become available, patients who do not meet these guidelines at the time of assessment may not be identified as having a cancer predisposition syndrome. This can have significant implications for personal risk and cancer management and the ability to capture unaffected relatives to potentially prevent cancer altogether. It is increasingly evident that we are approaching pathogenic variant carrier frequencies that argue for a more aggressive expansion of guidelines. This has been a catalyst for societies recommending germline genetic testing for all patients with history of certain cancer types. Aside from NCCN, one such society is the American Society of Breast Surgeons (ASBrS). Here, we evaluate how updated version guidelines, which expand patient eligibility, capture patients that were previously missed using the prior guidelines. Furthermore, we evaluate subjects with personal history of breast cancer who meet ASBrS criteria but do not meet NCCN criteria for genetic testing.

Methods: Patient data was obtained from the Informed Genetics Annotated Patient (iGAP) Registry, an IRB-approved, patient-consented, multi-center longitudinal, observational study designed to capture genetic and genomic test results and their utilization and impact on treatment practices and outcomes. One thousand four hundred thirty-nine subjects were assessed using the most up to date guidelines at the time of assessment (*NCCN 2.2021 (1207), 2.2020 (127), and 2.2019 (96) Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic (BOP) Guidelines*) and underwent germline genetic testing. Of these 1439 subjects, 704 met criteria for genetic testing while 735 did not meet criteria. These subjects were then reassessed using the recently updated *1.2022 BOP* guidelines. Five hundred twenty-eight subjects with personal history of breast cancer who did not meet NCCN BOP criteria at the time of assessment (2019-2022) were also evaluated. Descriptive statistics were used to assess and compare data of these populations.

Results: Of the 704 subjects who met *2.2021 BOP* criteria, the average age was 55.62 years. 81.53% had a personal diagnosis of any cancer, while 85.94% had a family history of cancer. Of the 735 subjects who did not meet criteria, the average age was 62.09 years. 84.90% had a personal diagnosis of any cancer, while 58.78% had a family history of cancer. These 1439 subjects were reassessed using the updated *1.2022 BOP* guidelines. For most subjects, this reflects a period of 7 months and 20 days between guideline versions (December 22, 2020, to August 11, 2021). An additional 71 subjects were identified as meeting criteria for genetic testing, of which 19.71% had at least one pathogenic or likely pathogenic variant in the germline. Variants were identified in the following genes: *ATM, BAP1, BRCA2, CHEK2, MSH3, MUTYH, NBN, PALB2* and 2 others. All genes have implications for medical management and/or reproduction. Of this cohort, 85.92% had a personal history of cancer and 91.55% had a family history of cancer. The average age was 64.54 years. Of the 528 subjects with personal history of breast cancer who did not meet NCCN BOP criteria at the time of assessment (2019-2022), 13.8% had a pathogenic/likely pathogenic test result. All these patients meet ASBrS criteria for genetic testing. These subjects did meet other guidelines, including United States Preventive Services Task Force (USPSTF) (20.8%), and NCCN Colorectal (3.41%).

Conclusion: This study demonstrates that expanded NCCN qualifying criteria allows for the identification of more patients with clinically actionable germline genetic variants. In this cohort alone, nearly 20% of subjects had a clinically actionable variant that would have been missed due to a failure to offer germline testing using the prior guideline version. For breast cancer subjects specifically, nearly 14% had a clinically significant variant that would have been missed using NCCN guidelines. Providers and payors who use these guidelines as gold standard to offer and cover germline testing, rather than other available guidelines or clinical intuition, miss an opportunity for personalized cancer risk management. This may affect both treatment and prevention strategies. As NCCN qualifies all patients with ovarian cancer, pancreatic cancer, and certain neuroendocrine/adrenal tumors, this study begs the question, how long will we wait before genetic testing is offered to all patients with cancer?

https://doi.org/10.1016/j.gim.2022.01.079

ePO42

Highly sensitive blocker displacement amplification-based qPCR approach in detecting low level JAK2 variant

Zheng Wang[1], Frank Mularo[1], Cailin Weller[2], Alessandro Pinto[2], David Yu Zhang[2], Yu-Wei Cheng[1]

[1]*Cleveland Clinic;* [2]*NuProbe USA, Inc*

Introduction: JAK2 exon 14 c.1849G>T, p.V617F (NM_004972.3) variant is commonly found in Myeloproliferative neoplasms (MPN); approximately 100% of polycythemia vera and 50% of essential thrombocytosis and primary myelofibrosis cases have this pathogenic variant. The pathogenic nature of JAK2 V617F in these disorders have been well-established in the past. The National Comprehensive Cancer Network (NCCN) guidelines for cancer care has indicated molecular testing using blood samples for JAK2 V617F as well as other mutant alleles is needed for MPN diagnosis. Many molecular genetic testing methods are available to detect this single nucleotide sequence change, including Sanger sequencing, quantitative PCR (qPCR), Next-generation sequencing (NGS), and droplet digital PCR (ddPCR). Variants with high allelic fraction (eg, >10%) can be easily determined, however, the low variant allelic fraction (VAF) may not be consistently identified. In this study, we describe a Blocker Displacement Amplification (BDA)-based qPCR approach that has the flexibility to use many existing qPCR platforms to deliver test results at the ddPCR level sensitivity. We are interested at evaluating and applying the BDA, to detect low level and rare gene sequence alterations. The clinical specimens were also tested using other molecular diagnostic methods such as NGS and ddPCR, to compare each method's detecting limitation of the V617F variant.

Methods: We tested 122 clinical specimens (extracted genomic DNA) where the JAK2 gene mutational status has previously been deep sequenced with NGS methods, including 11 samples of low level variants (eg, VAF =<2%), 14 samples of various mutant allelic proportions (eg, VAF>2%), and over 50 samples of no variant identified by NGS testing. A molecular blocker method has been introduced recently to detect gene variants. This novel technology, Blocker Displacement Amplification (BDA), uses competing molecular blockers to enrich low level mutant alleles. Customized BDA assays are designed targeting a specific JAK2 variant (ie, c.1849G>T, p.V617F). The NGS was performed on Illumina NextSeq 550. The sequencing reads of >Q30 score ranged from 81.9–87.9%, and the average read-depth was at least 1500x at this locus. The ddPCR assay was performed according to BioRad (Hercules, CA) applications guide. The JAK2 probe assay mix was purchased from BioRad.

Results: Our study shows that although NGS is able to identify the c.1849G>T, p.V617F variant at 1% VAF level, it is challenging to detect variants less than 0.5% VAF without ultra-deep sequencing. On the other hand, the BDA approach delivers comparable data as ddPCR, which identifies the variant down to 0.1% VAF. BDA provides a cost-effective alternative mean to identify low level variants without the purchase of capital equipment.

Conclusion: The BDA method gives the flexibility of simultaneously targeting multiple gene alteration events at one time using equipment that is usually present in most laboratories. The successful development of the molecular blocker method will give our pathologists and oncologists a more cost-effective alternative diagnostic tool to identify low level JAK2 c.1849G>T, p.V617F variant or other gene variants.

https://doi.org/10.1016/j.gim.2022.01.080