HbA\textsubscript{1c} for screening and diagnosis of Type 2 diabetes in routine clinical practice

Zhong X Lu (PhD)\textsuperscript{1,2}, Karen Z Walker (PhD)\textsuperscript{3,4}, Kerin O’Dea (PhD)\textsuperscript{6}, Ken A Sikaris (FRCPA)\textsuperscript{1} and Jonathan E Shaw (MD)\textsuperscript{5}

\textsuperscript{1}Melbourne Pathology Services, Melbourne, Australia; \textsuperscript{2}Department of Medicine, Monash Medical Centre and \textsuperscript{3}Department of Nutrition and Diabetes, Monash University, Melbourne; \textsuperscript{4}Preventative Health Unit and \textsuperscript{5}Clinical Diabetes and Epidemiology Unit, Baker IDI Heart and Diabetes Institute, Melbourne; \textsuperscript{6}Sansom Institute for Health Research, University of South Australia, Adelaide, Australia.

**Running title:** HbA\textsubscript{1c} a screening tool for diabetes

**Corresponding author:**
Dr Zhong X Lu
e-mail: zhong.lu@mps.com.au

Submitted 21 September 2009 and accepted 6 January 2010.

This is an uncopyedited electronic version of an article accepted for publication in *Diabetes Care*. The American Diabetes Association, publisher of *Diabetes Care*, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of *Diabetes Care* in print and online at http://care.diabetesjournals.org.
Objective—To evaluate HbA1c for screening and diagnosis of undiagnosed Type 2 diabetes defined by oral glucose tolerance testing in clinical and general populations.

Research design and methods—HbA1c cut-offs (≤5.5% to ‘rule-out’; ≥7.0% to ‘rule-in’ diabetes) were derived from a clinical group (MP: n= 2,494; undiagnosed diabetes 34.6%) and then evaluated in a population-based sample (AusDiab: n=6,015, undiagnosed diabetes 4.6%).

Results—For diabetes in MP and AusDiab, HbA1c at 5.5% gave sensitivities of 98.7% and 83.5%, while HbA1c at 7.0% gave specificities of 98.2% and 100%, respectively. Many (61.9 - 69.3%) with impaired HbA1c (IA1c: 5.6 - 6.9%) in both populations had abnormal glucose status.

Conclusions—HbA1c ≤5.5% and ≥7.0% predicts absence or presence of Type 2 diabetes while at HbA1c 6.5-6.9%, diabetes is highly probable in clinical and population settings. A high proportion of people with IA1c have abnormal glucose status requiring follow-up.
HbA\textsubscript{1c} a screening tool for diabetes

With current screening tools (fasting plasma glucose (FPG) ± oral glucose tolerance test (OGTT)), the prevalence of undiagnosed diabetes in Australia remains high (1). HbA\textsubscript{1c} provides a practical alternative for screening (2,3). It is more convenient and reproducible than is blood glucose (3,4). As optimal cut-offs are still in debate, we here explore HbA\textsubscript{1c} levels that confidently ‘rule-out’ and ‘rule-in’ diabetes in two different Australian populations.

RESEARCH DESIGN AND METHODS
We studied two populations: the clinical population (MP) included all patients referred by medical practitioners for an OGTT in 2003-2008 to a state-wide private pathology service (Melbourne Pathology Services, Australia); the AusDiab population comes from a national population-based study (2004-2005 AusDiab Follow-up) (5). Only people with concurrent HbA\textsubscript{1c} and OGTT results are included here (MP: n=2,494; AusDiab: n=6,014). Glucose status was classified by ADA criteria for OGTT (6).

HbA\textsubscript{1c} was determined either by DCCT-aligned (7) cation-exchange chromatography (MP population) or by boronate affinity chromatography with values converted to DCCT-aligned HbA\textsubscript{1c} (5) (AusDiab). Plasma glucose was measured using hexokinase.

RESULTS:

\textbf{HbA\textsubscript{1c} cut-offs defined from the MP population.} Among those with undiagnosed diabetes (34.6%) by OGTT criteria in the MP population, HbA\textsubscript{1c} at the 2.5\textsuperscript{th} percentile was 5.6%. HbA\textsubscript{1c} ≤5.5% was thus chosen to ‘rule-out’ diabetes. For those without diabetes (65.4%), HbA\textsubscript{1c} at the 97.5\textsuperscript{th} percentile was 6.9%. HbA\textsubscript{1c} ≥7.0% was thus chosen to ‘rule-in’ (diagnose) diabetes.

\textbf{Applying HbA\textsubscript{1c} cut-offs to the MP population (34.6% undiagnosed diabetes).} Applying the above cut-offs, a total 35.2% of the MP population had diabetes ruled-in or ruled-out (Figure 1A) while the remaining 64.8% had an ‘impaired’ HbA\textsubscript{1c} (IA\textsubscript{1c}) of 5.6 - 6.9%. From those with IA\textsubscript{1c}, 61.9% had abnormal glucose status.

For diabetes, HbA\textsubscript{1c} at 5.5% provided high sensitivity (97.8%) and high NPV (95.8%) while HbA\textsubscript{1c} at 7.0% gave high specificity (98.2%) and high PPV (92.9%). Using the recommended cut-off of 6.5% (3), specificity decreased to 88.8% and the PPV decreased to 76.8%.

\textbf{Applying HbA\textsubscript{1c} cut-offs to the AusDiab population (4.6% undiagnosed diabetes).} Applying the same cut-offs, a total 75.9% of the AusDiab population had diabetes ruled-in or ruled-out (Figure 1B) while the remaining 24.1% had IA\textsubscript{1c}. From those with IA\textsubscript{1c}, 69.3% had abnormal glucose status.

For diabetes, HbA\textsubscript{1c} at 5.5% provided moderate sensitivity (83.5%) but high NPV (99.0%) since diabetes prevalence was lower in the AusDiab than in the MP population. HbA\textsubscript{1c} at 7.0% gave 100% specificity and 100% PPV. By dropping the cut-off to 6.5%, specificity remained 99.9%, with PPV near 100%.

CONCLUSION
Our study supports recommendations to use HbA\textsubscript{1c} for diabetes screening and diagnosis (2,3). Using two, rather than one, cut-off values for HbA\textsubscript{1c} achieved high sensitivity for screening plus optimal specificity for diabetes diagnosis. We also show the high probability that those with IA\textsubscript{1c} have abnormal glucose status.

Single HbA\textsubscript{1c} cut-offs have limited clinical utility in identifying those people with abnormal blood glucose levels. The most commonly reported single HbA\textsubscript{1c} cut-off obtained from reported receiver operated characteristic (ROC) curves was ~6.1% which gives sensitivities of 78 - 81% with...
specificities of 79 - 84% (8). In our MP population, the ROC curve-identified optimal \( HbA1c \) of 6.2% gave a sensitivity 82.2% and specificity 78.8% (Lu, unpublished data) but yielded a reduced PPV (67.2%) and NPV (89.3%). We therefore apply proposed cut-offs. The lower was chosen for its high NPV as diabetes is ruled-out with high confidence (with \( HbA1c \leq 5.5 \), NPV was > 95% in both clinical and population settings). Applying \( HbA1c \) 5.5% to NHANES data generated sensitivity ~92% and specificity ~30% from the published ROC curve (9). This gave an NPV ~99% (assuming 3.4% undiagnosed diabetes) and agrees with our finding that diabetes is very unlikely in individuals with \( HbA1c \leq 5.5 \%. Our 7.0\% cut-off for diabetes diagnosis is higher than the 6.5\% recommended (2,3) but was chosen to optimize specificity.

For those with \( IA1c \), the prevalence of diabetes increases as \( HbA1c \) increases. \( HbA1c \) is a continuous variable for diabetes and any cut-off values chosen are somewhat arbitrary. From our data, people with \( HbA1c \) 5.6-6.0\% were more likely to have either normoglycemia or pre-diabetes (IFG and/or impaired glucose tolerance (IGT)) than diabetes, consistent with NHANES where \( HbA1c \) 5.5-6.0\% excluded diabetes in moderate but not high-risk individuals (10). Thus, those with \( HbA1c \) 5.6-6.0\% would probably require education and lifestyle modification to prevent progression to diabetes (11) plus retesting every 6-12 months. Also in our study, people with \( HbA1c \) 6.1 - 6.4\% were more likely to have pre-diabetes or diabetes than normoglycemia while among those with \( HbA1c \) 6.5-6.9\%, diabetes was highly probable. Thus, individuals with \( HbA1c \) 6.1 - 6.9\% may require an OGTT to confirm their glycemic status plus lifestyle education and regular monitoring as for people with pre-diabetes. For those with \( HbA1c \geq 6.5\%), screening for retinopathy is also necessary (2).

\( HbA1c \) as a screening/diagnostic tool has some limitations (3). The main issues are: method bias which is now being addressed by IFCC Standardization (12) and certain confounding medical conditions (hemoglobinopathies and anemia). Most new \( HbA1c \) methods can identify or are unaffected by hemoglobinopathies. Anemia is also readily identifiable.

The cost of \( HbA1c \) has also been raised as a concern. While \( HbA1c \) analysis *per se* is more expensive than for glucose, the overall differences are small once the costs for blood collection are accounted for. From our own estimates, total costs are AUD$10.20 for \( HbA1c \) compared with AUD$8.80 for FPG and AUD$12.10 for a two-point collection of OGTT. These cost comparisons are consistent with reports from other countries (13,14,15). Further, the time and inconvenience to patients in having to fast for an OGTT cannot be ignored. \( HbA1c \leq 5.5\% \) and \( \geq 7.0\% \) predicts with 97.5\% confidence the absence or presence of type 2 diabetes using the OGTT as a reference. Many with \( IA1c \) have pre-diabetes while diabetes is highly probable when \( HbA1c \) reaches 6.5-6.9\%. \( IA1c \) thus requires follow-up and lifestyle modification. Although the cost of \( HbA1c \) is slightly higher than for FPG, the overall efficiency of using \( HbA1c \) as first-line for diabetes screening may facilitate early diagnosis and reduce the health burden associated with diabetes complications.

**ACKNOWLEDGEMENTS**

The AusDiab study co-ordinated by the Baker IDI Heart and Diabetes Institute, gratefully acknowledges the generous support given by: National Health and Medical Research Council (NHMRC grant 233200), Australian Government Department of Health and Ageing, Abbott Australasia Pty Ltd, Alphapharm Pty Ltd, AstraZeneca, Bristol-Myers Squibb, City Health Centre-Diabetes.
Service-Canberra, Department of Health and Community Services - Northern Territory, Department of Health and Human Services – Tasmania, Department of Health – New South Wales, Department of Health – Western Australia, Department of Health – South Australia, Department of Human Services – Victoria, Diabetes Australia, Diabetes Australia Northern Territory, Eli Lilly Australia, Estate of the Late Edward Wilson, GlaxoSmithKline, Jack Brockhoff Foundation, Janssen-Cilag., Kidney Health Australia, Marian & FH Flack Trust, Menzies Research Institute, Merck Sharp & Dohme, Novartis Pharmaceuticals, Novo Nordisk Pharmaceuticals, Pfizer Pty Ltd, Pratt Foundation, Queensland Health, Roche Diagnostics Australia, Royal Prince Alfred Hospital, Sydney, Sanofi Aventis, Sanofi Synthelabo. The following individuals also made an invaluable contribution: A Allman, B Atkins, S Bennett, A Bonney, S Chadban, M de Courten, M Dalton, D Dunstan, T Dwyer, H Jahangir, D Jolley, D McCarty, A Meehan, N Meinig, S Murray, K O’Dea, K Polkinghorne, P Phillips, C Reid, A Stewart, R Tapp, H Taylor, T Whalen, F Wilson and P Zimmet.

Disclosure: None to declare

FIGURE LEGENDS
Figure 1: Application of the HbA1c cut-offs to screen or diagnose diabetes A) in a clinical group (MP population, n= 2,494, undiagnosed diabetes 34.6%) and; B) in a national population-based group (AusDiab population, n= 6,014, undiagnosed diabetes 4.6%).
REFERENCES:
**HbA$_1c$ a screening tool for diabetes**

**A**

HbA$_1c$ (MP population)  
\[ n = 2,494 \]

Diabetes 'ruled out'  
- n = 457  
  (18.3%)

Impaired HbA$_1c$ (IA$_1c$)  
- n = 1,616  
  (64.8%)

Diabetes 'ruled in'  
- n = 421  
  (16.9%)

Classified by ADA:
- Normal (n):
  - ≤5.5%: 275
  - 5.6-6.0%: 342
  - 6.1-6.4%: 123
  - 6.5-6.9%: 31
  - ≥7.0%: 2

- IFG and or IGT (n):
  - ≤5.5%: 163
  - 5.6-6.0%: 290
  - 6.1-6.4%: 255
  - 6.5-6.9%: 121
  - ≥7.0%: 28

- Diabetes (n):
  - ≤5.5%: 19
  - 5.6-6.0%: 100
  - 6.1-6.4%: 144
  - 6.5-6.9%: 210
  - ≥7.0%: 391

Total (n):  
- 457
- 732
- 522
- 362
- 421

Diabetes prevalence:  
- 4.2%  
- 13.7%  
- 27.6%  
- 58.0%  
- 92.9%

**B**

HbA$_1c$ (AusDiab population)  
\[ n = 6,014 \]

Diabetes 'ruled out'  
- n = 4,534  
  (75.4%)

Impaired HbA$_1c$ (IA$_1c$)  
- n = 1,449  
  (24.1%)

Diabetes 'ruled in'  
- n = 31  
  (0.5%)

Classified by ADA:
- Normal (n):
  - ≤5.5%: 3,388
  - 5.6-6.0%: 548
  - 6.1-6.4%: 4
  - 6.5-6.9%: 0
  - ≥7.0%: 0

- IFG and or IGT (n):
  - ≤5.5%: 1,100
  - 5.6-6.0%: 653
  - 6.1-6.4%: 39
  - 6.5-6.9%: 4
  - ≥7.0%: 0

- Diabetes (n):
  - ≤5.5%: 46
  - 5.6-6.0%: 102
  - 6.1-6.4%: 58
  - 6.5-6.9%: 41
  - ≥7.0%: 31

Total (n):  
- 4,534
- 1,303
- 101
- 45
- 31

Diabetes prevalence:  
- 1.0%  
- 7.8%  
- 57.4%  
- 91.1%  
- 100%

Figure 1