Pancreatic autoantibodies: who to test and how to interpret the results

Access to pancreatic autoantibody requests is increasing; however, deciding whom to test and how to interpret results remain unclear.

In this article, Dr Shivani Misra explores the evidence base and controversies around pancreatic autoantibody testing, and discusses some recommendations about how to use the test in a clinically meaningful way.

What are pancreatic autoantibodies?

Pancreatic autoantibodies form against components of the pancreatic beta-cell and may be detected in people with type 1 diabetes.\(^1\)\(^2\)

Antibodies to a variety of beta-cell components – including glutamate decarboxylase-65 (GAD-65), islet-antigen-2 (IA-2), zinc transporter-8 (ZnT8), insulin itself and, most recently, tetraspanin-7 – have been detected.\(^3\)\(^-\)\(^7\) (See Figure 1.)

The significance of pancreatic autoantibodies has been studied most in people without diabetes where the presence of multiple antibodies is a major risk factor for developing type 1 diabetes, with each additional detectable antibody conferring a higher risk.\(^8\)

The interpretation of pancreatic autoantibodies as a diagnostic test for type 1 diabetes in people with established diabetes is an emerging field of study, with many heterogenous observational studies assessing antibodies at and after diagnosis, in different age groups and using different antibodies and assays.

How are pancreatic autoantibodies measured?

Pancreatic autoantibodies can be measured in serum or plasma separated from a blood sample. Most laboratories use an immunoassay or radio-binding assay to detect titres of antibody, such that either a negative result or a numerical value indicating positivity can be reported. Previously, assays were used that gave a qualitative or semi-quantitative result, e.g. weakly positive or strongly positive, using immunofluorescence assays.\(^9\) The immunofluorescence-based islet cell antibody assay is still in use at some centres, but should be replaced as it is operator-dependent and non-specific, detecting inter-species antigen-antibody binding.\(^10\) The international antibody standardisation programme has also provided data showing the immunofluorescence technique lacks sensitivity in the detection of antibody positivity.\(^11\)\(^,\)\(^12\)

Reference ranges for immunoassays that measure pancreatic autoantibodies are poorly standardised. Work is being undertaken internationally to try to standardise measurement.

Who should be tested according to national guidelines?

At present neither adult nor paediatric UK national guidelines advocate routine testing of antibodies in adults or children with clinically suspected type 1 diabetes.\(^13\)\(^,\)\(^14\)

Vignette

A 27-year-old lady presented to her general practitioner (GP) with a short duration of thirst and urinary frequency. Her body mass index (BMI) was 29kg/m\(^2\) and she had lost some weight after starting to exercise and modifying her diet.

A fasting blood glucose was elevated at 22mmol/L and her Hba1c was 72mmol/mol. Urinalysis was negative for ketones. Her GP diagnosed type 2 diabetes and prescribed metformin. However, after two months her capillary blood glucose monitoring results were still elevated and she continued to lose weight, feeling lethargic.

She presented to the emergency department with hyperglycaemia and lethargy. Investigations revealed ketosis, but not acidosis. She was started on a fixed-rated insulin infusion and was reviewed by the diabetes team who requested pancreatic autoantibody testing. She was converted to a basal-bolus insulin regimen and was discharged home for review in clinic.

At her clinic appointment, results for antibody testing revealed that she was GAD-65 and IA-2 antibody positive. In view of her clinical presentation and autoantibody results, she was reclassified as having type 1 diabetes. She was provided with type 1 diabetes specific education and education around home blood glucose and ketone monitoring. She was followed up in the hospital type 1 diabetes service.

Comment. In this case the decision to start insulin was influenced by the clinical presentation. However, after the acute presentation, the results of pancreatic autoantibody testing supported the clinical presentation and made the diagnosis of type 1 diabetes clear, supporting lifelong insulin treatment.

The initial presentation with raised BMI and mild symptoms without ketones in the urine favoured type 2 diabetes, but these features may overlap between type 1 and type 2 diabetes. People with type 1 diabetes who are overweight may present earlier because of insulin resistance and may not necessarily be ketotic.

Following the reclassification of diabetes subtype, she was referred for type 1 specific education and follow up. Clarity around her diagnosis will prevent erroneous treatment choices and ensure she has access to appropriate technologies to support people with type 1 diabetes.

It is recommended that children presenting with diabetes should be assumed to have type 1 diabetes at diagnosis, so that there are no delays in initiating life-sustaining insulin treatment. Routine pancreatic autoantibody testing is not recommended and is reserved for cases where there may be uncertainty around diagnosis.\(^14\) However, there is emerging research that systematically checking antibodies in children may be an effective approach to identify those with a monogenic form of diabetes, for genetic testing.\(^15\)

In adults, the diagnosis of type 1 diabetes is based on clinical features but there is recognition of the increasing need to substantiate the diagnosis of type 1 diabetes. Antibody testing is recommended in the following circumstances.\(^13\)
• Atypical features of type 1 diabetes; what constitutes an atypical picture is down to the clinician making the diagnosis.
• In those where a monogenic form is suspected and antibody testing may guide decision-making around genetic testing.
• In cases where confirmation of type 1 diabetes may have implications for ongoing management, for example prior to consideration of bariatric surgery or access to type 1 diabetes specific technologies or management pathways.

What is known about pancreatic autoantibodies in type 1?

Studies that assess pancreatic autoantibodies in type 1 diabetes are variable in the populations studied, their design and in the antibodies tested. In general, the percentage of individuals positive to one or more antibodies at diagnosis is highest in children with type 1 diabetes with only ~4% negative at diagnosis.15 In adults, preliminary data from the ADDRESS-2 study show that 85% of those with a clinician-assigned diagnosis of type 1 diabetes tested positive for one or more antibodies at diagnosis.16

Data on antibody positivity in ethnic groups other than white are lacking and studies from around the world suggest variable positivity.17–20 Titres diminish with duration of type 1 diabetes; however, GAD-65 and IA-2 antibodies are likely to stay positive for many years, making them a useful measure, even if long after diagnosis.21

It is important to test several pancreatic autoantibodies, as a proportion of individuals may be negative to one but positive to another. When testing antibodies in adults, 60% of individuals were positive to GAD only; however, 80% were positive to GAD and/or IA-2.22 ZnT8 testing is not available in all laboratories, but data suggest that testing this antibody will further increase detection of autoimmunity.15 Therefore less than complete testing of all antibodies may result in erroneous interpretation.

Why not test pancreatic autoantibodies in everyone with diabetes?

There are several pitfalls related to pancreatic autoantibody testing of which clinicians need to be aware when interpreting results (see Box 1). These caveats underlie the reason that routine pancreatic autoantibody testing, particularly in adults, is not recommended currently.

The main issue relates to the sensitivity and specificity of these tests when used for diagnosis (see below).

What does a positive pancreatic autoantibody result mean?

A positive result is not necessarily diagnostic of type 1 diabetes, but may support a clinical diagnosis. A proportion of individuals in the population have background antibody positivity; for example GAD-65 positivity has been reported in between 0.4–4.7% of people who did not develop diabetes over the time period assessed, but had an affected relative.23–25 This means that a positive result should first be correlated with the clinical case. As more people with diabetes are tested, it is likely that some individuals who have 'background positivity' will be encountered. There is no way to differentiate people who have background positivity with diabetes, from people who have type 1 diabetes or from those with type 1 diabetes in evolution. However, this distinction may not have any clinical value, as we know from studies such as UKPDS that people who had a type 2 diabetes phenotype, but in whom an antibody was positive, required insulin sooner.26 The value of identifying this intermediary group is therefore still unclear. Confidence around interpretation of positive results can increase if more than one antibody is positive.

These limitations mean that a positive diabetes antibody result can be helpful in substantiating a diagnosis of type 1 diabetes; however, negative results in someone with suspected type 1 do not exclude the condition, particularly as it is recognised that a proportion of individuals with type 1 are antibody negative.

What does a negative pancreatic autoantibody result mean?

A negative result does not exclude type 1 diabetes. A proportion of people who have type 1 diabetes clinically do not have antibodies when measured at diagnosis.27–29 More antibodies are being discovered so it may be the case that some of these individuals have antibodies to, as yet, unknown antigens. Antibody negativity in type 1 diabetes may be more common in people from different ethnicities, but these studies are difficult to interpret due to incomplete antibody testing.29–30

People who are initially antibody positive at diagnosis may also become
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Test tips

- A positive titre may not be diagnostic of type 1 diabetes
- A negative result does not exclude type 1 diabetes
- When tested after diagnosis, initial positive titres may become negative
- Many laboratories do not offer a panel of autoantibodies and less than complete testing may miss people who are positive to another antibody
- Assays that measure pancreatic autoantibodies may not be standardised
- The significance and interpretation of pancreatic autoantibodies are understudied in people from non-white ethnic groups

Box 1. The pitfalls of testing pancreatic autoantibodies

Seronegative with time, so when measured after several years’ duration, interpretation is problematic. Therefore, negative results should be interpreted with the clinical context in mind. Somebody who is clearly insulin-requiring should not have therapy changed because pancreatic autoantibodies are negative. Equally, decision-making around starting insulin should not be delayed because pancreatic autoantibody results are awaited.

When might testing be helpful in clinical practice?

Although guidelines do not recommend routine testing, there are many clinical scenarios in which testing pancreatic autoantibodies may be helpful; some of these examples are provided:

- Positive results may support a clinical suspicion of type 1 diabetes to enable access to type 1 diabetes management pathways.
- A negative result might increase suspicion of an alternative diagnosis; for example ketosis-prone type 2 diabetes or maturity-onset diabetes of the young.
- A negative result may help stratify someone with young-onset diabetes for genetic testing.
- Diabetes presenting in pregnancy with an unusual phenotype may be incipient type 1 diabetes unmasked by insulin resistance of pregnancy. Testing antibodies may help direct appropriate follow-up, although the evidence base to support this is lacking.
- In cases where features of type 1 and type 2 diabetes overlap, for example young age at onset but obese, or older age at onset but lean.
- In ethnic groups where type 2 diabetes predominates but risk of type 1 diabetes may be underestimated (see below).

People from non-white ethnic groups are often assumed to have type 2 diabetes, even when presenting as young adults. There are compelling data to show that migrant populations adopt the local risk of type 1 diabetes in the country to which they move.\textsuperscript{31–33} In these cases, testing antibodies may support a definitive diagnosis of type 1 diabetes and some studies suggest that proportions of antibody positivity may be similar to those of white individuals.\textsuperscript{17,34,35}

Simple recommendations for safe testing

- Consider one key question before requesting the test: how will this result change management in the case? If the answer is that it won’t and there is confidence around the diagnosis of type 1 diabetes, it is not worth undertaking the test.
- Decision-making around commencing and replacing insulin therapy should primarily be based on clinical parameters.
- The antibody results should be supporting a diagnosis, directing a treatment pathway or stratifying people for genetic testing. More evidence is needed to demonstrate there is value within and outside these areas.
- Most laboratories offer GAD-65 antibodies but few offer IA-2 and ZnT8 antibodies. If pancreatic autoantibodies are requested but a full panel is not tested, be aware that you may be missing other positive results. Where possible, opt for the measurement of all three.
- If type 1 diabetes is suspected clinically, but the antibody results are negative, do not be put off: review the caveats above and remember insulin remains a safe and appropriate treatment in the majority of cases.
- Specialist centres provide diagnostic clinical support and can be contacted to discuss cases where diabetes subtype is unclear.

Future research areas

Although there has been a proliferation in pancreatic autoantibody requesting, the evidence base to support their measurement in classifying diabetes is limited and further work is needed to establish high-quality evidence that testing antibodies affects clinical pathways and changes patient outcome.

Very little is known about whether antibody titres influence clinical phenotype or if serial measurement has any utility. Further work is needed to identify any potential role for tetraspanin-7 antibody measurement – currently not available for routine measurement.

Conclusions

In clinical practice there is increasing heterogeneity in diabetes presentation. Diagnostic uncertainty can lead to misclassification of diabetes subtype and erroneous treatment decision making. Pancreatic autoantibodies may have a place in supporting assignment of diabetes subtype, but the evidence base to demonstrate this conclusively is lacking. There are many pitfalls related to testing that can make interpretation of both positive and negative results a challenge, and practitioners should be aware of these when requesting testing.

When undertaking testing, in line with guidance, there should be a clear clinical question in mind – how will this result influence treatment or diagnosis? Antibody testing is best reserved for cases where they may aid subtype assignment, provide clarity around follow up and access to services, and in stratifying people for genetic testing of monogenic diabetes. If a decision is made to test antibodies, be aware that less than complete testing of the three main autoantibodies may result in erroneous interpretation.

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Pancreatic autoantibodies: who to test and how to interpret the results

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