Commercial insulin immunoassays fail to detect commonly prescribed insulin analogues

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Hypoglycaemia

A common clinical condition...

...with potentially serious consequences
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A common clinical condition...

...with potentially serious consequences

• Poor management of diabetes mellitus
• Prolonged fasting
• Hyperinsulinaemia
  • Exogenous insulin administration
  • Insulinoma
• Sepsis

• Adrenal insufficiency
• Inborn errors of metabolism
• Maternal hyperglycaemia
• Alcohol-induced hypoglycaemia
• Hepatic, renal or cardiac failure
• Insulin autoimmune hypoglycemia
Hypoglycaemia

A common clinical condition...

...with potentially serious consequences

- Exogenous insulin administration
  - Accidental insulin overdose
  - Deliberate insulin overdose
  - Malicious administration of insulin
Exogenous insulin administration

- Rare presentation, but vital to identify
- Often apparent from patient history

They stated that the patient had appeared to be depressed during the past two weeks. He had recently separated from his wife and had not been eating well. They suspected that the patient might have taken excess insulin, noting that seven bottles of insulin (three regular and four NPH, 1,000 units each), believed to be full two days earlier, were found empty the evening of admission. The family

On arrival of the emergency medical team, three empty disposable syringes and an empty ampoule of insulin were found on the coffee table. The content of the syringes was later analysed and found to be human insulin.

day of admission, she injected multiple doses of glargine (100 units/ml, total = 20 ml, = 2000 units) sub-cutaneously in the back of the left arm. One to two hours later, she was found unresponsive and hypoglycemic [blood glucose was 1.4 mmol/l] alongside two empty vials of glargine and a used syringe. Intravenous glucose (IV) (7.5 g) was given by

A 51-year-old woman with insulin-dependent diabetes mellitus (DM) presented to the Emergency Department (ED) after an intentional overdose of her insulin glargine. At 5:00 p.m. the day before her ED presentation, she had an argument with her boyfriend and injected herself subcutaneously with 9 Lantus SoloSTAR® pens, which was approximately 2700 units total. She admitted this was a suicide attempt. The patient’s past medical history

A 37-year-old man presented to the Emergency Department (ED) after an intentional overdose of insulin glargine. He reported self-administering 150 units subcutaneously over multiple sites at 1:00 a.m. on the day of
Exogenous insulin administration

- Rare presentation, but vital to identify
- Surreptitious administration is known

For 5 days in the ICU under continuous observation. No additional hypoglycemic episode occurred. The girl’s mother, who had received a diagnosis of type 1 diabetes at the age of 10 years, was suspected to have injected subcutaneous insulin into her daughter. Both parents denied administration of insulin. Several days later, during a psychological evaluation, the child imitated insulin injections to her teddy bear. Several months later, the mother admitted that she had repeatedly injected insulin into her daughter.

Suspicion that the mother was injecting some of her insulin into the child’s i.v. access were triggered by the fact that her son’s abnormally low blood sugar levels occurred only when she was in the room. The mother also voiced concern that her child was becoming a diabetic just like her, and the child knew how to perform his own finger prick for glucose monitoring. Behavioral aberrations on the part of the mother were also noted, as evi-
Exogenous insulin administration

• Rare presentation, but vital to identify

Insulin administration should be considered in patients with unexplained hypoglycaemic episodes
Laboratory investigation

- Performed during hypoglycaemic attack
- Measure Insulin and C-peptide

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Insulin</th>
<th>C-Peptide</th>
<th>Insulin: C-peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulinoma</strong></td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>&gt; 1</td>
</tr>
<tr>
<td><strong>Ketotic hypoglycaemia</strong></td>
<td>↓</td>
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<td>↓</td>
<td>&gt; 1</td>
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<tr>
<td><strong>Exogenous administration</strong></td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

- Insulin measured by immunoassay
- MSIA technique (King’s) in development
Insulin analogues

- **Human insulin**
  - Short acting insulin
    - Duration: 3 hours
    - Plasma insulin level: 12 pmol/L
  - Long acting insulin
    - Duration: 12 hours
    - Plasma insulin level: 6 pmol/L
### Insulin analogues

<table>
<thead>
<tr>
<th></th>
<th>A chain</th>
<th>B chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>GIVEQCTSICSLYQENYCN</td>
<td>FVNQHLCGSHLVEALVLVCGERGFFYTPKT</td>
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<tr>
<td>Porcine</td>
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<td>Bovine</td>
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<td>FVNQHLCGSHLVEALVLVCGERGFFYTPKA</td>
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<tr>
<td>Glulisine</td>
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<td>FVKQHLCGSHLVEALVLVCGERGFFYTPET</td>
</tr>
<tr>
<td>Glargine</td>
<td>GIVEQCTSICSLYQLEN</td>
<td></td>
</tr>
<tr>
<td>Levmir</td>
<td>GIVEQCTSICSLYQLEN</td>
<td></td>
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<tr>
<td>Degludec</td>
<td>GIVEQCTSICSLYQLEN</td>
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Does changing the structure of the insulin molecule interfere with antibody binding in the insulin assay?
Previous work

- Previous work has shown that cross-reactivity is poor for certain platforms
  - Limitations include:
    - number of platforms tested
    - number of insulins tested
    - variable methodologies


This project aimed to assess cross-reactivity of all insulin analogues listed in the BNF on all commercial clinical platforms currently available in the UK
## Insulin analogues

<table>
<thead>
<tr>
<th>Human Insulin</th>
<th>One amino acid difference</th>
<th>Three amino acid difference</th>
<th>Complex insulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actrapid®</td>
<td></td>
<td>Hypurin® Bovine Neutral</td>
<td>Levemir® (insulin detemir)</td>
</tr>
<tr>
<td>Insulatard®</td>
<td></td>
<td>Hypurin® Bovine Isophane</td>
<td>Tresiba® (insulin degludec)</td>
</tr>
<tr>
<td>Humulin® I</td>
<td></td>
<td>Hypurin® Protamine Zinc</td>
<td></td>
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<tr>
<td>Humulin® S</td>
<td></td>
<td>Lantus® (insulin glargine)</td>
<td></td>
</tr>
</tbody>
</table>

### Three amino acid difference
- Hypurin® Bovine Neutral
- Hypurin® Bovine Isophane
- Hypurin® Protamine Zinc
- Lantus® (insulin glargine)

### One amino acid difference
- NovoRapid® (insulin aspart)
- Hypurin® Porcine Neutral
- Hypurin® Porcine Isophane

### Complex insulins
- Levemir® (insulin detemir)
- Tresiba® (insulin degludec)

### Two amino acid difference
- Humalog® (insulin lispro)
- Apidra® SoloStar (insulin glulisine)

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Insulins kindly supplied by Tessa Kaczorek at RDE Pharmacy
Methods

• Insulins were diluted in BSA (40 g/L)
• Diluted to stock solution in pooled serum from fasting volunteers
• Divided into aliquots and frozen (-80 °C)
• Sample sets comprised
  – 15 analogues (1000 nmol/L and 300 nmol/L)
  – 2 blanks (BSA alone/pooled serum + BSA)
• Samples sent blinded to 5 participating labs on dry ice
• Measured according to local lab protocols
Methods

- Each result was blank corrected
- Percentage recovery for each analogue/concentration was calculated
- The average of these values was used to assess cross-reactivity
- Classified as:
  - Poor (< 21 % CR)
  - Moderate (21 – 79 %)
  - Good (> 80 %)
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*CONTAINS ISOPHANE
**ADDITIVES NOT DETECTED

Results truncated at > 140 % recovery
Individual assay performance

- Commercial insulin assays in the UK are unable to detect the presence of several commonly prescribed insulin analogues.
Minor modification of insulin structure

- Commercial insulin assays in the UK are unable to detect the presence of several commonly prescribed insulin analogues
  - Even a single amino acid change can significantly limit cross-reactivity

Insulin aspart (NovoRapid®)
## Novorapid (insulin aspart)

### Average percentage recovery (1000 and 300 pmol/L)

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Measurement of a specific analogue

• Commercial insulin assays in the UK are unable to detect the presence of several commonly prescribed insulin analogues
  – Individual platforms vary in their ability to identify a particular analogue

Hypurin Bovine Neutral
### Hypurin neutral bovine

#### Average percentage recovery (1000 and 300 pmol/L)

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Overall cross-reactivity

- Mercodia Iso-insulin assay has good cross-reactivity with most insulin analogues
Complex insulins

• Neither insulin degludec or insulin levemir are consistently identified by any assay
  – Likely due to bulky groups blocking any antibody binding at C-terminal
Commercial insulin assays in the UK are unable to detect the presence of several commonly prescribed insulin analogues

- Manufacturers do not typically provide cross-reactivity information on kit inserts
- Reliance on established raised insulin/decreased C-peptide ratio may miss cases of exogenous insulin administration
- Biochemists must remain alert to the possibility of exogenous insulin administration and be prepared to advise clinicians accordingly
Acknowledgements

• RDE
  – Dr Tim McDonald
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  – Carol Maguire

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Questions?