

Laboratory Challenges to Diabetic Care
Larry Kaplan, Ph.D.
Beth Israel Medical Center



Laboratory Challenges to Diabetic Care: Case Hx of Type 2 Diabetic

A 43 year-old Hispanic woman enters a hospital's diabetes clinic for the first time, referred from her neighborhood clinic for suspected DM.

She is 5'5", 180 lb, unemployed, divorced, housekeeper.

She is P2G2, treated for mild hypertension.

For her 2 pm afternoon clinic visit the physician orders:

Initial Laboratory order

- **Hepatic profile:** AST, 21 U/L; ALT, 21 U/L, ALB 4.9 g/dL; T. bilirubin, 0.9 mg/dL; ALK PHOS, 71 U/L. **Basic metabolic profile (BMP):** Na, 141 mmol/L; K, 3.8 mmol/L; Cl, 101 mmol/L; glucose, 350 mg/dL; tCO₂, 29 mmol/L; BUN, 29 mg/dL; creatinine, 1.1 mg/dL. **Lipid profile:** T. cholesterol, 283 mg/dL; triglycerides, 353 mg/dL; HDL-cholesterol, 40 mg/dL; calculated LDL, 167 mg/dL. **Routine urinalysis, CBC. Thyroid:** TSH, 40.7 mIU/L; Free T₄, 1.1 ng/dL

Questions: Initial Laboratory order

- Are these profiles reasonable for this patient's problem? Why? Why not?
Given that the patient's immediate problem is likely DM (how likely?, What are the *a priori* risk factors?), the glucose (why a BMP) BMP and lipid profiles are not inappropriate and could help focus management decision.

Initial Laboratory order

- A routine urinalysis is one of the most frequently ordered “routine” profiles, and its uselessness in the absence of symptoms is well documented. The CBC is of very marginal use.
- The hepatic profile and the TSH are relatively benign, but do not address immediate problems. See later for discussion.

How reliable will these results be for interpretation?

Alternative glucose measures

The clinic nurse had performed a point-of-care testing (POCT) glucose measurement right before the patient was sent to phlebotomy and obtained a result of 250 mg/dL (lab=350 mg/dL).

When the main laboratory result is seen the doctor is concerned and demands a repeat glucose meter test; the repeat POCT glucose is 295 mg/dL. The doctor questions the laboratory director, wanting to know which of the results is accurate.

More questions

- Has the value of POCT to improved patient care been demonstrated?
- Does the POCT glucose have to be as accurate and precise as Central Lab's glucose?

Alternative glucose measures

- The POCT glucose is essentially a screening test to obtain a ballpark idea of glucose level; is the patient hypo-, normo-, or hyperglycemic. If the latter, is the blood glucose level high enough to suggest non-ketotic hyperosmolar coma or affect medication? No lit.-based demonstration of utility.
- When used to follow treatment, the precision should be sufficient to establish trends; *routine changes of therapy should be based on Central Lab results*. A study for an ISO document of glucose meter testing (ISO/TC 212 WD15196) recommended that **total error of $\pm 25\%$ was acceptable**; **CLIA'88 total error for Central Lab testing is \pm or 10%, although usually $\sim \pm 4\%$.**

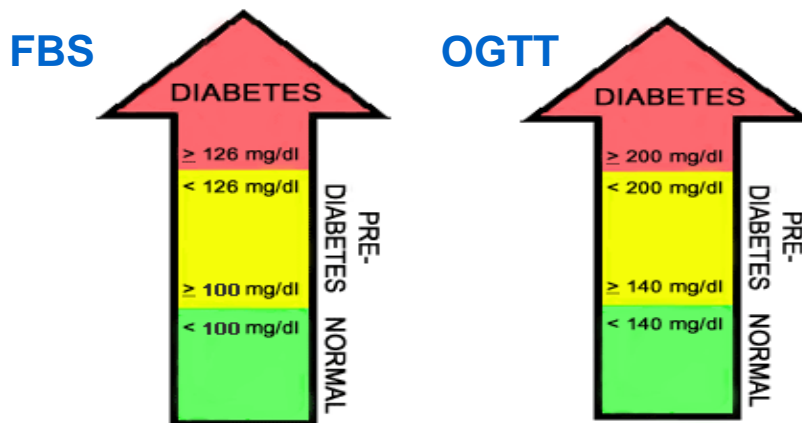
Follow up clinic visit

- The patient's physician reviews the laboratory's results and decides to order a 2-hour Glucose tolerance test (GGT), a lipid profile, and a TSH. The latter tests are drawn at the time of the FBS taken at the time of her next clinic visit, at 8 am. The results of these tests are:
 - **Lipid profile:** T. cholesterol, 240 mg/dL; triglycerides, 220 mg/dL; HDL-cholesterol, 39 mg/dL; calculated LDL, 157 mg/dL.
 - **TSH:** 35.2 mIU/L; **Free T4,** 1.4 ng/dL
 - **Fasting blood sugar (FBS):** 135 mg/dL; 0.5 hrs, 1 hr, and 2 hr GGT samples not received

More, more questions

- What are the recommended tests to diagnose a diabetic?
- What are the problems associated with obtaining valid GGT samples?
- What is the reliability of an abnormal GGT for the diagnosis of diabetes?
- What is the reliability of an elevated FBS for the diagnosis of diabetes?
- What additional tests should be used to follow-up the elevated TSH?

Answers, more answers: Dx



<http://www.diabetes.org/pre-diabetes/pre-diabetes-symptoms.jsp>

Answers, more answers: FBS/OGTT

- The major problem hurdle for getting a reliable FBS is getting the patient to fast. An overnight fast is usually not a difficulty, but since many clinics are scheduled for the late morning or afternoon, fasting until blood drawing becomes difficult and people slip.
- The difficulties for obtaining the samples for the OGTT are getting the patient to remain in the phlebotomy area for the assigned times and the emesis that is frequently associated with the glucose challenge drinks. The latter problem occurred with this patient.

Answers, more answers: problems

- **Illness** reduces glucose tolerance. Abnormalities in thyroxin, growth hormone, cortisol, and catecholamines interfere physiologically.
- **Drugs and medications** such as oral contraceptives, salicylates, nicotinic acid (found in cigarettes, cigars, pipe tobacco, chewing tobacco), diuretics (including caffeine), and hypoglycemic agents (insulin, sulfonylureas) interfere.

Answers, more answers: problems

- **Testing time affects the test.** The best time to conduct the test is between 7 am and noon, something rarely achieved in clinics. Evaluation criteria should also be adjusted for age.
- If adjustments for age are not made, about 80% of persons over 60 years of age will be judged diabetic
- The OGTT has been criticized because variables affecting test results are difficult to control and the reproducibility of the test is poor (Nelson RL: Subspecialty clinics: endocrinology: oral glucose tolerance test: indications and limitations, *Mayo Clin Proc* 63:263, 1988).

Answers, more answers: problems

For these reasons the ADA recommends repeated elevated FBS to diagnosis DM.

Answers, more answers: Thyroid

There is discordancy between the repeatedly very elevated TSH and the free T4 value that is repeatedly with the reference range. One can perform additional tests:

- free T4 by dialysis, free T3 by dialysis
- TSH by another method thyroid Ab
- CAT scan, MRI, etc, etc

or assume severe hypothyroidism and treat. More later.

Treatment and Monitoring

- The physician decides that, based on the patient's physical condition (obesity with a Body Mass Index [BMI] >30 <http://www.nhlbisupport.com/bmi/>), race, age, and elevated blood-pressure (http://www.joslin.harvard.edu/news/pre_diabetes.shtml) that this patient is diabetic and treatment should be initiated.
- The program includes nutritional counseling with the goals of weight reduction, a hypoglycemic agent, and a statin. He instructs the patient to be seen at a clinic in 3 weeks. For the next clinic visit the physician orders the following tests: **lipid profile, FBS, hemoglobin A1c**.
- Do you agree with the tests ordered? Any unneeded? Any missing? What is HEDIS?

Treatment and Monitoring

- One could question the value of the FBS. We have the same problems with unreliability of the sample and it has no recommended value for monitoring the control that diabetics have over their blood sugar. Picking out for 6 weeks and fasting well overnight can give a relatively low FBS.
- The lipid profile is OK, although <1 month might be pushing it for expecting results.

Treatment and Monitoring

The tests that are *missing* are even more important; recommended by the DCCT and are required by regulatory agencies working for governments and managed care organizations. An example of one is **HEDIS** (*Health Plan Employer Data and Information Set*, (<http://www.ncqa.org/Programs/HEDIS/> , <http://www.cmeoncd.com/CD/CD0010/AB0060/contact.html> [this is very good], www.opm.gov/insure/health/hedis2002/index.asp), audits hospital charts to determine not only if the required tests are being ordered, but also that a specified percentage of patients have results that reflect quality of care.

Treatment and Monitoring

The FY 2000 HEDIS required monitors are:

- % who had a **GHb** measurement (at least 1/year),
- %<9.5% **Hb A1c**,
- % with an **LDL-C** screen, % with LDL-C <130 mg/dL,
- %with a **dilated eye exam** (checking for retinopathy),
- monitoring early kidney changes with a measured **urine albumin** ('microalbuminuria').

Failure to meet HEDIS requirements may result in dire consequences- no \$\$.

Treatment and Monitoring

- The results at the next clinic visit are as follows:

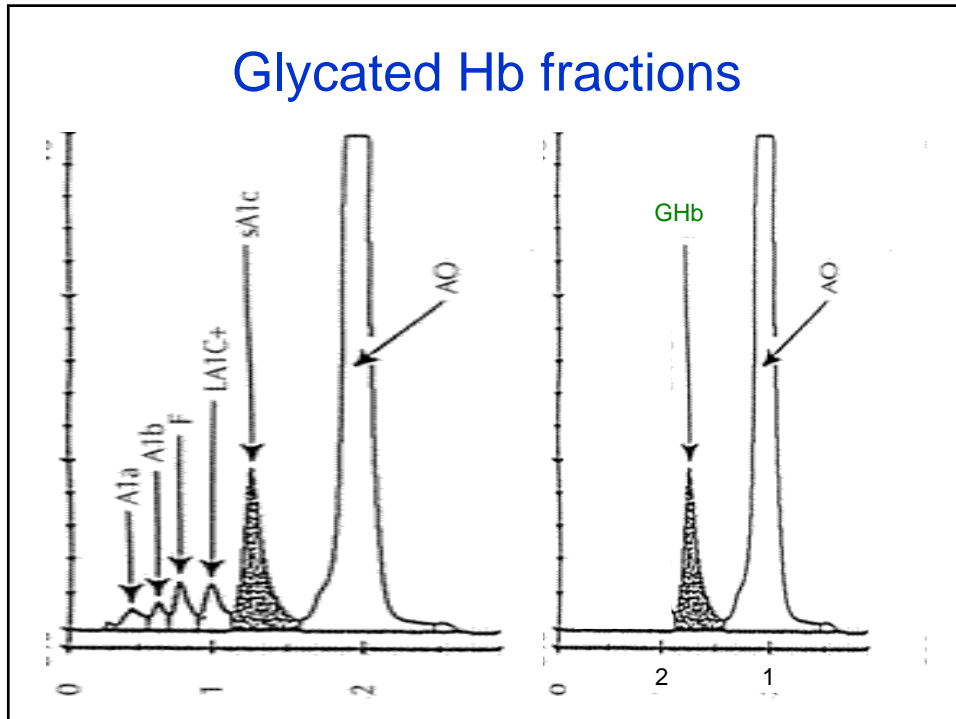
FBS: 135 mg/dL, *Lipid profile*: T. CHOL: 230 mg/dL, TRIGS: 210 mg/dL, HDL-cholesterol: 41 mg/dL, calculated LDL: 147 mg/dL.

- *Glycohemoglobin*: 18.7% (4.8-7.8%)
- The physician is confused and concerned about the very high Hb A1c and he calls the laboratory director asking what the reported result means.

Glycated hemoglobin

- Glycated hemoglobin, Hb A1, actually consists of four principal components, called Hb A1a1, Hb A1a2, Hb A1b, and Hb A1c.
- Each species represents either a different carbohydrate reacting with the hemoglobin molecule or a different type of amine group. For normoglycemic persons, Hb A1a1, Hb A1a2, and Hb A1b constitute 0.4% to 0.8% of the total hemoglobin.
- Hb A1c constitutes 4% to 5% of total hemoglobin. Total glycated hemoglobin, GHb, ~5.0% to 7.5%.

Glycated Hb fractions



Glycated Hb fractions

- Three ways to assess glycated hemoglobin levels: ion-exchange chromatography, immunoturbidity, and affinity chromatography. The first two procedures measure Hb A1c while the last measures total GHb.
- The anion-exchange method depends on separating hemoglobin molecules of differing charges and variant hemoglobins can often co-chromatograph with HbA1c and falsely increase the reported value. On the other hand, the affinity chromatography procedure has no known interferences.
- Because all the diabetes literature is based on studies using HbA1c, many labs report out a highly accurate calculated HbA1c results along with the measured GHb level.

Case Hx: A HbA_{1c} problem

- A 56 y/o West Indian male has been newly diagnosed as a Type 2 diabetic. Aside from being overweight he is in fairly good health.
- His physician, a recent medical school graduate, strongly encourages his patient to go on a weight reduction program. He is placed on a statin, and a hypoglycemic agent.
- His initial HbA_{1c} is 10.6%

A HbA_{1c} problem

- Over the next 9 months, the patient's HbA_{1c} never gets lower than 9.5%. His [microalbumin] and retina are WNR.
- Using the HbA_{1c} to drive therapy, the clinician gradually increases the dosage of hypoglycemic agents.
- Ten months after initial Dx, the patient is brought to the ER in hypoglycemic coma.

A HbA_{1c} problem

- While his patient is recovering, the physician speaks with the lab director.
- A blood sample is sent to a neighboring hospital's lab where the total GHb is 5.8% (reference interval, 4.5-6.5%).
- A sample of blood is analyzed by HPLC for variant Hb and the patient is found to have ~5.5% of HbAC, HB C trait, which is a known interferent in the hospital's HbA_{1c} procedure.



Diabetic DKA and the Laboratory

- A resident calls the laboratory, concerned about some apparently discordant laboratory results obtained for a 15 year old white female who came into the emergency room dehydrated and in moderate diabetic ketoacidosis.
- Laboratory results obtained following admission to the emergency room were as follows: pH, 7.17; Pco₂, 9 mmHg; glucose, 483 mg/dL; serum ketones (by dipstick), trace to small; potassium, 2.8 mmol/L; and sodium, 131 mol/L.

Diabetic DKA and the Laboratory

The patient was treated with fluids, saline, potassium, phosphate, and insulin and, after a number of hours, glucose. Why this treatment?

There are repeated laboratory analyses over the next 48 hours: K, IP, glucose, and blood-gases.

She responded well, her glucose dropping to 156 mg/dL at 48 hours.

Diabetic DKA and the Laboratory

Why this treatment?

- As insulin drives glucose into cells, glucose must be phosphorylated and plasma IP will drop; **monitor.**
- As plasma $[H^+]$ drops with insulin therapy, potassium will re-enter cells, dropping plasma K, in a patient whose total body K might be low; **monitor.**
- DMs typically have lost free water with urinary glucose and electrolytes; **need saline.**
- Glucose to have slow landing; **monitor.**

Diabetic DKA and the Laboratory

What confuses the resident is that her serum ketone levels, after 48 hours of treatment, are now reported as *moderate* (initial ketones was small), although her glucose has decreased considerably. The resident questions whether the laboratory has mixed up or mis-analyzed the specimen.

Diabetic DKA and the Laboratory

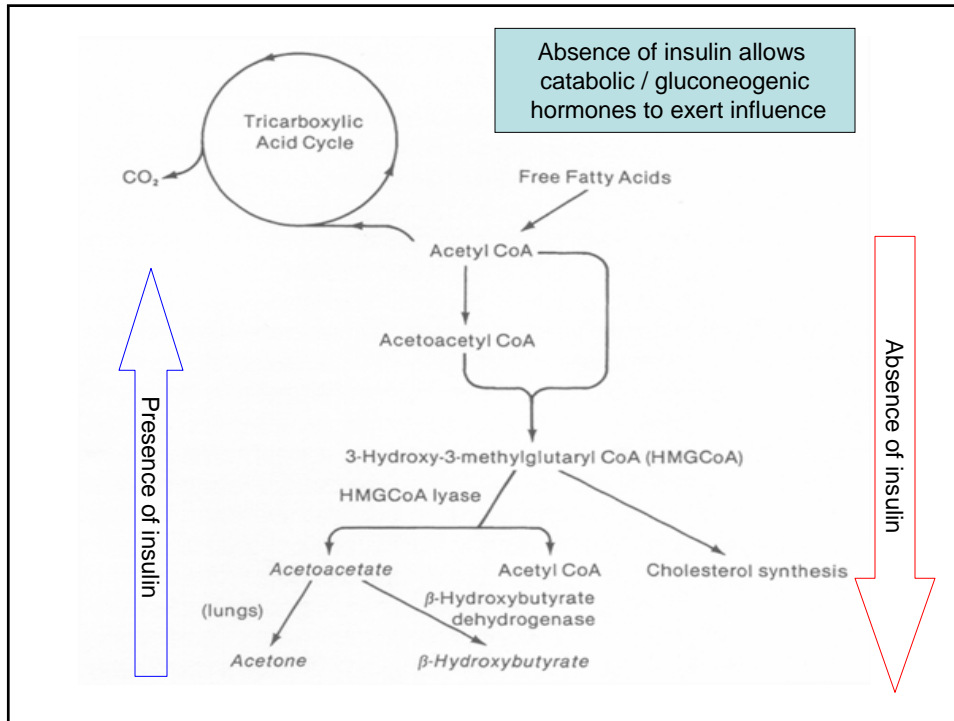
What are “ketone bodies?”

Ketone bodies” is a name given to a group of metabolites of fatty acid oxidation. They are **acetoacetic acid**, **acetone**, and β -hydroxybutyric acid. Only the first two are true ketones.

- What are the pathways involved for the production of ketone bodies?

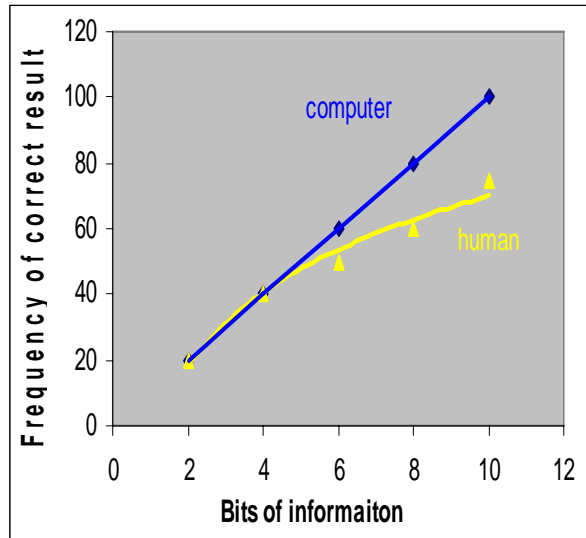
Why are ketone bodies produced during diabetic ketoacidosis?

During starvation or diabetic ketoacidosis low insulin levels cause fat to be mobilized from adipose tissue, resulting in the release of free fatty acids. The fatty acids are oxidized by the liver to acetyl CoA and NADH. However, the large amounts of fatty acids being metabolized in ketoacidosis soon exceed the liver cells' capacity to recycle coenzyme A (CoA) and NAD⁺. This prevents additional metabolism of fatty acids. To allow continued fatty acid metabolism, the acetyl CoA reacts to form the ketone bodies and free CoA and NAD⁺.



What's the downside of excess test ordering?

- Increase costs to society
- Inability to handle excess data (overload), which can lead to confusion



What's the downside of excess test ordering?

- Effect of statically erroneous results.
- Most tests' reference interval have 95% confidence; the likelihood for a false result (low or high) is 5/100. If a physician orders 10 unrelated tests, the chance that *all* the tests will be normal is $(0.95)^{10}$, or only 60%.
- That is, a 40% chance of some abnormal result. Another way of looking at this is of all patients screened with 10 unrelated tests, 40% will have an abnormal result; for 20 unrelated tests the percentage of patients having one or more falsely result is ~66%.

What's the downside of excess test ordering?

- The Canadian physician Dr. Mercer Rang (The Ulysses syndrome M. Rang CMAJ 1972;106: 122-123.) coin the term "Ulysses syndrome" to describe the effect of false positives.
- **Ulysses' Syndrome:** A long and trying journey by a patient and his physician consequent to the discovery of a falsely positive finding on routine screening.

What's the downside of excess test ordering?

Ulysses returned to Ithaca after his harrowing ten-year odyssey.

The spurious finding based on a false positive test result can initiate a series of equally wearing diagnostic adventures and misadventures for both patient and physician, with ultimate return to the point of departure, that is, nothing wrong with the patient.

One Ulysses Adventure

- A 43 y/o Hispanic woman was seen by her general practitioner in a City hospital diabetes clinic. This is the same woman we discussed earlier.
- He ordered: CMP (14 tests), a thyroid panel (2 tests), a lipid profile, a urinalysis with microscopic, and a CBC with manual diff.
- The patient had no complaints other than the likely diabetes..

One Ulysses Adventure

- All the results were WNR except for...
- *Thyroid: TSH, 40.7 mIU/L; Free T4, 1.1 ng/dL, free T3, 175 pg/dL, anti-TPO, negative.*
- Repeat thyroid profile essentially repeated the original results.
- Patient was placed on Synthroid therapy, doctor pleased to detect occult hypothyroidism.
- Follow-up TSH, 35.2 mIU/L.

One Ulysses Adventure

- Physician increased dosage of Synthroid for a few weeks and repeated TSH; 39 mIU/L.
- Kept patient on dosage until she complained of increased bowel frequency, heart palpitations, headaches, and sweating.
- Her TSH was 43 mIU/L.
- CAT scan of pituitary; negative findings.

One Ulysses Adventure

- Finally took patient off of Synthroid and spoke with laboratory director about the case.
- Repeated TSH in two labs: reference lab, 0.3 mIU/mL; hospital lab, 40 mIU/mL.
- PROBLEM: While screening for thyroid disease would not be inappropriate for a patient with clinical problems possible related to thyroid dysfunction or >60 y/o (http://www.nacb.org/Impg/thyroid_Impg_pub.stm), it was appropriate here.
- Patient was put in harm's way for no clinical reason.

One Ulysses Adventure

HAMA, an acronym for Human AntiMouse Antibodies, which is the most commonly encountered heterophile antibody, may be present in as many as 10% of patient's serum.

The incidence increases in people who have received or have been treated with radiolabelled mouse monoclonal antibodies.

It is increasingly felt on circumstantial evidence that these heterophile antibodies are natural antibodies in normal people, though they could also represent autoantibodies.

Affects different assays differently.

