H26. Complex Chromosomal Rearrangements in B-Cell Lymphoma: Evidence of Chromothripsis?

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Introduction: Genomic instability is a well-known hallmark of cancer. Recent genome sequencing studies identified a novel phenomenon called chromothripsis in which complex genomic rearrangements are thought to be derived from a single catastrophic event rather than by several incremental steps. Chromothripsis suggests an evolutionary modality for cancer cells to circumvent individual mutational events with one simultaneous shattering of chromosomes resulting in the random reassembling of segmented genetic material to form complex derivative chromosomes. Although chromothripsis is well documented in solid tumors and leukemias, chromothripsis in lymphoma is rarely reported. **Methods:** A 58-year old presented with swelling in the neck which upon CT scan showed extensive infiltration and enlargement of the thyroid gland with significant effect on the trachea. The mass was biopsied and sent to lab for histological, immunostaining, chromosome analysis, fluorescence in situ hybridization and microarray (Cytochip 60K custom oligo array) studies. **Results:** Histology and immunostaining revealed that the mass contained characteristic pattern of diffuse large B-cell lymphoma. Chromosome analysis from the biopsy showed a complex karyotype with multiple numerical and structural rearrangements involving the BCL6 and IGH and BCL2 gene regions. The karyotype was interpreted as 51~56,XX,+X,+2,t(3;7)(q29;p11.2),der(7)t(3;7)t(14;7;22)(q32;p11.2;q12), +der(7)t(14;7;22),der(8)t(8;18)(p12;q21),+der(9)t(5;9)(q13;q22),+13,der(14) t(14;7;22),+21,+1~4r[cp20]. FISH analysis with B-cell lymphoma probe panel confirmed the BCL6 gene rearrangement, IGH gene rearrangement or 4-5 copies of IGH and 3 copies of BCL2. To further characterize this complex rearrangement, array comparative genomic hybridization studies were performed with Cytochip 60K custom oligo array. The results showed multiple complex copy number variations including a previously unidentified chromosome 12 abnormality, the complexity of which appears to confirm the phenomenon of chromothripsis. However, array analysis did not reveal any imbalance involving the BCL6, BCL2 or IGH gene regions whose rearrangements were observed by FISH, thus suggesting that these rearrangements are balanced in nature. **Conclusions:** Although the criteria used to identify possible chromothripsis remains unclear, this pathway is said to occur more often than expected. Our patient's genomic abnormalities show characteristics suggestive of chromothripsis and provides initial evidence that chromothripsis is not confined to solid tumors, but can also be seen in B-cell lymphomas with well characterized one or two-step lymphomagenesis. Our case further illustrates that lymphomagenesis can be complex and may arise from a catastrophic event resulting in multiple complex chromosome rearrangements.

OTH02. Implementing Lean Methods to Improve the HER2 Fluorescence *in Situ* Hybridization Process in the Diagnostic Molecular Pathology Laboratory

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Introduction: Lean and Six Sigma process improvement methods have been utilized in the manufacturing industry for decades, and are now being applied to healthcare settings. HER2 FISH result turnaround times (TAT) were not routinely meeting some customers' needs. To provide better service to our providers the current HER2 FISH processes in the Molecular Pathology Laboratory were evaluated using Lean Six Sigma analysis tools. Opportunity to improve the workflow was identified. **Methods:** The evaluation of the HER2 FISH assay had multiple phases: documentation of the current state to establish a baseline measurement of TAT; workflow observations to determine areas for improvement; and, pilot studies to test theories to decrease overall TAT. A flow chart was constructed to record the baseline processes from the time of order to result reporting and current TAT was generated from the LIS. In the analysis phase, time points for all exchanges that occurred for each HER2 case were collected over the course of 10 consecutive clinical runs. Following 10 cycle analysis, multiple user to user variations in processing times were identified. A single location for pick up and drop off of all HER2 slides was implemented within this phase. From the analysis phase, it was evident that the time of day that the technologist read the slides impacted when the Pathologist could complete a case review. The improvement phase of the project included a pilot study in which two technologists analyzed and drafted reports for all HER2 slides by 12pm daily. This allowed sufficient time for slide review, report entry by a technologist and verification of the results, thus decreasing the possibility of an overnight delay. **Results:** The baseline TAT for the HER2 FISH assay was measured at 7 business days. In the analysis phase, with a centralized slide pick-up location, the TAT improved to 5 business days and the number of hand-offs, misplaced slides and recuts decreased (waste). After the improvement

phase, the early read pilot was implemented as standard procedure resulting in overall TAT decreasing to 3 business days, fully satisfying customer expectations. **Conclusions:** Within six months, the implementation of Lean processes to improve the workflow of HER2 FISH testing in our molecular pathology laboratory resulted in improved overall patient care and reduced assay TAT and waste. Process improvement strategies, like Lean, should be embedded into all workflow analyses in a clinical laboratory.

ST62. *BRAF* and *RAS* Mutation Analysis for Thyroid Nodules on Direct Cytology Smears Using Multiplexed Single Nucleotide Primer Extension Assay

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Introduction: Fine needle aspiration (FNA) is commonly used for primary evaluation of thyroid nodules. Although the majority of nodules are classifiable, up to 30% of aspirated nodules fall into the category of indeterminate thyroid lesions. Specific gene mutations have been found to be associated with malignant thyroid nodules. The presence of *BRAF* V600E mutation is known to be highly specific for papillary thyroid carcinoma (PTC). *RAS* mutations, although can be seen in benign nodules, carry a 74% to 87% positive predictive value for malignancy. To maximize the utility of thyroid FNA material, we developed a multiplexed single nucleotide primer extension assay (SNu-PE) using DNA obtained from direct FNA smears to simultaneously detect 52 mutations in *BRAF* and the *RAS* genes. **Methods:** Thirty-nine thyroid FNAs (Romanowsky-type and Pap slides) and paired formalin-fixed, paraffin-embedded (FFPE) tissue from resection specimens from 28 cases were tested. The areas of interest were circled by a surgical pathologist and cytopathologist, microdissected, and genomic DNA was isolated from cytology smears and FFPE tissue using the Pinpoint Slide DNA Isolation System (Zymo Research, Irvine, CA) or QIAamp FFPE DNA Tissue kit (Qiagnen, Valencia, CA), respectively. Mutation analysis for *BRAF* V600E, K601E and K601Q, *KRAS* and *NRAS* codons 12, 13 and 61 and *HRAS* codon 61 was performed using a laboratory developed two-tube multiplex PCR assay followed by SNu-PE using the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA). The products were analyzed by capillary electrophoresis. **Results:** All *BRAF* V600E-positive cases (5) carried a final diagnosis of PTC on resection. Two follicular adenomas (FA) were positive for *KRAS* mutation. *NRAS* mutations were detected in 5 cases including 2 FAs, 2 adenomatoid nodules and a case of columnar variant of PTC. An *HRAS* mutation was identified in a young patient with the follicular variant of PTC. 100% concordance was observed between FNA smears and FFPE tissue. The limit of detection of the assay is 5%. **Conclusions:** The FNA smear is a reliable source of DNA for molecular testing of thyroid nodules.Our laboratory-developed SNu-PE assay worked well on DNA isolated from both Romanowsky-type and Papanicolaou stained slides. This approach allows molecular as says to be performed on specific cells of interest and maximizes the utility of FNA materials. It may also save patient from an additional, unnecessary procedure.