Biomarkers of Autoimmune Diabetes

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Disclosures

• Athena Diagnostics
• Lab Corp.
• Abbott
What is a biomarker?

A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease.

A biomarker may be used to see how well the body responds to a treatment for a disease or condition.

Also called molecular marker and signature molecule.

Source: NCI dictionary of cancer terms: http://www.cancer.gov/dictionary/?CdrID=45618
What is type 1 diabetes?

**Insulinopenic diabetes** = type 1 diabetes (2 subtypes)

**Type 1A** – autoimmune
  common
  CD8 T cells & macrophages destroy beta cells

**Type 1B** – non-autoimmune
  uncommon to rare
  viral (?)

In this presentation: **Type 1A -- > Type 1 (T1DM)**
How can biomarkers of type 1 diabetes be classified?

**Beta cell function markers**
- *Not* unique to T1DM
- Useful in studies of other forms of diabetes
- *Not* used for diagnosis
- Important for research: functional assessment & assessment of interventions

**Genetic markers**
- Do *not* serve as diagnostic markers

**Islet autoantibodies**
- *Unique* to autoimmune diabetes
What are the uses of biomarkers of beta cell function?

Assesses the health status of the beta cells
Declining insulin secretion -- > predicts T1DM
Decreased insulin action -- > central pathophysiology in T2DM

*Used in research; Not used in clinical practice*

* Beta cell function assessed in clinical practice via:
  Glucose measurements
  Glycated protein measurements
  A1c
  Fructosamine
What are genetic biomarkers of T1DM?

**Polymorphisms are associated with T1DM:**

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Comment:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA class II</td>
<td>- HLA-DR3 &amp; DR4</td>
</tr>
<tr>
<td></td>
<td>- HLA-DQB1*0201, *0302</td>
</tr>
<tr>
<td></td>
<td>- DR2 (DQB1**0602): protective</td>
</tr>
<tr>
<td>Insulin gene</td>
<td>- variable #’s of: 5’ VNTRs</td>
</tr>
<tr>
<td>PTPN22</td>
<td>- involved in T-cell receptor signaling</td>
</tr>
<tr>
<td>CTLA4</td>
<td>- down regulates T cells</td>
</tr>
<tr>
<td>IL2RA</td>
<td>- IL-2R alpha chain</td>
</tr>
<tr>
<td>IFIH1</td>
<td>- Modifies response to viral triggers (?)</td>
</tr>
</tbody>
</table>
What are the considerations regarding genetic biomarkers of T1DM?

**Note:** Genetic studies only define “susceptibility”

- Genetic typing is not diagnostic by itself.
- **No** alleles are specific for T1DM
- Provides only “supportive” information of dx of T1DM

<table>
<thead>
<tr>
<th></th>
<th>T1DM pts</th>
<th>General pop.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR3 and/or DR4</td>
<td>~95%</td>
<td>~50%</td>
</tr>
</tbody>
</table>
What is the **usefulness** of islet autoantibody testing?

The presence of islet autoantibodies **defines** T1DM

But . . .

. . . . Islet autoantibodies need *not* be measured to dx T1DM

**Dx of T1DM:**
- Age at onset
- Lean
- Severe hyperglycemia
- Ketosis (+/- DKA)
What are the major islet autoantibodies that are used in clinical practice and research studies that are commercially available?

<table>
<thead>
<tr>
<th>Islet autoantibody markers</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>Islet cell cytoplasmic autoantibodies</td>
</tr>
<tr>
<td>GADA</td>
<td>Glutamic acid decarboxylase autoab.</td>
</tr>
<tr>
<td>IA-2A</td>
<td>Insulinoma associated-2 autoab.</td>
</tr>
<tr>
<td>IAA</td>
<td>Insulin autoantibodies</td>
</tr>
<tr>
<td>ZnT8A</td>
<td>Zinc transporter 8 autoantibodies</td>
</tr>
</tbody>
</table>
Fluorescent microscopic appearance of ICA
ICA  Islet cell cytoplasmic autoantibodies

**Autoantigens - Multiple:** sialoglycoconjugate; GAD, IA-2 & sialoglycoconjugate; cytoplasmic autoantigens*

**Methodology - Indirect immunofluorescence:** substrate: 4 um, cryo-cut sections of group O pancreas (<45 y/o)

**Frequency at onset of T1DM:** 70-80%

**Comments**  
- First islet autoab test  
- Technically demanding  
- Basis for DPT-1 study.

---

* Islet cell surface autoantibodies (ICSA) have been described using indirect immunofluorescence and islet suspensions: specific autoantigen(s): unknown.
GADA  Glutamic acid decarboxylase autoab.

**Autoantigen - GAD65** (more commonly the autoantigen than GAD67)

Glutamic acid ---GAD--- > glutamate
(inhibitory neurotransmitter)

**Methodology** - *Immunoprecipitation* assay (IPA; $^{35}$S-methionine-labeled GAD); nonradioactive ELISA method is available

**Frequency at onset of T1DM**: 70-80% (similar to ICA)
GADA Glutamic acid decarboxylase autoab.

Comments

- Assay: highly automated

- Most persistent autoab following dx (LADA)*

- Target autoantigen in rare cases of neurological dis. (e.g., stiff-person syndrome)\*

* ZnT8A also marker of LADA
IA-2A  Insulinoma associated-2 autoab.

**Autoantigen** - IA-2: a protein tyrosine phosphatase (PTP); cytoplasmic domain is autoimmunogenic (therefore not a surface molecule)

**Methodology** - same as GADA (IPA)

**Frequency at onset of T1DM** - 60%

**Comments** - Can be automated
IAA  Insulin autoantibodies

Autoantigen – insulin

Methodology - Immunoprecipitation of A14-\(^{125}\text{I}\)-monoiiodinated insulin

Frequency at onset of T1DM - 50-60% in children; uncommon in adults

Comments –
Comments – IAA should not be measured after 7-10 days of exogenous insulin because of the development of insulin antibodies (can not be distinguished from autoantibodies)

Micro versus macro assays (macro assay is superior; however 200 uL versus 10 uL)

Because of low [__]'s: difficult to measure

May be 1st autoantibody to appear

Insulin - Only beta-cell specific autoantigen
Evidence for insulin on beta-cell surface
ZnT8A Zinc transporter 8 autoantibodies

**Autoantigen** - Zn transporter 8 - transports Zn into insulin-containing granules

**Methodology** – Immunoprecipitation assay (IPA)

**Frequency at onset of T1DM** - ~60%

[Diagram of Beta cell, Insulin granule, and Zn++]
ZnT8A  Zinc transporter 8 autoantibodies

Comments –

ZnT8A frequency rises as the age at onset of T1DM rises

ZnT8A – marker of LADA

ZnT8A – in the setting of pancreas transplants -- > predicts graft failure
When should islet autoantibodies be measured?

Confirmation of T1DM when the etiological diagnosis is *unclear*:

- Positivity for an islet autoab in the setting of biochemically diagnosed diabetes is *diagnostic* for autoimmune, T1DM

*Importance*: aggressive therapy with insulin to normalize glucose can prolong endogenous beta-cell function
- better beta-cell function: fewer complications (DCCT data)
CASE NJ - History: NJ was an 8 y/o girl who complained of burning with urination for a "few days." She had also been drinking and urinating more than normal for the past "few days."

When seen in her LMD's office, her glucose was found to be elevated. Therefore she was referred to the university hospital emergency department (ED). Upon presentation to the ED, she complained of abdominal pain and had vomited that morning.

**Vital signs** | **Results** | **Comment**
--- | --- | ---
BP | 117/62 mmHg | No hypotension
Pulse | 143 | Tachycardia (nl: <120 bpm)
Resp | 20 | No tachypnea (nl 18-30)
Temp | 37.1 °C (98.8 °F) | Afebrile
Weight | 52.8 kg | (No height was recorded)

**PE:** White discharge on labia with raised lesions
Weight-for-age percentiles: Girls, 2 to 20 years

97th %tile

Obese

NJ
She is obese. Does the patient have T2DM?

<table>
<thead>
<tr>
<th>Units</th>
<th>Pt result</th>
<th>RI</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>JDF</td>
<td>320</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IAA</td>
<td>nU/mL</td>
<td>344</td>
<td>&lt;126</td>
</tr>
<tr>
<td>GADA</td>
<td>U/mL</td>
<td>10.8</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>IA-2A</td>
<td>U/mL</td>
<td>3.00</td>
<td>&lt;0.76</td>
</tr>
</tbody>
</table>
What type of diabetes does she have?

Islet autoantibodies (+) x 4 -- >

Answer: T1DM

- Despite obesity --- >

- *Classic findings of polyuria and polydipsia in a child (+) hyperglycemia and possible DKA (+) positive islet autoantibodies x 4*
CASE BC

**History:** BC was a 25 y/o female who was seen by an endocrinologist for thyromegaly and elevated glucose blood levels (e.g., 195 mg/dL). Her hemoglobin A1c was elevated 9.4% (=<6.0% reference interval).

Two years previously, she had a Roux-en-Y pancreatico-jejunostomy to drain a pancreatic pseudocyst that developed following an episode of severe pancreatitis.
Was her diabetes due to her pancreatic disease and previous pancreatitis?

<table>
<thead>
<tr>
<th>Units</th>
<th>Pt result</th>
<th>RI</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>JDF</td>
<td>Negative</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IAA</td>
<td>nU/mL</td>
<td>3</td>
<td>&lt;126</td>
</tr>
<tr>
<td>GADA</td>
<td>U/mL</td>
<td>7.6</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>IA-2A</td>
<td>U/mL</td>
<td>0.23</td>
<td>&lt;0.76</td>
</tr>
</tbody>
</table>
Diagnosis: T1D

Learning objective:

All markers need *not* be positive!

- Number of positive markers can be: 1, 2, 3 or 4
- Any combination of markers
CASE BH

History: BH was an obese (101.4 kg; Wt >95th percentile for age) 17 year old Caucasian male who presented to the ED acutely ill with a 2 week history of polyuria, nocturia, polydipsia, 20-lb weight loss, headache, abdominal pain, nausea, vomiting and decreased appetite.

At the office of his LMD, his blood glucose was 419 mg/dL (nl random: <200 mg/dL). He was referred to the university hospital ED.
Weight-for-age percentiles: Boys, 2 to 20 years

Obese

Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).
CASE BH - Continued: At the university hospital ED:

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>Pt result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>478 mg/dL</td>
<td>NL random: &lt;200 mg/dL</td>
</tr>
<tr>
<td>VBG pH</td>
<td>7.24</td>
<td>RI: 7.35-7.45</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>4 meq/L</td>
<td>RI: 24-32 meq/L</td>
</tr>
<tr>
<td>U-ketones</td>
<td>Strongly (+)</td>
<td>RI: Negative</td>
</tr>
<tr>
<td>A1c</td>
<td>8.9%</td>
<td>RI: &lt;6.0%</td>
</tr>
</tbody>
</table>

(drawn in ED; reported later)

What do these findings indicate?
Clinical course: He was treated for diabetic ketoacidosis.

Based upon hyperglycemia and DKA, what type of diabetes does he have?
<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Units</th>
<th>Pt result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>JDF</td>
<td>Negative</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IAA</td>
<td>nU/mL</td>
<td>36</td>
<td>&lt;126</td>
</tr>
<tr>
<td>GADA</td>
<td>U/mL</td>
<td>&lt;0.1</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>IA-2A</td>
<td>U/mL</td>
<td>&lt;0.20</td>
<td>&lt;0.76</td>
</tr>
</tbody>
</table>
Islet autoantibody results

Negative x 4

**Diagnosis:** T2DM (with diabetic ketoacidosis)

- Obese teenagers w/ T2DM can present w/ DKA

*Why is the diagnosis of T1DM versus T2DM so critically important?*
### T1DM versus T2DM

<table>
<thead>
<tr>
<th><strong>Therapy is different</strong></th>
<th><strong>T1DM</strong></th>
<th><strong>T2DM</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin</td>
<td>Metformin (+ many other possible drugs)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Severity</strong></th>
<th><strong>T1DM</strong></th>
<th><strong>T2DM</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>++++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Associated diseases</strong></th>
<th><strong>T1DM</strong></th>
<th><strong>T2DM</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>AITD, Pernicious anemia, Celiac dis., Addison dis.</td>
<td>Metabolic syndrome - Hyperinsulinism - Dyslipidemia - Hypertension - Hyperandrogenism - Polycystic ovary syn. - Gout</td>
<td></td>
</tr>
</tbody>
</table>

AITD = autoimmune thyroid disease.
CASE RK

History: RK was 13 y/o African American girl. Her mother had noticed that RK had polyuria, polydipsia and nocturia 3x per night for the previous 1-2 months. She did not have blurry vision, nausea or vomiting. Over the previous 2 weeks RK was reported to have lost 10 pounds. A random afternoon blood glucose was elevated at 424 mg/dL (nl: <200 mg/dL).

<table>
<thead>
<tr>
<th>Vital signs</th>
<th>Results</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP:</td>
<td>123/74</td>
<td>No hypertension</td>
</tr>
<tr>
<td>Pulse:</td>
<td>95</td>
<td>High normal pulse</td>
</tr>
<tr>
<td>Temp:</td>
<td>36.6 °C (97.9 °F)</td>
<td>Afebrile</td>
</tr>
<tr>
<td>Height:</td>
<td>1.485 m</td>
<td></td>
</tr>
<tr>
<td>Weight:</td>
<td>53.3 kg</td>
<td></td>
</tr>
<tr>
<td>BMI:</td>
<td>24.2 Kg/M²</td>
<td>90th %tile for age &amp; sex</td>
</tr>
</tbody>
</table>
"RK has new onset diabetes with a presentation most consistent with type 1 diabetes mellitus.

There is a strong family history of type 2 diabetes so mother had many questions about why RK must be on insulin and could not start on pills.

Even after repeated explanations, mother spoke to the paternal grandmother by phone during the clinic visit asking why pills couldn't be used.

We explained in detail insulin requirements and the difference between type 1 and 2 diabetes."
Does RK have T1DM?

Islet autoantibody test results

<table>
<thead>
<tr>
<th>Units</th>
<th>Pt result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA JDF</td>
<td>Negative</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IAA nU/mL</td>
<td>6</td>
<td>&lt;126</td>
</tr>
<tr>
<td>GADA U/mL</td>
<td>&lt;0.1</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>IA-2A U/mL</td>
<td>&lt;0.20</td>
<td>&lt;0.76</td>
</tr>
</tbody>
</table>

How should these results be interpreted?
Islet autoantibodies (-) x 4

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Units</th>
<th>Pt result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>JDF</td>
<td>Negative</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IAA</td>
<td>nU/mL</td>
<td>6</td>
<td>&lt;126</td>
</tr>
<tr>
<td>GADA</td>
<td>U/mL</td>
<td>&lt;0.1</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>IA-2A</td>
<td>U/mL</td>
<td>&lt;0.20</td>
<td>&lt;0.76</td>
</tr>
</tbody>
</table>

**Diagnosis:** T1DM is *unlikely*
Endocrinologist's follow-up:

Note: "Saw her in clinic yesterday. . . I think she (has) type 2 (DM) based on (her) phenotype, but she required significant insulin in the early stages of treatment (as she presented with a) HgbA1c over 12% and large ketones …… She was started on Metformin yesterday and we are in the process of weaning her insulin dose."
CASE TB

History: TB was a 64 y/o year old male who presented with a recent history of polyuria and weight loss. The frequency of urination and polyuria had been noted for ~1-2 month and was worsening. He also reported increasing fatigue. Because of his weight loss and he had intentionally began to eat more fatty foods.

PE: Metric English
Ht 0.152 m 6"
Wt 73.528 kg 162 lb 1.6 oz
BMI 22.2 kg/M² - - - -
<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>Pt result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose mg/dL</td>
<td>260 mg/dL</td>
<td>NI random: &lt;200 mg/dL</td>
</tr>
<tr>
<td>A1c</td>
<td>13%</td>
<td>NI: =&lt;6.0%</td>
</tr>
</tbody>
</table>

**Units** | **Pt result** | **Reference interval**
--- | --- | ---
GADA U/mL | 19.1 | <1.1 |

**Diagnosis:**
- Latent autoimmune diabetes of adulthood (LADA)
- Slowly progressive form of T1DM
- GADA & ZnT8A -- > most common markers of LADA

**Implication:**
- Aggressive insulin therapy is advised.
## Summary

Islet autoantibodies: Most useful biomarkers for T1DM

<table>
<thead>
<tr>
<th></th>
<th>Present at Dx/</th>
<th>Ease of detection</th>
<th>Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>GADA</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IA-2A</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IAA</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ZnT8A</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>
Notes on islet autoantibody testing:

- Testing for **multiple** autoantibodies is important
- No one autoab is always (+)

Islet Autoantibody Panel

<table>
<thead>
<tr>
<th></th>
<th>ICA</th>
<th>GADA</th>
<th>IA-2</th>
<th>ZnT8A</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pediatric</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Adult</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Notes on islet autoantibody testing:

- **GADA & ZnT8A** - most common autoabs (+) in LADA

- **IAA** - uncommon in adults

- **Comment**: Islet autoantibody positivity *confirms* an autoimmune etiology;
- absence of islet autoantibodies does *not* exclude an autoimmune etiology or confirm a non-autoimmunity etiology.
Notes on endocrine autoantibody testing:

Multiple endocrinologic and non-endocrinologic autoimmune disorders are associated w/ T1DM:

Basic Endocrine Autoantibody Panel (ICA, TPOA, TGA, Gastric Parietal Cell Autoantibodies, Adrenal Cortical/Ovarian/Testicular/Placental Autoantibodies)

Comprehensive Endocrine Autoantibody Panel (Endocrine Autoantibody Panel and GADA, IA-2A, IAA and ZnT8A)
What are future uses of islet autoantibody testing?

1. **Confirmation of T1DM** in new-onset patients undergoing *intervention therapy* to preserve/salvage beta cell mass

2. **Prediction of T1DM** in non-diabetic subjects undergoing therapies for the *prevention* of T1DM

   - Experimental uses (no safe & effective tx’s yet available to preserve/salvage beta cells or prevent T1DM).

   - Testing in non-diabetic subjects: research only
THANK YOU FOR LISTENING