**MetBioNet Workshop Report**

**Support for remote/virtual IMD clinics - IMD monitoring on patient collected samples**

**Background**

In response to NHSE & NHSI’s call to capture and build on improvements and innovations to clinical pathways that have arisen from the COVID-19 pandemic, the Metabolic CRG and BIMDG have identified the provision of remote/virtual clinics as a key priority to develop and embed within service delivery. A limiting factor for the provision of such clinics is the availability and access to routine and specialist blood tests local to the patient and easy access to the results. In order to address this, two key work streams have been identified:

1. To identify an IT solution(s) for remote requesting, reporting and reviewing of patient results (both metabolic & routine tests) – this is progressing with support from Pathology Lead for Get It Right First Time, GIRFT
2. To increase the availability of testing of patient collected samples for IMD monitoring

**The Workshop**

To support this work a MetBioNet Workshop was organised to explore and discuss the provision of IMD monitoring on patient-collected samples. A virtual workshop was held on Wednesday August 26th 2020. Representatives from 14 MetBioNet laboratories attended the workshop. The programme was as follows:

Setting the scene

*Elaine Murphy, National Hospital, Queen Square, London*

Results of the lab survey (current provision) & Aims of the workshop

*Helena Kemp, Southmead Hospital, Bristol*

The use of dry blood spots (DBS) to monitor patients with IMDs

– factors to be considered

*Stuart Moat, University Hospital of Wales, Cardiff*

Manchester experience of blood spot amino acid analysis - reference ranges, workflows, IQC/EQA, and reporting strategy including monitoring for homocystinuria

*Alistair Horman, Willink Biochemical Genetics Laboratory, Manchester*

Other considerations (including sample collection devices, results of the ERNDIM EQA scheme)

*Rachel Carling, Viapath, St Thomas’ Hospital London*

Discussion and next steps

Copies of the presentations will be circulated with this report.

**Results of the MetBioNet Laboratory survey**

* Responses were received from 15 MetBioNet Stakeholder labs. The survey results, outlining the current provision of testing on patient-collected samples, are summarised below.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test** | **No of labs** | | **Analytical platform** | | **Disorders monitored** |
|  | DBS | Finger-prick capillary blood | DBS | Finger-prick capillary blood |  |
| Phenylalanine | 13 | 2 | LC-MS/MS, HPLC,UPLC-UV | HPLC, IEC | PKU |
| Tyrosine |  |  | LC-MS/MS, HPLC,UPLC-UV | HPLC, IEC | PKU, Tyrosinaemia 1 & 2, AKU |
| Branch-chain amino acids (BAA) | 5 | 5 | LC-MS/MS, uHPLC, UPLC-UV, Biochrom | Biochrom, HPLC, IEC | MSUD |
| Hcy (with methionine) | 3 (1) | 1 | Chromsystems kit, LC-MS/MS | HPLC | Homocystinuria, MTHFR |
| Full or limited amino acid profile | 1 | 2 | UPLC –UV | Biochrom, IEC | MSUD, PKU, Tyrosinaemia, UCDs, gyrate atrophy, AKU |
| Free carnitine & acylcarnitines | 4 |  | FIA-MS/MS  LC-MS/MS |  | Patients on carnitine, FAODs, MMA, PA, GA1, Primary carnitine deficiency, ketogenic diets |

* All labs are currently providing phenylalanine and tyrosine testing on patient-collected samples, there is variable provision for BCAA (both DBS & capillary) and limited provision of homocysteine (only one lab providing with methionine) and of a full or limited amino acid profiles (only 3 labs). All responding labs provide DBS carnitine and acylcarnitines profiles but very few currently provide these on patient collected samples.
* Testing is provided using a variety of different analytical platforms. Additional information was requested regarding methodology, responses highlight the following variations in practice.
  + IQC – in house, CDC, commercial (Chromsystems, Clin Check, Neobase)
  + EQA – ERNDIM (blood spot, αα, carnitine), NEQAS (αα),
  + Reference ranges – local, published guidelines, none (dependant on IMD clinician’s treatment targets), one lab reports plasma equivalents for blood spot results and one lab reports blood spot results with plasma ranges for reference.
  + Many labs reported problems with these processes including poor quality DBS samples, use of out of date cards, capillary samples that are too small or haemolysed and postal delays.
* A variety of sample collection devices and lancets are in use for blood spot (newborn screening cards, Perkin Emmer 226 card) and capillary samples (Sarsted, Microvette tubes). Collection kits are sent out by some labs often with the support of dieticians.

**Use of DBS samples to monitor IMD patients**

Advancements in analytical technology, in particular mass spectrometry, have led to the development of methods to measure numerous different analytes in dried blood spots (measurement of over 2000 different DBS assays reported in the literature). DBS analysisoffers clear advantages for patient care including minimally invasive sampling, small sample volumes, simple logistics and sample storage, potential cost efficiencies and the opportunity for patients to collect their own samples and post them to the laboratory.

There are however many factors that need to be considered in order to optimise the quality of blood spot analysis to ensure accurate, reliable and clinically applicable results which were highlighted by all speakers and are summarised below:

* The perceived benefit of DBS for testing is dependent upon the assumption that a sub-punch of a defined volume can be obtained from a DBS from a non-volumetrically applied blood sample.
* Pre-analytical factors
  + DBS collection devices (defined as Class II Medical devices) are required with defined performance criteria of filter paper (CLSI NBS01) – new devices are now on the market (see later)
  + DBS sample collection (guidance CLSI NBS01-A6) & blood spot quality have a significant influence on the accuracy of results – issues include sample volume, multi-spotting & compression – standardised acceptance criteria are essential
  + Training of sample takers (including patients/carers) and maintaining competency is crucial.
  + Blood spot storage conditions including during transport to the lab (temperature, humidity), can significantly affect results – see presentations for stability data.
* Analytical
  + Results are significantly influenced by blood spot volume, haematocrit, punch size & punch location.
  + Results are also affected by the quality of analyte extraction, choice of internal standard & methods of calibration. There are no matrix matched certified reference materials (CRM) available for DBS assays
  + IQC material for DBS analysis and EQA schemes are very limited in terms of the number of analytes included and the frequency of distributions.
  + Multiple analytical methodologies for amino acid analysis are in use, even within the same laboratory, with differing sensitivity, specificity and linearity.
  + As a consequence of the above factors there is significant between-lab analytical variation (CVs >20%) - see presentations for ERNDIM DBS performance data.
* Post-analytical issues
  + Interpretation of DBS results is a challenge as the availability of reference range data is limited and consensus management guidelines, where available (PKU, MSUD & HCY), largely relate to plasma/serum values rather than DBS results.
  + Correlation between plasma and DBS is often not straightforward and varies between analytes.
  + Measurement of uncertainty is rarely considered in the context of clinical guidelines

**Blood spot collection devices**

As highlighted above, pre-analytical issues have a significant impact on the accuracy of DBS results. Alternatives to standard filter paper collection devices e.g. HemaXis DB10, Capitainer B and Mitra tips, are now available which potentially offer some advantages over current practice. See Rachel Carling’s presentation for a detailed overview of the above devices. The potential benefits and disadvantages can be summarised as follows:

Benefits: collect a precise and accurate volume of blood, reduce the effect of haematocrit, easy to use, ease of transport, CE marked & FDA approved.

Disadvantages: Different punch size than in current use so manual punching required, manual transfer of samples currently needed, lack of automation, increased costs of collection devices

A study (Viapath, Evelina and UHW, Cardiff) to compare the performance of three commercial DBS collection devices (HemaXis, Capitainer and Mitra BS) including laboratory assessment, use in the clinic setting and use for home monitoring was due to commence earlier this year but has been delayed due to COVID-19. There is also some experience in the use of Mitra tips for immunosuppressant drugs and creatinine at Viapath and preliminary experiments for the IMD markers phenylalanine, tyrosine, BCAA & acylcarnitines have been promising. It is noted that both HemaXis and Capitainer have plasma collection devices in development.

**Summary**

* Analysis of patient collected samples offers practical, clinical and potentially financial advantages for the monitoring of IMD patients.
* The Metabolic laboratories currently provide a variety of tests, principally amino acids, for monitoring IMD on patient collected samples mainly on DBS samples.
* The workshop has summarised the key issues and challenges associated with DBS monitoring including pre-analytical, analytical and post analytical factors.
* The need for optimal sample collection, transport and storage is crucial to the quality of results achieved. The use of commercial sample collection devices would address some of the pre-analytical variables and should be explored. Regular training of sample takers is essential.
* The variation in practice across laboratories in terms of the analytical techniques and methodologies used, along with the lack of a commercially available CRM to facilitate assay standardisation, explains the significant between-laboratory variation in the reporting of metabolites demonstrated by the ERNDIM blood spot EQA scheme.
* Such variation influences the reliability of applying results to European and international consensus clinical guidelines and highlights the need to standardise current practice across laboratories as the first step towards increasing access and the repertoire of tests available on patient collected samples.

**Next Steps**

MetBioNet and BIMDG will continue to work with GIRFT on finding IT solutions to support remote specialist metabolic clinics.

A joint MetBioNet/BIMDG working group is proposed to take forward the following:

1. Promote the clinical understanding of the utility and limitations of DBS testing for the monitoring of IMD e.g. through the organisation of a joint BIMDG/MetBioNet workshop.
2. Agree an approach to the development of blood spot assays for the monitoring of IMDs
3. Support the work required to standardise and optimise the blood spot assays including addressing the reporting requirements to meet the clinical need.
4. Support the work assessing the performance and utility of the new sample collection devices.
5. Identify the resources required to implement the above.