NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT)

This LOP is developed to guide clinical practice at the Royal Hospital for Women. Individual patient circumstances may mean that practice diverges from this LOP. This LOP has been developed in partnership with the Haematology team at Sydney Children’s Hospital and the Australian Red Cross.

1. AIM
   - To identify potential cases of neonatal alloimmune thrombocytopenia (NAIT)
   - To assist in immediate clinical management of the neonates affected with NAIT
   - To arrange appropriate follow up

2. BACKGROUND
   - Neonatal alloimmune thrombocytopenia or “NAIT” or fetomaternal alloimmune thrombocytopenia (FMAIT) is a rare but serious condition
   - It is associated with significant neonatal morbidity and mortality, including a 20% risk of intracranial haemorrhage (ICH)
   - Platelet antigens, inherited from the father and expressed on neonatal platelets, are destroyed by maternal alloantibodies, which cross into the neonatal circulation via transplacental transfer
   - Immediate management includes confirmation of diagnosis, use of specific platelets (mostly HPA-1bb) to keep the platelet count above 30 x 10^9/L and immune-modulatory treatment
   - There are risks to subsequent offspring, which are higher if the first offspring with identified NAIT experienced an ICH

3. CLINICAL PRACTICE
   When to suspect NAIT:
   - Severe thrombocytopenia in an otherwise well neonate even if no history of NAIT in previous pregnancies (< 50 x 10^9/L) although NAIT can occur with mild/moderate thrombocytopenia (< 150 x 10^9/L)
   - Exclude alternative diagnoses, such as infection, collection error, maternal auto-antibodies (especially maternal ITP), maternal medications, neonatal liver disease
   - NAIT in a prior pregnancy (although NAIT can occur in the first pregnancy)

Diagnosis and immediate management:
   - Arrange neonatal full blood count if thrombocytopenia is suspected on history or clinical examination
   - If the diagnosis is suspected, treat as NAIT (confirmatory tests may take a few days)
   - Contact Paediatric Haematology Fellow/Consultant
   - Call Australian Red Cross Blood Service (ARCBS) – 1300 478 348
     o 24 hour phone line, request the Medical Officer on call
     o Request rare platelets type HPA-1bb, unless prior platelet genotyping on the parents is available to suggest use of another HPA group
     o Transfuse to keep neonatal platelet count above 30, preferably above 50 x 10^9/L
     o Rare platelets will always be available from ARCBS (usually HPA-1bb platelets)
     o Rare HPA-1bb platelets are also genotyped for other HPA types
     o It may be possible to provide another HPA-type if the HPA antibody is known
   - Urgently collect blood from both parents
     o Samples required are indicated on the ARCBS cover page for requests (see appendix A) or can be downloaded from the ARCBS website www.transfusion.com.au

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RHW NICU version: Tim Schindler, Srinivas Bolisetty, Antonia Shand
Original: 11 January 2013
Approved by the Newborn Quality Committee 4th February 2013
Mother – 20ml clot tube (no gel) and 30ml EDTA tube, Father – 40ml whole blood EDTA tube only
- The form is available below (see appendix B) or can be downloaded from the ARCBS website www.transfusion.com.au
- Please use a form for each parent and indicate in “Tests Required Section” that NAIT testing is needed
- Fresh samples (collected within the last 24 hours) must be delivered, transported at ambient temperature, to ARCBS within working hours Monday to Friday (testing will not occur on a weekend)
- Advise SEALS Blood Bank and ARCBS about urgency and transport of samples
- Blood can be couriered to ARCBS, to arrive as early as possible. Preliminary results will be available on the same day if samples arrive by mid-morning

- If preliminary results are positive (for example if maternal serum alloantibodies to HPA-1a are present) then the neonate needs URGENT TREATMENT to reduce the risk of ICH
- Solid Phase platelet (intact platelet panels) antibody investigations and HPA genotyping are performed urgently and results sent out as a preliminary result, which is followed up with definitive MAIPA assays (Monoclonal Antibody-specific Immobilisation of Platelet Antigens assay)
- MAIPA assays have been developed to allow identification and characterisation of antibodies directed against platelets (with platelet glycoproteins)

Treatment:
It is important to maintain platelet counts above 30 x 10^9/L and preferably above 50 x 10^9/L
- Platelet transfusions are the mainstay of initial therapy, followed by IVIG and possibly steroids (platelets are ideally CMV negative and irradiated – if CMV negative platelets are not available, then discuss with the haematologist)
- Volumes advised are 10-20ml/kg
- HPA antigen negative platelets are preferred, but random donor platelets (HPA antigen positive) can also be used safely and effectively if HPA antigen negative platelets are not available
- Repeat testing for platelet level is required (for neonates with severe NAIT, a one-hour post-platelet increment may be useful)
- Please advise ARCBS of the neonate’s clinical status and likely need for further rare platelets
- Maternal platelet collection is also a possibility however this procedure is rarely performed (please speak to Blood Bank and ARCBS)
- In addition to platelet transfusion, treatment with IVIG is strongly recommended – the dose is 1g/kg x 2 days (ordered from ARCBS and administered intravenously)
- Consider addition of steroids for severe/ refractory NAIT (suggested dose: IV methylprednisone 1-2mg/kg/ day in 2-3 divided doses x 5 days)
- Seek further advice from Haematology in severe/refractory NAIT cases
- Treatment side effects to be discussed with the parents include (but are not limited to):
  - IVIG – allergic/anaphylactic reactions, fever, headache, aseptic meningitis (rare)
  - Steroids – hypertension (that may require additional treatment), hyperglycaemia, irritability, mood changes, avascular necrosis, transient adrenal suppression
- Arrange head ultrasound to exclude ICH (further imaging may be required)

Follow up:
- As NAIT is purely a consequence of maternal antibodies directed against paternal platelet antigens NAIT will resolve after 1-3 weeks
- Monitoring of platelet counts for at least 2-6 weeks post delivery is recommended
- Consider reporting the case to the Australian NAIT registry to inform epidemiological studies
- Arrange haematology follow-up as an outpatient
Arrange referral for parents to a maternal foetal medicine specialist for follow up
Advise parents that subsequent pregnancies are at risk and early antenatal, or preferably pre-conception, counselling with a maternal foetal medicine subspecialist is recommended

4. EDUCATIONAL NOTES
Epidemiology:
• NAIT accounts for 3% of all fetal and neonatal thrombocytopenia (defined as platelets < 150 x 10^9/L) and 27% of severe cases (platelets < 50 x 10^9/L)
• NAIT is associated with significant neonatal morbidity and mortality, including a 20% risk of intracranial haemorrhage (ICH)
• NAIT can occur in the first pregnancy

Risk factors:
• NAIT in a prior pregnancy is a risk factor for NAIT in subsequent pregnancies, especially where there is known discordance between parental HPA (human platelet antigen) types (see table below)
• HPA types vary in frequency across racial groups. The most frequent cause of NAIT in a Caucasian population is anti-HPA-1a antibodies and in Asian populations are anti-HPA-4a antibodies
• Other HPA antibodies implicated in NAIT include anti-HPA-3a, anti-HPA-5b, anti-HPA-15a and anti-HPA-15b
• Severe NAIT and ICH in a prior pregnancy greatly increases the risk of ICH in subsequent pregnancies

Future pregnancies:
• Through NAIT testing, parental HPA typing is determined and the risk to future pregnancies can be predicted (see table below)
• In-utero HPA genotyping can be performed on DNA extracted from amniocytes (DNA extraction, and confirmatory testing that DNA is of foetal origin, is performed by the Molecular Genetics Unit at Prince of Wales Hospital) –the DNA is then referred to the Tissue Typing Department at the ARCBS for HPA-genotyping
• Amniocytes should also be cultured in the cytogenetic laboratory to allow for subsequent retesting if needed
• If NAIT is considered likely either from history and/or prenatal invasive diagnosis, antenatal therapy may be instituted (this may include IVIG and/or steroids and possible in-utero platelet transfusion)
• Treatment depends on the previous history of severity of thrombocytopenia, including any history of previous ICH
• ARCBS can be contacted peri-partum to ensure availability of specific platelets. A follow-up antibody screen post-partum is often useful, to confirm genotyping and determine if additional HPA antibodies have developed
### REFERENCES


<table>
<thead>
<tr>
<th>Causative Maternal HPA allo-antibody</th>
<th>Maternal HPA type</th>
<th>Paternal HPA type</th>
<th>Offspring HPA type</th>
<th>Platelets required for transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HPA-1a antibody</td>
<td>HPA-1bb</td>
<td>HPA-1aa or HPA-1ab</td>
<td>If father HPA-1aa, then 100% of offspring are HPA-1ab, therefore 100% affected</td>
<td></td>
</tr>
<tr>
<td>These maternal antibodies are directed against HPA-1a antigens expressed on the neonatal platelets</td>
<td></td>
<td></td>
<td>If father HPA-1ab, then 50% of offspring affected (HPA-1ab)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Offspring who are HPA-1bb will be unaffected</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HPA-1bb</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>These are negative for HPA-1a antigens, to which the maternal alloantibodies are directed</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX A: Parental blood sample information

REQUEST FORM FOR TISSUE TYPING INVESTIGATIONS

REQUEST FORM:
The request form must be completed accurately and legibly. Ensure that all fields are completed and legible. Samples should be clearly labeled with:
- patient's full name (first name and last name)
- date of birth

SPECIMENS:
The quality of typing cannot be guaranteed for samples that are not received at room temperature. Samples should be sent within 24 hours of collection. Samples (other than frozen samples) should be maintained at room temperature.

Pediatric patients/donors: initial and confirmatory testing (1:100/1:1000 ratio) within 24 hours of birth or up to 4 years of age. (4/100 ratio for patients at CT).

Samples sent for flow cytometry crossover screening for initial screening (three times the initial testing) prior to living renal transplantation must be pre-booked (please email transplan@redcrossblood.org.au).

DELIVERIES:
Samples should be sent to:
Transplantation & Immunogenetics Services (NSW)
Australian Red Cross Blood Service
Clic A
17 O'Riordan St ALEXANDRIA NSW 2015
For any concerns or queries, please contact the laboratory for sample requirements transplan@redcrossblood.org.au

SPECIMEN REQUIREMENTS

<table>
<thead>
<tr>
<th>Testing</th>
<th>Specimen</th>
<th>Volume</th>
<th>Collection</th>
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<tbody>
<tr>
<td>Initial patient testing</td>
<td>Whole blood</td>
<td>40 mL</td>
<td>ACU tubes</td>
</tr>
<tr>
<td>Heart donor testing</td>
<td>Whole blood</td>
<td>25 mL</td>
<td>ACU tubes</td>
</tr>
<tr>
<td>Recipient donor testing</td>
<td>Whole blood</td>
<td>20 mL</td>
<td>ACU tubes</td>
</tr>
<tr>
<td>Confirmatory patient testing</td>
<td>Whole blood</td>
<td>40 mL</td>
<td>ACU tubes</td>
</tr>
<tr>
<td>Confirmatory related donor testing</td>
<td>Whole blood</td>
<td>40 mL</td>
<td>ACU tubes</td>
</tr>
<tr>
<td>Cellular assays</td>
<td>Patient donor</td>
<td>50-100 mL</td>
<td>ACU tubes</td>
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</tbody>
</table>

Solid Organ Transplantation

<table>
<thead>
<tr>
<th>Testing</th>
<th>Specimen</th>
<th>Volume</th>
<th>Collection</th>
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</thead>
<tbody>
<tr>
<td>Recipient entry- NDMA/CAGT testing list</td>
<td>Whole blood</td>
<td>7 mL</td>
<td>EDTA tubes</td>
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<tr>
<td>Monitoring serum samples for specific organ transplantation</td>
<td>Whole blood</td>
<td>10 mL</td>
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Live Donor Kidney Workups

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<th>Specimen</th>
<th>Volume</th>
<th>Collection</th>
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<tr>
<td>Whole blood</td>
<td>60 mL</td>
<td>ACU tubes</td>
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</tr>
<tr>
<td>Whole blood</td>
<td>7 mL</td>
<td>EDTA tubes</td>
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Platelet Immunology

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<th>Testing</th>
<th>Sample details</th>
<th>Volume</th>
<th>Collection</th>
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<tbody>
<tr>
<td>Anti-HLA Multiple Antigen Panel (MAP)</td>
<td>Whole blood and red cells</td>
<td>30 mL</td>
<td>EDTA tubes</td>
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<tr>
<td>Anti-HLA Multiple Antigen Panel (MAP)</td>
<td>Whole blood and red cells</td>
<td>30 mL</td>
<td>EDTA tubes</td>
</tr>
<tr>
<td>Platelet WBC</td>
<td>20 mL</td>
<td>EDTA tubes</td>
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<tr>
<td>Platelet WBC</td>
<td>30 mL</td>
<td>EDTA tubes</td>
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For technical questions, please contact the laboratory transplan@redcrossblood.org.au
APPENDIX B: Parental sample request form

NEW SOUTH WALES TRANSPLANTATION AND IMMUNOGENETICS SERVICE

Australian Red Cross Blood Service

Delivery: Dock A, 17 O’Riordan Street
Alexandria, Sydney NSW 2015

Dr P Coghlan

SURNAME (Please print)

GIVEN NAMES

DOB / / 

SEX

ADDRESS

TELEPHONE

UR No

Name of Collector, Date and Time

TRANSPLANT CENTRE

REQUESTING DOCTOR

SURNAME AND INITIALS

ADDRESS

POSTCODE

TELEPHONE

FACSIMILE

REPORTS TO BE SENT TO

NAME

ADDRESS

TELEPHONE

FACSIMILE

NAME

ADDRESS

TELEPHONE

FACSIMILE

CLINICAL NOTES

PROVISIONAL DIAGNOSIS

REASON FOR REQUEST/TYPE OF TRANSPLANT

TEST/S REQUESTED (Please see reverse for code list)

PLEASE COMPLETE RECIPIENT DETAILS BELOW IF SPECIMEN ABOVE IS FROM A POTENTIAL DONOR

PATIENT NAME

PATIENT DOB / / 

RELATIONSHIP OF DONOR TO PATIENT

FOR NON-TRANSPLANT/TRANSFUSION TESTING

ACCOUNT TO BE SENT TO (please tick)  N/A  PATIENT  INTERHOSPITAL  PRIVATE PATH

ORIGINAL COPY
# PLEASE USE THE FOLLOWING TEST CODES WHEN REQUESTING TESTS

<table>
<thead>
<tr>
<th>Code</th>
<th>Test Description</th>
<th>Specify EG.</th>
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<tbody>
<tr>
<td>CL1</td>
<td>Class I Typing</td>
<td>HLA-A, B or C</td>
</tr>
<tr>
<td>CL2</td>
<td>Class II Typing</td>
<td>DR, DQ, DP</td>
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<tr>
<td>CYT</td>
<td>Cytotoxic Antibody Screening</td>
<td>Monthly dry tube</td>
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<tr>
<td>LUM</td>
<td>Luminox HLA Antibody Screen</td>
<td>If Class I and/or Class II antibody specificity is required</td>
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<tr>
<td>XM</td>
<td>Crossmatch</td>
<td>CDC XM or Flow XM</td>
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<td>NAB</td>
<td>Neutrophil Antibody</td>
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<td>PAB</td>
<td>Platelet Antibody</td>
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<td>PGEN</td>
<td>Platelet Genotyping</td>
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<td>B27</td>
<td>Specific Class I Typing</td>
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<td>GM</td>
<td>Gene Mutation Testing</td>
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<td>Cellular Assays</td>
<td>CTLp</td>
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</tbody>
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**FOR MORE INFORMATION ON TESTING, CODES OR BLOOD VOLUMES:**

- **PHONE**: 02 9234 2322
- **EMAIL**: ttcbo@redcrossblood.org.au
- **WEB**: transplantservices.com.au

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**PLEASE DO NOT DETACH THIS PAGE FROM ORIGINAL REQUEST AND FORWARD WITH SAMPLE.**