How accurate is haematological neoplasms chromosome microarray (CMA) testing and interpretation? – the EQA experience

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K. Rack[1], F. Morgan[2], R. Hastings[2], Z. Deans[2]

1. GenQA, Oxford University Hospitals NHS Foundation Trust, Oxford, OX3 9DU, United Kingdom, 2. GenQA, NHS Lothian, Royal Infirmary of Edinburgh, Edinburgh, EH16 4UX, United Kingdom

Introduction

Chromosomal microarray analysis (CMA) is a high resolution, genome-wide assessment of copy number aberrations (CNA) that can be used to identify recurrent small gains and losses of genomic material, as well as large genomic copy number imbalances, which are often characteristic of specific haematological disease entities. In addition, CMA can identify poor prognostic complex genomic signatures such as chromothripsis and chromoanasynthesis, and where SNP-arrays are used it can inform on ploidy levels and regions of copy neutral loss of heterozygosity.

Depending on the diagnostic pathway CMA are used to replace or complement other methods such as chromosome banding analysis, FISH and MLPA.

GenQA has delivered laboratory external quality assessments (EQAs) for CMA testing of haematological neoplasms, assessing the quality of testing, interpretation and clinical reporting since 2014. Neoplasms covered include CLL, MDS, ALL and Myeloma.

Methods

Laboratories were provided with DNA samples extracted from blood or bone marrow specimens, obtained from patients with a variety of haematological neoplasms, for genome wide copy number analysis using microarrays or low pass sequencing. Where appropriate, an additional fixed sample for complementary FISH analysis was provided or, in the absence of this, the results were provided as a summary report.

Laboratories were expected to analyse the samples distributed and interpret the clinical significance of any CNA detected, together with any complementary results provided, in the context of the disease referral according to current guidelines.

Laboratories submitted the results as a genetic laboratory report that was assessed by a panel of assessors against peer-reviewed marking criteria.

Results

In all EQAs there was a high level of accuracy in the identification of the essential CNA present. There was a high adherence to the European recommendations[1] for the interpretation and reporting of results and a very small minority of laboratories also cited the American guidelines.[2]

Participation in this EQA remains static across the different neoplasms (see Table 1).

Overall, there were few critical errors. However, there was notable variation in the approach to reporting of array results, in the following aspects:

- The total number of abnormalities detected (Example 1);
- The description of genes involved (Example 2);
- Interpretation of complex profiles (Example 3).

Example1: Total number of abnormalities reported

In 2021, the Acquired array EQA included a DNA sample from a CLL patient with a complex genome and array profile, including a 39Mb loss of 11q (*ATM* gene), and a 39.11Mb region of copy neutral loss of heterozygosity (CN-LOH) of 11p. In addition, there were nine copy number aberrations <5Mb across chromosomes 6, 14 and 17.

All 30 participants reported the 39Mb loss of 11q including *ATM* gene, with approximately one third reporting this alongside an 11p CN-LOH. There was large variation in the number of additional abnormalities reported by the remaining two thirds of participants, ranging from 1-15 CNA reported. The range of CNAs reported was dependent on whether the participant considered consecutive CNAs in the same state to be one single event or whether the CNA were counted independently, and how strictly the participant adhered to the recommendations for reporting1.

With regards to interpretation of the results reported, there was also variation in which abnormalities were considered prognostic in this case. Where laboratories reported multiple abnormalities of chromosome 11, the clinical significance of these findings were interpretated in the following ways:

- not considered to constitute a complex profile or determined as being of unknown clinical significance;
- potentially indicative of genomic complexity but not considered as such in the final prognostication;
- consistent with a complex karyotype and poor prognosis.

This variation in both the number of CNAs reported and interpretation of their clinical significance, highlights the lack of guidance with respect to complex karyotypes determined by chromosome banding.

Example 2: Reporting genes of interest

Review of EQA submissions from 2014-2021 also highlighted the variation in which genes of interest were considered significant and therefore subsequently described in the EQA reports. The majority of EQA reports only included a description of the genes of interest which were specifically related to the referral reason indicated in the EQA scenario which is helpful to the clinician. However, some reports included an extensive list of genes, referencing any known cancer gene located within the given CNA detected.

Although the latter approach is permitted in the current reporting recommendations, this approach often results in long reports, which may detract from the essential result of the analysis.

This example highlights that further guidance on how to identify relevant genes for inclusion in the clinical report would be beneficial.

Table 1. EQA participation per neoplasm 2014-2022

EQA Year	Number of participations			
	CLL*	MDS**	Myeloma	ALL***
2014	12			
2015	23			
2016	31	31		
2017	30	31		
2018	27	28		
2019	28	28	11 (11/72, 15% of participants)	24
2020	28	28	Array case not provided due to Covid	
2021	30	30	14 (14/81, 17% of participants)	
2022	20 (20/92, 22% of participants)	29 (29/118, 25% of participants)	13 (13/85, 15% of participants)	30 (30/81, 37% of participants)

* Included in Acquired Array EQA 2014-2021, and CLL EQA 2022 (optional case), ** Included in Acquired Array EQA 2016-2021, and Myeloid EQA 2022 (optional case 2022), *** Educational case in Acquired Array EQA 2019, and ALL EQA 2022 (optional case)

Example 3. Interpretation of chromothripsis

In 2021, the Multiple myeloma EQA involved a DNA sample from a patient with a complex genome and array profile, including a region of 1p with alternating copy number states. Of the 14 laboratories that participated in this EQA case, five chose to report this finding as cth1p (chromothripsis 1p).

The International System for Human Cytogenomic Nomenclature 2020[3] defines chromothripsis as a complex pattern of alternating number changes (commonly alternating disomy and heterozygous loss) clustered along a chromosome or chromosomal segment. However, the wider description of chromothripsis is variable across the literature and the lack of precision about both the number of altered copy number states and size of the region involved made the determination of whether this was chromothripsis or not challenging4.

Chromothripsis is relatively common in some haematological malignancies and has been reported to be associated with a poor prognosis[4], therefore accurate determination is important.

This example highlights inconsistency in the interpretation of complex regional profiles detected by cytogenomic techniques.

Conclusion

The CMA EQA cases demonstrate that despite an overall high accuracy of testing, there remains considerable variation in the interpretation and reporting of CNAs in haematological malignancies. Although most laboratories stated that they followed current recommendations for CMA testing in haematological neoplasms, these were interpretated and therefore applied in different ways by participants within EQAs.

These results highlight areas where further guidance would be beneficial to ensure consistent reporting of haematoligical neoplasms across diagnostic laboratories.

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References

1. Schoumans J *et al.* (2016) Guidelines for genomic array analysis in acquired haematological neoplastic disorders. Genes Chromosomes Cancer 55(5):480-91.
2. Mikhail, F. M., *et al.* (2019)Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). Genetics in Medicine 21(9):1903-1916.
3. McGowan-Jordan, J., Hastings, R.J., Moore, S. (2020), ISCN 2020: An International System for Human Cytogenomic Nomenclature. S. Karger, Basel.
4. Kolsova A.S., et al, 2019. On the Complexity of Mechanisms and Consequences of Chromothripsis: An Update. https://doi.org/10.3389/fgene.2019.00393

info@genqa.org • www.GenQA.org • +44 (0)131 242 6898