Perinatal Loss elearning – Investigations section

Investigations

Aim:

To describe the core investigations recommended following perinatal death.

Objectives:

Following completion of this module you will be able to:

1. Understand the investigations necessary for thorough assessment of fetal death.
2. Understand investigations required for neonatal deaths.

Stillbirth Investigations

Recommended investigations following a diagnosis of fetal death.
It is recommended that all perinatal deaths are thoroughly investigated. Even if the cause of death appears obvious, additional information can be obtained that may assist in the management of the woman and her future pregnancies. [1]

Thorough investigation into cause of fetal death comprises:

- Review of antenatal and peripartum history
- Pathology investigations
- Ultrasound and amniocentesis
- Postmortem examination
- Placental histopathology

Due to the wide range of aetiological, clinical and geographic circumstances the nature of investigations following death vary widely. Decisions regarding appropriate investigations should be made by the clinical team providing care, on an individual basis, in consultation with specialist expertise. [2]

A comprehensive maternal medical and social history should be taken following all perinatal deaths.

History includes:

- Medical
- Obstetric
- Family
- Possible infections
- Exposure to environmental contacts (animals or toxic chemicals).
- Smoking and substance use
- Current pregnancy and any complications
- Review of routine antenatal blood tests and infectious disease screening tests
**Ultrasound**

- Ultrasound confirming fetal death in utero should also include examination for possible fetal abnormalities, fetal biometry and the assessment of amniotic fluid volume.
- Ultrasound may be particularly useful for assessment of the brain which is frequently difficult to examine at autopsy due to autolysis.\(^1\)

Liaise with local radiology units to determine the availability of amniocentesis facilities. Refer to Maternal Fetal Medicine Service (KEMH [WA]) for further advice.

Where possible it is recommended that a midwife be available to support patients with a suspected fetal death whilst attending ultrasound.\(^3\)

**Amniocentesis**

- Amniocentesis samples (tissue, amnion, fluid and placental villi) are recommended for karyotyping and microbiology. It is estimated that 6.9%-20% of all stillbirths have a fetal chromosomal abnormality.\(^2\)
- Growth of tissue samples taken at postmortem have a high failure rate (60% placenta, 30% skin) for chromosomal studies and therefore samples obtained during amniocentesis may be more successful (82%-92%).\(^1,2\)
- Amniotic fluid collected prior to labour may provide useful samples for microbiological culture.
Although the woman may be asymptomatic for infections, micro-organisms such as Group B Streptococcus (GBS), may be present. Detection of GBS is optimised by the use of a low vaginal and perianal swab. This is obtained by using a single dry swab stick first inserted into the introitus and then the anorectal region.

Blood group and antibody screen

To exclude haemolytic disease due to maternal sensitisation, eg Rh D and Kell.

Kleihauer-Betke test

- The sample must be taken prior to labour to allow a diagnosis of fetomaternal haemorrhage to be made with confidence.
- Fetal cells in maternal blood are counted to determine the amount of fetal haemorrhage.
- A fetal haemorrhage of 50ml is considered significant, but fetal impact is dependent on fetal age, weight and total blood volume.
- Chronic small fetomaternal haemorrhage may result in compensatory fetal changes, whereas acute fetomaternal haemorrhage is more likely to present as an apparently unexplained fetal death.

HbA1c

- Reflects average glucose concentration over the life of red blood cells in the previous 3 months.
- Increased risk of fetal morbidity and mortality with maternal diabetes.
Thrombophilia investigations
- Anticardiolipin antibodies, lupus anticoagulant and activated protein C resistance (APC).

Renal function tests including uric acid
- Abnormal renal function is found in some medical conditions associated with pregnancy and perinatal loss (e.g., PE, SLE, chronic renal failure).
- Elevated uric acid levels in pre-eclamptic women have been associated with perinatal death.

Liver function tests and bile acid
- Liver test abnormalities may indicate viral hepatitis, CMV, toxoplasmosis, acute fatty liver of pregnancy, HELLP syndrome (Haemolysis, Elevated Liver function, Low Platelets), and obstetric cholestasis.

Thyroid function test
- Pregnancy is associated with physiological changes in thyroid function.
- Thyroid disorders have been associated with increased risk of miscarriage, gestational hypertension, low birth weight and fetal death.

Cytomegalovirus (CMV), Toxoplasma, Parvovirus B19, Rubella and Syphilis
- Maternal-fetal transmission of toxoplasmosis is dependant on the time of maternal infection. Transmission is more likely to occur later in the pregnancy. [2]
- Parvovirus B19 may cause severe fetal anaemia, non-immune hydrops and fetal death.
- Rubella and syphilis are associated with fetal abnormalities and fetal death.

Click the links for KEMH [WA] blood test examples.
For a thorough work up of stillbirth, the following are recommended:

- External examination of the baby
- Clinical photographs
- Cord or cardiac puncture blood samples, blood samples for chromosomal analysis, routine guthrie test
- Postmortem examination
- Detailed macroscopic examination of placenta and cord
- Placental microbiological cultures
- Placental and amnion biopsy for chromosomal analysis
- Placental histopathology

Examination of the baby, placenta, and autopsy examination will be covered in separate modules.

Infection may be sub-clinical in the mother and therefore important when the cause of death is not obvious.

Intrauterine infections are reported as the cause of death in 15%-24% of stillbirths with premature rupture of membranes.

Swabs are taken from the ear and throat of the stillborn baby and sent for aerobic and anaerobic bacterial culture (by pathologist as part of autopsy).
A blood sample (cord blood) from the baby should be collected to:

- Investigate signs of infection (culture and inflammatory response).
- Allow haematologic karyotyping (if not already performed).
- Perform the routine Guthrie/newborn screening test.
- Assess the haematological status (full blood count, group and antibody screen, red cell count).

If a cord blood sample cannot be collected a cardiac puncture may be performed by the pathologist during the postmortem.

Thorough investigation into the cause of death is recommended.

These investigations are undertaken on diagnosis of fetal death and following birth.

A non-selective approach to all core investigations is recommended and should be adopted for all stillbirths if available. [2]

Alternative approaches to autopsy are possible if required.

Copies of all investigations should be sent to all health care providers involved with the case.

Baby, placenta/swabs, cord bloods, and documentation should be sent together via the perinatal transport system.
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Click the following link to read the PSANZ Investigation of Stillbirth algorithm. [2]

Neonatal Death Investigations
Recommended investigations to be performed following a neonatal death.
Neonatal deaths can result from disorders of the neonate, placenta or mother. [2]

The majority of neonatal deaths are due to major congenital abnormalities and complications of preterm birth.

Due to the wide range of aetiological, clinical and geographic circumstances the nature of investigations following death vary widely. [2]

Decisions regarding appropriate investigations should be made by the clinical team providing care, on an individual basis, in consultation with specialist expertise as required. [2]

High risk newborns include:

- Admissions to neonatal intensive care.
- Preterm birth less than 32 weeks gestation.
- Suspected fetal compromise including growth restriction.
- Severe cardiorespiratory depression at birth.
- Signs consistent with congenital infection.
- Severe growth restriction.
- Hydropic babies.
- Suspected severe anaemia.
- Suspected or known major congenital abnormalities.
- Other circumstances where a liveborn baby dies shortly after birth in the birth suite.
The recommended investigations for all neonatal deaths are:

- Detailed external examination of the baby
- Autopsy
- Guthrie/Newborn screening blood sample

Assessment of all high risk newborns includes:

- Detailed external examination of the baby.
- Comprehensive maternal, medical, social and antenatal history including the results of investigations documented by obstetric staff.
- Cord blood gas analyses (arterial and venous samples).
- Detailed macroscopic examination of the placenta and cord with documented findings by obstetric staff.
- Placenta, cord and membranes sent fresh and unfixed for histopathology examination.

Further investigations at the time of birth may provide valuable information, particularly where consent for autopsy is not obtained. [2]

Specific clinical presentations require specific investigations, such as:

- Suspected clinical infection (clinical chorioamnionitis, spontaneous preterm labour and birth)
- Suspected congenital abnormalities, hydrops and severe growth restriction.
- Severe cardiorespiratory depression.
- Suspected thrombophilic disorders; pre-eclampsia, fetal growth restriction.
- Macrosomic infant.
Suspected clinical infection (chorioamnionitis, spontaneous preterm labour and birth):

- Maternal low vaginal/anorectal culture for GBS and culture for other common bacterial pathogens (e.g. E coli, Klebsiella).
- Maternal serology for CMV, Toxoplasma, Parvovirus B19, Rubella and Syphilis (if TPHA not taken in this pregnancy).
- Infant blood samples for haematological assessment: full blood count (with nucleated red cell count), blood group, Direct Coombs test (DCT), antibody screen and microbiological culture.
- Placental swabs for aerobic and anaerobic culture.
- If viral infection is suspected a placental biopsy should be sent for PCR or viral culture.

Suspected congenital abnormalities, hydropic and severely growth restricted:

- Maternal serology for CMV, Toxoplasma, Parvovirus B19, Rubella and Syphilis.
- Infant blood samples—full blood count, blood group, DCT and antibody screen, microbiological culture, CRP.
- Placental swabs for aerobic and anaerobic culture.
- Placental biopsy if suspected viral infection.
- Infant cord or peripheral sample for chromosomal analysis.
- Hydropic infants—blood tests for Transferrin Isoforms for Carbohydrate deficient glycoprotein disorders (CDG).
- Fibroblast culture (storage for future testing)
- Clinical photographs are recommended for infants with congenital abnormalities.
Severe cardiorespiratory depression:

- Maternal low vaginal/anorectal culture for GBS and other common bacterial pathogens.
- Maternal serology for CMV, Toxoplasma, Parvovirus B19, Rubella and Syphilis.
- Infant blood samples for haematological assessment, blood group, Direct Coombs Test and antibody screen and microbiological culture.
- Infant surface swabs from the ear and throat for microbiological culture.
- Placental swabs for aerobic and anaerobic culture and fungal infections.
- Consider investigations for genetic metabolic disorders and chromosomal analysis.

Suspected thrombophilic disorders

- At birth: anticardiolipin antibodies, lupus anticoagulant, activated protein C (APC) resistance.
- 8-12 weeks postpartum: if antiphospholipid positive at birth a repeat test, fasting homocysteine, protein C and S deficiency, prothrombin mutation G20210A, anti-thrombin III.
- APC resistance is a positive test for Factor V Leiden gene mutation.
- If homocysteine test is positive, Methyleneetetrahydrofolate (MTHFR) testing.
- MTHFR3 testing should also be performed if fetal anomalies are identified (e.g. cleft lip/palate, neural tube defects, cardiac defects).

Investigations to identify possible thrombophilic disorders should be considered in women with pre-eclampsia, personal/family history of thrombosis and following the birth of a severely growth restricted infant.

This testing allows planning of future pregnancies and considerations of the risks and benefits of subsequent pregnancy anti-thrombotic therapy.
Macrosomic infant:

- Investigations for maternal diabetes if not already undertaken.
- Maternal HbA1c level (as soon as possible following birth).
- If HbA1c level is raised - fasting blood glucose, with consideration to a Glucose Tolerance Test 6-8 weeks postnatally.

Suspected Genetic Metabolic Disorders

To ensure a precise diagnosis, perimortem examination of baby is recommended.

Parental consent for postmortem and blood/tissue samples prior to death is required.

Due to the complexity and number of different possible causes it is recommended that clinicians discuss individual cases with the State Laboratory to determine optimum tests required.

All tissue samples should be stored and transported to a specialist laboratory.
Clinical or biochemical presentations which may precede neonatal death include:

- Acute encephalopathy
- Hypoglycaemia
- Hyperammonaemia
- Ketosis
- Acid-base balance disorders
- Seizures
- Acute hepatocellular disease
- Severe hypotonia
- Non-immune hydrops fetalis
- Facial dysmorphism with or without congenital malformations
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Prior to death:
- Consult a metabolic physician or histopathologist before collecting samples.
- Blood sample (0.8ml) in a lithium heparin tube and refrigerate (green top tube).
- 5-10ml urine sample.
- Skin biopsy (3x2mm punch biopsies collected by a qualified clinician).

Immediately following death, a qualified clinician (pathologist) should:
- Obtain blood sample by cardiac puncture if not previously obtained.
- Obtain liver and muscle biopsies for electron microscopy, histopathology and enzymology (wrap in aluminium foil, snap freeze, store at -70 degrees celsius). Collect within 2-4 hours.
- Contact the laboratory to ensure all blood or urine specimens are retained.

The investigation into sudden unexpected death of a neonate should include:
- A thorough maternal and infant medical history.
- A full autopsy performed by a trained pathologist.
- An investigation of the various scenes where incidents leading to the death might have occurred including the sleeping environment.
- Investigations for genetic metabolic disorders.
- Aspects of Sudden Infant Death Syndrome (SIDS) and Sudden Unexpected Death in Infancy (SUDI) investigation protocols.

Fitroy Crossing WA
If permission for autopsy has not been obtained other less invasive testing may assist in establishing cause of death.

The alternative investigations for neonatal death are the same as for a stillbirth.

Click the following link to read the PSANZ high risk newborn investigation checklist.

Click the following link to read the PSANZ suggested screening tests for genetic metabolic disorders.

In Summary:

1. Due to the wide range of circumstances surrounding neonatal death, investigations vary considerably and are assessed on an individual basis.
2. Decisions concerning appropriate investigations are made by the attending clinical team in consultation with specialists.
3. Investigations should commence at the birth of a high risk infant (ideally).
4. The autopsy examination following neonatal death is an important investigation.
5. The investigation of neonatal deaths involves a multi-disciplinary team approach with consultation between clinicians and specialists involved in the case.
You have now completed the Investigations Module

Click the link to view the references for this module.

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