Haematological Malignancy Diagnostic Service

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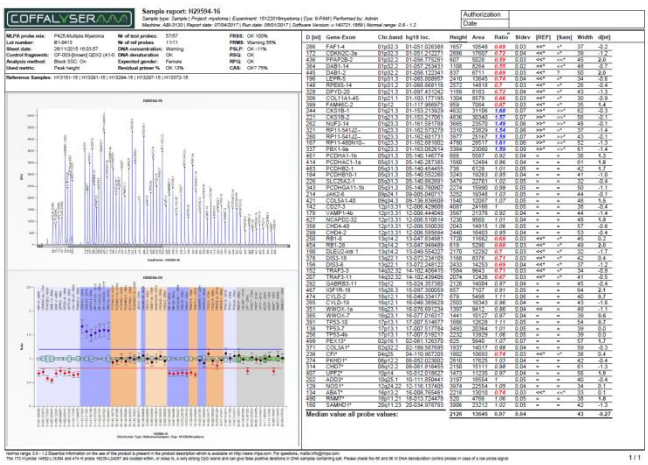
Welcome back

We have had a very busy last six months dealing with our first UKAS ISO:15189 inspection and the resulting findings, but we are happy to report that we had our accreditation confirmed in March. This is as a result of lots of hard work from all members of staff so thanks to everyone. This month we'd like to highlight some diagnostic processes which we are planning to change to improve investigation in these areas and shorten turn-around times. Assessment of chromosomal abnormalities in myeloma is being up-dated to use a PCR-based assessment for copy number abnormalities with FISH still been used to look for relevant translocations. We have also improved our internal processes to deal with requests to investigate eosinophilia and suspected myeloproliferative neoplasms.

Changes to investigation of copy number abnormalities in myeloma

Copy number variations (CNVs) are one of the main sources of genetic variation in pathological conditions. There are several key CNVs in myeloma which have been shown to have prognostic significant. These abnormalities are currently investigated within HMDS using FISH but more recently a commercial PCR-based assay has been trialled. Multiplex Ligation-dependent Probe Amplification (MLPA) is a semi-quantitative technique to assess the relative copy number of up to 60 DNA sequences in a single reaction. This has been validated within HMDS for use on CD138+ selected plasma cells and is due to be put into routine use in the next few weeks. Relevant translocations will still be investigated using FISH.

The assay will be run on all newly presenting myeloma cases with sufficient sample to select plasma cells and extract DNA. A new section on HILIS will generate a MLPA results section on the report. Copy number variations (CNVs) will be described as losses or gains of specific chromosomal regions. This will also be supplemented by a MLPA report (see example below) and a comment on the prognostic significance, if known.



MLPA in myeloma

There have been numerous publications in the literature on the use of MLPA in the genetic characterisation of myeloma. Martin Kaiser's group at the ICR applied this technique to the Myeloma IX trial cohort at presentation and are also analysing samples from Myeloma XI currently. The range of abnormalities covered includes all those currently assessed by FISH within HMDS and some additional chromosomal regions of interest. These CNVs include:

- Chromosome 1 abnormalities, including gain of 1p and loss of 1q.
- Loss of TP53 on chromosome 17p.
- Loss of regions of 13q

*Boyle EM, Proszek PZ, **Kaiser** MF, et al. A molecular diagnostic approach able to detect the recurrent genetic prognostic factors typical of presenting myeloma. Genes Chromosomes Cancer. 2015 Feb;54(2):91-8*

Paperless reporting

Following feedback from users about how our reports are most frequently viewed, HMDS is planning to stop printing and posting reports in the near future. To help us ensure that reports still get delivered successfully, we are asking users to supply a nominated nhs.net e-mail address for reports. This must be a generic, departmental e-mail, although copies will still be e-mailed to personal nhs.net accounts if requested. Please send details of nominated accounts to **hmds.lth@nhs.net**.

It is also possible to register for email notifications via the HILIS website, click 'Resources', then 'user function' and then select 'register for new report notifications'. You'll need to enter your GMC number to register. You can then click 'all' or 'new' reports. 'All' reports is the recommended default option.

DLBCL diagnoses with important outstanding tests

In order to get the soonest possible diagnosis for your patients, HMDS often makes an initial diagnosis which may have further additional tests outstanding. These tests will not radically change the diagnosis but may allow more appropriate sub-classification or prognosis, which may in turn impact on patient management. If cases do have tests outstanding when released by HMDS this is always mentioned on the report. All cases of DLBCL diagnosed within HMDS have FISH performed for MYC/BCL2/BCL6 abnormalities and may also have other molecular assessment, such as ASO-PCR or sequencing. The terminology for some DLBCL classifications was changed in the recent WHO classification up-date and as a result we introduced the term **High grade B-cell lymphoma, NOS** until MYC results are available. The diagnosis may then be up-dated to **Burkitt lymphoma** or **High grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements** or remain as **High grade B-cell lymphoma, NOS** if no chromosomal rearrangements are identified. Following incorporation of the additional results, a new report is issued and this should always be reviewed by the referring clinician before final decisions about patient management are made. Recent feedback from users has suggested that this review does not always occur and this is particularly critical in the case of **High grade B-cell lymphoma**, where subclassification as **Burkitt lymphoma** would necessitate different therapy.

We are therefore introducing modifications to our practice to make this process clearer and better highlight important changes to the final diagnosis to the referring clinician.

- An additional term 'High grade B-cell lymphoma, further tests pending' has been introduced to make it clearer that investigations are still on-going.
- A statement will also be added to the reports explaining that the further tests may lead to refinement of the final diagnosis and these changes may impact on treatment decisions
- Updated diagnoses will now trigger additional e-mail alerts

Although these changes to our practice will make it more obvious if an update to the diagnosis has occurred, we ask that all clinicians still refer to the most recent HMDS report when making decisions about patient management and consider if any outstanding tests may have implications for therapy choices.

Investigation of suspected MPN

HMDS regularly receives peripheral blood samples requesting JAK2 and CALR which have completely normal full blood counts. In most cases this occurs as a result of a resolution of abnormal counts in the intervening period between request of the test and blood taking, meaning testing is no longer indicated. This is having a significant impact on the molecular workload of the department and is prolonging the turn-around of all molecular tests. It is also conferring unnecessary cost on the referring hospital. We have therefore introduced a change to our screening procedures to reject requests which do not meet the criteria from the WHO guidelines for the diagnosis of MPN. To be processed for JAK2 and CALR testing, samples will have to meet one or more of the following criteria:

- Hb >165g/l or Hct >49% for males and Hb >160g/l or Hct >48% for males OR Plt ≥450 x 10^9/l based on HMDS count
- WBC leukocytosis or neutrophilia/monocytosis
- Morphological evidence of teardrop poikilocytes, left-shifted neutrophil series or Fe deficiency in context of normal RBC
- Relevant clinical details (known MPN on treatment, splenomegaly, suspected myelofibrosis, VTE)

In the absence of these features, the sample will not be processed and a report will be issued with an explanatory comment. Billing will be adjusted to reflect the full work-up has not been done.

Investigation of eosinophilia in peripheral blood is also requested relatively often in samples which do not have a high enough eosinophil count to perform the full range of investigations, which could lead to false negative results. An eosinophil count of >2 x 10^9/l is required for high enough sensitivity to detect PDGFR abnormalities by FISH. If there is clinical suspicion of a clonal eosinophilia in a patient with a peripheral count of < 2 x 10^9/l a bone marrow biopsy should be performed. Please discuss with the lab if in doubt.

HMDS study day

Places are going quickly for the 2017 HMDS study day on Friday, 30th June. We've recently added a myeloma session to the already interesting programme.

Organisation of the meeting is been provided by Hartley-Taylor this year so for registration visit their website:

http://www.hartleytaylor.co.uk.

Places are only £24 and are limited so register soon to secure a place.

Farewell to Dr Goodlad

Unfortunately, we are having to say goodbye to John this week following his short time with us. John is leaving due to a change in personal circumstances which require a move back up to Scotland. We wish him all the best with his new post. You may still see/hear John at MDT meetings in the short term until a permanent replacement has been appointed. We hope to be able to introduce a new consultant to you in the next edition.

Myeloid sequencing

In the previous edition of this newsletter, we described our high through-put sequencing panels for myeloid disorders. We have now analysed and reported more than 1,000 samples and this is ready for use in the routine setting. Unfortunately, we cannot continue to supply this without cost so from April 2017, targeted sequencing for myeloid malignancies is a costed laboratory investigation in HMDS.