Sickle cell and thalassaemia screening programme support service

Frequently Asked Questions –19/03/19

Antenatal queries: Alpha Thalassaemia

Q1: How do I find out if a particular country is high risk for alpha zero thalassaemia?

A: The following areas of the world are considered high risk for alpha zero thalassaemia: South East Asia, including Hong Kong, Taiwan, Thailand, Cambodia, Laos, Vietnam, Burma, Malaysia, Singapore, Indonesia, Philippines, Cyprus, Greece, Sardinia, Turkey, or if family origins are unknown. These areas are identified with a red hash on the family origin questionnaire. For screening purposes all other areas of the world are classified as low risk.

Q2: I have a couple who are both from an area at high risk of alpha zero thalassaemia. One has an MCH below 25 and therefore possibly has alpha zero thalassaemia, the other partner has an MCH between 25 and 27 and therefore possibly has alpha plus thalassaemia. Do I need to carry out DNA testing to exclude the risk of Hb H disease?

A: No, testing for the risk of Hb H disease is not included in the sickle cell and thalassaemia antenatal screening programme. However if fetal anaemia/hydrops is seen on ultrasound scanning or if there is a family history of hydrops fetalis further investigations may be needed to exclude the possibility of the very rare condition of Hb H hydrops fetalis.

Q3: What do I do about alpha thalassaemia if one parent is from an area which is high risk for alpha zero thalassaemia and the other isn't?

A: For screening purposes alpha zero thalassaemia only needs to be considered if both biological parents are from high risk areas.

Q4: When do I need to consider masked alpha zero thalassaemia?

A: The presence of the carrier state for alpha zero thalassaemia can be masked by other co-existing haemoglobinopathies such as beta thalassaemia or Hb E. Therefore alpha zero thalassaemia must be considered whenever both biological parents' family origins are from high risk areas, regardless of any other haemoglobinopathy identified.

Q5: I have a couple from a high alpha zero thalassaemia risk area who both have an MCH below 25 and therefore might have alpha zero thalassaemia. What do I do next?

A: Alpha zero thalassaemia can only be definitively diagnosed by DNA testing, therefore parental samples should be sent for molecular confirmation. However to avoid unnecessary delay the counselling /clinical team must be contacted straight away to let them know that a couple possibly at risk for homozygous alpha thalassaemia has been identified. Do not wait for the DNA confirmation results to come back before alerting the counselling /clinical team that the couple should be offered counselling.

Q6: What do I do about alpha thalassaemia testing when one or both partners have mixed family origins?

A: If both biological parents' family origins include any ancestry from any area at high risk of alpha zero thalassaemia then alpha thalassaemia must be considered.

Q: We tested a South East Asian lady three years ago and reported her as normal, but repeat testing in this pregnancy has found her to have an MCH of 24.7 suggesting possible alpha zero thalassaemia status. Based on her previous history I consider this to be unlikely and therefore do not want to cause the couple unnecessary alarm. What should I do?

A: Repeat testing has resulted in several instances of lady's results changing in subsequent pregnancies. These are screening results and do not typically provide a concrete diagnosis; any system with action limits will inevitably result in different results being issued if the values shift slightly over the thresholds. Each scenario is unique and needs to be treated on a case by case basis but in general you should consider the following:

1) Could the discrepancy have resulted from a sampling error on either this or the previous sample? If so further investigation and possibly repeat sampling may be required.
2) Is the previous report and the new report similar in terms of conclusions (e.g. beta thalassaemia carrier to possible beta thalassaemia carrier?). In this case the new reporting format can be used without causing too much confusion.
3) In general you have to follow the current reporting guidelines based on the sample you have received, even if this contradicts previous results (although it may be advisable to add extra comments onto the report or to include "possible" if you have reason to doubt the conclusion). However, in this scenario it is advisable to contact the screening coordinator before releasing the result so that they understand why this report might cause confusion and are able to clearly explain the situation to the couple.
4) In the scenario above, further investigation established that another previous pregnancy was also normal which further reduced the likelihood of the lady being an alpha thalassaemia carrier. Testing of the baby's biological father was activated based on this lady's current result but the antenatal screening coordinator was able to reassure the couple during this process that the risk was low so that they were not unduly alarmed. The lady was also referred for investigation of iron deficiency.

Antenatal queries: Beta thalassaemia

Q7: We are a high prevalence area and we have identified a lady who is of White British origin whose results suggest beta thalassaemia. Her partner is White British do we need to test him?

A: Yes, if a risk is identified in the mother then the baby's biological father should always be tested regardless of family origins. The exception to this is alpha thalassaemia where the family origins of both biological parents are considered.

Q8: We are a low prevalence area and we have been notified about a White British antenatal lady who has previously been identified outside the screening programme as being a carrier for beta thalassaemia. As White British ladies are not normally included in our programme; do we need to test her partner?

A: Yes, if a risk is identified or known about in the mother then the baby's biological father should always be tested regardless of the family origins of either biological parent. The exception to this is alpha thalassaemia where the family origins of both biological parents are considered.

Q9: I have a lady that we have run twice on our HPLC system. On one occasion the A2 level was 3.5% on the 2nd it was 3.4%, her MCH is 26.5pg. I have 3 questions:

1) **Should I test her partner?**
2) **Should I report her as beta thalassaemia trait?**
3) **Should I be re-running these borderline A2 levels on our HPLC system?**

A:

1) You obtained an A2 value of 3.5% for this lady which is the screening programme action level when the MCH is below 27pg, therefore screening should be offered to the baby's biological father.
2) These parameters could be indicative of a beta thalassaemia carrier which is why screening must be offered to the baby's biological father. However these indices are borderline and are not typical of beta thalassaemia carriers, therefore it is also possible the lady is normal with respect to beta thalassaemia. She should therefore be reported as a possible beta thalassaemia carrier (reporting format 4b in the Antenatal Laboratory Handbook). DNA testing would be required to give a definitive diagnosis, although this will normally only be necessary if the baby's biological father is found to have a condition which interacts with beta thalassaemia.
3) As long as you are confident your HPLC system is functioning efficiently and is properly calibrated and controlled there is no need to repeat borderline A2 levels. Please remember these are screening not diagnostic tests and they will not identify all haemoglobinopathies. The laboratory handbook contains risk assessments for rare situations where conditions will not be detected by the screening tests.

Q10: What do I do when we have a clearly raised A2 (>4.0%) but the red cell indices are normal (>27.0).

A: This could be indicative of a beta thalassaemia carrier which is why screening of the baby's biological father must be offered. However these indices are not typical of beta thalassaemia carriers, therefore it is also possible the lady is normal with respect to beta thalassaemia and other factors are increasing her A2 level. She should therefore be reported as a possible beta thalassaemia carrier (reporting format 4b). DNA testing would be required to give a definitive diagnosis, although this will normally only be necessary if the baby's biological father is found to have a condition which interacts with beta thalassaemia.

Q11: When do we need to send a sample for DNA confirmation of beta thalassaemia?

A: Usually this only needs to be done in the context of an at risk couple, either to confirm that one or both of the biological parents actually have beta thalassaemia i.e. when the haematological indices are ambiguous (e.g. borderline A2 or normal indices) or to identify the mutations involved so prenatal diagnosis can be offered.

Q12: The A2 level is very low (~1%) or even absent. Do I need to do anything?

A: Assuming everything else is normal (i.e. no 2nd small peak indicating a delta chain variant, normal Hb F levels and normal MCH) then this is most likely delta thalassemia which is of no clinical significance. No further action will be required.

Antenatal queries: HPFH/delta-beta thalassaemia

Q13: I have a lady who has a reduced MCH and an increased Hb F. Is this delta-beta thalassaemia?

A: If the Hb F% is greater than 5% it certainly could be and testing must be offered to the baby's biological father. However it could be other things as well (e.g. alpha thalassaemia and co-existing HPFH). DNA studies are required to give a definitive diagnosis and should be undertaken if the baby's biological father is found to have a possibly interacting haemoglobinopathy.

Q14: I have a lady with an Hb F of 6% but her MCH and red cell indices are completely normal. Do I need to test her partner?

A: No, if MCH is normal, testing of the baby's biological father only needs to be carried out when the Hb F level is greater than 10%.

Q15: We have identified a case of what looks like typical beta thalassaemia but the Hb F level is 9%. I know that beta thalassaemia is often associated with small increases in Hb F level but this seems very high. Should I report as beta thalassaemia trait and HPFH?

A: Carrying beta thalassaemia can also be associated with quite high increases in Hb F. As long as the Hb level is normal or only slightly reduced and the A2 is clearly raised this can be reported as straightforward beta thalassaemia carrier.

Q16: I have an adult who appears to have sickle cell disease but the F level is very high (~20%). Is this S/HPFH?

A: It could be, or it could be Hb SS with a high F level. S/HPFH is where you have Hb S on one chromosome and deletional HPFH on the other. This can look like SS disease as there is no Hb A present but it is usually a very benign condition similar to a sickle cell carrier. However some SS individuals can also have Hb F levels as high as 20%. DNA analysis or family studies are usually required to definitively distinguish between the two.

Antenatal queries: Variants

Q17: I have a variant (not S, C, D or E) which runs at a retention time/zone on HPLC/capillary electrophoresis and position on electrophoresis which matches a variant described in my reference book or manufacturer's manual. Is it OK to report it as this variant?

A: No, over a thousand structural Hb variants have now been reported so some are bound to run in the same positions on HPLC and electrophoresis. Definitive identification of rare haemoglobin variants can only be made by DNA sequencing or mass spectrophotometry. If you are unable to definitively identify the Hb variant testing of the baby's biological father should be recommended.

Q18: We have an unusual variant, how do I get it identified?

A: Definitive identification of rare haemoglobin variants can only be made by DNA sequencing or mass spectrophotometry.

Q19: When do I need to get an unusual variant identified?

A: For antenatal screening purposes a precise identification of a rare variant is usually only required in the context of an at risk couple i.e. if the other biological parent has is a carrier of a haemoglobinopathy. In these cases it is vital that the Hb variant is properly identified so any risk to the pregnancy can be assessed.

Q20: How do I find out about the clinical significance/possible interactions of rare variants?

A: Refer such cases to a consultant haematologist. If the consultant is unsure we may be able to provide some guidance in some cases, although for many rare variants or rare interactions there is no clear information available.

Q21: Should I get a variant identified to find out if it is clinically significant before testing the partner?

A: No, test the baby's biological father first to avoid delay.

Q22: We are a low prevalence area but a possible Hb variant has been identified in an antenatal White British lady having A1c testing for diabetes. How do I proceed?

A: Check the FOQ, if the lady has not declined testing, then check the variant on your system and if confirmed, proceed with testing of the baby's biological father as normal. If the lady has declined testing then no further action can be taken.

Antenatal queries: Prenatal Diagnosis

Q23: Can we do prenatal diagnosis when the partner is unavailable?

A: Yes, although the testing is likely to take longer and the results may be issued on a risk basis. Always consult the prenatal diagnosis lab for advice on individual cases prior to taking any samples.

Q24: We have a couple who want prenatal diagnosis do we need to send parental samples ahead for mutation identification?

A: This is not normally required when the biological parents are carriers of Hb S or Hb C. In these cases parental samples can be sent with the CVS or amniotic fluid sample to the prenatal lab at the time of the PND. For all other carrier states, whenever possible, parental samples should be sent ahead to identify/confirm the mutations involved. This is particularly important for couples who are possible alpha zero thalassaemia carriers to avoid an unnecessary invasive fetal sampling procedure if one of the biological parents is found not to be a carrier following genetic testing. If in any doubt or when this is not possible contact the PND lab for advice.

Q25: Is an amniotic fluid sample or a CVS better for PND?

A: Both types of sample are suitable for prenatal diagnosis. However it is slightly more likely that the PND results from an amniotic fluid sample will be delayed than from a CVS sample. This is because although individual samples vary, on average there is more DNA in a CVS sample than in an amniotic fluid sample. If there is insufficient DNA in a sample for all of the DNA tests to be completed then the

PND lab will have to wait for the cultured back-up cells to grow so they can obtain more DNA. Cultured cells take 10-14 days to grow so this will considerably delay the PND result.

Q26: Do I need to contact the PND lab before sending the samples?

A: Yes, this is very important so that the lab can make sure the correct samples are being sent and are alerted that a sample is on its way. If the sample doesn't arrive at the expected time, the PND lab can then immediately start locating the samples whereabouts.

Newborn

Q27: How do we get a variant (which is not S,C,D ,E, or O-Arab) identified?

A: These variants are no longer reported by the new-born screening programme, therefore identification is not required.

Q28: Variants which are not S,C,D,E or O-Arab are not being reported any more, therefore when I see them on my first line screening system do I still need to send them for second line testing, or can I just ignore them?

A: Second line testing is not required if the variant elutes before A on HPLC (to the left of Hb A on capillary electrophoresis). However second line testing must be performed if the variant runs after Hb A on HPLC (to the right of Hb A on capillary electrophoresis. This is to ensure Hb S (or one of the other designated haemoglobins) is not missed even if it falls outside the predefined analytical window, and Hb O-Arab, which has no defined analytical window, will still be identified.

Q29: We have identified a baby who is a Hb D carrier but both parents are normal. What should we do?

A: In the first instance double check that there have been no mix-ups in the laboratory. Then prior to reporting, alert the counselling/clinical team of the discrepancy. Guidelines for dealing with these situations can be found in the laboratory handbook.

Q30: What do we do when we identify a baby with greater than 25% Bart's?

A: Identification of Hb H disease is no longer part of the new-born screening programme therefore no action is required.

Q31: We have received a liquid sample on a baby for haemoglobinopathy testing. We don't receive these very often and are not really used to looking at them, can you help?

A: We recommend that if you are not confident about reporting these samples they should be referred to a lab that regularly processes capillary blood samples from babies. If in doubt, please consult the screening programmes best practise guidelines found in the antenatal laboratory handbook appendices.

OTHER

Q32: What should I do about issuing haemoglobinopathy cards?

A: The screening programme does not issue guidance on the use of haemoglobinopathy cards. Other organisations such as the British Committee on Standards in Haematology have. This can be found in

"Significant haemoglobinopathies: guidelines for screening and diagnosis" https://doi.org/10.1111/j.1365-2141.2009.08054.x

Q33: What do we do about antenatal screening in cases of assisted conception (donor eggs/donor sperm)?

A: In cases of egg donation the baby's biological father should be tested, if he is found to carry a haemoglobinopathy then the IVF unit should be contacted to identify the origin of the eggs. For sperm donation, the biological mother should be screened as normal and if she is found to be a carrier then the sperm bank should be contacted to identify the origin of the sperm. The women carrying the baby should always be tested for her own health. More detailed guidelines on this matter are available in the laboratory handbook.

Q35: We have someone in the lab that we would like to send for haemoglobinopathy training, can you help?

A: The programme provides support for laboratories via e-learning and training days. Further information can be found at https://www.gov.uk/guidance/sickle-cell-and-thalassaemia-screening-education-and-training

References:

More information regarding Sickle Cell and Thalassaemia screening can be found at the following website:
https://www.gov.uk/guidance/sickle-cell-and-thalassaemia-screening-programme-overview

If you have a specific query you would like to discuss, please contact us:
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