tests corresponded to FPG + GCT (AROC=0.8512), followed by FPG + RPG, FPG + A1c, RPG + GCT, GCT + A1c, and lastly RPG + A1c (Table 9). When evaluating combined tests with single tests, all comparisons were significantly different (all $p<0.03$; Table 11). A significant negative difference was observed between FPG + A1c vs. RPG + A1c ($p<0.0001$; Table 12). FPG + GCT had a significantly higher AUC value than FPG + A1c ($p<0.0001$). All other comparisons with FPG + A1c did not demonstrate statistical

significance. RPG + A1c was significantly different from all other combined tests with the lowest area under the curve (Table 12). Similarly in Table 12, with the highest area under the curve FPG + GCT was statistically different from all other tests ($p<0.0004$).

High-Risk prediabetes was predicted best by the test combination FPG + GCT and was used to test for the maximum diagnostic effectiveness. The cutoff selected was FPG ≥ 90 and GCT ≥ 130 with maximized sensitivity and specificity 75% and 72%, respectively (Table 13). This cutoff correctly identified 220 individuals with prediabetes but incorrectly classified 74 people as negative. Three hundred and thirty nine subjects were wrongly labeled as positive for high-risk diabetes but 868 people were accurately recognized as not having diabetes.

Diabetes Definition as 2h-OGTT>199 or A1c ≥ 6.5%

Seventy-one individuals were identified diabetic through the outcome 2h-OGTT > 199 mg/dL or A1c ≥ 6.5%, a different definition of diabetes combining glucose and hemoglobin A1c levels. Considering all subjects (n=1573), 2h-OGTT had the highest area under the curve with 0.95, followed by GCT equaling 0.89 and A1c at 0.88. The AROC was 0.87 for FPG and 0.79 for RPG to identify diabetes (Table 14). This differs from others outcomes analysis since A1c produced a higher AUC value than FPG. Additionally, GCT had the second highest AUC value whereas FPG did with the first outcome. With analysis restricted to only individuals with A1c values higher than 5.7%, a similar order was observed with the previous diabetes definition with 2h-OGTT the highest followed by FPG, GCT, and then RPG. Further, area under the ROC curve for subjects with FPG values greater than 100 were arranged identically to the previous outcomes order: 2h-OGTT, GCT, A1c, and RPG. Also, tests were ordered in decreasing manner for those with GCT > 140 mg/dL as 2h-OGTT, FPG, A1c, and RPG.

Examining c-statistics for the single tests revealed FPG was no different from A1c or GCT, but was significantly different from RPG ($p=0.001$; Table 15). Further, RPG was significantly lower than all single tests ($p<0.04$; Table 15). From Table 15, GCT wasn't significantly different from A1c. When comparing tests to the "gold standard", 2h-OGTT, each test was found to have a significantly lower AUC (Table 15). Unlike the analysis for 2h-OGTT>199 or FPG > 125, the highest AUC for combined tests was 0.9565 from GCT + A1c (Table 14). The next highest value was 0.924 from FPG + A1c followed by 0.917 from FPG + GCT, RPG + A1c (0.909), RPG + GCT (0.898), and RPG + FPG (0.87). Only two paired tests were found not significantly different from single tests. These were FPG versus FPG + RPG and GCT compared with GCT + RPG (Table 16). Comparing combined tests with each other, GCT + A1c, the highest AUC, was no different from FPG + A1c or FPG + GCT but significantly higher than the remaining pairs (Table 17). Additionally with FPG + RPG as the reference category, FPG + A1c and FPG + GCT were both statistically significant with higher area under the curve (Table 17).

Diabetes Definition as 2h-OGTT>199 or A1c≥6.5 or FPG>125

Eighty-two subjects were identified positive from the complete ADA definition of diabetes. As expected, 2h-OGTT (AROC=0.9212) produced the highest area under the curve. FPG, GCT, A1c, and RPG followed with their respective c-statistics values, 0.8941, 0.8645, 0.8435, and 0.7809 (Table 18). Within these results, 2h-OGTT was always the highest AUC while RPG was consistently the lowest. 2h-OGTT was significantly higher than all other single tests except FPG; RPG was significantly lower than all other single tests with the exception of A1c (Table 20). FPG was only statistically different when compared to RPG; when analyzing A1c as the reference test, 2h-OGTT was the only significant difference found.

If the population had been initially screened with the A1c values greater than or equal to 5.7%, 370 individuals would be identified for further classification. 2h-OGTT had the highest area under the curve; FPG, GCT, and RPG were the results following (Table 19). For the 599 individuals examined first with GCT>140, the tests were ordered 2h-OGTT, FPG, A1c, and RPG in decreasing value.

Among the 82 subjects, the highest AUC for combined tests was FPG + A1c, 0.9335, closely followed by FPG + GCT with area under the curve 0.9241. The subsequent tests were GCT + A1c, RPG + FPG, RPG + GCT, and finally RPG + A1c. The majority of combined tests were significantly different from the single tests, with the exception of FPG + RPG vs. FPG and RPG + GCT vs. GCT (Table 20). The test FPG + A1c compared to other paired options was significantly higher than FPG + RPG and RPG + A1c, but not when evaluated against the remaining pairs (Table 21). RPG + A1c, the lowest AUC, was no different from RPG + GCT, FPG + GCT, and FPG + RPG.

Discussion

Principal Findings

While the 2h-OGTT is the standard to identify diabetes with maximum sensitivity and specificity, it is inconvenient and time-consuming and hence may not be suitable for screening, in particular opportunistic screening in primary care. Currently, there is no consensus as to which screening strategy is best for detecting individuals with high-risk prediabetes or diabetes in the general population. This analysis demonstrated a suitable screening strategy using a glucose challenge test first followed by fasting plasma glucose. An AROC of 0.85 and 0.96 to detect high-risk prediabetes and the standard diabetes status (2h-OGTT>199 or FPG>125) respectively resulted from this study. However, with the other two outcomes (2h-OGTT > 199 mg/dl or A1c ≥ 6.5% and 2h-OGTT > 199 mg/dl or A1c ≥ 6.5% or FPG > 125), different paired tests were revealed as the highest area under the curve. Paired tests in these two outcomes became harder to distinguish while FPG + GCT was clearly superior in the first two diabetes definitions.

Fairly consistent results were obtained from all four outcomes. Regardless of the diabetes definition, 2h-OGTT had the highest AROC analyzing all subjects, followed by FPG and GCT. However, in the outcome combing glucose and A1c measurements (2h-OGTT > 199 or A1c ≥ 6.5%), A1c was ranked higher than FPG but was not found significantly different from FPG or GCT. In general for the outcomes, 2h-OGTT performed the best against the other single tests. Only in the two categories defined as 2h-OGTT >199 or FPG > 125 and 2h-OGTT > 199 or A1c ≥ 6.5% or FPG > 125 was the "gold standard" test not significantly different when compared to FPG. Further, there was no difference between FPG and GCT in any of the outcomes. Based on predictive accuracy, 2h-OGTT and FPG were the two strongest tests. These two tests are

currently endorsed by the ADA as diagnostic tools for both diabetes and high-risk prediabetes. [13] RPG and A1c, routinely the lowest AROCs, were no different when compared in all but one diabetes status.

Review of A1c

Generally, A1c had one of the lowest areas under the curve when analyzing all patients. The performance of other tests was not improved by pairing them with A1c since all paired tests that included A1c were not statistically different when compared to FPG, RPG, or GCT. But when comparing these paired tests to A1c, all were found to have a significantly higher area under the curve. Interestingly, A1c has been recently recommended by the ADA as a diagnostic test for diabetes. [20] Although the ADA endorses A1c as a diagnostic test, our study evaluated it also as a screening option. Conclusive results regarding A1c as a suitable screening test were not found. While it is convenient, A1c continually ranked as one of the lowest predictors of diabetes or high-risk prediabetes in the current analysis, which is consistent with many recent findings. One study noted that fifty million people previously identified with high-risk prediabetes would be missed using A1c in an analysis of the NHANES 1999-2006. [29] Another study also noted the large number of individuals incorrectly diagnosed when using A1c criteria and questioned its validity as a screening test. [19] Additional weaknesses reported for A1c are low specificity and racial variability. [22] A1c is a simple and convenient test but may perform poorly for identifying the undiagnosed portion of Americans with diabetes. Further evaluation of A1c is needed to determine its suitability as a possible screening test.

Glucose Challenge Test

The glucose challenge test is administered on pregnant women for diagnosis of gestational diabetes. However, using this test might be beneficial since it involves glucose measurements but is more convenient than performing a 2h-OGTT. Our study found the glucose challenge test to be an accurate method for identifying diabetes at the various definitions. GCT, when compared with other single tests, repeatedly performed better than RPG and A1c in all but 1 case. In addition, GCT was close in performance when compared with FPG, a diagnostic test used widely now. Another study also identified GCT as an appropriate screening method noting the tests consistent measurements, non-fasting nature, and inexpensive cost as advantages. [10] Additionally, when analyzing the paired tests for highest area under the curve, three of the four diabetes outcomes identified a combination with GCT as the test with the best performance. Applying GCT to the general population would not only identify people with diabetes but be a resource efficient way to do so. It would easily be performed in a doctor's office at any time of day and requires no fasting.

Paired Tests in Previous Literature

Studies previously examining screening strategies have not concentrated on the idea of pairing tests together to replace 2h-OGTT but rather on already existing ideas, such as risk factor questionnaires, glucose measurements or a combination of the two. [9] Some questionnaires report an AROC of about 0.80, which in our study is well below what some of the single tests and paired tests offer. [10] Depending on the disease status, the following paired tests had the highest area under the curve: FPG + GCT, GCT + A1c, and FPG + A1c. One questionnaire based on information gathered from the National Health and Nutrition Examination Survey resulted in sensitivity of 79% and specificity of 65%. Comparing these results to our study findings, the paired test chosen to represent a potential screening test for diabetes resulted in

higher values for sensitivity and specificity, 81% and 94% respectively. Furthermore, typically the paired tests all had a significantly higher AUC than the matching single tests. This supports the idea that by combining information from both measurements a better diagnostic test may be obtained rather than each test alone. Using a multiple-step screening strategy allows the more convenient and less expensive test to be administered first, followed by the more sensitive and costly test. One main concern for screening is being able to identify individuals with undiagnosed diabetes. Using a simpler test at the beginning may help discover this group of individuals through a more widespread application. Utilizing a paired test increases the prevalence of disease in the second screening, meaning not as many subjects undergo the more expensive second test.

Limitations

The SIGT population included individuals in the Atlanta area and therefore is not a nationally diverse group of individuals. It would be very important to repeat the study and show whether the results from SIGT can be replicated in a nationally diverse population. This dataset only included black and white individuals; therefore, these results may not be appropriate for application to all ethnicities. Additionally, this research did not examine the tests prediction variability with age or racial differences, which are known risk factors for diabetes. Stratifying by these categories may offer beneficial insight regarding how well certain tests perform in different populations. Analyzing these risk factors may help eliminate unnecessary testing by decreasing the number of individuals screened.

Future Research

Although this analysis identified a potential screening method, its cost effectiveness is yet to be investigated. Screening has been supported as cost-saving and one study recognized GCT as the most economical test. [30] It would be beneficial to complete a cost analysis of these results, evaluating the expenses associated with the paired test chosen. Additionally, future research should investigate algorithms using the tests discussed throughout this paper. The algorithms could be adapted to certain races, age groups, genders, etc. by establishing test cut-offs corresponding to each of these factors. Doing this would provide a distinct and accurate screening method for high risk individuals.

Conclusions

Research projects diabetes to affect 29 million people or have a disease prevalence of 7.2% by the year 2050 in the United States. [31] This estimate acknowledges the serious public health problem diabetes presents. Advances in primary care screening and prevention may help reduce the burden and it is crucial to identify optimal screening strategies resulting in a more methodical screening process and also reducing associated cost. Through a screening strategy, individuals could be detected earlier in the diseases' natural history and complications could be alleviated. Our data and results do suggest that strong consideration should be given to screening. Performing this through a paired test such as FPG + GCT would be an accurate and simple technique to perform in general practices. Additionally, this study has potential to raise awareness of the importance in developing a screening test for identifying subjects at risk for diabetes.

References

1. Centers for Disease Control and Prevention. *2011 National Diabetes Fact Sheet*. 2012; Available from: http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf.

2. El Bassuoni, E.A., et al., *The "metabolic syndrome" is less useful than random plasma glucose to screen for glucose intolerance.* Primary care diabetes, 2008. **2**(3): p. 147-53.

3. *Harrison's Principles of Internal Medicine*. 18 ed, ed. D.L. Longo, et al. Vol. 1. 2012: The McGraw-Hill Companies, Inc.

4. *Executive summary: standards of medical care in diabetes--2011.* Diabetes care, 2011. **34 Suppl 1**: p. S4-10.

5. Ziemer, D.C., et al., *Random plasma glucose in serendipitous screening for glucose intolerance: screening for impaired glucose tolerance study 2.* Journal of general internal medicine, 2008. **23**(5): p. 528-35.

6. American Diabetes Association. *Diabetes Basics: Type 2*. [cited 2012 January 24]; Available from: http://www.diabetes.org/diabetes-basics/type-2/.

7. American Diabetes Association. *Living with Diabetes: Complications*. Available from: http://www.diabetes.org/living-with-diabetes/complications/.

8. Li, R., et al., *Cost-effectiveness of interventions to prevent and control diabetes mellitus: a systematic review.* Diabetes care, 2010. **33**(8): p. 1872-94.

9. Engelgau, M.M., K.M. Narayan, and W.H. Herman, *Screening for type 2 diabetes.* Diabetes care, 2000. **23**(10): p. 1563-80.

10. Phillips, L.S., et al., *Glucose challenge test screening for prediabetes and undiagnosed diabetes.* Diabetologia, 2009. **52**(9): p. 1798-807.

11. Ko, G.T., et al., *Combined use of a fasting plasma glucose concentration and HbA1c or fructosamine predicts the likelihood of having diabetes in high-risk subjects.* Diabetes care, 1998. **21**(8): p. 1221-5.

12. Lu, Z.X., et al., *A1C for screening and diagnosis of type 2 diabetes in routine clinical practice.* Diabetes care, 2010. **33**(4): p. 817-9.

13. *Standards of medical care in diabetes--2011.* Diabetes care, 2011. **34 Suppl 1**: p. S11-61.

14. Saudek, C.D., et al., *A new look at screening and diagnosing diabetes mellitus.* The Journal of clinical endocrinology and metabolism, 2008. **93**(7): p. 2447-53.

15. American Diabetes Association. *Diabetes Basics: Prevention - Pre-diabetes*. Available from: http://www.diabetes.org/diabetes-basics/prevention/pre-diabetes/.

16. *Standards of medical care in diabetes--2010.* Diabetes care, 2010. **33 Suppl 1**: p. S11-61.

17. Luijf, Y.M., et al., *The added value of oral glucose tolerance testing in pre-diabetes.* Current diabetes reviews, 2011. **7**(1): p. 56-60.

18. Bartoli, E., G.P. Fra, and G.P. Carnevale Schianca, *The oral glucose tolerance test (OGTT) revisited.* European journal of internal medicine, 2011. **22**(1): p. 8-12.

19. Bersoux, S., et al., *Hemoglobin A1c testing alone does not sufficiently identify patients with prediabetes.* American journal of clinical pathology, 2011. **135**(5): p. 674-7.

20. *International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes.* Diabetes care, 2009. **32**(7): p. 1327-34.

21. Kramer, C.K., M.R. Araneta, and E. Barrett-Connor, *A1C and diabetes diagnosis: The Rancho Bernardo Study.* Diabetes care, 2010. **33**(1): p. 101-3.

22. Olson, D.E., et al., *Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria.* Diabetes care, 2010. **33**(10): p. 2184-9.

23. Cowie, C.C., et al., *Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006.* Diabetes care, 2010. **33**(3): p. 562-8.

24. Carson, A.P., et al., *Comparison of A1C and fasting glucose criteria to diagnose diabetes among U.S. adults.* Diabetes care, 2010. **33**(1): p. 95-7.

25. Pepe, M., *The Statistical Evaluation of Medical Tests for Evaluation and Prediction*. Oxford Statistical Science Series2003: Oxford University Press.

26. Ziemer, D.C., et al., *Age, BMI, and race are less important than random plasma glucose in identifying risk of glucose intolerance: the Screening for Impaired Glucose Tolerance Study (SIGT 5).* Diabetes care, 2008. **31**(5): p. 884-6.

27. Perkins, N.J., E.F. Schisterman, and A. Vexler, *ROC curve inference for best linear combination of two biomarkers subject to limits of detection.* Biometrical journal. Biometrische Zeitschrift, 2011. **53**(3): p. 464-76.

28. Perkins, N.J. and E.F. Schisterman, *The Youden Index and the optimal cut-point corrected for measurement error.* Biometrical journal. Biometrische Zeitschrift, 2005. **47**(4): p. 428-41.

29. Mann, D.M., et al., *Impact of A1C screening criterion on the diagnosis of pre-diabetes among U.S. adults.* Diabetes care, 2010. **33**(10): p. 2190-5.

30. Chatterjee, R., et al., *Screening adults for pre-diabetes and diabetes may be cost-saving.* Diabetes care, 2010. **33**(7): p. 1484-90.

31. Boyle, J.P., et al., *Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence.* Population health metrics, 2010. **8**: p. 29.

Tables

Table 1. Descriptive statistics for study participants.

Variable	Overall (n=1573) Mean (SD) or n (%)
Sex ***Female*** ***Male***	 914 (58.1%) 659 (41.9%)
Age (years)	47.9 (12.2)
Race ***Black*** ***White***	 912 (57.98%) 661 (42.02%)
BMI (kg/m^2)	30.3 (6.8)

Table 2. Analysis of Receiver Operating Characteristic Curve with Diabetes defined as OGTT > 199 or FPG > 125 (N = 72).

	Test	**Area under the ROC curve**	**95% CI**	**P-value (AROC = 0.5)**
All Subjects (N=1573)	*FPG*	0.93	0.885-0.975	<0.0001
	RPG	0.826	0.779-0.873	<0.0001
	A1C	0.8179	0.7548-0.8811	<0.0001
	GCT	0.8951	0.8566-0.9366	<0.0001
	OGTT	0.9483	0.9127-0.9839	<0.0001
A1C >= 5.7 (N=370)	*FPG*	0.9399	0.8857-0.9822	<0.0001
	GCT	0.8850	0.8353-0.9348	<0.0001
	RPG	0.8073	0.7459-0.8686	<0.0001
	OGTT	0.9585	0.9201-0.997	<0.0001
FPG > 100 (N=423)	*A1C*	0.7878	0.7163-0.8594	<0.0001
	GCT	0.8192	0.7596-0.8787	<0.0001
	RPG	0.7553	0.6862-0.8243	<0.0001
	OGTT	0.9127	0.8601-0.9654	<0.0001
GCT > 140 (N=599)	*A1C*	0.8118	0.7438-0.8798	<0.0001
	RPG	0.7751	0.7152-0.835	<0.0001
	FPG	0.9138	0.8641-0.9635	<0.0001
	OGTT	0.9554	0.9186-0.9923	<0.0001
All Subjects (N=1573)	*FPG + A1C*	0.932	0.8875-0.9765	<0.0001
	FPG + RPG	0.9323	0.8885-0.9761	<0.0001
	FPG + GCT	0.9633	0.9353-0.9913	<0.0001
	RPG + GCT	0.9188	0.8868-0.9508	<0.0001
	RPG + A1C	0.8635	0.8081-0.9189	<0.0001
	GCT + A1C	0.9191	0.8816-0.9567	<0.0001

Table 3. Diabetes Prevalence by American Diabetes Criteria (Overall n=1573)	
Diagnosis of Diabetes	N (%)
A1C Pre-Diabetes: 5.7% ≤ A1C ≥ 6.4%	336 (21.4)
A1C Diabetes: A1C ≥ 6.5%	34 (2.2)
FPG Pre-Diabetes: 100 ≤ FPG ≥ 125	434 (27.6)
FPG Diabetes: FPG ≥ 126	42 (2.7)
OGTT Pre-Diabetes: 140 ≤ OGTT ≥ 199	251 (16)
OGTT Diabetes: OGTT ≥ 200	57 (3.6)
RPG Diabetes: RPG ≥ 200	6 (.4)

Table 4. P-Values from Comparisons of Single Tests: OGTT > 199 or FPG > 125					
	FPG	**RPG**	**A1C**	**GCT**	**OGTT**
FPG	———	0.0001	0.001	0.25	0.55
RPG	0.0001	———	0.80	0.01	<0.0001
A1C	0.001	0.80	———	0.02	<0.0001
GCT	0.25	0.01	0.02	———	0.002
OGTT	0.55	<0.0001	<0.0001	0.002	———

Table 5. P-Values from Comparisons of Paired Tests vs. Corresponding Single Tests: OGTT > 199 or FPG > 125				
	FPG	**RPG**	**A1C**	**GCT**
FPG + A1C	0.52	———	0.0002	———
FPG + RPG	0.21	<0.0001	———	———
FPG + GCT	0.01	———	———	0.002
RPG + A1C	———	0.11	0.001	———
RPC + GCT	———	<0.0001	———	0.04
GCT + A1C	———	———	<0.0001	0.05

Table 6. P-Values from Comparisons of Paired Tests: OGTT > 199 or FPG > 125

	FPG + A1C	**FPG + RPG**	**FPG + GCT**	**RPG + A1C**	**RPG + GCT**	**GCT + A1C**
FPG + A1C	———	0.94	0.02	0.01	0.62	0.64
FPG + RPG	0.94	———	0.01	0.01	0.60	0.63
FPG + GCT	0.02	0.01	———	0.0002	0.02	0.03
RPG + A1C	0.01	0.01	0.0002	———	0.03	0.01
RPG + GCT	0.62	0.6	0.02	0.025	———	0.98
GCT + A1C	0.64	0.62	0.03	0.01	0.98	———

Table 7. Sensitivity and Specificity Calculations for Various Test Cutoffs (OGTT > 199 or FPG > 125).

FPG→ GCT↓	≥ 80	≥ 90	≥ 100	≥ 110	≥ 120
≥ 120	94.4% / 44.8 %	93.1% / 56.2 %	88.9% / 78.6 %	80.6% / 93.9%	66.7% / 98.8%
≥ 130	91.7% / 54.6 %	90.3% / 62.8 %	86.1% / 81.1%	77.8% / 94.4%	63.9% / 99%
≥ 140	90.3% / 64.8 %	88.9% / 70.2%	84.7% / 84.5%	76.4% / 95.2%	62.5% / 99.2%
≥ 150	83.3% / 73.6 %	81.9% / 77.2%	77.8% / 87.8%	70.8% / 95.9%	56.9% / 99.2%
≥ 160	80.6% / 81.1 %	79.2% / 83.4%	75% / 90.4%	68.1% / 96.5%	54.2% / 99.3%

Numbers shown are Sensitivity/Specificity

Table 8. Youden Index Calculation for OGTT > 199 or FPG > 125.

GCT/FPG	≥ 80	≥ 90	≥ 100	≥ 110	≥ 120
≥ 120	0.393	0.493	0674	0.744	0.655
≥ 130	0.463	0.53	0.672	0.722	0.629
≥ 140	0.55	0.591	0.693	0.716	0.617
≥ 150	0.57	0.591	0.656	0.668	0.561
≥ 160	0.616	0.626	0.654	0.645	0.534

Table 9. Analysis of Receiver Operating Characteristic Curve with High Risk Pre-Diabetes defined as OGTT > 140 or FPG > 100 (N = 294).				
	Test	**Area under the ROC curve**	**95% CI**	**P-value (AROC = 0.5)**
All Subjects (N=1501)	*FPG*	0.7993	0.7681-0.8305	<0.0001
	RPG	0.6978	0.6651-0.7305	<0.0001
	A1C	0.6745	0.6392-0.7099	<0.0001
	GCT	0.7879	0.7604-0.8155	<0.0001
	OGTT	0.9252	0.9018-0.9486	<0.0001
A1C >= 5.7 (N=317)	*FPG*	0.7954	0.7417-0.8492	<0.0001
	GCT	0.7912	0.7402-0.8442	<0.0001
	RPG	0.6675	0.6054-0.7297	<0.0001
	OGTT	0.9192	0.8772-0.9612	<0.0001
FPG > 100 (N=357)	*A1C*	0.6535	03.5966-0.710	<0.0001
	RPG	0.6466	0.5893-0.7039	<0.0001
	OGTT	0.8336	0.7877-0.8795	<0.0001
	GCT	0.7064	0.6531-0.7597	<0.0001
GCT > 140 (N=534)	*A1C*	0.6619	0.6134-0.7104	<0.0001
	RPG	0.6126	0.5634-0.6619	<0.0001
	FPG	0.7379	0.6924-0.7824	<0.0001
	OGTT	0.9435	0.9186-0.9684	<0.0001
All Subjects (N=1501)	*FPG + A1C*	0.8092	0.7791-0.8394	<0.0001
	FPG + RPG	0.8098	0.7795-0.8401	<0.0001
	FPG + GCT	0.8512	0.8264-0.8759	<0.0001
	RPG + GCT	0.805	0.7783-0.8314	<0.0001
	RPG + A1C	0.7298	0.6981-0.7616	<0.0001
	GCT + A1C	0.804	0.7774-0.8305	<0.0001

Table 10. P-Values from Comparisons of Single Tests: OGTT > 140 or FPG > 100

	FPG	RPG	A1C	GCT	OGTT
FPG	———	<0.0001	<0.0001	0.57	<0.0001
RPG	<0.0001	———	0.33	<0.0001	<0.0001
A1C	<0.0001	0.33	———	<0.0001	<0.0001
GCT	0.57	<0.0001	<0.0001	———	<0.0001
OGTT	<0.0001	<0.0001	<0.0001	<0.0001	———

Table 11. P-Values from Comparisons of Paired Tests vs. Corresponding Single Tests: OGTT > 140 or FPG > 100

	FPG	RPG	A1C	GCT
FPG + A1C	0.01	———	<0.0001	———
FPG + RPG	0.001	<0.0001	———	———
FPG + GCT	<0.0001	———	———	<0.0001
RPG + A1C	———	0.03	<0.0001	———
RPC + GCT	———	<0.0001	———	0.003
GCT + A1C	———	———	<0.0001	0.02

Table 12. P-Values from Comparisons of Paired Tests: OGTT > 140 or FPG > 100

	FPG + A1C	FPG + RPG	FPG + GCT	RPG + A1C	RPG + GCT	GCT + A1C
FPG + A1C	———	0.92	<0.0001	<0.0001	0.82	0.77
FPG + RPG	0.92	———	<0.0001	<0.0001	0.79	0.76
FPG + GCT	<0.0001	<0.0001	———	<0.0001	0.0004	0.0003
RPG + A1C	<0.0001	<0.0001	<0.0001	———	<0.0001	<0.0001
RPG + GCT	0.82	0.79	0.0004	<0.0001	———	0.9
GCT + A1C	0.77	0.76	0.0003	<0.0001	0.9	———

Table 13. Sensitivity and Specificity Calculations for Various Test Cutoffs (OGTT > 140 or FPG > 100).					
FPG GCT	≥ 80	≥ 90	≥ 100	≥ 110	≥ 120
≥ 120	87.1% 52.6%	80.3% 65.1%	56.1% 87%	31.3% 100%	6.1% 100%
≥ 130	80.9% 63.3%	74.8% 71.9%	52% 89.2%	28.6% 100%	5.1% 100%
≥ 140	71.1% 73.5%	66% 79%	45.2% 91.8%	24.5% 100%	4.1% 100%
≥ 150	58.2% 81.4%	54.1% 84.8%	37.4% 94%	20.8% 100%	4.1% 100%
≥ 160	45.9% 87.7%	42.9% 89.8%	31.6% 95.8%	18% 100%	3.7% 100%
Numbers shown are Sensitivity/Specificity					

Table 14. Youden Index Calculation for OGTT > 140 or FPG > 100.					
GCT/FPG	≥ 80	≥ 90	≥ 100	≥ 110	≥ 120
≥ 120	0.397	0.454	0.431	0.313	0.061
≥ 130	0.442	0.467	0.412	0.286	0.051
≥ 140	0.446	0.45	0.37	0.245	0.041
≥ 150	0.395	0.388	0.314	0.207	0.041
≥ 160	0.336	0.327	0.274	0.180	0.037

Table 15. Analysis of Area under the ROC curve with diabetes defined as OGTT>199 or A1C >= 6.5

	Test	ROC Curve Area	95% CI	P-Value (AROC = 0.5)
All Subjects (n=1573)	*A1C*	0.8773	0.8229-0.9317	<0.0001
	RPG	0.7926	0.733-0.8522	<0.0001
	FPG	0.8733	0.8165-0.9301	<0.0001
	GCT	0.8845	0.8379-0.931	<0.0001
	OGTT	0.9489	0.914-0.9837	<0.0001
A1C >=5.7 (n=370)	*RPG*	0.742	0.6648-0.8192	<0.0001
	FPG	0.8493	0.7789-0.9198	<0.0001
	GCT	0.8304	0.7653-0.8955	<0.0001
	OGTT	0.9083	0.8542-0.9625	<0.0001
FPG>100 (n=423)	*RPG*	0.7896	0.7209-0.8584	<0.0001
	A1C	0.8568	0.7929-0.9207	<0.0001
	GCT	0.8701	0.8196-0.9207	<0.0001
	OGTT	0.9609	0.928-0.9938	<0.0001
GCT>140 (n=599)	*RPG*	0.7914	0.7315-0.8513	<0.0001
	FPG	0.8923	0.8402-0.9444	<0.0001
	A1C	0.8529	0.7904-0.9153	<0.0001
	OGTT	0.9734	0.9537-0.9931	<0.0001
All Subjects (n=1573)	*FPG + A1C*	0.9236	0.879-0.9681	<0.0001
	RPG + A1C	0.9088	0.862-0.95555	<0.0001
	RPG + FPG	0.8726	0.8152-0.93	<0.0001
	FPG + GCT	0.917	0.8713-0.9628	<0.0001
	RPG + GCT	0.8982	0.8511-0.9453	<0.0001
	GCT + A1C	0.9565	0.9336-0.9795	<0.0001

Table 16. P-Values from Comparisons of Single Tests: OGTT>199 or A1C >= 6.5

	FPG	RPG	A1C	GCT	OGTT
FPG	———	0.001	0.9	0.68	0.005
RPG	0.001	———	0.03	0.0003	<0.0001
A1C	0.9	0.03	———	0.85	0.0001
GCT	0.68	0.0003	0.85	———	0.04
OGTT	0.01	<0.0001	0.04	0.0001	———

Table 17. P-Values from Comparisons of Paired Tests vs. Corresponding Single Tests: OGTT>199 or A1C >= 6.5

	FPG	RPG	A1C	GCT
FPG + A1C	0.03	———	0.003	———
FPG + RPG	0.87	0.001	———	———
FPG + GCT	0.01	———	———	0.03
RPG + A1C	———	0.001	0.003	———
RPC + GCT	———	<0.0001	———	0.2
GCT + A1C	———	———	0.0003	0.002

Table 18. P-Values from Comparisons of Paired Tests: OGTT>199 or A1C >= 6.5

	FPG + A1C	FPG + RPG	FPG + GCT	RPG + A1C	RPG + GCT	GCT + A1C
FPG + A1C	———	0.04	0.81	0.16	0.43	0.05
FPG + RPG	0.04	———	0.004	0.23	0.25	0.002
FPG + GCT	0.81	0.004	———	0.79	0.1	0.07
RPG + A1C	0.05	0.23	0.79	———	0.75	0.01
RPG + GCT	0.43	0.25	0.01	0.75	———	0.02
GCT + A1C	0.16	0.002	0.07	0.008	0.02	———

Table 19. Analysis of ROC Curve Area with diabetes defined as OGTT>199 or A1C >= 6.5 or FPG > 125 (n=82)

	Test	ROC Curve Area	95% CI	AROC test p-value
All Subjects (n=1573)	*A1C*	0.8435	0.7866-0.9005	<0.0001
	RPG	0.7809	0.7263-0.8355	<0.0001
	FPG	0.8941	0.8440-0.9441	<0.0001
	GCT	0.8645	0.8195-0.9094	<0.0001
	OGTT	0.9212	0.8817-0.9608	<0.0001
A1C >= 5.7 (n=370)	*RPG*	0.7383	0.6644-0.8121	<0.0001
	FPG	0.8654	0.7989-0.9313	<0.0001
	GCT	0.8365	0.7745-0.8984	<0.0001
	OGTT	0.9007	0.8474-0.954	<0.0001
FPG>100 (n=423)	*RPG*	0.7494	0.6806-0.8181	<0.0001
	A1C	0.8058	0.7374-0.8743	<0.0001
	GCT	0.8259	0.7693-0.8826	<0.0001
	OGTT	0.9054	0.8547-0.956	<0.0001
GCT>140 (n=599)	*RPG*	0.7647	0.7047-0.8247	<0.0001
	FPG	0.9102	0.8629-0.9574	<0.0001
	A1C	0.8303	0.7661-0.8945	<0.0001
	OGTT	0.9426	0.9059-0.9793	<0.0001
All Subjects (n=1573)	*FPG + A1C*	0.9335	0.8951-0.9718	<0.0001
	RPG + A1C	0.8762	0.8244-0.9280	<0.0001
	RPG + FPG	0.894	0.8435-0.9444	<0.0001
	FPG + GCT	0.9241	0.8826-0.9657	<0.0001
	RPG + GCT	0.8816	0.8374-0.9258	<0.0001
	GCT + A1C	0.923	0.8876-0.9584	<0.0001

Table 20. P-Values from Comparisons of Single Tests: OGTT > 199 or A1C >= 6.5 or FPG > 125

	FPG	RPG	A1C	GCT	OGTT
FPG	———	<0.0001	0.15	0.27	0.34
RPG	<0.0001	———	0.09	0.001	<0.0001
A1C	0.15	0.09	———	0.55	0.02
GCT	0.27	0.001	0.55	———	0.001
OGTT	0.34	<0.0001	0.02	0.001	———

Table 21. P-Values from Comparisons of Paired Tests vs. Corresponding Single Tests: OGTT > 199 or A1C >= 6.5 or FPG > 125

	FPG	RPG	A1C	GCT
FPG + A1C	0.02	———	0.0003	———
FPG + RPG	0.95	<0.0001	———	———
FPG + GCT	0.01	———	———	0.002
RPG + A1C	———	0.003	0.001	———
RPC + GCT	———	<0.0001	———	0.09
GCT + A1C	———	———	<0.0001	0.005

Table 22. P-Values from Comparisons of Paired Tests: OGTT > 199 or A1C >= 6.5

	FPG + A1C	FPG + RPG	FPG + GCT	RPG + A1C	RPG + GCT	GCT + A1C
FPG + A1C	———	0.024	0.65	0.01	0.07	0.65
FPG + RPG	0.024	———	0.006	0.58	<0.0001	0.33
FPG + GCT	0.65	0.01	———	0.14	0.59	0.96
RPG + A1C	0.001	0.58	0.14	———	0.86	0.007
RPG + GCT	0.07	0.59	0.01	0.86	———	0.06
GCT + A1C	0.65	0.33	0.96	0.007	0.06	———