Paediatric Cardiomyopathies EMCHC Investigation Guideline

Staff relevant to:	Medical staff within EMCHC, Cardiac PICU services and wider EMCH Network
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Related Guidelines and Policies:	
C39/2016	Muscle and Skin Biopsy UHL Childrens Medical Guideline

1. Introduction

Cardiomyopathies can be:

- Dilated
- Hypertrophic
- Restrictive
- Left ventricular non-compaction
- Arrhythmogenic Right Ventricle

By far the commonest to present to paediatric cardiology are dilated and hypertrophic cardiomyopathies. Presentations are variable, causes are numerous and, in many cases, rare. The diagnosis is made and confirmed on ECG and echocardiogram. The rationale for identifying the causes of cardiomyopathies is to try to:

- Identify a possible condition
- Confirm the cause.
- Provide any condition specific treatments / prognosis / advice as appropriate

This guideline is for the use of medical staff to summarise the basic investigations required to identify a possible cause of cardiomyopathy and provide direction towards confirmatory tests.

2. Screening Tests: Please note several tests require special measures - read through the list and make sure you have everything necessary before bleeding the patient.

Table 1 Screening Tests

1st line screening tests	Sample Requirements (Bottles, special requirements, etc)	Date Sent	Opted out / why	Test Result & date
Blood				
Full blood count	0.5ml EDTA			
Urea and electrolytes Liver function tests Bone profile Magnesium CK Uric Acid Cholesterol, triglyceride Creatinine Kinase	1.2ml LiHep			
Coagulation screen	Filled Citrate			
Blood gas Blood glucose Bicarbonate Chloride Anion gap Lactate				
Ketones	Ketostix			
Ammonia	1ml LiHep, on ice, to lab within 30 minutes			
Lactate (if no facilities for a blood gas)	1ml Fluoride, on ice, to lab within 30 minutes			
Pyruvate: please check comments from Biochemistry below	Special bottle, call lab on 16559			
Free fatty acids	Fluoride, ideally hypoglycaemic sample			
Amino Acids	0.5ml LiHep			
Acylcarnitine profile	0.5ml LiHep			
Thyroid function	1.2 ml LiHep			
Blood cultures				
Viral PCR: *Enterovirus, echovirus, coxsackie virus, Parvovirus B19, consider HIV*	1.2ml EDTA			
ASOT Rheumatic fever	0.5ml serum			
Vitamin D	0.5ml LiHep			
Selenium	0.5ml serum			
Thiamine (Vit B1)	1ml EDTA, protect from light, to lab within 30 minutes			
Urine				
Amino acids	1ml plain bottle			
Organic acids	2ml plain bottle			
Urine Mucopolysaccharide Screen (MPS I, II, VI) Oligosaccharides Sialic Acid Glycosaminoglycans GAG electrophoresis	5ml plain bottle, request MPS screen			
Ketones	Dipstick			
Glucose	Dipstick			
Reducing substances	5ml plain bottle			
NB: A full set of 1st line blood investigations requires: **3 EDTA, 4 Lithium heparin, 1 citrate, 1 fluoride and 2 serum bottles and One blood gas**				

2nd line testing	Sample requirements	Date sent	Opted out / why	Result & date
Muscle and Skin Biopsy for mitochondrial disorders				
Histology	See muscle biopsy instructions			
Electron microscopy				
Enzyme assays				
Mitochondrial DNA analysis				
Blood				
Cardiolipin Levels (Barth Syndrome)	See proforma and letter below. EDTA 1-3ml			Monolysocardiolipin /cardiolipin phospholipid ratio
White cell enzymes *For lysosomal storage disorders (MPS II & III), if abnormal urinary oligosaccharides, GAG's, elevated CK or ALT/AST*	EDTA 5ml, to lab before 1pm, Mon-Thurs			
Transferrin and Apoliporotein isoforms *if Congenital Disorder of Glycosylation suspected*	1ml Clotted			
Phytanic Acid if Refsum Disease suspected	Call lab on 16559			
Fabry Disease *Alpha glucosidase*	1ml EDTA blood, request Fabry Screen			
Pompe's Screen *Acid-alpha glucosidase*	1ml EDTA blood, request Pompe Screen			
Brain MRI				
Suspected mitochondrial disorders				

NB: Testing for specific genetic conditions should be on the basis of the eligibility criteria according to the national genomic test directory as advised by Professor Pradeep Vasudevan Consultant Clinical Geneticist. Please see the link below.

https://www.england.nhs.uk/wp-content/uploads/2018/08/Rare-and-inherited-disease-eligibility-criteria-version-5.2.pdf

2.1 Pyruvate

(Advice from Dr Lorna Maddocks - Clinical Bio-Chemist, Mon-Wed 01162586553)

- The laboratory provides a pre-weighed sample bottle containing an acid precipitant, which is needed to be in the laboratory **at LRI within 30 minutes on ice** in order to reweigh on a sensitive a balance which is located at LRI and also centrifuge a.s.a.p.
- **A precise measurement of 1 ml of blood** to be added immediately into the sample bottle (if less than 1 ml – insufficient sample for analysis, if significantly greater than 1 ml – insufficient precipitant present to precipitate all the proteins and the assay result will be unreliable)
- The pyruvate result if only of clinical value when paired with simultaneously lactate measurement and **when the lactate level is persistently raised**.
- The blood lactate to pyruvate (L:P) ratio can be used to distinguish between pyruvate dehydrogenase deficiency and other causes of congenital lactic acidosis. In conjunction with an elevated lactate, an L:P ratio greater than 30 suggests inherited disorders of the respiratory chain complex or tricarboxylic acid cycle disorders. In conjunction with an elevated lactate, an L:P ratio less than 25 suggests a defect in pyruvate metabolism.
- An artifactually high L:P ratio can be observed in acutely ill individuals.

Abnormal concentrations of lactate, pyruvate, and the L:P ratio are not diagnostic for any single disorder and must be interpreted in the context of the individual's clinical presentation and other laboratory studies.

2.2 Muscle biopsy for histochemistry, immunochemistry and electron microscopy and for the investigation of mitochondrial disorders.

See Muscle and Skin Biopsy UHL Childrens Medical Guideline C39/2016

Contacts:

1. Chemical Pathology, Dr Elaine Maddocks; Dr Virginia Lee ext 16553.
2. Histopathology – an advice on additional sample requirements before any muscle biopsy sample is collected. Peter Wells-Jordan, ext 16590.
3. In cases of possible mitochondrial cardiomyopathy, Dr Robert McFarland, Consultant Paediatric Neurologist (robert.mcfarland@ncl.ac.uk), can be contacted via:

   Newcastle NCG Rare Mitochondrial Disease Service
   4th Floor, The Medical School, Framlington Place, Newcastle University
   Newcastle upon Tyne, NE2 4HH
   Tel +44 (0)191 2820340 Fax +44 (0)191 2824373

   Clinical advice:*Dr Robert McFarland: via bernadette.caygill@nuth.nhs.uk*
   Catherine Feeney: catherine.feeney@nuth.nhs.uk

   Diagnostic Laboratory: *Prof Robert Taylor: robert.taylor5@nuth.nhs.uk*

2.3 Barth Syndrome Testing:

Routine blood testing: Cardiolipin Analysis

Cardiolipin analysis is temporarily unavailable in the UK. The laboratory at Bristol Royal Infirmary continues however to facilitate testing for Barth syndrome by forwarding samples to the Academic Medical Centre (AMC) in Amsterdam for analysis. Analysis of samples sent via the Bristol laboratory to the AMC are free of charge.

Please take 1-3 ml of whole blood collected into a K-EDTA (full blood count) tube, Monday to Thursday only and send within 24 hours of collection by first class post to the:

Metabolic, Neuroendocrine and Nutrition Laboratory
Department of Clinical Biochemistry
Bristol Royal Infirmary
Bristol
BS2 8HW

Please label the box 'Urgent clinical sample - for immediate delivery to the Metabolic, Neuroendocrine and Nutrition Laboratory'.

Alternatively a blood spot can be sent directly to The Academic Medical Centre (AMC), Amsterdam (see https://www.amc.nl).
Please note that samples sent directly to the AMC will incur a charge.

Post mortem testing
If a patient is suspected to have died as a result of Barth Syndrome, cardiolipin analysis can be performed in cultured fibroblasts (skin cells). If fibroblasts are unavailable, please contact Vicki Powers to discuss details on 0117 342 2590 or by email at: victoria.powers@UHBristol.nhs.uk

Genetic testing by TAZ Gene Sequencing
Further information on genetic testing in Bristol, and a proforma for referral of samples are given here.

The information on this page is taken from the NHS Barth Syndrome Service site – for further details please visit Barth Syndrome Service - Testing

Test for it in the following situations:

- **All boys with DCM, especially neonates/infants with CM**
  (several large studies suggest that 3-7% of this cohort will have Barth Syndrome; 70% develop CM during their first year)
- **Any boy with unexplained LVNC or HCM with other features suggestive of Barth Syndrome** (e.g. neutropenia, growth failure, feeding problems, myopathy, lactic acidosis, hypoglycaemia) **or X-linked family history**
- **Fetal tissue (fetal fibroblasts or DNA) from recurrent male stillbirth or single stillbirth with suspicion of cardiomyopathy**

3. Interpretation of Investigations

Interpretation of the results of the tests outlined above relies on an understanding of the causes of cardiomyopathies.

Causes of Hypertrophic Cardiomyopathy

Genetic

Gene mutation not always apparent, but definite heritability

- Sarcomeric/other cardiac genetic abnormalities
  - Sarcomeric protein disease
    - Commonest causes of adult HCM
    - 50-60% have mutations in 1 of 11 sarcomere protein genes
    - Also account for over 50% of idiopathic HCM cases in children
  - Z-disk protein disease
    - Small number of patients
  - Calcium-handling disease
    - Small number of patients

Inborn Errors of Metabolism

- <10% of paediatric HCM
- Most cases of HCM due to IEM are due to Glycogen Storage Disorders

- Can be classified as:
    - Accumulation of Toxins
        - Protein metabolism
            - Amino acidopathies
            - Organic acidopathies
            - Urea Cycle Defects
        - Carbohydrate intolerance
        - Lysosomal storage disorders
            - Mucopolysaccharidoses
                - I, II, III, IV, VII
            - Gangliosidoses
            - GM1 and 2
            - Danon Disease (LAMP-2)
            - Fabry Disease
    - Disorders of energy utilization
        - Fatty Acid Metabolism Defects
            - Carnitine transport defects
                - Systemic
                - Muscle
                - CPT II
                - CAT
            - Fatty Acid Oxidation Defects
                - VLCADD
                - LCHADD
                - Glutaric Acidaemia Type II
        - Carbohydrate utilization defects
            - Glycogen storage disorders
                - GSD II (Actually a lysosomal storage disorder, Pompe)
                - GSD III
                - GSD IX
                - AMP KInase Disease (PRKAG2)
            - Gluconeogenetic disorders
            - Glycogenolytic disorders
        - Mitochondrial disorders
            - Respiratory Chain Enzyme Deficiencies
                - Complexes I - V
            - MELAS
            - MERRF
            - Kearns-Sayre
            - Pyruvate dehydrogenase deficiency (Leigh Disease)
            - Barth Syndrome
            - Senger Syndrome
                - Mitochondrial DNA depletion syndrome
        - Peroxisomal disorders

Neuromuscular conditions

- Friedrich's Ataxia
- Myotonic dystrophy

Malformation Syndromes

- RAS/MAPK Diseases
  - Noonan Syndrome
  - Noonan Syndrome with Multiple Lentigines (Previously LEOPARD syndrome)
  - Costello Syndrome
  - Beckwith-Wiedemann Syndrome
  - Swyer Syndrome (Pure gonadal dysgenesis)

Other causes

- Obesity
- Infant of Diabetic Mother
- Amyloidosis
- Conditions that mimic HCM
  - Athletic training
  - Steroid-induced LVH

Causes of Dilated Cardiomyopathy

- Idiopathic (66%)

- Known causes (34%)
  - Mycocarditis
    - Infectious agent
      - Enterovirus, echovirus, coxsackie, HIV
      - Diphtheria, Rheumatic fever, Chagas Disease

  - Neuromuscular
    - Duchenne Muscular Dystrophy
    - Becker Muscular Dystrophy
    - Emery-Dreifuss Muscular Dystrophy

  - Familial
    - AD
    - AR
    - X-linked

  - IEM
    - Mitochondrial
    - Barth Syndrome
    - Primary carnitine deficiency

Causes of Restrictive Cardiomyopathy

- **Idiopathic 90% of childhood causes**

- Fabry's disease – Decreased lysosomal 1 galactosidase A in WBC. Pain hands/feet, papules on waist/buttocks

- Gaucher's disease – Hepatosplenomegaly/ Hypersplenism/ Bone marrow infiltrate, flask deformity of distal femur, decreased beta glucosidase in WBC/Skin fibroblasts

- Haemochromatosis- Diabetes/ Bronze skin/ Cirrhosis/ Abdominal pain/ hypogonadism/ : Raised Iron, Ferritin, Increased transferring saturations > 62%.Do liver biopsy

- Glycogen storage disorder : Lactate/Glucose/ raised TGL/ Raised cholesterol

- Hypereosinophilic (Loeffer) Syndrome : Blood film , raised eosinophil count

- Carcinoid : In appendix. Do 5 HT
- Metastatic malignancy
- Pseudoxanthoma elasticum : Yellow papules and plaques / Eyes- angiod streaks
- Scleroderma- ANA (Anti Scl 70 specificity), Skin signs
- Amyloidosis- in childhood sec to other inflammatory process, Low voltage QRS, Thickened myocardium (sparkling echogenicity)
- Sarcoidosis – Eyes/ Hepatosplenomegaly / erthema nodosum/ polyarthritis/ lungs/ thoracic L.N – Serum ACE level, T cells in BAL

Table 2 **Interpretation of Investigations**

1st line screening tests	Interpretation
Blood	
Full blood count	Neutrophils low in Barth Syndrome
Urea, creatinine, electrolytes	Allows calculation of anion gap
Liver function tests, including bilirubin	Elevated transaminases in fatty acid disorders, GSD II, III May be raised or normal in virtually all other disorders
Coagulation screen	Abnormal in hepatic dysfunction associated with e.g. Lysosomal storage disorders
Blood gas *To allow calculation of anion gap*	pH, glucose, lactate Allows assessment of metabolic acidosis *Primary metabolic acidosis always present in organic acidaemia, usually present in mitochondrial disorder and fatty acid disorder* Allows calculation of anion gap
Blood glucose	Hypoglycaemia in *Some mitochondrial disorders associated with diabetes* *Fatty acid metabolism defects* *GSD III and IX (phosphorylase kinase defect)*
Ketones	Raised in GSD III, can be raised in organic acidaemias, low or absent in fatty acid disorders Can be raised in unwell child – *non-diagnostic pattern on urinary organic acid analysis*
Bicarbonate	Assessment of metabolic acidosis
Ammonia	Raised in Urea cycle defects, organic acidaemias, fatty acid defects, May be raised in aminoacidopathies, mitochondrial disorders
Lactate	Sometimes elevated in mitochondrial disorders With abnormal organic acids, suggests fatty acid disorder Raised in Barth syndrome, Senger's Syndrome Allows calculation of lactate:pyruvate ratio – *elevated in respiratory chain defects, normal in e.g. pyruvate dehydrogenase deficiency, Normal is <15:1, high is >25:1*
Pyruvate	Normal or low in respiratory chain defects Elevated in pyruvate metabolism disorders
Creatine kinase	Raised in GSD II
Free fatty acids	Fatty acid metabolism defects Plasma FFA:beta hydroxybutyrate (intermediate metabolites)
Cholesterol	Smith-Lemli-Opitz
Amino Acids	Amino acidaemias
Acylcarnitine profile	Carnitine transport defects Fatty acid metabolism
Thyroid function	Exclude hypothyroidism
Blood cultures	
Viral PCR *Enterovirus, echovirus, coxsackie virus, consider HIV*	Myocarditis
ASOT *Rheumatic fever*	Rheumatic fever
Vitamin D	Vitamin D deficiency
Selenium	Nutritional deficiencies
Thiamine (Vit B1)	Nutritional deficiencies
Urine	
Amino acids	Elevated in amino acidaemias
Organic acids	Abnormal in organic acidaemias, fatty acid oxidation defects, Barth Syndrome
Oligosaccharides	Raised in GSD II, other lysosomal disorders

Glycosaminoglycans	Elevated in MPS, lysosomal disorders
Ketones	Elevated in organic acidaemias
Glucose	Exclude DM, abnormal in GSD's (some)
Reducing substances	Galactosaemia
2nd Line Testing	
Muscle and Skin Biopsy for mitochondrial disorders	
Histology	
Electron microscopy	
Enzyme assays	For respiratory chain enzymes, Complexes I to V
Mitochondrial DNA analysis	For specific mitochondrial DNA mutations, may also look at nuclear DNA
Brain MRI	
Suspected mitochondrial disorders	May see basal ganglia changes, general cortical changes
Blood	
Cardiolipin Levels (Barth Syndrome)	
White cell enzymes *For lysosomal storage disorders (MOS II & III), if abnormal urinary oligosaccharides, GAG's, elevated CK or ALT/AST*	Specific enzyme assays
Transferrin and Apoliporotein isoforms *if Congenital Disorder of Glycosylation suspected*	Abnormal in CDG Type I or II respectively
Phytanic Acid *if Refsum Disease suspected*	

Anion gap raised in

- organic acidaemias,
- fatty acid oxidation defects,
- respiratory chain disorders,
- pyruvate disorders

Differentiating requires assessment of lactate and pyruvate levels

4. Education and Training

No training required for this guideline

5. Monitoring Compliance

None currently identified

6. Supporting References

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7. Key Words

Barth syndrome, Biopsy, Cardiomyopathy, Dilated cardiomyopathy, Hypertrophic cardiomyopathy, Inborn errors of metabolism , Mitochondrial disorders, Muscle biopsy Myopathy, Restrictive cardiomyopathy, Skin Biopsy

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The Trust recognises the diversity of the local community it serves. Our aim therefore is to provide a safe environment free from discrimination and treat all individuals fairly with dignity and appropriately according to their needs.
As part of its development, this policy and its impact on equality have been reviewed and no detriment was identified.

CONTACT AND REVIEW DETAILS	
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Details of Changes made during review: **August 2023: updated muscle biopsy and Barth Syndrome and shortened the document**	