A1C Analyzer Bias Can Skew Prevalence and Sensitivity/False Positive Rate When Screening for Type 2 Diabetes

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Abstract

A1C is now an accepted test for screening and diagnosis of prediabetes and type 2 diabetes. The difference between a normal A1C (5.6%) and diabetes (6.5%) is only 0.9%. The College of American Pathologists biannual survey of central laboratory A1C (GH2 Survey Data 5/10) shows that analyzer biases can range between -0.3% and 0.4% for the 5.9% reference sample (GH2-01). Given the 0.9% Δ between a normal A1C and diabetes, we wanted to quantify the potential effect of A1C analyzer bias on A1C defined diabetes risk and the relationship of A1C to glucose-based diabetes screening tests.

The NHANES, 2007-2008 data include measurements of A1C, fasting plasma glucose (FPG) and a 75 gm, two hour oral glucose tolerance test (OGTT) on a subset of participants. Adults with valid OGTT, FPG and A1C that met the ADA guidelines for diabetes screening were included in the analysis. The extracted data were weighted using the WTSOG2YR variable to be nationally representative. Prevalence of A1C≥6.5%, 5.7≤A1C≤6.4% and 6.0≤A1C≤6.4% were determined for the baseline data and by either adding 0.4% or subtracting 0.3% from the decision thresholds to simulate analyzer bias. Abnormal glucose tolerance (AGT) was defined as an OGTT≥140 mg/dL at 2 hours and abnormal fasting glucose (AFG) was defined as an FPG≥100 mg/dL.

The prevalence of A1C-defined diabetes or diabetes risk varies widely across the range of analyzer biases (Table 1).

Table 1	Prevalence (%)		
	-0.3% Bias	0.0% Bias	+0.4% Bias
5.7 ≤ A1C ≤ 6.4%	6.8	20.2	57.7
6.0 ≤ A1C ≤ 6.4%	2.0	5.9	25.7
A1C ≥ 6.5%	0.5	1.4	5.2

Table 2	AGT		AFG	
Analyzer Bias	FPR	SEN	FPR	SEN
-0.3%	0.04	0.20	0.03	0.13
0.0%	0.16	0.42	0.12	0.33
+0.4%	0.58	0.82	0.50	0.77

By ROC curve analysis, the sensitivity (SEN) and false positive rate (FPR) at the A1C≥5.7% threshold for detection of AGT and AFG also vary significantly (Table 2).

A1C analyzer bias alone skews the prevalence of abnormal A1C and A1C-determined diabetes. Further, this variability due to the assay itself significantly varies the estimated SEN and FPR of A1C for detection of AGT and AFG. A1C analyzer bias should be considered when interpreting A1C results for prediabetes and diabetes screening both in clinical decision making and in epidemiologic data interpretation.

Background

- Hemoglobin A1c (A1c) is now recommended by the American Diabetes Association for the screening and diagnosis of pre-diabetes and type 2 diabetes
- Fasting plasma glucose (FPG) and a 75 gm, two hour oral glucose tolerance test (OGTT) are also accepted methods for screening and diagnosis of pre-diabetes and diabetes
- The difference between a normal A1c (5.6%) and diabetes (6.5%) is only 0.9%[1]
- The College of American Pathologists biannual survey of reference laboratory A1c (April, 2011) shows an average bias of 0.1% ± 0.4% (95% CI) across the 32 commonly used laboratory analyzers for the 5.4% and 6.4% reference samples[2]
- Limited dynamic range of A1c between normal and diabetes may make analyzer bias a potentially significant error source when screening for and/or estimating prevalence of pre-diabetes and diabetes
- Glucose analyzer bias quantified at 0.7 mg/dL ± 2.4 mg/dL (95% CI) across 12 types of laboratory glucose analyzers[3]

Study Aims

- Using the ADA guidelines for diagnosis of type 2 diabetes[1] and the NHANES, 2007-2008 data set[4], investigate the effects of A1c and glucose analyzer bias on:
  - Pre-diabetes and undiagnosed type 2 diabetes disease prevalence estimates
  - Test threshold sensitivity (SENS) and false positive rate (FPR) for pre-diabetes and undiagnosed type 2 diabetes

Inclusion Criteria

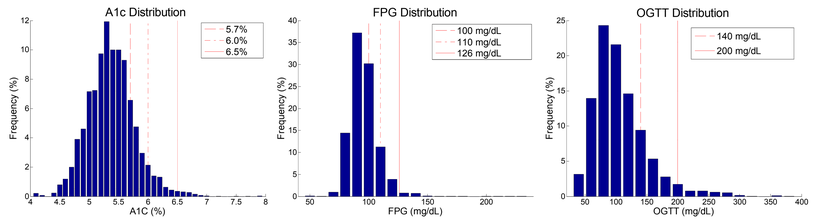
All must be true

- Age ≥ 18 yrs
- No pre-existing diagnosis of any type of diabetes
- Overnight fast ≥ 8 hours
- A1c ≥ 3.5% and FPG ≥ 40 mg/dL and OGTT ≥ 40 mg/dL

Population Demographics

- Gender: 48.8% male, 51.2% female
- Age: 44.5 ± 16.8 yrs, range= 18 to 80 yrs
- BMI: 27.5 ± 6.5 kg/m^2, range = 15.3 to 72.6 kg/m^2
- Race/Ethnicity: 69.7% Non-Hispanic White, 10.6% Non-Hispanic Black, 8.7% Mexican American, 5.0% Other Hispanic, 5.9% Other

A1c, FPG and OGTT for Subjects w/o Known Diabetes



Statistical Methods

- NHANES, 2007-2008 subjects that met inclusion criteria weighted using WTSOG2YR (OGTT Subsample 2 Year MEC Weight) variable
- Disease prevalence thresholds
  - Abnormal glucose tolerance (AGT) defined as OGTT ≥ 140 mg/dL[1]
  - Abnormal fasting glucose (AFG) defined as FPG ≥ 100 mg/dL[1]
  - Abnormal A1c (AA1c) defined as A1c ≥ 5.7%[1]
  - Diabetes by OGTT defined as OGTT ≥ 200 mg/dL[1]
  - Diabetes by FPG defined as FPG ≥ 126 mg/dL[1]
  - Diabetes by A1c defined as A1c ≥ 6.5%[1]
- Prevalence estimated for no bias, mean bias, mean bias ± bias standard deviation (1 SD), mean bias ± twice standard deviation (2 SD) added as an offset to NHANES, 2007-2008 measurements of A1c, FPG and OGTT
- Sensitivity and false positive rates calculated by standard method

Results - Prevalence

- NHANES, 2007-2008 disease prevalence estimates for A1c, FPG and OGTT defined pre-diabetes and type 2 diabetes are shown in Table 1

Dysglycemia Criteria	NHANES, 2007 - 2008 Prevalence (%)						
	No Bias	Mean Bias[A]	-1 SD[B]	+1 SD[B]	-2 SD[B]	+2 SD[B]	Relative Change[C]
5.7 ≤ A1c ≤ 6.4%	20.2	19.8	9.2	47.1	4.7	65.3	13.9
6.0 ≤ A1c ≤ 6.4%	5.9	8.4	2.7	17.8	1.3	33.6	25.8
6.5% ≤ A1c	1.4	1.9	0.8	3.8	0.4	7.3	18.3
100 ≤ FPG ≤ 125	44.4	48.9	44.4	52.7	37.2	57.0	1.5
126 ≤ FPG (mg/dL)	2.8	3.1	2.8	3.3	2.4	3.8	1.6
140 ≤ OGT ≤ 199	17.5	18.0	17.5	18.7	16.8	19.0	1.1
200 ≤ OGT (mg/dL)	4.8	4.9	4.8	4.9	4.6	4.9	1.1

Table 1: Prevalence estimates for pre-diabetes and type 2 diabetes as determined by A1c, FPG and OGTT using 2011 ADA criteria

[A] A1c mean bias = 0.1%, glucose mean bias = 0.7 mg/dL
[B] A1c standard deviation = 0.2%, glucose standard deviation = 1.2 mg/dL
[C] Relative Change = +2 SD / -2 SD

Results – Dysglycemia Detection

- A1c detection of AGT and AFG is shown in Table 2
- A1c detection of type 2 diabetes is shown in Table 3
- FPG detection of AGT and AA1c is shown in Table 4
- FPG detection of type 2 diabetes is shown in Table 5

Table 2 A1C Threshold + Analyzer Bias (%)	AGT (OGT ≥ 140 mg/dL)		AFG (FPG ≥ 100 mg/dL)	
	FPR (%)	SENS (%)	FPR (%)	SENS (%)
5.7%	15.7	42.0	11.9	32.4
6.0-5.2 (+Mean[A]±2 SD[B])	3.6 - 68.9	20.3 - 85.5	2.2 – 62.5	13.1 - 83.9

Table 3 A1C Threshold + Analyzer Bias (%)	Diabetes (OGT ≥ 200 mg/dL)		Diabetes (FPG ≥ 126 mg/dL)	
	FPR (%)	SENS (%)	FPR (%)	SENS (%)
6.5%	0.6	17.7	0.6	30.0
6.8-6.0 (+Mean[A]±2 SD[B])	0.0 – 5.4	10.1 – 46.2	0.0 – 5.9	16.9 – 65.9

Table 4 FPG Threshold + Analyzer Bias (mg/dL)	AGT (OGT ≥ 140 mg/dL)		Abnormal A1c (A1c ≥ 5.7%)	
	FPR (%)	SENS (%)	FPR (%)	SENS (%)
100	39.7	73.5	40.7	70.9
103-98 (+Mean[A]±2 SD[B])	28.7 – 49.4	61.4 – 79.1	28.4 – 50.2	63.4 – 77.5

Table 5 FPG Threshold + Analyzer Bias (mg/dL)	Diabetes (OGT ≥ 200 mg/dL)		Diabetes (A1c ≥ 6.5%)	
	FPR (%)	SENS (%)	FPR (%)	SENS (%)
126	1.1	35.3	2.0	58.0
129-124(+Mean[A]±2 SD[B])	0.5 – 1.5	29.1 – 38.3	1.2 – 2.5	48.8 – 61.3

Summary

- Laboratory A1c analyzer bias can skew disease prevalence estimates for abnormal A1c and A1c defined diabetes by factors of 13.9 to 25.8 in the US population without diagnosed diabetes
- Laboratory glucose analyzer bias skews disease prevalence estimates an order of magnitude less than A1c analyzer bias (factors of 1.1 to 1.6)
- Laboratory A1c analyzer bias can also result in radically different estimates of A1c sensitivity and FPR for diabetes screening and diagnosis
- Laboratory glucose analyzer bias has a less pronounced effect on FPG sensitivity and FPR estimates for diabetes screening and diagnosis

Conclusion

A1C analyzer bias is a significant error source and should be considered when interpreting A1C results for pre-diabetes and diabetes screening and diagnosis both in clinical decision making and in epidemiologic data interpretation.

References

1. American Diabetes Association, Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2011; 34:S62-S69.
2. College of American Pathologists (CAP) GH2 Survey Data: (updated 4/11).
3. Miller et al, State of the Art in Trueness and Interlaboratory Harmonization for 10 Analytes in General Clinical Chemistry, Arch Pathol Lab Med. 2008;132:838–846.
4. http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/nhanes07_08.htm