H022. **Chromoanagenesis: A Common Mechanism That Leads to Highly Complex Karyotype and Extensive Clonal Heterogeneity in Hematological Malignancies**

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Introduction: Complex karyotype and clonal heterogeneity are associated with therapy resistance and poorer prognosis in leukemia patients. Chromoanagenesis (CAG) is a group of genomic catastrophic events, including chromothripsis, chromoanasynthesis, and chromoplexy; these events feature clusters of chromosomal rearrangements and segmental copy number alternations involving one or a few chromosomes. Chromothripsis has been detected in 10% to ~15% of cases with acute myeloid leukemia (AML) or T-cell acute lymphoblastic leukemia (T-ALL) in earlier studies, but recent advances of technology have revealed a higher prevalence of CAG than initially appreciated. In this study, we utilized optical genome mapping (OGM) to evaluate for CAG in patients with hematological malignancies, and to assess the association of CAG with karyotypic complexity and clonal heterogeneity. **Methods:** The study group included 178 patients with various types of hematologic malignancies. All patient samples were subjected to OGM, karyotyping, fluorescence *in-situ* hybridization, and next-generation sequencing. Here we define highly complex karyotype (HCK) as five or more cytogenetic abnormalities (with at least one structural abnormality), and extensive clonal heterogeneity (ECH) as the presence of five or more clones/subclones in one specimen, including a composite karyotype. **Results:** CAG was detected by OGM in 33 (18.5%) cases in this cohort. Mature T-cell lymphoid neoplasms had the highest frequency of CAG (58%), followed by mantle cell lymphoma (43%), myeloma (29%), B-cell acute lymphoblastic leukemia (27%), T-ALL (22%), AML (15%) and myelodysplastic syndromes (MDS, 15%). None of the 28 patients with myeloproliferative neoplasm (MPN) or MDS/MPN showed CAG. Most (n = 26, 79%) cases of the CAG were limited to one to about three chromosomes but could involve up to nine chromosomes. Chromosome 17 was the most frequently involved chromosome (n = 9); otherwise there was no predilection for particular chromosomes. *TP53* deletion and/or mutation was detected in 23 (70%) patients who had CAG. HCK was detected in 40 (22%) patients, 26 (65%) of whom showed CAG. Conversely, CAG was uncommon in cases without HCK, present in 5% of 138 cases. ECH was observed in 26 (15%) cases, 16 (62%) of whom had CAG; among the 152 patients without ECH, 11% had CAG. **Conclusions:** CAG is present in approximately two-thirds of hematologic neoplasms that exhibit a highly complex karyotype and/or extensive clonal heterogeneity, which are indicators of genomic instability. CAG is much more prevalent in lymphoid neoplasms than in myeloid neoplasms, and cases of MPN or MDS/MPN did not exhibit CAG. This understanding of the prevalence and impact of CAG across various hematological malignancies paves the way for further research into its role in disease progression and patient outcomes, as it may have potential therapeutic implications.

ID054. **Dual Digital PCR Coupled with High-Resolution Melt for Bacterial-Fungal Coinfections Detection**

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Introduction: Bacterial-fungal coinfections in clinical settings pose diagnostic challenges. Culture-based methods, the gold standard for diagnosis, have limitations due to separate growth requirements and prolonged turnaround times. To overcome these challenges, our study introduces a novel approach: dual digital PCR coupled with high-resolution melt analysis (2d PCR-HRM). This method enables simultaneous detection of fungal and bacterial DNA in a single assay, allowing for the identification of pathogens from different taxonomic levels in a single run. **Methods:** Our approach for simultaneous detection of bacterial and fungal DNA involves using two sets of broad-range primers within a single digital PCR reaction. The universal bacterial primer targets the ITS region between the 16S and $tRNA^{ala}$ domains, offering diverse melt curve patterns for detecting various bacterial species. Meanwhile, the universal fungal primer amplifies the ITS1-5.8S-ITS2 region, effective for broad-range fungal detection. Melt curves are acquired for each bacterial and fungal species, and a one-versus-one binary support vector machine (ovo-SVM) machine-learning classifier identifies the melt curves. In this study, we detect 16 bacterial and fungal pathogens from the World Health Organization's (WHO) priority lists using the 2d PCR-HRM platform. **Results:** The dual HRM assay was characterized by subjecting a panel of 16 pathogens to bulk-based PCR-HRM using bacterial or fungal primers. The results demonstrated a high level of primer specificity within their respective kingdoms. Comparative analysis of single and dual HRM assays showed similar efficiency, validating the robustness of the dual HRM assay for targeted pathogen detection. Subsequently, the 2d PCR-HRM platform successfully generated digital melt curves for all 16 pathogens, displaying distinct melting temperatures, peak numbers, and shapes, indicative of their potential for accurate identification. Implementation of a machine-learning-assisted workflow with an ovo-SVM classifier achieves >99.8% accuracy for the 16 pathogens. The 2d PCR-HRM platform showcases quantification and identification capabilities, effectively detecting and distinguishing four pathogens at different taxonomic levels within a single polymicrobial mixture. Clinical bronchoalveolar lavage samples are also detected using the 2d PCR-HRM platform. With a turnaround time of less than 2.5 hours and a cost per test of less than $3, this platform offers rapid and cost-effective pathogen detection in clinical settings. **Conclusions:** The 2d PCR-HRM platform demonstrates rapid and cost-effective detection of bacterial-fungal coinfections, making it feasible for use in clinical settings. The use of broad-range primers facilitates detection of additional pathogens, enhancing its utility as a comprehensive diagnostic tool.

G056. **Identification of a Novel *RPS7* Non-Coding Variant in a Rare Form of Diamond-Blackfan Anemia by Whole-Genome Sequencing**

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Introduction: Diamond-Blackfan anemia (DBA) is a rare, genetically heterogenous disorder, typically characterized by profound anemia that develops in the first year of life and may require transfusion support or bone marrow transplantation (BMT). However, the phenotypic spectrum of DBA is wide, ranging from anemia of variable severity, congenital malformations, and growth retardation, to fetal hydrops and pregnancy loss. Notably, incomplete intrafamilial penetrance is well documented, with 20% of cases genetically unsolved. *RPS7* encodes a 40S ribosomal protein, whose variants account for ~1% of DBA cases. We evaluated a 16-month-old male proband clinically diagnosed with DBA but genetically unsolved despite DBA gene panel and exome sequencing. Family history was notable for multiple individuals in the maternal lineage with a history of anemia. The proband's clinical findings included profound normocytic anemia at diagnosis (two-month, Hb = 4.4 g/dL) requiring regular transfusions, and markedly increased adenosine deaminase (ADA) activity. A steroid trial was successful for an increase in Hb but was discontinued due to side effects. He is otherwise developmentally normal without obvious physical differences. Bone marrow evaluation showed 30% hypocellularity with left shifted erythropoiesis. **Methods:** Bone marrow failure gene panel, karyotyping, chromosome breakage analysis, and trio whole-exome sequencing (WGS) were negative. Quad WGS was performed from buccal samples from proband, parents, and unaffected sibling. WGS data were analyzed by Emedgene software with special attention to DBA-related genes. **Results:** We identified a maternally inherited splicing variant at the end of the first non-coding exon in *RPS7* (NM_001011.4 RPS7 c.-19G >C). The location is highly conserved. The variant is predicted by multiple algorithms to result in a 50% reduction in splicing donor strength. The adjacent c.-19+1 and c.-19+2 variants have been recently reported in patients with DBA and are considered pathogenic. With multiple additional maternal family members possibly affected, and the mother having mild anemia and elevated ADA activity, we initiated cascade testing in the family and RNA sequencing to substantiate the finding. The clinical team is considering BMT after confirming that the unaffected matched sibling donor does not carry this variant. **Conclusions:** This study highlights the clinical utility of WGS to transform the care and treatment of undiagnosed rare genetic disorders. The variant evades prior testing because such approaches did not include non-coding region, indicated by this and prior studies as a novel hotspot for pathogenic *RPS7* variants. This study also underscores the value of WGS when critical care decisions must be made in a timely fashion.