Efficient Investigation and Differential Diagnosis of Childhood Onset Niemann-Pick Type C

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Abstract

Niemann-Pick disease type C (NPC) is a fatal, neurodegenerative, lysosomal storage disorder. It is rare with a broad phenotypic spectrum and variable age of onset. This complicates diagnosis, which is often delayed by several years after presentation of the first symptoms. It is a treatable condition if detected early, therefore reliable means of diagnosis are essential. Clinical diagnosis of NPC involves identifying characteristic neurological features, taking a detailed history of the patient’s details, and must be confirmed by biochemical and/or genetic testing. The key laboratory diagnostic test for NPC is filipin staining of cultured skin fibroblasts, which shows free cholesterol accumulation in lysosomes resulting from impaired intracellular cholesterol transport. Genetic testing for mutations in the NPC1 and NPC2 genes is also important for confirmation of the diagnosis. However, there is an unmet need for cheaper diagnostic tests with greater specificity and sensitivity.

Keywords

Filipin, lysosomal storage disorder, Niemann-Pick type C (NPC), NPC1, NPC2, progressive intellectual, neurological deterioration, genetic analysis

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Niemann-Pick disease type C (NPC) is a rare, autosomal recessive, neurodegenerative disorder occurring in all ethnic groups, with an estimated minimal incidence of 0.82 per 100,000 live births. This figure is likely to be an underestimation due to failure to recognise the clinical characteristics and initiate appropriate tests. NPC is a devastating disorder, characterised by a variety of progressive and disabling neurological/psychiatric symptoms and leading to premature death. In the terminal phase, patients are immobile and require tube feeding. The emotional and economic burden imposed by NPC on patients, families and society is disproportionate to the rarity of the disease.

The wide clinical spectrum of NPC was not recognised until the early 1990s, particularly regarding rapidly fatal cases in infants, and no specific laboratory tests had been available making accurate diagnosis challenging. Delayed presentation of NPC in adolescents and adults has further added to the misdiagnosis of this disorder. Since awareness of the disease and diagnostic techniques have improved, what was once considered to be a childhood condition is now increasingly recognised as an illness affecting individuals of all ages.

The biochemistry behind NPC was elucidated in 1984 following studies on cholesterol metabolism. It is a cellular lipid trafficking disorder characterised by lysosomal accumulation of low-density lipoprotein (LDL)-derived, unesterified cholesterol. Genetic studies found that mutations in two genes, NPC1 (reported in 90–95 % of patients) and NPC2 (~5 % of patients), play a role in the disrupted transport of unesterified cholesterol, sphingolipids and glycosphingolipids. When there is mutation within one of these genes, accumulation of cholesterol in late endosomes and lysosomes within the spleen, liver and brain occurs.

NPC is a heterogeneous condition with an age at onset ranging from the perinatal period to as late as 50 years of age or older; juvenile onset (ages 6–15 years) is the most common. The age of onset has a major impact on the severity and the course of the disease although progression is linear and independent of this variable (see Figure 1). The different ages of disease onset can be used to define the disorder: the generally accepted categories are pre/perinatal, early-infantile, late-infantile, juvenile, and adolescent/adult. Apart from a subset of patients that die at birth, most patients follow a progressive and ultimately fatal neurological disease course. The lifespan of affected individuals can vary from days to years with many dying between 10 and 25 years of age.

While diagnosis of this rare condition is not difficult, mis-/delayed diagnoses are common – possibly due to poor clinical examination and inappropriate tests being performed. Timely detection of NPC is
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Table 1: List of Symptoms Associated with Age at Onset

<table>
<thead>
<tr>
<th>Age at Onset</th>
<th>Systemic Manifestations</th>
<th>Neurological Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre/perinatal period (&lt;3 months)</td>
<td>• Foetal hydrops</td>
<td>• Usually not recognised</td>
</tr>
<tr>
<td></td>
<td>• Hepatosplenomegaly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Foetal ascites with or without persistence after birth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Prolonged cholestasis (frequent)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Respiratory failure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Hepatic failure</td>
<td></td>
</tr>
<tr>
<td>Early-infantile period (3 months to &lt;2 years)</td>
<td>• Isolated hepatosplenomegacy or hepatosplenomegaly (usually present)</td>
<td>• Delayed developmental motor milestones</td>
</tr>
<tr>
<td></td>
<td>• Central hypotonia</td>
<td>• Hearing loss</td>
</tr>
<tr>
<td></td>
<td>• Hearing loss</td>
<td>• VSGP &lt;i&gt; (usually not recognised)</td>
</tr>
<tr>
<td>Late-infantile period (2 years to &lt;6 years)</td>
<td>• Isolated organomegaly or organomegaly (usually present)</td>
<td>• Frequent falls, clumsiness</td>
</tr>
<tr>
<td></td>
<td>• Central hypotonia</td>
<td>• Progressive ataxia, dystonia, dysphagia, dysarthria</td>
</tr>
<tr>
<td></td>
<td>• Hearing loss</td>
<td>• Myoclonus</td>
</tr>
<tr>
<td></td>
<td>• Seizures (partial or generalised)</td>
<td>• Cataplexy</td>
</tr>
<tr>
<td></td>
<td>• VSGP &lt;i&gt; (usually present)</td>
<td></td>
</tr>
<tr>
<td>Juvenile (classical) (6–15 years)</td>
<td>• Isolated organomegaly or organomegaly (not always present)</td>
<td>• School failure, learning disability</td>
</tr>
<tr>
<td></td>
<td>• Central hypotonia</td>
<td>• Behavioural problems</td>
</tr>
<tr>
<td></td>
<td>• Hearing loss</td>
<td>• Frequent falls, clumsiness</td>
</tr>
<tr>
<td></td>
<td>• VSGP &lt;i&gt; (usually present)</td>
<td>• Progressive ataxia, dystonia, dysphagia</td>
</tr>
<tr>
<td></td>
<td>• Seizures (partial and/or generalised)</td>
<td>• Myoclonus</td>
</tr>
<tr>
<td></td>
<td>• VSGP &lt;i&gt; (usually present)</td>
<td>• Seizures (partial and/or generalised)</td>
</tr>
<tr>
<td>Adolescent and adult (&gt;15 years)</td>
<td>• Organomegaly (not always present) or isolated splenomegaly in adults has been described in exceedingly rare cases</td>
<td>• Clumsiness</td>
</tr>
<tr>
<td></td>
<td>• Central hypotonia</td>
<td>• Cataplexy</td>
</tr>
<tr>
<td></td>
<td>• Myoclonus</td>
<td>• Psychiatric signs †</td>
</tr>
<tr>
<td></td>
<td>• Seizures (partial and/or generalised)</td>
<td>• Cognitive decline, dementia, learning disability</td>
</tr>
<tr>
<td></td>
<td>• VSGP &lt;i&gt; (usually present)</td>
<td>• Slowly progressing motor symptoms ‡</td>
</tr>
<tr>
<td></td>
<td>• Myoclonus</td>
<td>• Psychosis, depression, Fatigability</td>
</tr>
<tr>
<td></td>
<td>• Seizures (partial and/or generalised)</td>
<td>• Cognitive decline, dementia, learning disability</td>
</tr>
<tr>
<td></td>
<td>• VSGP &lt;i&gt; (usually present)</td>
<td></td>
</tr>
</tbody>
</table>

<i>VSGP = vertical, supranuclear gaze palsy – increased latency in initiation of vertical saccades, with gradual slowing and eventual loss of saccadic velocity; †schizophrenia (psychosis), depression; ‡ataxia, dystonia, dysarthria, dysphagia. Source: Wraith JE et al., 2009. 3</i>

Figure 1: Relationship Between Age at Onset and Niemann-Pick Disease Type C Severity Based on the Natural History of the Disease Without any Treatment

essential, however, because this disorder responds better to disease-modifying pharmacological and supportive treatments when they are administered at an early stage. This article will review the clinical manifestations of NPC as well as discuss current strategies for the efficient diagnosis of this rare disease.

Signs and Symptoms

The broad clinical manifestations of NPC and their association with age at onset are presented in Table 1, although there is considerable overlap between the different categories. The wide range of signs and symptoms includes cerebellar ataxia manifesting as language delay, gait problems, frequent falls and clumsiness; this leads to dysarthria (slurred speech). There is also dysphagia, and dysphagia is a pronounced symptom. Dystonia commonly begins with the turning inwards of one foot when walking, but hand posturing is also often evident early on. Later in the disease course, spasticity and progressive dementia become more obvious. Children of school age frequently exhibit learning and/or behavioural difficulties. Seizures are also common and psychiatric symptoms are typically seen in late-onset patients.

Moreover, NPC is associated with some very specific signs. Vertical supranuclear gaze palsy (VSPG) beginning with subtle abnormal saccadic eye movements is more commonly detected from late infancy onwards. However, it is a sign that is mostly overlooked and
Challenges in Diagnosing Niemann-Pick Disease Type C

For many neurometabolic conditions, no disease-modifying therapies are available. However, treatment is available for NPC, which can arrest and/or slow disease progression, although improvement of symptoms is unlikely. As a result, early diagnosis is imperative to allow for prompt application of management strategies. Unfortunately, NPC is often misdiagnosed for various reasons leading to a delay between initial symptoms and appropriate therapy.

Childhood-onset NPC is frequently missed as it tends to start insidiously with an inconspicuous steady progression that diverges increasingly from the normal population. Moreover, the rate of disease progression varies greatly, further confusing matters. Lack of awareness among clinicians and paediatricians (perhaps due to the rarity of NPC) also contributes to the mis/underdiagnosis of this disease.¹

Furthermore, NPC is a multifaceted and heterogeneous condition, so not all classical signs may be present at any given time.² Symptoms might be quite common and/or spread over time (e.g. unexplained prolonged jaundice as neonates followed by developmental delay later in life) and there is often a ‘wait and see’ attitude among physicians when no obvious cause is found, which is not appropriate when NPC is a possible diagnosis.

As a result of these obstacles, the average time from initial symptoms to a definite diagnosis is approximately four years.³ Routine tests (even for metabolic dysfunctions) do not normally include NPC. Consequently, it is often overlooked.

Clinical Investigation and Differential Diagnosis

The clinician is faced with a broad spectrum of symptoms in suspected NPC, as a consequence efficient and correct diagnosis is often not made. Paediatricians need to be able to effectively distinguish conditions such as NPC, which are fully treatable, from simple ‘developmental delay’ or static neuropsychiatric disorders in children. Moreover, they need to ensure efficient investigation and application of appropriate tests for degenerative disorders. While there remains no standard procedure to follow for NPC diagnosis, there are key symptoms that should signal the need for further examination. The following details the steps to take in the diagnostic work-up of patients who potentially have NPC.

An essential part of the initial investigation is a thorough history taking of the patients’ details, usually through the parent(s). This will reveal any major milestones experienced in the past and processes that may have affected the brain. Obtaining their family histories in terms of neurological illnesses such as prolonged neonatal jaundice and seizures should alert healthcare professionals to the possibility of an NPC diagnosis. In addition, specifically asking for consanguinity in the family history is essential at this stage. Table 2 highlights the key information that should be elucidated during this history taking. A complete history of the patient is vital in determining the next steps of the assessment process.

With a clear understanding of the individual’s history, a detailed examination can be performed to separate the developmental delay or dyspraxia from other neurological signs. Table 3 summarises the signs that should raise red flags in the physician’s mind regarding an NPC diagnosis. The clinical assessment must include ophthalmologic evaluation, with specific examination of spontaneous vertical saccadic eye movements. The impairment of these movements is specific for VSGP, a key sign of NPC. Neurological examination should include assessment of muscle strength, muscle tone, motor reflexes, movement, speech, swallowing, and cognition.¹ Psychometric testing is also advisable. When there are focal neurological signs and/or a suggestive history, additional specific testing using biochemical techniques and genetic analyses should be performed; these are discussed in the next section.

A frequent error is to base differential diagnoses on a deficient examination. Systemic signs such as neonatal jaundice/isolated splenomegaly, and neurological symptoms such as dystonia, ataxia,
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Figure 2: The Niemann-Pick Disease Type C Suspicion Index Tool

<table>
<thead>
<tr>
<th>Signs and Symptoms</th>
<th>Visceral Score</th>
<th>Neurological Score</th>
<th>Psychiatric Score</th>
<th>Category scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicators</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Strong</td>
<td>40 points per item</td>
<td>Prolonged unexplained neonatal jaundice or cholestatis</td>
<td>Vertical supranuclear gaze palsy</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Isolated unexplained splenomegaly (historical and/or current with/without hepatomegaly)</td>
<td>Gelastic cataplexy</td>
<td>Positive</td>
</tr>
<tr>
<td>Strong</td>
<td>20 points per item</td>
<td>Ataxia, clumsiness or frequent falls</td>
<td>Pre-senile cognitive decline and/or dementia</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Prolonged unexplained neonatal jaundice or cholestatis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>10 points per item</td>
<td>Ataxia, clumsiness or frequent falls</td>
<td>Psychotic symptoms (hallucinations, delusions and/or thought disorder)</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dystonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>5 points per item</td>
<td>Acquired and progressive spasticity</td>
<td>Treatment-resistant psychiatric symptoms</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Isolated unexplained splenomegaly (historical and/or current with/without hepatomegaly)</td>
<td>Other psychiatric disorders</td>
<td>Negative</td>
</tr>
<tr>
<td>Ancillary</td>
<td>1 point per item</td>
<td>Hydrops fetalis</td>
<td>Disruptive or aggressive behaviour in adolescence and childhood</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Siblings with foetal ascites</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Category scores

- Visceral & psychiatric
- Visceral & neurological
- Neurological & psychiatric

NPC family relationship

- Parent or sibling with NPC
- Cousin with NPC

Risk Prediction Score

= sum of scores

NPC = Niemann-Pick type C. Source: Patterson MC et al., 2012.

spasticity and supranuclear gaze palsy can appear in other diseases. Therefore other conditions that involve these symptoms need to be considered during diagnosis.

In neonatal and infantile presentation, differential diagnosis should be made between NPC and idiopathic neonatal hepatitis. It must be emphasised that isolated splenomegaly or hepatosplenomegaly can present long before neurological symptoms; the latter being seen in up to 50% of cases of perinatal presentation.

In juvenile NPC, all of the neurodegenerative diseases that present during teenage years, such as Wilson’s and Huntington’s, need to be excluded. In general with juvenile disease, because there are minimal psychiatric symptoms, NPC will tend not to be confused with other psychiatric diseases. Instead, NPC is more likely to be confused with diseases showing progressive intellectual and neurological deterioration (PIND). In adult-onset NPC, on the other hand, psychosis is often the initial manifestation of NPC and differential diagnosis includes schizophrenia and multiple sclerosis.

The other lysosomal storage disorders such as the gangliosidoses and lipofuscinoses, mitochondrial diseases, and dysmyelination syndromes such as Pelizaeus-Merzbacher, Rett’s and organic acidemias (particularly glutaric aciduria) should be taken into consideration for differential diagnosis. GM2 gangliosidosis is probably the most frequently diagnosed similar disease, although it tends not to have such a slow deterioration. A study from the UK and reported by Verity et al, summarises the epidemiology of the majority of PIND disorders and is a very good basis for consideration of differential diagnoses.

Niemann-Pick type B (NPB) results in similar foam cells in the bone marrow. However, patients with NPC tend to have acid sphingomyelinase activity within the normal range for leucocytes. This is an important factor for excluding Niemann-Pick type A (NPA) and NPB. Moreover, retinal pigment abnormalities, which are characteristic of certain other lysosomal storage disorders, are not associated with NPC and macular cherry-red spots are not seen in NPC, in contrast to NPA and NPB.

The differentiation of NPC from static developmental disorders requires care. NPC is very slowly progressive and year on year assessment can give the impression that there is no significant deterioration. Attention is therefore needed to establish incontrovertible developmental steps and where evident – regression.
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**Currently Available Diagnostic Tools**

At present, there is a lack of a good non-invasive and definitive screening method for NPC. Most standard blood, urine and magnetic resonance imaging (MRI) tests employed for diagnosis of degenerative conditions are not useful in the diagnosis of NPC.

The NPC Guidelines Working Group comprises a panel of experts that has convened regularly since 2009, and most recently in 2011, to discuss best care practices for NPC.2 Current recommendations on the screening and diagnosis of NPC are an update to the original international guidelines published three years ago.1 They describe a new tool, the NPC suspicion index, which can be used to aid in diagnosing cases of suspected NPC, particularly within non-specialist centres where experience with the disease is limited (See Figure 2).2 By assigning points to each symptom based on their association with NPC and combining these with the individual's family history, a final risk prediction score can be generated indicating whether the patient should be referred to an NPC centre (≥70 points), further follow-up is required (40 to 69 points) or the likelihood of NPC is low (<40 points).

Specific analyses (biochemical/histological and genetic testing) are the only means of a definitive diagnosis of NPC and should be performed according to the algorithm illustrated in Figure 3.19 The key laboratory test is filipin staining of living fibroblasts to reveal any accumulation of cholesterol. For this technique, a skin biopsy is taken under local anaesthetic. Fibroblasts are cultured in an LDL-enriched medium, a process which takes four to six weeks, after which the cells are fixed and stained with filipin, a compound that binds specifically to unesterified cholesterol. Characteristic, strongly fluorescent cholesterol-filled perinuclear vesicles are detected by fluorescence microscopy in approximately 80–85 % of NPC cases20 Although the filipin test is diagnostically valuable, the requirement for a skin biopsy excludes it from routine metabolic screening protocols. Furthermore, it is complex, expensive and should only be carried out at specialist centres with experienced staff, since assessment can be difficult in cell lines presenting only minor abnormalities.

Molecular genetic testing of NPC1 and NPC2 mutations is also important and provides confirmation of an NPC diagnosis.19 It is offered at a number of specialist laboratories and has become offered at a number of specialist laboratories and has become widely available.21 It is important to note that genetic testing is not usually performed until after the filipin test has been attempted.

*Numerous polymorphisms – check allele segregation from parental study; conclude quickly on NPC2 in children <8–10 months; **Profile also seen in heterozygote subjects. aNew test under clinical development; bTwo subsequent filipin tests (not duplicates); cCell disease gives a false positive result; dASM deficiency can give a similar filipin pattern; eNot certainly pathogenic; fDNA is usually needed to study the effect of splice mutations. ASM = acid sphingomyelinase; EDTA = ethylene-diamine-tetra-acetate acid; EM = electron microscopy; GD = Gaucher disease; MLPA = multiplex ligation-dependent probe amplification; NPC = Niemann-Pick type C. + and - signs denote positive and negative, respectively.

Source: Patterson MC et al., 2012.19

**Figure 3: Laboratory Diagnostic Algorithm**

![Laboratory Diagnostic Algorithm Diagram]

* * *
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Figure 4: Mechanisms of Action of Therapeutic Interventions in Niemann-Pick Disease


considerably less expensive over the last few years. Identification of NPC1 mutations can be difficult, however, because of polymorphism. Still, genetic testing is considered essential for prenatal diagnosis at 10–12 weeks for families at risk and to identify carriers in blood relatives. In some patients mutations have only been identified in one allele and, in a few, no mutations at all have been detected, raising the possibility that a third gene may be involved in NPC.1

Chitotriosidase may also be normal or slightly elevated in NPC, although with poor sensitivity and specificity.23 Other non-specific methods of analysis may include measurement of plasma lipid profiles.23

Following a diagnosis of NPC, patients may be started on treatment with the goal of stabilising disease or reducing the rate of disease progression.19 Due to the nature of NPC, it can take six months to a year before clinical benefits are seen and in adults with slower progressive forms of NPC, these effects may take even longer to become evident.

Overview of Treatment and Management Options

At present, treatment strategies for NPC rely on limiting, rather than reversing, the damage. Several disease modification strategies are broadly discussed, although only one is currently approved. Their modes of action are illustrated in Figure 4.24

Miglustat, (N-butyldeoxynojirimycin, NB-DNJ, Zavesca®), inhibits glucosylceramide synthase, a key component of the glycosphingolipid biosynthetic pathway. It is currently the only approved disease-modifying therapy for NPC and slows progression with limited side effects.25 It is generally well tolerated and side effects are reversible.8 Because the treatment stabilises rather than reverses disease progression, it is important to start treatment early. Once the disease is at an advanced stage, treatment with miglustat should be regularly re-evaluated, as the therapeutic effect on increasing quality of life may be limited.5

Various other potential therapeutic approaches are in clinical development. Pharmacological or molecular chaperone therapy is a relatively new therapeutic concept that may be applicable to NPC.25 These include effective delivery, long-term expression, and avoidance of an immune response.26 Further investigational treatments are in discussion, such as cyclodextrin that is hypothesised as promoting cholesterol efflux from the lysosomal compartments by an unknown mechanism, bypassing the defective NPC1 and NPC2 proteins.27,28 Histone deacetylase inhibitors are another group of agents for which an effect on cholesterol accumulation in NPC mutant human fibroblasts could be observed.29

Besides pharmacological options, supportive strategies are also essential for minimising symptoms; physio, occupational and speech/language therapy, as well as psychological, family, social and educational support can be helpful.7 There is no single approach for managing NPC and among the many issues to consider are mobility difficulties, fine motor control, pain, boredom, speech/swallowing ability, cognition and psychiatric signs. Symptomatic treatments are available for catalepsy, seizures and dystonia and should be employed when necessary.27 Management of infections and feeding difficulties is also of importance.

Future Developments

There is a clear need for less-expensive, quicker, specific, and sensitive methods in NPC diagnosis. In future, there will likely be a trend towards wider genetic testing that will cover all the neurological disorders that present at certain ages. Genetic testing is advantageous in terms of turnaround time, cost, invasiveness and the number of different conditions that can be screened for simultaneously. However, a simple diagnostic blood test would represent a significant advance in the field of NPC.

At present there is considerable research into the development of screening tools, both with clinical algorithms and biochemical analyses. Several cholesterol oxidation products have been found to be elevated in mouse models, which were detectable before the onset of symptoms, and were associated with disease progression. Cholesterol oxidation products were similarly increased in the plasma of human NPC subjects and correlated with age of onset and disease severity. Recent data suggest that these plasma oxysterols may be sensitive and specific biomarkers for NPC. An LC-MS/MS assay has therefore been developed to quantify these oxysterols. This assay was able to discriminate with high sensitivity and specificity between control and NPC subjects and would offer clinicians a non-invasive, rapid, and highly effective method for diagnosing NPC.

In addition, researchers at Oxford University, UK, have developed a microtitre-based assay utilising lysotracker as a measure of general lysosomal storage, rather than storage of a specific substrate, which can be adapted for use on any fluorescence plate reader. The assay has been validated using a macrophage model system as well as human NPC fibroblasts. An early report of it being applied to the screening of novel natural compounds for possible therapeutic benefits30 shows that it may be valuable in diagnosing NPC.

Concluding Remarks

The need for prompt diagnosis of NPC is evident, but the current lack of a simple blood-based biochemical test with sufficient specificity to be used for screening is a major limitation. Physicians need an increased understanding of NPC, with better identification of sensitive and specific clinical symptoms, in order to make an early diagnosis, which would lead to appropriate treatment and avoid unnecessary tests. Current diagnostic techniques are relatively invasive, costly and time-consuming. However, new techniques are under development that should improve the range of diagnostic tests available.