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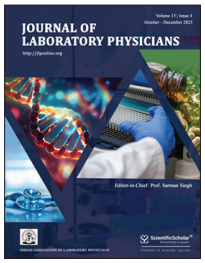

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P-1

MET exon 14 skipping mutation opens new doors in non-small cell lung carcinoma: Single-institutional experience with lung panel next-generation sequencing

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Introduction: Somatic alterations in exon 14 of MET gene (METex14), specifically splice junction mutations, result in loss of exon 14, causing fusion of exons 13 and 15, leading to synthesis of an aberrant MET receptor protein, activating downstream signaling pathways promoting tumor development.[1] METex14 skipping in non-small cell lung cancer (NSCLC) is a recently recognized crucial target for capmatinib or tepotinib therapy, emphasizing the need to identify this genomic alteration for effective treatment.

Aims and Objectives: (1) Characterize METex14 skipping mutation using next-generation sequencing (NGS) and to determine its frequency in NSCLC. (2) Determine the clinicopathologic characteristics of NSCLC with METex14 skipping mutation.

Methods: NGS was performed using DNA and RNA extracted from formalin-fixed paraffin-embedded tissues. The entire coding region of 52 actionable genes (including mutations, copy number variations, and fusions) was targeted. The sample was sequenced using the NGS platform (Ion GeneStudio S5 plus, Thermo Fisher, USA), and the data were analyzed using the Ion Reporter™ software 5.20. 1000× depth and 5% variant allele frequency (VAF) were the minimum requirements to identify significant variants.

Results: In 123 NSCLC cases, six had this pathogenic METex14 skipping mutation (AMP class IA), indicating a frequency of 4.8%. All cases showed chromosome 7:116411708 (exon 13)- chromosome 7:116414935 (exon 15) fusion with deletion of chromosome 7:116412044 locus (exon 14). VAF ranged from 248 to 100359 read counts; no concomitant driver mutations were found. Demographically, five cases were in males (45–70 years, four smokers, one non-smoker) and one in a never-smoker female (72 years). They presented in T3/T4 stage with lymph node involvement. Biopsy revealed adenocarcinoma (n = 2), squamous cell carcinoma (n = 2), and adenosquamous carcinoma (n = 2). Four cases had chemotherapy and were advised tepotinib for further treatment, while two received palliative care due to comorbidities.

Conclusion: METex14 skipping mutation is found in ~4% of NSCLC cases, often without other driver mutations[2,3] as seen in our study. Its incidence rivals or exceeds many actionable oncogenic drivers in NSCLC.[4] Notably, this mutation is also seen in squamous NSCLC, underscoring the importance of first-line testing in such cases.[5] NGS could therefore be the rational choice as a testing platform.

Keywords: METex14 skipping, Non-small cell lung cancer, Next-generation sequencing.

P-2

Spectrum of mutations by next-generation sequencing among non-consecutive metastatic liver lesions: An experience from a tertiary care center

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Aim & Objectives: (1) To study the spectrum and frequency of mutations among metastatic liver tumors using next-generation sequencing (NGS). (2) To correlate the mutation status with clinicopathological findings.

Methods: NGS was performed using DNA and RNA extracted from formalin-fixed paraffin-embedded tissues. Entire coding region of 52 genes actionable genes (including mutations, copy number variations, and fusions) was targeted. Th sample was sequenced using the NGS platform (Ion Gene Studio S5 plus, Thermo Fisher, USA), and the data were analyzed using the Ion Reporter™ software 5.20. 1000× depth and 5% variant allele frequency were minimum requirements to identify significant variants.

Results: Twenty-one cases of metastatic tumors in liver were included

in this study (August–December 2023) and 76% (n = 16) were mutated. The largest subset (n = 8;38.1%) included was metastasis from primary gallbladder malignancy, where 80% were found to harbor mutations or copy number variations in KRAS, ERBB2, EGFR, MET, and CCND1 genes. Other primary tumor sites included pancreas (n = 5; 23.8%), with KRAS gene mutation (100%), and lung (n = 4; 19%) which had EGFR mutation (1 case), ERBB2 mutation (1 case), and CD74-ROS1 fusion (1 case). Four cases were from the rectum (RAF1 gene mutation), urinary bladder (ERBB2 mutation), retroperitoneal leiomyosarcoma (no variants), and osteosarcoma (no variants). KRAS gene (n = 8; 38.1%) was the predominant mutation in this study, in which 6 cases (75%) had G12D, one case (12.5%) with G12V, and one had copy number variation (12.5%). Some KRAS mutant samples also had concomitant molecular alterations including MYC amplification (n = 2), CTNNB1 exon 3 mutation (n = 1), and JAK2 mutation (n = 1). Other mutations were ERBB2 mutation (n = 3), EGFR mutation (n = 2), MET and CCND1 amplification (n = 1), CD74-ROS1 fusion (n = 1), and RAF1 gene mutation (n = 1). Lung cancer patients-one with EGFR mutation responded to osimertinib and other with ROS1 fusion was treated with Crizotinib. The rest received standard chemotherapy. KRAS G12C mutation lung cancer, Food and Drug Administration-approved treatments such as Sotorasib or Adagrasib are used post-chemotherapy. However, our patients had KRAS G12D and G12V mutations, lacking targeted treatment. fam-trastuzumab deruxtecan-nxki offers a promising therapy for ERBB2-mutated non-small cell lung cancer after chemotherapy, but is not yet available in India. MET amplification and RAF1 mutations can be targeted with therapies such as capmatinib or trametinib, respectively, but these are out of reach due to exorbitant cost.

Conclusion: (1) 80% of the metastatic lesions harbored mutations. (2) KRAS mutations (38.1%) were predominant with gallbladder as a primary tumor site (38.1%) being the largest subset. (3) ~62% of mutations detected appeared targetable. (4) Mutations in the metastatic tumor mimicked that expected from its primary tumor site.

Keywords: Liver metastasis, Molecular septum, Chemotherapy.

P-3

Paraoxonase 2 gene polymorphism in patients with coronary artery disease

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Aim: To study the association between paraoxonase 2 (PON2) gene polymorphism and risk of coronary artery disease (CAD).

Materials and Methods: It was a case–control study. Fifty diagnosed patients of CAD of either sex and any age were taken as cases. Venous blood sample (5 mL) was collected from patients and controls in ethylenediaminetetraacetic acid vial for DNA extraction using phenol-chloroform extraction method. The DNA was visualized by agarose gel electrophoresis after ethidium bromide staining and pictures were taken. The detection of PON2 gene polymorphism was done by polymerase chain reaction-restriction fragment length polymorphism.

Results: Three genotypes of PON2 gene were separated on gel electrophoresis CC, CS, and SS. The proportion of SS genotype was higher in CAD cases as compared to controls. Patients with SS genotype had 1.5-fold increased risk as compared to CC genotype and 2.6-fold increased risk as compared to CS genotype for developing CAD. The proportion of S allele was higher as compared to C allele in CAD patients. The level of cardiac biomarkers in CAD patients across the three genotypes was also compared. There was a significant association between the SS genotype and CPK-NAC levels in the CAD patients. No significant difference was observed in the age distribution, lipid profile, and clinical and biochemical parameters in the cases and the controls across the genotypes. However, the maximum number of subjects with deranged lipid profile had SS genotype. The present study indicated that PON2 gene polymorphism may not be associated with the development of CAD in the Indian population; however, the observations of this study need to be replicated on a larger population based on ethnicity and geographic distribution.

Conclusion: The present study found no significant association of PON2 gene polymorphism with CAD India.Distribution of the genotypes between CAD patients and healthy control group showed a predominance of SS genotype and healthy control group.Also,the SS genotype was significantly associated with the lipid profile and CPK-NAC levels in CAD patients.

P-4

Establishment of good-quality control practice for routine next-generation sequencing-based diagnostic solid tumor formalin-fixed paraffin-embedded samples (DNA and RNA)

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Aims and Objectives: Next-generation sequencing has helped advance the era of precision medicine through massively parallel high-throughput sequencing. Targeted sequencing has further reduced the cost of next-generation sequencing (NGS) to an affordable level. However, the challenges associated with formalin-fixed paraffin-embedded (FFPE) are intrinsic and depend on multiple factors such as age of the block, fixation, tissue morphology, and the type of biopsy. This study provides an elaborate quality control (QC) guideline that would help characterize optimum quality and quantity of FFPE nucleic acid from poor ones, so that poor specimens can be avoided for sequencing that do not provide adequate information.

Methods: A total of 394 FFPE specimens (March 2021 till November 2023) were processed with targeted NGS panel Oncomine Focus™. The DNA/RNA QC and library QC were performed using the Qubit 3.0 fluorometer™, Agilent 4200 Tape station™, and Ion Taqman Quantification kit™.

Results: Out of the 394 cases processed, a failure rate of 9.39% (n = 37) was observed for DNA targets. Of these 37 cases, 70.27% (n = 26) were outside referral blocks and 23.07% (n = 11) were in house prepared blocks. Outside blocks with a mean DNA integrity number (DIN) value <2.0 (76.93%) failed at the DNA QC checkpoint, whereas the mean DIN of 2.67 (23.07%) failed at the library QC checkpoint. In house FFPE blocks with the mean DIN 3.53 failed at the DNA QC (54.54%) and library failure was observed with FFPE samples having a mean DIN 3.78 (45.45%). 1.77% (n = 7) of the samples failed at the RNA target level. The analysis of the RNA samples indicated that the % Dv200 required for an efficient RNA target amplification was more than 65%. Thus, a benchmark of DIN >4 (DNA) and % Dv200 >65% (RNA) was set for samples to be included in the assay at our center.

Conclusion: Due to the intrinsic nature of fragmented genomic profile in the FFPE samples, it is important to have a routine regimen of including DIN >4, fragment size >1500bp for successful DNA libraries and >65% Dv 200 for successful RNA libraries respectively.

Keywords: Solid tumor, Next-generation sequencing, Targeted sequencing, Quality control.

P-5

Mutational landscape of core-binding factor–acute myeloid leukemia at a tertiary center in Kolkata

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Introduction: Acute myeloid leukemia (AML) associated with t(8;21) or inv(16)chromosomal abnormalities is termed as core-binding factor-AML (CBF-AML), characterized by a heterodimeric transcription factor complex. The cytogenetic identity of CBF-AML involves the balanced translocation t(8;21)(q22;q22) and the pericentric inversion (inv) (16)(p13q22)/t(16;16)(p13;q22). An array of coexisting mutations along with these translocations contributes to the disease spectrum.

Aims and Objectives: We investigated the mutation landscape of CBF-AML.

Methods: A retrospective study was done with 251 AML cases referred for next-generation sequencing (NGS) from March 2019 to March 2023. NGS was performed using Oncomine™ myeloid assay on ION GeneStudio S5.

Results: Out of 215 AML patients, only 33 harbored CBF-AML. The t(8;21) translocation represented 8.8% and inv(16) accounted for 6.5%; thus, CBF comprises 15% (33) in our cohort. Mutations activating tyrosine kinase signaling pathways, including C-KIT, NRAS, KRAS, and CSF3R, were evident in both CBF-AML subtypes. NRAS mutations were prevalent, encompassing about 21% in t(8;21) and 42% in inv(16) cohorts and primarily involving exon2 and exon 3, C-KIT mutations were observed in 15% in t(8;21) and 42% in inv(16) AML with the majority located in exon 17, followed by exon 8. KRAS (Exon 2) and CSF3R (Exon 14) mutations embrace 23% and 19.5%, respectively. Conversely, PTPN11 mutations regulating RAS/MAPK signaling were exclusive to inv(16) AML (14%). Moreover, mutations associated with chromatin conformation regulation (EZH2), calcium regulation (CALR), transcription factor activity (ETV6), and DNA methylation/hydroxymethylation (DNM3TA, IDH1) were detected exclusively in t(8;21) AML. Our study highlights the importance of recognizing the complex genetic landscape of CBF AML in both prognosis assessment and therapeutic planning.

Conclusion: Our study highlights the importance of recognizing the complex genetic landscape of CBF AML in both prognostic assessment and therapeutic planning.

P-6

HER2 gene amplification in a series of 115 biliary tract cancer cases: A Western Indian tertiary cancer center experience

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Aims and Objectives: To assess the frequency of human epidermal growth factor receptor 2 (HER2) amplification in biliary tract cancers (BTC) and correlate with demographic and pathological parameters.

Methods: We retrospectively evaluated consecutive cases of BTC that underwent HER2 testing by fluorescence *in situ* hybridization (FISH) at our institution between January 2020 and December 2023. All the cases were first subjected to HER2 immunohistochemistry (IHC) and the cases with equivocal HER2 underwent FISH testing.

Results: A total of 1,110 BTC were evaluated by IHC, out of which 127 (11.4%) were positive (score 3+) and 791 (71.3%) were negative (score 0, 1+). Out of the remaining 192, FISH was performed on 115 (10.4%). The cases predominantly comprised females (n = 72; 62.6%) with an age ranging from 15 to 75 years (mean 51.6 years, median 51 years). All the cases were metastatic at presentation, except 8 which were locally advanced gallbladder (GB) cancers. The samples comprised biopsies (n = 101; 87.8%) and resections (n = 14; 12.2%). The majority of cases were primary GB cancers (n = 102; 88.7%), followed by intrahepatic cholangiocarcinoma (IHCC) (n = 10; 8.7%) and extrahepatic biliary adenocarcinoma (n = 3; 2.6%). Histopathology showed adenocarcinoma (moderately differentiated [73.9%], poorly differentiated [23.5%], and adenosquamous carcinoma [2.6%]). HER2 FISH results were amplified (44; 38.3%) and non-amplified (71; 61.7%). Among GB, 38% were amplified; whereas, among IHCC, 50% were amplified. The degree of differentiation was not significantly correlated with HER2 amplification. Intermixed genomic heterogeneity (GM-I) was seen in 10 (8.7%) cases and cluster type (GH-C) in 4 (3.5%) cases. The results of HER2 FISH in GH-I (n = 10): 5 (50%) amplified and 5 (50%) non-amplified; all the GH-C were reported as amplified for HER2.

Conclusion: The prevalence of HER2 amplification is significant in BTC, and hence, routine assessment is essential in all the locally advanced and metastatic cases, in view of the proposed benefit of anti-HER2 therapy in these cases.

P-7

Flow cytometry and real-time polymerase chain reaction-based detection and comparison of HLA-B27 in suspected cases of ankylosing spondylitis

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Aims and Objectives: To identify sensitivity and specificity for borderline positive and negative human leukocyte antigen (HLA)-B27 by flow cytometry and compare with gold standard method real-time polymerase chain reaction (PCR) on suspected cases of ankylosing spondylitis.

Methods: This was a cross-sectional study carried out in our multidisciplinary research unit. Blood sample of 5 mL was collected in ethylenediaminetetraacetic acid vial from all the patients who gave consent. Within 24 h of collection, one part of every sample underwent an initial flow cytometry test using BD flow cytometry system. The whole blood samples were stained with anti-HLA-B27 antibody conjugated with fluorescein and with anti-CD3 antibody conjugated with phycoerythrin. Samples were incubated for 20 min in the dark, at room temperature, and then analyzed using FACSCanto II (BD BIOSCIENCE), and another part was kept for RT-PCR. The RT-PCR test kit described is a qualitative one that relies on amplifying the allelic gene region using primers and probes that are designed specifically for HLA-B27 in Quantstudio 5 RT-PCR machine.

Results: Out of 14 cases done in both flow cytometry and RT-PCR, all have similar results in both. We will further carry out the study and present in 2–3 months.

Conclusion: All the HLA-B27 positive cases in flow cytometric analysis were found to be positive for RT-PCR analysis resulting in 100% sensitivity and 100% specificity. The HLA-B27 analysis can be performed by both the techniques and depending on the availability of the respective technique in the institutions.

Keywords: HLA-B27, Flow cytometry, RT-PCR.

P-8

Investigation of the visceral leishmaniasis outbreak in the endemic region of India

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Background: Leishmaniasis, a neglected tropical disease transmitted by female sand flies, is caused by the intracellular protozoan parasite Leishmania. In India, only anthroponotic transmission is reported. The disease manifests as chronic cutaneous leishmaniasis, mucocutaneous leishmaniasis, and the lethal visceral leishmaniasis (VL), known as kala-azar, with a high fatality rate.

Objective: (1) To describe in detail the outbreak of VL in the selected villages. (2) To determine whether the VL outbreak in these villages can be attributed to local transmission. (3) To devise appropriate control measures in case local transmission is suspected.

Method:

Study Population: 2503 samples were acquired from seven villages, encompassing a total population of 3603 individuals aged 2 years and above.

Serology: 2503 blood samples were collected on filter papers, testing them for Rk-39 RDT and enzyme-linked immunosorbent assay positivity. For infection tracing, index cases from the seven villages underwent epidemiological investigations, including a 6-month travel history before VL diagnosis.

Quantification of parasite load: 1337 blood samples were collected in citrate tubes for quantitative polymerase chain reaction (qPCR) analysis, enabling this quantitative approach aids in gauging disease prevalence in endemic regions.

Result: Among 2503 samples, 2.7% (68) tested positive for rK-39 enzyme-linked immunosorbent assay (ELISA), with 39.7% linked to a history of VL and 60.3% asymptomatic. Only two of seven VL cases had recent travel. Of 1337 tested with qPCR, 55.55% had more than one parasite genome/mL, and 44.44% had five or more parasites.

Conclusion: Local transmission evidence is minimal, as only 1.6% tested positive for rK-39 ELISA, and merely two of seven patients had recent travel to endemic areas. Those in VL endemic areas with high antibody titers or positive qPCR are more prone to developing VL. Combining rk-39 and real-time PCR can unveil asymptomatic VL cases, indicating their significant role in the transmission cycle in resource-poor rural Bihar.

P-9

Molecular classification of endometrial carcinoma with an emphasize on polymerase epsilon gene status in an Indian cohort

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Introduction: The era of precision medicine highlights the importance of identifying polymerase epsilon (POLE) mutations to personalize treatment approaches, potentially improving outcomes for patients with endometrial cancers (EC).

Background: The POLE gene has gained attention in EC because of its better prognosis, favorable clinical outcome, despite being associated with high-grade EC.

Aim: Our study aims to explore POLE gene for a detailed understanding of the observed histological features, prevalence, and associations with other molecular markers such as mismatch repair gene (MMR) and p53 in an Indian cohort.

Methods: This was a retrospective and prospective study of 145 patients in a diagnosed primary EC carried out at Agilus Global Reference Lab. The molecular profile of POLE gene was done by PCR amplification and Sanger sequencing, and nine hotspot areas including P286R, V411L, S297F, L424V/L424I, P436H, A456P, S459F, D462Y, and A465V were studied. MMR and p53 were performed by immunohistochemistry method.

Results: We found 3-POLE gene mutation out of total 145 patients (2.06%), with the common mutation seen in P286R (exon 9), c.857 C>G. One patient showed P436S, C>T exon 13, c.1306, which showed no clinical significance on Clinvar and was reported as negative. Out of three, two patients showed similar histology findings of high-grade endometrioid carcinoma, with no lymphovascular invasion and <50% myometrial invasion. Mixed histological feature of endometrioid (70%) and serous (30%) with >50 % myometrial invasion was seen in one patient who was given three cycles of radiation. None of the patients showed lymph nodal metastasis. All three cases showed MMR proficient and wild-type p53, and no coexistence was found in our study.

Conclusion: Our study is the first investigation on an Indian cohort, to the best of my knowledge, investigating the POLE gene for a better understanding of its implications, frequency, and potential correlations that would lead to better management of EC.

Keywords: Polymerase epsilon gene, Mismatch repair gene, p53, Endometrial carcinoma.

P-10

Exploring the modulation of inflammatory cytokines in osteoporosis: Unveiling the anti-inflammatory potential of bioactive compounds such as punicalagin and quercetin

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Aims and Objectives: To study and probe the control and mitigation of reactive oxygen species (ROS) and tumor necrosis factor-alpha (TNF-α) in osteoporosis patient monocytes grown in osteoclastogenic media using coculturing with naturally occurring antioxidants punicalagin, which is abundant in pomegranates, and quercetin, which is abundant in onion.

Methods: Effects of antioxidants were evaluated in the treatment of osteoporosis by employing cell culture study, enzyme-linked immunosorbent assay, glutathione peroxidase (GPx) activity determination, glutathione (GSH) assay, TNF-α, and interleukin-1 beta (IL-1β) levels in monocytes culture supernatants.

Results: The results have shown that there is a suppressed GPx activity in osteoporosis patients (n = 30; $P < 0.001$) indicating impairment of H_2O_2 neutralization mechanism. Intramonocyte GSH levels were also observed to be decreased, again an indication of a weak antioxidant system. Levels of by-product of lipid peroxidation, i.e., malondialdehyde (MDA), were high in monocyte culture of osteoporosis patients, pointing to the increased oxidative stress. Punicalagin and quercetin (0–20 μg/mL) increased GSH levels and GPx activity dose dependently to almost normal values. MDA levels were decreased dose dependently by these antioxidants (0–25 μg/mL). ROS-mediated activation of monocytes of osteoporosis patients resulted in the induction of enhanced/augmented basal levels of TNF-α and IL-1β. TNF-α and IL-1β levels in 24 h monocytes culture supernatants were downregulated dose dependently (0–100 μg/mL) in patients with the administration of the above natural antioxidants.

Conclusion: Punicalagin from pomegranates and quercetin from onions exhibit anti-inflammatory and antioxidant properties, reducing TNF-α and IL-1β levels, enhancing GPx activity, and decreasing oxidative stress markers. These natural compounds show promise in mitigating inflammation and oxidative damage associated with osteoporosis.

Keywords: Glutathione peroxidase activity, Glutathione, Tumor necrosis factor-alpha, Natural antioxidant.

P-11

Diatech tackles cobas EGFR testing challenges: Salvaging the uninterpretables and exon 20 insertion

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Aims and Objectives: Epidermal growth factor receptor (EGFR) mutation detection is one of the hallmark assays for clinical management of patients with lung adenocarcinomas. There are several assays, of which Cobas® EGFR mutation test v2 provides rapid qualitative results for non-small cell lung cancer (NSCLC) patients, although it has limitations such as false positive calls for Exon20 Insertion and invalid calls for samples with suboptimal DNA quality. To preserve precious patient samples, especially from small lung biopsies, an alternative diagnostic approach is imperative for offering the right diagnosis. This study aims to salvage uninterpretable/invalid results and address false-positive interpretations by employing an alternative robust method on a different platform. It also assesses the utility of this approach in clinical management.

Material and Methods: Present study included 217 NSCLC patient samples. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues and liquid biopsies using COBAS Sample Preparation Kit and were tested for EGFR mutations during (2021-2023). The study consisted of two parts: 1. Invalid cases: Reassessment of these cases reported (n=146); 2. False Positive cases. Exon 20 Insertion false positive (n=71) on the COBAS Z480 were reassessed. All samples were analyzed using Easy EGFR Diatech Pharmacogenetic kit on Quant Studio 12k Flex system

Results: The 146 cases which were rendered invalid by COBAS system could be successfully interpreted in 79 cases (54.10%), of these 30 (20.5%) were reported as *EGFR* mutation positive and 49 (33.56%) were wild type by Diatech kit. The remaining 45.8% of samples (n = 67) were uninterpretable by Diatech kit as well. Out of 71 samples considered as false positive for Exon 20 Insertion, 67.6% of samples (n = 48) were found to be *EGFR* wild type, while the remaining 32.3% of samples (n = 23) were true positive.

Conclusion: This study highlights the importance of using an orthogonal method for assessing EGFR mutations by addressing false positive and inconclusive outcomes to ensure appropriate therapeutic management of patients.

P-12

Choosing between Sanger sequencing and droplet digital polymerase chain reaction for the detection of polymerase epsilon mutations in endometrial carcinomas: A tertiary cancer center perspective

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Aims and Objectives: DNA polymerase epsilon (POLE) gene in endometrial carcinomas has gained attention in the past few years implicating a better prognosis for the disease. Mutations in 9, 13, and 14 exons of POLE have been considered as primary target regions for testing. Sanger Sequencing is used extensively for detection, although next-generation sequencing and Droplet digital polymerase chain reaction (ddPCR) methods are also explored. The current study is a comparative analysis of Sanger sequencing and ddPCR assay to detect efficacy, assay concordance, and applicability for routine clinical use.

Material and Methods: From 100 routine diagnostic cases of POLE mutations that were reported by Sanger sequencing, 25 cases were selected and ddPCR was performed. Sequencing was performed on hotspot spanning regions of exons 9, 13, and 14 using Big Dye v3.1 terminator cycle sequencing kit (Thermo Fisher). DroplexPOLE mutation ddPCR kit (covering only 5 hotspot regions) was setup on Qx200 Droplet Digital PCR System (Bio-Rad). Data were analyzed using ChromasLite (2.6.1) and Quantasoft (1.7) software, respectively.

Results: Among the 25 cases, 3 samples (12%) consisting of Ex.14 (D462Y) and two Ex.9 (P286R, P286L) mutations were detected and 7 cases (28%) were wild type by Sanger sequencing. Suboptimal DNA quality resulted uninterpretable data for all exons in 3 cases (12%) and 7 cases (28%) were noisy for exon 14 and 5 cases (20%) for exon 13. ddPCR results were interpretable in all 25 cases; 1 case was exon 9 (P286R) mutant, while the remaining 24 (96%) cases were identified as wild type.

Conclusion: ddPCR is more effective in identifying only hotspot mutations for samples with poor quality DNA and low tumor allele fractions. Factors like total turnaround time, cost, trained expertise, interpretation of data

and its use needs to be taken into consideration before implementing any method in diagnostic use.

P-13

Gray platelet syndrome with severe thrombocytopenia: A novel NBEAL2 gene variant from India

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Introduction: Autosomal mutations in the neurobeachin-like receptor 2 (NBEAL2; MIM 614,169) are identified to be causative for gray platelet syndrome characterized by platelet α-granule and cargo deficiency. Data on NBEAL2 gene variants and their phenotypic manifestations are manifold and evolving.

Case Report: A 4-year-old female born out of non-consanguineous marriage presented with petechiae, ecchymosis over the extremities of 2-month duration. On laboratory workup, the child was severely thrombocytopenic with an unremarkable coagulation profile. Peripheral smear revealed scant scattered pale gray-pink staining macroplatelets with neutrophils lacking inclusion bodies. Bone marrow aspiration and biopsy and autoimmune serology are unremarkable. The child did not respond to steroid and immunomodulatory drugs. Clinical exome sequencing on peripheral blood which revealed novel variants in the NBEAL2 gene at exon 8. This was confirmed on parental Sanger sequencing.

Conclusion: NBEAL2 variants and their phenotypic manifestations are manifold and evolving, and identifying other novel variants expands the known repertoire of published pathogenic variants, thus further helping in gene curation to future research.

Keywords: Thermbocytopenia, Gray Platelet Syndrome, NBEAL2 gene.

P-14

Significance of HLA-DR and HLA-B27 typing in juvenile idiopathic arthritis

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Introduction: Many studies of human leukocyte antigen (HLA) association with juvenile idiopathic arthritis (JIA) have reported conflicting results, which were probably related to ethnic differences.

Objective: The aim of our study was to reveal the frequency of HLA-B27 and HLA DR types in a cohort of polyarticular JIA in northern India.

Methods: Forty-one polyarticular JIA patients were included as per the recent International League of Associations for Rheumatology classification. HLA-DR typing was performed in 21 patients (18 rheumatoid factor [RF]+ and three RF) by a DNA-based polymerase chain reaction method for the determination of HLA alleles using sequence-specific primers. The results were compared with that of 23 healthy controls of the same age and sex.

Results: HLA-B 27 was present in 20 cases. HLA-DR4 was present in five cases (23%) in the diseased group, while only in one case (4.3%) in the control group with a relative risk of 5.47, but when compared with only RF+ polyarticular JIA, HLA-DR4 was found to be significantly high (27.7% vs. 4.43%; $P < 0.05$) with a relative risk of 6.3. Further, DR4, DR1, DR2, DR9, and DR10 were also non-significantly high in these patients with a relative risk of 3.2 for DR9 and 1.8 for DR10. In contrast, HLA-DR6 was seen only in 5.5% of polyarticular JIA cases, whereas it was present in 39% of controls ($P < 0.05$), showing a negative association.

Conclusion: HLA-DR4 codes for susceptibility to RF+ polyarticular JIA with a six-fold risk, whereas HLA-DR6 offers protection.

Keywords: HLA-DR, HLA-B27, Juvenile idiopathic arthritis, Polyarticular juvenile idiopathic arthritis.

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Detection of types of BRAF and NRAS gene mutations in papillary carcinoma thyroid by real-time polymerase chain reaction

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Introduction: Thyroid carcinoma is an endocrine malignancy that affects the thyroid gland. It is the most common endocrine cancer, accounting for only 1–2% of all cancers. The most common type of thyroid cancer is papillary thyroid cancer (PTC), which makes up 80–90% of all thyroid malignancies.

Aims and Objectives: To study the types of BRAF and NRAS gene mutations in various histological variants of papillary carcinoma thyroid by real-time polymerase chain reaction (RT-PCR).

Material and Methods: We took 40 patients from the endocrinology department. Histopathological confirmed cases of PTC were screened for at least 15–20% tumor cells in H&E-stained tissue sections. After fulfilling the adequacy criteria, tissue sections were cut into 6–10 µm thickness from the formalin-fixed paraffin-embedded tissue blocks by microtome. DNA was extracted from freshly cut FFPE tissue using TRUPCR® FFPE Tissue DNA Extraction Kit. The extracted DNA was quantified via a spectrophotometer for a satisfactory DNA quality. DNA samples were run by RT-PCR using TRUPCR® BRAF and TRUPCR® NRAS kits, as per the manufacturer's protocol: BRAF mutation in codon 600 and NRAS mutation in codon 12, 13, 59, 61, 117, and 146 using RT-PCR assay. Mutation analysis was performed from multicomponent plots by calculating cycle threshold values and comparing them with cutoff values.

Results: *BRAF* and *NRAS* mutations are the most common in PTC. In this series, we found that *BRAF* mutation incidence was 42.50% and V600E was the most common mutation. Notably, the classic variant showed a BRAF mutation rate of 48.48%, while the columnar variant exhibited 100% mutation. While *NRAS* was approximately 15%, Q61R was the most common mutation. Comparably, we found that BRAF mutations were more prevalent than *NRAS* mutations in cPTC (48.48% vs. 50%). Whereas, the analysis demonstrates that NRAS mutation is significantly higher in the follicular variant of PTC, with a mutation rate of 75%, compared to other variants.

Conclusion: While RAS mutations are less common, BRAF V600E is the most common mutation and is highly prevalent in Indian patients with PTC.

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Detection of types of epidermal growth factor receptor gene mutation in non-small cell lung carcinoma by real-time polymerase chain reaction

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Introduction: Lung carcinoma is the leading cause of cancer death in 2020 and represents one in five deaths due to cancer. Recently, epidermal growth factor receptor (EGFR) mutations have gained a lot of attention due to promising clinical trials of tyrosine kinase inhibitors in patients of non-small cell lung carcinoma (NSCLC) harboring these mutations.

Aims and Objectives: This cross-sectional observational study aimed at establishing the prevalence of EGFR mutation in different histological subtypes of NSCLC and its association with smoking.

Material and Methods: Our study involved 40 treatment-naïve primary NSCLC patients. After histopathological diagnosis, tissue sections were cut into 6–10 μm thickness from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, and DNA extraction was done using FFPE Tissue DNA Extraction Kit and was quantified by a spectrophotometer. DNA samples were run by RT-PCR using a TRUPCR EGFR kit, which could qualitatively detect 32 somatic mutations in exon 18–21 of EGFR gene from tumor tissue DNA. Mutation analysis was performed from multicomponent plots by calculating cycle threshold values.

Results: We detected a 15% mutation rate in this study cohort involving 32 males and 08 females with a median age of 59 years. EGFR mutation was present in 10 and 22% of cases with a diagnosis of adenocarcinoma and squamous cell carcinoma, respectively. Patients of squamous cell carcinoma showed only EXON20 (T790M, exon20 insertion) mutation, whereas cases of adenocarcinoma showed both EXON20 (T790M) and EXON21 (L858R) mutation. 31 patients who had smoking history showed mutation in 16% of them, whereas non-smokers showed mutation only in 11%.

Conclusion: When compared to limited Indian studies on EGFR mutation, ours showed a lower mutation rate but more frequent EGFR mutations in squamous cell carcinoma than adenocarcinoma. A comparable prognosis and higher frequency of EGFR mutations in squamous cell carcinoma lung with that of adenocarcinoma warrants a definite screening of these mutations in them.

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Association of human papillomavirus and Epstein–Barr virus infection with epithelial-mesenchymal transition markers in oral cancer

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Aims and Objectives: Our study aimed to assess the prevalence of Epstein–Barr virus (EBV) and human papillomavirus (HPV) in oral cancer cases using real-time polymerase chain reaction (RT-PCR) in tissue sections and to correlate the results with the expression of epithelial-mesenchymal transition (EMT) markers, including E-cadherin, N-cadherin, vimentin, and β-catenin.

Methods: This was a prospective study conducted over a period of 2 years, with a sample size of 137 cases. RT-PCR was used to detect viral DNA. Primers for the EBNA-1 (213 bp) region and BHRF-1 (208 bp) region were used for EBV detection, while E6/7 was used for HPV 16 (96 bp) and 18 (115 bp) detection. Beta globin gene (286 bp) was used as a housekeeping gene. The expression of four EMT markers, vimentin, E-cadherin, N-cadherin, and β-catenin, was studied using immunohistochemistry on formalin-fixed paraffin-embedded tissue samples. E-cadherin and β-catenin were assessed using an immunoreactivity score based on the proportion of positive cells and staining intensity, while vimentin was assessed using a label index score. N-Cadherin was considered positive when at least 5% of the cells were positive. The correlation between EBV and HPV expression and the expression of the above-mentioned EMT markers was analyzed using SPSS software. $P < 0.05$ was considered statistically significant.

Results: Based on 137 cases, 16 (1.17%) had HPV 16, while 36 (26%) were positive for EBV. Coexpression was found in 7 (5.1%) cases. No significant relationship was observed between HPV and EBV positivity and factors such as age, gender, tumor grade, depth of invasion, tumor infiltrating lymphocytes, tumor size, lymph node status, and immunohistochemistry markers. Out of 36 EBV-positive cases, only 10 (27.7%) expressed LMP1 positively, while the remaining 26 (72.3%) did not. These results suggest that EBV is relatively common but not associated with EMT marker expression. LMP1 might not be an effective EBV detection marker.

Conclusion: EBV positivity by PCR is higher than HPV in OSCC but as there is no significant correlation with any of the clinico-pathological parameters and expression of EMT markers, EBV may be a by-stander in OSCCC.

Keywords: Oral cancer, Human papillomavirus, Epstein–Barr virus, Polymerase chain reaction, Epithelial-mesenchymal transition markers.

P-18

Soft tissue tumor caused by capillary morphogenesis gene 2 mutations: A case report of Juvenile Hyaline fibromatosis

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Aims and Objectives: To report a rare case of Juvenile Hyaline fibromatosis (JHF) and summarize the findings of JHF cases reported in Indians to date.

Case Report: A 19-year-old male presented to the department of general surgery with complaints of multiple swellings over the back and gluteal region, present since 2 years of age, gradually increasing in size and associated with mild pain. There were no significant symptoms pertaining to internal organs. On examination, the swellings ranged from 3 cm to 11 cm in size, were nodular, well-circumscribed, firm, and projecting over the skin with foci of ulceration. There was no apparent gingival involvement. Regional lymph nodes were not enlarged. With a clinical differential diagnosis of neurofibromatosis, two of the lumps were excised for histopathological examination. Formalin-fixed paraffin-embedded sections examined microscopically showed large nodular subcutaneous masses composed predominantly of PAS-positive diastase-resistant eosinophilic matrix. Cords and nests of oval to spindle fibroblastic cells, many within a halo formed by retraction, were seen embedded within the matrix, simulating chondroid tissue on histomorphology. Considering the clinical presentation and histomorphology, a diagnosis of JHF was rendered and Sanger sequencing of Exon 3, 13, and 14 of CMG2 gene was recommended.

Conclusion: JHF is a rare soft tissue tumor and forms part of the spectrum of Hyaline fibromatosis syndrome, caused by mutations in the CMG2 gene. Of the 84 published cases of this syndrome with genetic data in 2018,[1] 10 patients were of Indian ethnicity. Symptomatic treatment and supportive care is the mainstay of therapy.

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B/Monocytoid mixed phenotype acute leukemia with BCR: ABL P190 minor fusion transcript: A case report

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Introduction: Mixed phenotype acute leukemias (MPAL) are acute leukemias of ambiguous lineage composed of more than or equal to 20% blasts or abnormal progenitors showing differentiation along more than one lineage, detected by morphology and reliably by flow cytometric immunophenotyping, respectively. They account for <4% of all leukemias and can occur in both children and adults. MPAL with BCR: ABL1 fusion in a *de novo* acute leukemia, which fulfills the criteria for MPAL and harbors BCR: ABL1 fusion at initial diagnosis, without evidence of chronic myeloid leukemia. It accounts for <0.5% of acute leukemias and 20–25% of MPAL. Here, we report a rare case of a 74-year-old female diagnosed with B and monocytoid lineage MPAL with BCR: ABL1 p190 minor fusion transcript who on follow-up relapsed.

Case Details: A 74-year-old female, a known diabetic, hypertensive, and dyslipidemia on treatment, presented with complaints of high-grade fever, dry cough, generalized weakness, and fatigue. On examination, she was febrile. Her general and systemic examination was normal. Laboratory investigations revealed hyperleukocytosis (white blood cells count - 80,000 cells/µL), anemia (hemoglobin - 9 g%), and a normal platelet count. Peripheral smear had 63% of blasts with scanty to moderate cytoplasm, with occasional granules and cytoplasmic vacuolations, convoluted nuclei with open chromatin and two to three nucleoli. Myeloperoxidase cytochemistry was negative. Flow cytometry confirmed presence of two populations of CD45 dim positive cells. It showed positivity for B-lymphoblastic lineage (20.13%)- CD34, Tdt, CD19, CD22, CD79a and the other monocyte lineage (48.62%) - MPO, CD14, CD64, CD11c, CD13, CD33, HLA-DR, and CD38. FISH and RT-PCR confirmed the presence of BCR: ABL1 fusion with p190 minor transcript. Cytogenetics - 46,XX,del(7)(q22) and t(9;22)(q34;11.2)[5]. Next-generation sequencing showed BCR: ABL fusion [total read depth 1855×] with no other significant Single nucleotide variants (SNVs), small (INDELs) small insertions and deletions, and Copy number variations (CNVs). The patient received vincristine, steroid, and dasatinib induction and follow-up remission assessment showed morphological complete response with BCR: ABL RQ-PCR of 1.31%. Subsequently, she was put on palliative azacytidine, dasatinib, 6MP, and methotrexate. However, 8 months later, she relapsed with flow cytometry indicating 55.38% blasts with B cell lineage markers, HLA-DR, and CD 13 positivity and a reduction in the monocyte lineage. BCR: ABL1 minor transcripts were raised to 22.8%. Imatinib resistance mutation analysis was negative and next-generation sequencing revealed no other mutations. The patient discontinued treatment.

Discussion: B/myeloid leukemia is considered a high-risk leukemia. The BCR: ABL1 fusion transcript is the most common cytogenetic abnormality in MPAL. This chimeric fusion protein is a constitutively active tyrosine kinase. The frequency of p210 and p190 BCR: ABL1 transcripts varies, with one study reporting p190 as more common, while another study showing p210 predominance. Although B/myeloid is the subtype of MPAL that is seen more commonly, this case highlights B/Monocytic MPAL, which is extremely rare and the presence of BCR: ABL p190 minor transcript which is even rarer. Detecting this transcript helps supplement the treatment with a tyrosine kinase inhibitor, which can help in improving prognosis and overall survival.

Conclusion: B/myeloid(monocytic lineage) leukemia with p190 BCR: ABL1 fusion transcript is extremely rare. The prognosis may be improved by adding a tyrosine kinase inhibitor to the treatment in addition to what is provided by detection of this transcript.

Keywords: Mixed phenotype acute leukemia, B/Monocytic, BCR: ABL fusion, p190 minor transcript.

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A rapid and accurate SNP detection assay for the detection of unexplained recurrent pregnancy loss: Methylenetetrahydrofolate reductase polymorphism (A1298C and C677T)

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Objectives: Women with a methylenetetrahydrofolate reductase (MTHFR) gene mutation may be at a higher risk for miscarriage, preeclampsia, and having a baby with birth defects. Risk factors for MTHFR gene mutations include recurrent pregnancy losses, giving birth to babies with certain birth defects, and a history of preeclampsia. Numerous studies have investigated the associations between MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss (RPL); however, the results remain controversial. The aim of this study is to drive a more precise estimation of the association between MTHFR gene polymorphisms and risk of RPL.

Material and Methods: In our laboratory, we did a thrombosis genetic study to detect SNPS by real-time polymerase chain reaction method by the kit Anyplex™ II thrombosis SNP panel assay by see gene. The kit covers Factor V Leiden mutation (R506Q, H1299R, Y1702C), Factor II G20210A, and MTHFR (A1298C, C677T). Qia amp DNA extraction kit was used for the extraction procedure. Allelic frequencies for the cases and controls were calculated from corresponding genotypes from the sample which we tested in our laboratory for the last 1 year. To date, many studies have investigated the association between MTHFR A1298C polymorphism and RPL risk; however, the result is still controversial and inconclusive.

Conclusions: The results of our test results indicate that maternal and paternal MTHFR gene C677Tand A1298C polymorphisms are associated with RPL. We also observed a significant association between MTHFR A1298C polymorphism and RPL.

Keywords: Methylenetetrahydrofolate reductase, A1298C and C677T Mutation, Pregnancy loss, Polymorphism.

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Unraveling the immune checkpoint regulators and immune microenvironment in ocular lymphoma: Prognostic and therapeutic implications

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Aims and Objectives: To explore the expression of Immune checkpoint regulators (PD-1, PDL1, and CTLA-4) and components of the immunosuppressive microenvironment (tumor-associated macrophages and T regulatory cells) in ocular lymphoma patients and correlate with therapeutic outcome.

Methods: Seventeen histopathologically confirmed ocular lymphoma cases and 15 controls were included in this study. Clinical features were noted, histopathological classification was done (World Health Organization classification 2011 and comprehensive quantitative polymerase chain reaction gene expression profiles of PD-1, PD-L1, and CTLA4 were generated in all the cases and controls. Expression of tumor-associated macrophages (CD68, CD163, and Inducible NO synthase [iNOS]) and T regulatory cells (CD4 and FOXP3) was done by immunohistochemistry. Patients were followed up for 40.4 ± 23.58 years. Kaplan–Meier survival analysis was applied to identify the clinical significance of the immune checkpoint regulator in ocular lymphoma patients.

Results: The mean age of the 17 ocular lymphoma patients was 51.35 ± 13.56 years (range 21–82 years); marked male preponderance (76%) was observed in this study. Eleven (65%) lesions were located in the orbit, 4 (23%) in the eyelid, and 2 (12%) in the conjunctiva. Follow-up data were available in 14/17 (82%) patients. Recurrence developed in 2 and two patients died of the disease. On histopathological analysis, 14 cases were classified as small cell type (low-grade, extranodal marginal zone B-cell lymphomas), 3 were high-grade diffuse large B-cell lymphomas, and 1 follicular lymphoma and NK/T-cell lymphoma each. Messenger RNA overexpression of PD1 was seen in 76%, PDL1 in 64%, and CTLA4 in 59% of cases. Of the 3 immune checkpoint regulators analyzed, overexpression of CTLA4 gene was significantly associated with the worst disease-free survival ($P = 0.008$) in ocular lymphoma patients. All the cases showed positivity for CD68 immunoexpression and 53% (9/17) cases showed immunoexpression of iNOS. However, all the cases were negative for CD163 expression. CD4 immunoexpression was seen in all the cases and FOXP3 positivity was seen in 12% (2/17) cases. Of all the TAMs and Tregs markers analyzed, we observed that only iNOS expression was significantly associated with worst disease-free survival ($P = 0.03$) of ocular lymphoma patients.

Conclusion: CTLA4 gene overexpression and iNOS immunoexpression could prove to be useful biomarkers for identifying high-risk ocular lymphoma patients. Overexpression of CTLA4 and iNOS in patients with poor prognosis suggests the need for further investigation of anti-CTLA-4 immunotherapy that includes iNOS as a combination as potential partners in the treatment of refractory/relapsed ocular lymphoma.

Keywords: Ocular lymphoma, Immune checkpoint regulators, Immune suppressive tumor microenvironment, Prognostic and therapeutic markers.

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The potential therapeutic and prognostic impacts of the MET signaling pathway in orbital rhabdomyosarcoma

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Aims and Objectives: To evaluate the expression pattern of receptor tyrosine kinases (RTKs) and mutation status of its downstream pathways in orbital rhabdomyosarcoma patients. Their association with clinicopathological features and survival will also be done.

Methods: Eighteen patients with histopathologically confirmed orbital rhabdomyosarcoma and 5 skeletal muscles as normal controls formed part of this study. The mean age of patients was 7.7 ± 6.9 years, and 50% of the patients were male. Comprehensive quantitative polymerase chain reaction gene expression profiles of 19 RTKs were generated in all the 18 cases and 5 controls. Mutational status of 11 genes of RAS-MAPK and PI3K pathway was analyzed using amplification refractory mutation system (ARMS) technology. Protein expression of the most significant RTKs was confirmed by immunohistochemistry. Follow-up data were available in 72% of patients, over a period of 28–80 months (mean: 59.53±20.93 months). Four patients died of the disease. Kaplan–Meier analysis was done to identify the clinical significance of RTKs in orbital rhabdomyosarcoma patients.

Results: Overexpression of RTKs including MET, AXL, and EGFR was seen in 60–80% of cases. MET gene expression was associated with the worst overall survival ($P = 0.03$) in orbital RMS patients. A significant association ($P = 0.0001$) was also observed between MET messenger RNA expression and its immunoexpression and all the cases had mutated MET gene (c.3029C>T, c.2942_3082del141, c.3757T>G, and c.3803T>C). Expression of other RTKs including EGFR3, IGFR2, FGFR1, RET, PDGFR1, VEGFR2, PDGFR2 EGFR4, FGFR3, VEGFR3, ROS, IGFR1, EGFR1, FGFR2, and VEGFR1 was observed in <60% of cases. On ARMS analysis, PTPN11 (c.1504T>C,c.1508G>C,c.1508G>T,c.179G>T, c.181G>T,c.182A>T,c.215C>A,c.218C>T,c.226G>A,c.227A>T,c.227A>G) and KRAS gene mutation (c.182A>T,c.183A>T,c.34G>A, c.34G>C,c.35G>A,c.35G>C,c.35G>T, c.37G>A,c.37G>C,c.37G>T,c.38G>A) were detected in all the cases. Mutation of BRAF (c.1406G>C,c.1798G>A,c.1799T>A,c.1799T>C,c.1799T>G) and PIK3CA (c.1624G>A,c.1633G>A,c.1634A>G,c.1635G>T,c.3140A>G,c.3140A>T) in 70%, HRAS (c.181C>A, c.182A>G, c.182A>T, c.34G>A,c.34G>T,c.35G>A, c.35G>T) and PTEN (c.389G>A,c.388C>G,c.388C>T,c.517C>T,c.697C>T) in 60%, NRAS (c.182A>G, c.34G>A,c.35G>A, c.35G>C,c.37G>C, c.38G>A) in 50%, AKT (c.49G>A) in 40%, VHL (c.254T>C, c.266T>A, c.388G>C), and MEK1 (371C>T,199G>A, 171G>T,167A>C) in 30% cases. Mutations in RAS-MAPK pathway genes (BRAF, PTPN11, KRAS, and NRAS) were significantly associated with poor prognosis in ocular RMS patients. However, no mutation was seen in the P13-AKT pathway genes (PTEN, AKT1, and VHL) in patients with poor prognosis.

Conclusions: Overexpression of MET could be a useful biomarker for identifying patients of orbital rhabdomyosarcoma at high risk. Mutationally activated MET plays an important role in the pathogenesis of rhabdomyosarcoma. Effect of inhibitors targeting MET and mutations in RAS-MAPK pathway genes (BRAF, PTPN11, KRAS, and NRAS) should be evaluated for the better management of high-risk rhabdomyosarcoma patients.

Keywords: Orbital/ocular rhabdomyosarcoma, Receptor tyrosine kinases (RTKs) Amplification Refractory mutation system technology.

P-23

BRAFV600 E mutation in thyroid lesions: A study on Giemsa-stained fine-needle aspiration archival smears

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Introduction: Thyroid fine-needle aspiration (FNA) is an important diagnostic modality and is an integral part of thyroid nodule evaluation.

BRAF gene is a proto-oncogene and is located on chromosome 7. BRAF V600E mutations in the thyroid have been shown to be almost exclusive to thyroid cancer; therefore, it is a highly specific marker of thyroid carcinoma with the prevalence in conventional PTC ranging between 29% and 83%.[1]

Aim and Objectives: To evaluate the BRAFV600E mutation in thyroid lesions performed on giemsa stain FNA cytology (FNAC) smears.

Methods: A retrospective study was conducted from January 2019 to July 2023. A total of 1778 thyroid FNAC were performed during this period. Out of which 90 cases diagnosed as Bethesda, category III–VI were included. Slides were screened for cellularity and extra Giemsa-stained smears were used for DNA extraction by standard protocol. After DNA isolation, concentration was assessed using the Eppendorf BioPhotometer-plus nano spectrophotometer. BRAFV600E mutation was amplified by Droplet digital polymerase chain reaction (ddPCR) for absolute quantification of mutation observed by the software.

Results: Out of 90 cases, genomic DNA analysis was done in 55 cases, out of which according to the Bethesda system, 23 cases (41.8%) were Category VI, 05 cases (09%) were category V, 06 cases (20%) were category IV, and 21 cases (38.2%) were category III. The age ranged from 30 to 55 years and male-to-female ratio was 1:28. The size of the nodule was 2–8 cm and duration ranged between 02 and 05 years. Genomic DNA extracted for BRAF V600F was found in 13 cases (23.6%); out of which, 01 case of category III was benign, while 11 cases were malignant confirmed on histopathology.

Conclusion: MGG/Giemsa-stained smears can be utilized for DNA isolation. BRAF-positive cases must be closely followed up and subsequently managed.

Keywords: Thyroidlesion, BRAFmutation, Fine-needle aspiration smears.

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Association of human papillomavirus and Epstein–Barr virus expression with epithelial-mesenchymal transition markers in breast cancer

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Breast cancer is the most frequent type of cancer in women. 20% of malignancies are caused by oncoviruses, human papillomavirus (HPV), and Epstein–Barr virus (EBV) being most frequently encountered. These oncoviruses may coexist and contribute to epithelial-mesenchymal transition (EMT) via signaling pathways involved in cancer progression. Targeting these malignancies with medications that inhibit EMT may be used to slow tumor development. The aim of this study was to look for coexpression of EBV and HPV in breast cancer and their association with EMT markers. Objectives were (1) to screen for EBV and HPV by real-time polymerase chain reaction in tissue sections of histologically proven breast cancer and (2) to see an association of EBV and HPV with markers of EMT (E-cadherin, N-cadherin, vimentin, and β-catenin) by immunohistochemistry (IHC). In this prospective observational study carried out in King George's Medical University, Lucknow, 130 patients with histologically proven breast carcinoma were included during 2018–2020. After detailed histology, paraffin block with infiltrative tumor was selected for further analysis. Molecular testing included detection of beta-globin as housekeeping gene and HPV 16, 18, and EBV were detected by specific primer sequences. Evaluation of immunoreactivity of E-cadherin and beta-catenin was done as per immunoreactivity score method, for Vimentin, Label Index scoring was used and for N-cadherin staining, 5% membranous/cytoplasmic immunoreactivity rule was followed. Statistical analysis was done using SPSS Version 21.0 Statistical Analysis Software. Most of the patients studied were diagnosed with Infiltrating Ductal Carcinoma. A total of 25/130 cases of breast carcinoma had positive EBV polymerase chain reaction results (19.2%). HPV 16 and 18 were not detected in any of the 130 cases. The immunoreactivity of E-cadherin and beta-catenin were low and N-cadherin was negative in most of the cases, but a significant correlation between molecular and EMT IHC results was shown in immunoexpression for vimentin with a maximum expression seen between 20%- and 9%.

Keywords: Human papillomavirus, Epstein–Barr virus, Breast cancer, Epithelial-mesenchymal transition, Viral oncogenesis.

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Variant philadelphia chromosome translocations in chronic myeloid leukemia

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Aims and Objectives: To study variant philadelphia chromosome translocations in a series of 5 cases of chronic myeloid leukemia (CML) by conventional cytogenetic analysis for understanding complicated molecular mechanisms associated with CML. CML is characterized by the presence of Philadelphia chromosome in >90% of patients. 5–10% of CML patients showed variant Ph translocation, involving one or more chromosomes in addition to chromosome 9 and 22.[1] Some studies have associated it with poorer prognosis, while others found no difference in the prognosis between standard and variant translocations.[2]

Methodology: Cytogenetic analysis was performed on 5 CML patients using the GTG-banding technique. Bone marrow/peripheral blood specimens were subjected to unstimulated overnight or 48 h cultures. The karyotypes were classified using ISCN 2020.

Results: Cytogenetic analysis of the five CML patients revealed the presence of complex three-way translocations involving chromosomes 6, 7, 17, and 18 along with chromosomes 9 and 22. to 46,XX,t(9;22;18)(q34;q11.2;p11.2) chromosomal complement was observed in two patients. The other three patients exhibited translocations involving chromosomes 7, 6, and 17.

Conclusion: Our study emphasizes the relevance of conventional cytogenetic techniques in identification of complex chromosomal abnormalities. More such studies are required to identify the additional oncogenes that might be located on the additional chromosomes involved in variant translocations and their association with the prognosis of the disease.

Keywords: Chronic myeloid leukemia, Philadelphia, Translocations.

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Dedifferentiated chordoma with hotspot KRAS p.Gly13Asp (Missense) mutation: A possible cause of resistance to the novel Anti-EGFR therapy

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Introduction: Dedifferentiated chordoma (DDC) is a rare aggressive subtype of chordoma, with a biphasic appearance, comprising a high-grade sarcoma, juxtaposed to a conventional chordoma. Although molecular characteristics of DDC are not well studied, aberrant epidermal growth factor receptor (EGFR) signaling is described in the pathogenesis of chordoma, justifying EGFR antagonists in therapy. It is known that metastatic colorectal carcinoma, with KRAS mutation in codon 12 or 13, is resistant to anti-EGFR therapy. The same may be implicated in DDC.

Case Report: A 63-year-old male from Iraq presented to the neurosurgery outpatient department with insidious onset, non-radiating lower back pain since 2017, along with lower back swelling and constipation for the last 6 months. On contrast-enhanced computed tomography, a tumor measuring 12.3 × 3.7 × 2.2 cm was found at the sacrum with the involvement of the rectum. Incisional biopsy confirmed the diagnosis of conventional chordoma. Sacrectomy with tumor excision and diversion sigmoid colostomy was performed. Final histopathology showed a biphasic tumor having a conventional chordoma, juxtaposed to a fibrosarcomatous component, confirming a DDC. A sarcoma panel covering a total of 109 key sarcoma-related genes was done by RNA extraction and complementary DNA sequencing, validated Illumina sequencing platform. KRAS missense mutation as well as RPSAP52:HMGA2(Fusion) were observed in our case, which have not been reported before in literature.

Conclusion: Novel genetic alterations found in this case may explain the aggressiveness and prognosis. Further studies are needed for realizations of diagnostic, prognostic, and therapeutic biomarkers.

P-27

Beyond Cytomegalovirus and Epstein–Barr virus: Detection of emerging viruses in transplant recipients using molecular methods – Experience from South India

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Introduction: Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) have been recognized as potential drivers of morbidity and mortality of patients undergoing solid organ and stem cell transplantation for years. Specific protocols for pretransplant workup, monitoring, prophylaxis, and treatment for these viruses are already established. However, owing to emerging techniques of molecular testing, other viruses like BK (polyoma virus), adenovirus, and human herpes virus-6 (HHV-6) have been recently detected with increased frequency in transplant recipients. Given the less frequent detection of these infections, treatment outcome of these patients remains unsatisfactory owing to viremia-induced complications such as secondary infections and graft failure and rejections.

Aims and Objectives: To estimate the incidence of emerging viral infections other than EBV and CMV in samples of post-transplant recipients.

Methodology: Retrospective data analysis of viral transplant panel samples processed in various centers of microbiological laboratory across the four South Indian states (Tamil Nadu, Kerala, Andhra Pradesh, and Karnataka) from January 2023 to December 2023 using real-time polymerase chain reaction kit (Artus Kit, Qiagen).

Results: Apart from CMV and EBV viruses, which are the already established transplant-associated viruses, new viruses such as BK virus, adenovirus, and HHV-6 have shown to have increased incidence in both renal and stem cell transplant recipients in all the centers.

Conclusion: Multiplex PCR assays for transplant infection monitoring should include viruses such as adeno, BKV, and HHV-6, which are being frequently detected from various transplant recipients. Vigilance in diagnosing emerging viral infections and continued study of potential antiviral therapies in the transplant population will likely improve patient survival. Inclusion of these in pretransplant workup might be very useful in improving the posttransplant morbidity and long-term survival rates.

Keywords: Post-transplant recipients, Emerging viruses, Adeno virus, BK virus, Human herpes virus-6.

P-28

One Stop Solution for HLA-B27 Testing: Extraction – PCR – Sequencing Grade Data

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Aims and Objectives: (1) To evaluate HiMedia's Magnetic Bead Based Extractor and HiMedia's Pre-filled Cartridges kit, for extraction of High Quality, Sequencing Grade Nucleic Acid from blood samples, for detection of human leukocyte antigen (HLA-B27) using quantitative Polymerase Chain Reaction (qPCR). (2) To evaluate HiMedia's highly specific and sensitive qPCR assay for the detection of HLA-B27 allele using sequence-specific primers (SSP).

Methods: 25 positive and 50 negative samples of HLA-B27 were used for this study. (The specimens used were leftover blood samples by health-care practitioner available at a testing center in Mumbai, India). For both the objectives, IVD-approved competitor kits were used for comparison.

For Objective 1: Extraction of the samples was performed using HiMedia'sHiPurA® Pre- filled Cartridges for Blood DNA Extraction (MB504PC16) and Thermo's MagMAX™ DNA Multi-Sample Ultra 2.0 Kit. PCR of the eluates, from both the extraction methods, was performed using TRUPCR® HLA-B27 real-time PCR Kit to compare the efficiency of two extraction methods used. Further, the eluates were subjected to whole-genome sequencing (WGS) to check sequencing efficiency from the extracted eluates.

For Objective 2: Extraction of the samples was performed using TRUPCR® Blood DNA Extraction Kit. The eluate obtained was further used for PCR using HiMedias's Hi-PCR® HLA-B27 Probe PCR Kit and TRUPCR® HLA-B27 real-time PCR Kit comparing the efficiency of the two PCR kits used. HiMedia's InstaQ96® (LA1074) Real-time PCR system was used for setting up the PCR assays.

Results: For Evaluation of Extraction Kit (Objective 1): PCR was performed using TRUPCR® HLA-B27 Real-Time PCR Kit. Negative samples extracted using HiMedia's extraction kit gave no amplification for target gene and proper amplification of internal control (IC) implying proper extraction compared to that of the competitor extraction kit. At par Ct. values for Target Gene was observed for DNA extracted using HiMedia's Extraction kit when compared against Thermo's Extraction Kit.

For Evaluation of PCR Kit (Objective 2): Negative samples extracted using competitor's extraction kit were subjected to PCR using HiMedia's Hi-PCR®

HLA-B27 Probe PCR Kit and TRUPCR® HLA-B27 Real-Time PCR Kit. No amplification of target and proper amplification of IC was observed, implying proper specificity of the kit. At par Ct. Values for Target Gene was observed for both the PCR kits used. Hence HiMedia's Hi-PCR® HLA- B27 Probe PCR Kit gave comparable result to the competitor PCR kit used.

Sequencing: DNA Extracted using HiMedia'sHiPurA® Pre-filled Cartridges for Blood DNA Extraction (MB504PC16) and competitor extraction kit were subjected to sequencing. The Q30 values for both the extraction kits were found to be comparable (above 30).

Conclusion: HiMedia's magnetic bead based extractor & HiMedia's pre-filled cartridges is a bonus for laboratories. Features include-Time saving, Offer results with higher accuracy, No pre-processing required. Upto 16 different blood samples can be processed in a time span of 48 minutes. Usage of cartridges for small number of sample prevents wastage of resources. Around 200-600µl of blood sample is enough to provide a good, PCR amplifiable, sequencing grade DNA. The nucleic acid can be further used for downstream applications like NGS. PCR Kit :BPCR202- Hi-PCR® HLA-B27 Probe PCR Kit give Ct values better than TRUPCR® HLA-B27 Real Time PCR Kit thereby defining its specificity and sensitivity for HLA- B27. HiMedia's qPCR kits are a green standard.

P-29

Mutation landscape of homologous recombination repair genes in ovarian cancer: A 50 case study

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Background: Homologous recombination repair (HRR) enables fault-free repair of double-stranded DNA breaks. HRR deficiency is predicted to occur in around half of high-grade serous ovarian carcinomas. Ovarian cancers harboring HRR deficiency typically exhibit sensitivity to poly-ADP ribose polymerase inhibitors (PARPi). Current guidelines recommend a range of approaches for genetic testing to identify predictors of sensitivity to PARPi in ovarian cancer and to identify genetic predisposition.

Aims and Objectives: The status of HRR gene mutations and their impact on the survival of patients with epithelial ovarian cancer (EOC) are still unclear. In this study, we retrospectively analyzed the mutations of HRR genes in tumor tissues and evaluated their values for predicting the survival of ovarian cancer patients.

Methods: A total of 50 primary EOC patients between 2022 and 2023 were screened. All patients received after staging surgeries combined with systemic platinum-based chemotherapy. DNA was extracted from formalin-fixed, paraffin-embedded sections and analyzed for mutations using a 15-gene panel by next-generation sequencing. We performed amplicon sequencing using designed gene panel. Amplicon-based barcoded library was prepared using the Oncomine HRR Pathway DNA Panel. Library preparation was performed using the Ion AmpliSeq™ Library Kit Plus and loaded onto Ion Torrent S5 NGS platform to perform massive parallel sequencing. The FASTQ reads were aligned against the hg19 in the Torrent suite software (v5.10). Variant calling was done using Ion Reporter (v5.18). This Panel analyzed nucleotide substitutions, small insertions, small deletions, indels, and duplications. Variants were called using Torrent Variant Caller plugin and were annotated using Thermo Fisher's Ion Reporter software. Classification of variants and their clinical significance were identified by searching the variants against ClinVar database. These variants were further classified as pathogenic or benign or variant of uncertain Significance on the basis of American College of Medical Genetics guidelines.

Results: A total of 34% of cases had deleterious mutations in 6 HRR genes, namely, BRCA1 (18%), BRCA2 (8%), ATM (2%), RAD51C (2%), CHEK2 (2%), and RAD54L (2%). There is a strong mutual exclusion between HRR genes. The mutation landscape revealed several unappreciated deleterious variants in BRCA1/2 and other HRR genes reported previously. Estimated according to the mutation allele frequency, about 4.8% of the patients had potential somatic HRR gene mutations, which might be underestimated.

Conclusion: This study revealed the distribution of HRR gene mutations in EOC tissues. BRCA1/2 accounts for the majority of HRR gene mutations and predicts long prognosis in HGSOC. Non-BRCA HRR mutations also account for a very important proportion and might be associated with poor prognosis in HGSOC. It is suggested that HRR gene mutations need to be detected in EOC tissues and germline status be further clarified in clinical algorithm for potential targeted therapy, genetic screening, and prognosis prediction.

P-30

KRAS mutation profiling in an institutional cohort of colorectal cancer from a tertiary care center in North India

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Aims and Objectives: Genetic alteration in the KRAS gene in colorectal cancers (CRCs) is one of the most common genetic alterations.[1] KRAS mutation status helps clinicians in deciding the targeted therapy in CRCs.[2] The objective of this study is to identify the prevalence of KRAS mutation in our CRC cohort.

Materials and Methods: In this retrospective study, we analyzed somatic mutations in the codons 12, 13, 59, 61, 117, and 146 of the KRAS gene from tissue samples collected from colectomy specimens that underwent upfront surgery without neoadjuvant therapy. Total DNA was extracted from formalin-fixed tissue, allele-specific primers were used, and real-time polymerase chain reaction was performed using TRUPCR® KRAS PCR Kit.

Results: In our study population (n = 99), 58 cases (58.59%) had KRAS mutations. KRAS mutation on codon 12 and/or codon 13 of exon 2 was present in 52.53% of cases out of all codons examined. In the study, while 34% of cases exhibited one or more of the recognized common mutations such as G12D (35G>A), G13D (38G>A), and G12V (35G>T) within codons 12 and 13 of exon 2, the prevailing mutation among the study participants was G12S (34G>A) at codon 12 of exon 2, accounting for a prevalence of 41%. In total, the KRAS mutant type displayed a significant correlation with a lesser number of lymph node metastasis (P = 0.03). The presence of KRAS mutation on codon 12 and/or codon 13 of exon 2 was seen in 76.47% of CRCs with <4 lymph node metastasis, while in cases with ≥4 metastatic nodes, 35.71% mutants were seen (P = 0.03). Furthermore, the presence of one or more of the known common KRAS mutations G12D(35G>A), G13D(38G>A), and G12V(35G>T) on codon 12 and 13 of exon 2 showed a significant correlation with the lesser number of lymph node metastasis (P = 0.029) and absence of LVI (P = 0.027) than in non-mutant cases.

Conclusions: In our institutional cohort, the prevalence of KRAS mutation was 58.59%, while G12S(34G>A) of codon 12 was the most common. In this

cohort, KRAS mutant type had lesser nodal metastasis and lymphovascular emboli.

P-31

Antineoplastic activity of an endangered Himalayan medicinal plant Pushkarmool on liver cancer cell line

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Introduction: Hepatocellular carcinoma (HCC) is one of the deadliest cancers worldwide due to high recurrence rate after surgical resection and lack of effective chemotherapeutic drugs. Recent evidence indicated that the chemoradiation resistance of HCC might be due to the prevalence of cancer stem cells (CSC) and deregulation of self-renewal pathways.

Aims and Objectives: Considering the hepatoprotective role of *Inula recemosa* (Pushkarmool) suggested before, the aim of our study was to understand the role of *Inula recemosa* on Hepatocellular carcinoma progression and cancer stemness.

Material and Methods: The cytotoxicity effect of active component from n-hexane extract of Inula recemosa (IRE) was evaluated by MTT assay on HepG2 liver cancer and WRL68 liver normal cell lines. Wound healing assay was performed to evaluate the antimigratory effect. Flow cytometry method was used to investigate cell cycle by PI, apoptosis by annexin V-PI, surface stem cell marker by fluorescence tagged antibody, and mitochondrial transmembrane potential by JC-1 probes. Effect of IRE on cancer stemness transcription factors such as SOX-2 and OCT-4 was determined by Semi-Q polymerase chain reaction method. Post-treatment protein expression levels of stemness markers were checked by immunocytochemistry method.

Results: IRE showed comparatively low toxicity on normal liver cell line WRL68 but potent antiproliferative effect on HepG2 cell line by induction of cell cycle arrest, apoptosis, and disruption of mitochondrial transmembrane potential. Scratch wound healing assay showed restriction of migratory property in a dose-dependent manner after 24 h of treatment. Stemness markers' expression was also decreased.

Conclusion: Our study can conclude that IRE might open up new therapeutic approaches on advanced therapies of HCC in near future.

Keywords: Pushkarmool, Hepatocellular carcinoma, Antiproliferative effect, Cancer stemness.

P-32

Unveiling genetic complexity: Hereditary pyropoikilocytosis masked as congenital dyserythropoietic anemia – Implications for genetic counseling and phenotypic diversity

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Introduction: Hereditary pyropoikilocytosis (HPP) comprise rare red blood cell membrane disorders, often confused with congenital dyserythropoietic anemias (CDA), or enzymopathies like pyruvate kinase deficiency. Accurate diagnosis is challenging due to their rarity and complex genotype-phenotype associations.

Aims and Objectives: To highlight the use of multigene panel-based targeted next-generation sequencing (NGS) in enhancing the diagnostic precision of rare hemolytic anemias, especially with indistinct phenotypes.

Methods: We encountered three unrelated children with severe transfusion-dependent anemia, hemolytic features, reticulocytopenia, and hepatosplenomegaly. Tests for common hemolytic anemias (G6PD deficiency, autoimmune hemolytic anemia, thalassemia syndromes, and unstable hemoglobins) were negative. Incubated osmotic fragility test showed increased red cell fragility. Flow cytometric eosin-5′-maleimide dye-binding assay was normal. Bone marrow examination raised suspicion of CDA. Sanger sequencing excluded the common SEC23B exon 12 missense variant c.1385A>G. Targeted NGS was performed using the custom panel for genes associated with inherited hemolytic, dyserythropoietic, and sideroblastic anemias.

Results: Targeted NGS revealed no pathogenic variants in CDA-associated genes but identified variants linked to red cell membrane defects. Case 1 had a novel potentially deleterious variant NM_001355436.2: c.6223G>C(p.Glu2075Gln) in exon 31 of SPTB gene. Cases 2 and 3 exhibited complex SPTA1 genotypes. Both were homozygous for αLELY variant NM_003126.4:c.[5572C>G;6531-12C>T]. Case 2 additionally coinherited two novel heterozygous likely pathogenic variants: splice donor variant c.1112+2T>A and missense variant c.6145G>C(p.Ala2052Pro). Case 3 showed two novel heterozygous variants: likely pathogenic splice acceptor variant c.4876-2A>G in intron 34 and missense variant of unknown significance c.140A>C(p.Gln47Pro) in exon 2.

Conclusion: These cases underscore the rarity of molecularly confirmed cases of HPP/spectrin-linked hemolytic anemia in India, highlighting the value of NGS for precise genetic diagnosis in complex blood disorders, aiding appropriate treatment, family counseling, and prenatal diagnosis. It also brings out the inherent overlap in phenotypic differentiation between hemolytic and dyserythropoietic anemias and underscores the non-specific nature of bone marrow dyserythropoiesis.

Keywords: Hereditary pyropoikilocytosis, Congenital dyserythropoietic anemia, Rare red blood cell membrane disorders, Targeted next-generation sequencing.

P-33

EGFR quandary in lung adenocarcinoma: A dive into the mutation types and its impact on patient survival in the Southern part of Assam

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Lung cancer stands as the predominant cause of cancer-related mortality globally, with the epidermal growth factor receptor (EGFR) mutation emerging as a pivotal factor influencing the management and survival outcomes for afflicted individuals. This study aims to know the different spectrums of EGFR mutation in adenocarcinoma of the lung and their implication on patient survival. This is a retrospective analysis of 96 patients diagnosed histologically with adenocarcinoma who underwent lung biopsy and subsequent real-time polymerase chain reaction analysis to determine EGFR mutation status. Our findings revealed that 22 (22.92%) of cases exhibited EGFR mutations. Specifically, 11 (50%) of these mutations occurred in exon 19, 7 (31.82%) featured the L858R mutation

in exon 21, 2 (9.09%) displayed the L861Q mutation in exon 21, and 2 (9.09%) presented other mutations. Notably, patients with EGFR mutations exhibited a significantly extended progression-free survival, averaging 14 months, compared to a mere 4 months for those without EGFR mutations. These results underscore the critical role of EGFR as a predictive marker for the survival status of lung cancer patients within the Indian population. This study marks a crucial step in unraveling the molecular intricacies of lung adenocarcinoma, emphasizing the need for further exploration on a larger sample size. The comprehensive analysis of EGFR mutations and their impact on survival not only contributes valuable insights to the current understanding of lung cancer but also lays the foundation for potential advancements in personalized treatment strategies.

Keywords: Lung cancer, Adenocarcinoma, Epidermal growth factor receptor, Mutation spectrum.

P-34

Early onset triple-negative breast cancer clinching Li-Fraumeni syndrome: A rare diagnostic perplexity

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Introduction: Li-Fraumeni syndrome (LFS) is a rare autosomal dominant inherited cancer susceptibility disorder with a wide tumor spectrum, particularly in young adults. LFS patients have life-long cancer risk, and the most commonly encountered tumors include soft tissue sarcoma, breast cancer, brain tumors, osteosarcoma, leukemia, and adrenocortical carcinoma. It is associated with germline mutations in the tumor suppressor gene TP53 (pathogenic/likely pathogenic variants). Women harboring a TP53 pathogenic variant carry a lifetime risk of developing breast cancer of 80–90%. However, the diagnosis of LFS is currently based on recognized clinical criteria regardless of the genetic mutation status.

Aims and Objectives: To highlight the significance of early detection and appropriate management of such a challenging and rare LFS syndrome by conducting genetic testing for inherited risk.

Methods: A detailed clinical history, breast cytological, and radiological examination were followed by genetic screening by next-generation sequencing for an accurate patient assessment.

Results: A 29-year-old female presented with unilateral breast cancer; her detailed history revealed a soft tissue sarcoma of the left thigh at the age of 15 years. Due to the early onset of breast cancer and a history of childhood malignancy, a suspicion of LFS was made. Her daughter was also diagnosed with osteosarcoma, left femur at the age of 8 years. Genetic testing of both revealed a TP53 pathogenic variant mutation, further confirming the diagnosis of LFS.

Conclusion: Genetic testing and counseling are paramount for the timely diagnosis and management of LFS cases. Special considerations regarding breast cancer treatment options are warranted and secondary cancer periodic surveillance is required for a favorable outcome.

Keywords: Hereditary breast cancer, Li-Fraumeni syndrome, TP53 mutation.

P-35

A study of intra- and intertumoral heterogeneity in gallbladder carcinoma

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Objectives: Poor overall survival and treatment response in gallbladder carcinoma (GBC) can be due to intratumoral heterogeneity (ITH).

Material and Methods: Twenty-one cases of resected GBCs were divided into 18 tumor regions following a systematic protocol like a grid including tumor border, suborder, and tumor center. Tissue microarray blocks were prepared by sampling a 2 mm core from each of these 18 regions, followed by histological examination, immunohistochemical (IHC) evaluation of epithelial-mesenchymal transition (EMT), and flow cytometry for DNA aneuploidy of tumor cells. Spatial heterogeneity was assessed and correlated with tumor outcome. Sections from 17 cases of resected gallbladder with minimal inflammation were included as IHC control.

Results: Among the histological parameters, spatial heterogeneity of intratumoral budding was noted between tumor border and tumor center (P = 0.04). Among the EMT markers, spatial heterogeneity of Snail-1 expression was noted between tumor border and tumor center (P = 0.04). Spatial heterogeneity of complete membranous E-cadherin expression was noted between tumor border and center (P = 0.01), as well as tumor suborder and center (P = 0.01). Spatial heterogeneity of membranous expression of Claudin-1 was significant between tumor border and suborder (P = 0.03). Ki-67 proliferative index was higher in mucinous tumor areas (P = 0.02). Loss of membranous E-cadherin and Claudin-1 stains and increased Ki67 proliferation index were more in GBC than in controls. DNA aneuploidy was detected in all cases of GBC, with the majority showing hyperdiploidy (11/15, 73.33%) and the rest hypodiploidy (4/15, 26.67%).

Conclusion: This study highlights spatial intertumoral and intratumoral heterogeneity of histology, EMT, and cell proliferation activities in GBCs. Proximity of peritumoral stroma and tumor cells determines mesenchymal phenotype including tumor budding and EMT.

P-36

Unlocking VEXAS syndrome in India: Deciphering clinical and laboratory profiles through a multifaceted case series

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Introduction: VEXAS syndrome (vacuoles, the E1 enzyme, X-linked inheritance, autoinflammatory tendencies, and somatic mutations) is a recently recognized, acquired monogenic adult onset hemato-inflammatory syndrome, marked by novel somatic mutations within the UBA1 gene.

Aims and Objectives: We describe the clinical characteristics, laboratory features, and outcomes of patients diagnosed with VEXAS syndrome at our institute.

Materials and Methods: Six patients with VEXAS syndrome were identified between 2020 and 2023. Clinical details including treatment history and laboratory parameters were recorded.

Results: Six male patients were identified with UBA1 gene mutation with an age range from 42 to 77 years. Prominent clinical characteristics noted were a history of fever (6/6, 100%), arthritis/arthralgia (6/6, 100%), inflammatory skin lesions (5/6, 83%), vasculitis (3/6, 50%), ocular inflammatory conditions (3/6, 50%), relapsing polychondritis (2/6, 33%), unprovoked venous thrombosis (2/6, 33%), and ear chondritis (1/6, 16%). Generalized lymphadenopathy (2/6, 33%) and mild splenomegaly (1/6, 16%) were also seen. Persistent unexplained cytopenia was present in all the cases in the form of anemia (5/6, 83%), thrombocytopenia (2/6, 33%), and neutropenia (2/6, 33%). Bone marrow examination was performed in 5/6 (83%) cases, 4 of which had hypercellular bone marrow. All five cases showed dyshemopoiesis, in the form of dyserythropoiesis (4/5), followed by dysmegakaryopoiesis (3/5) and dysgranulopoiesis (1/5). All five cases also showed cytoplasmic vacuolations in the hematopoietic precursor cells. UBA1 somatic mutations included p.Met41Val (c.121 A>G Exon 2) (2/6, 33.3%) and p.Met41Thr (c.122 T>C Exon 2) (4/6, 66.6%). 4/6 (66.6%) died within a median follow-up of 3 months.

Conclusions: We highlight the spectrum of clinical and laboratory features of the newly described VEXAS syndrome. Identification of this entity, early in the course of the disease, is important as it is associated with a significantly high mortality.

P-37

HPV genotyping in cervical carcinoma using real-time polymerase chain reaction: Association with histopathological diversity

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Introduction: Human papillomavirus (HPV) plays a pivotal role in cervical squamous cell carcinoma (SCC) and adenocarcinomas (AC). While shared risk factors implicate HPV infection, a subset of aggressive HPV-negative AC challenges conventional understanding. The rising AC incidence relative to SCC reflects the evolving landscape of cervical cancers. HPV's role in adenocarcinoma pathogenesis, especially in histological subtypes like mucinous adenocarcinoma, remains unclear, necessitating a nuanced approach for refining interventions.

Aim: To unravel correlations between prevalent high-risk HPV genotypes and histopathological subtypes of cervical carcinoma, focusing on adenocarcinoma.

Objectives: (1) Discern prevalent high-risk HPV genotypes among cervical carcinoma patients through meticulous genotyping analysis. (2) Association between high-risk HPV genotypes and histopathological subtypes of cervical carcinoma.

Methods: This cross-sectional observational study involved 59 biopsy-confirmed cervical carcinoma cases. High-risk HPV DNA genotyping using real-time polymerase chain reaction, histopathological classification, and statistical analyses were conducted to explore association between HPV genotypes and histopathological subtypes.

Results: Among 59 cases, SCC dominated (74.58%) and AC represented 25.42%. HPV positivity was universal in SCC, with diverse genotypes. In the adenocarcinoma subset, 66.67% exhibited HPV positivity, predominantly HPV16. Within positive cases, usual type AC showed higher HPV16 prevalence than mucinous types. Importantly, 33.33% of adenocarcinomas were HPV-negative, introducing complexities, particularly in a case expected to be HPV positive but testing negative.

Conclusion: The study underscores the significant association between HPV16 and SCC/adenocarcinoma. Distinct HPV genotype patterns in adenocarcinomas, particularly the usual type, suggest potential variations, prompting further investigation. Identification of HPV-negative cases, notably within adenocarcinomas, prompts ongoing research. Existing HPV vaccine's limitations in covering all high-risk genotypes highlight the need for continued studies to enhance vaccine effectiveness.

Keywords: Cervical adenocarcinoma, Molecular study, High-risk human papillomavirus, Human papillomavirus 16 prevalence.

P-38

MYD88 L265P mutation in intraocular lymphoma: A potential diagnostic tool

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Aims and Objectives: Vitreous biopsies for the presence of MYD88 L265P mutation were retrospectively screened to evaluate its diagnostic utility and to compare with cytological light microscopic diagnosis.

Methods: Cytospin analysis and molecular studies including MYD88 L265P mutation by allele-specific real-time polymerase chain reaction (PCR) (True PCR assay) were carried out on vitreous biopsies collected from 11 patients and 5 controls. Patients on chemotherapy (2) were excluded from the study. The results of MYD88 L265P mutation analysis were compared with the cytological diagnosis made by may grünwald and giemsa (MGG), Papanicolaou (PAP) and immunocytochemistry.

Results: This study included 11 patients, 8 had clinical suspicion of intraocular lymphoma (IOL). Out of these 8 clinically suspected cases, five were diagnosed as diffuse large B-Cell IOL, based on cytological features. Two cases received chemotherapy and were excluded for molecular studies. The remaining 3 were diagnosed as reactive/inflammatory. MYD88 L265P mutation analysis was undertaken in all 9/11. MYD88 L265P mutation was detected in only 2/3 cases diagnosed by cytological features as lymphoma; it was negative in 6 patients which were cytologically reactive lymphoid infiltrates and in one patient diagnosed as IOL cytologically. The mean age of the 4 IOL patients was 56.4 ±10.76 years (range, 38–65 years). There was a marked female preponderance (75%) with a male-to-female ratio of 1:3 (1 male and 3 females). Duration of disease ranged between 2 months and 3 years. The left eye was involved in all the 4 cases. Diagnostic utility of MYD88 L265P mutation analysis revealed a sensitivity of 100%, specificity of 100%, and positive and negative predictive value of 100% each, respectively. Diagnostic accuracy of 100% was achieved with the mutation analysis as compared to 91% with conventional cytological analysis, which shows the superiority of MYD88 in confirming or ruling out IOL patients diagnosed by light microscopy.

Conclusion: The diagnostic utility of MYD88 L265P mutation may be superior to conventional cytological analysis, especially in cytologically

ambiguous IOL cases. MYD88 L265P mutation could provide a reliable alternative diagnostic modality in conjunction with conventional light microscopy in such cases.

Keywords: Intraocular lymphoma, MYD88 L265 mutation, Allele-specific real-time polymerase chain reaction.

P-39

Uncommon epidermal growth factor receptor mutation p.L747P in non-small cell lung cancer: Six case series from National Reference Laboratory

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The most common epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) are exon 19 deletions and exon 21 point mutations, which are both sensitive to EGFR tyrosine kinase inhibitors (TKI's). However, rare EGFR mutations such as S768I, G719X, L861Q, and L747P are uncommonly reported. Sparse evidence is available about the activity of EGFR TKI's in the setting of uncommon EGFR mutations and effective treatment decision making-process is challenging, Also robust data comes from large randomized clinical trials of EGFR TKIs in patients carrying common EGFR mutations, few and heterogeneous prospective data are available in the uncommon mutations setting. In this sense, there is a clinical need for literature report of these rare molecular alterations and their predictive role for response to EGFR TKIs in large updated databases to guide clinicians to personalize therapeutic strategies for NSCLC patients. Here, we report 6 cases diagnosed with Stage IV lung adenocarcinoma carrying EGFR exon 19 L747P, one of the uncommon mutations reported as a variant with uncertain significance in most of the databases. In this study, we discuss correlations between this mutation and other comutations and patient outcomes of treatment administered.

Keywords: Non-small cell lung cancer, Epidermal growth factor receptor tyrosine kinase inhibitor, Uncommon mutations.

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Polycythemia vera

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Introduction: Polycythemia vera belongs to the BCR-ABL-negative myeloproliferative neoplasm. It is more common in men than women (1.5-2:1) and is a disease of old age (55–80 years). It is characterized by increased red cells, increased blood volume, splenomegaly, and JAK- 2 mutation. There is panmyelosis with associated thrombocytosis.

Case Description: We present a case of polycythemia vera in a 65-year-old female who presented with complaints of generalized weakness for 01 month and cough for 2 weeks. On physical examination, there was no fever, jaundice, or cyanosis. On per abdominal examination, there was splenomegaly supported by USG findings, which also showed splenomegaly with thrombosis of splenic vein, coeliac artery, and its branches. Other baseline investigations revealed hemoglobin = 17g/dL, hematocrit = 51, total leukocyte count = 68000/cumm, and platelet count =5.2lac/cumm. On peripheral blood film examination, diagnosis of neutrophilic leukocytosis with mild shift to left was given. Bone marrow examination revealed hypercellular marrow with trilineage hyperplasia with greater percentage of segmented forms. Trephine biopsy show normal topography of cells. Therefore, based on the PBF, BM aspiration, and trephine biopsy findings, a diagnosis of myeloproliferative disorder was made with a possibility of chronic neutrophilic leukemia. Further, the following battery of tests was recommended for confirmation:-

- NAP score
- BCR-ABL translocation
- JAK-2 mutation
- CSF3R mutation

All were done and it was found NAP score was mildly increased, BCR-ABL translocation was negative, CSF3R mutation was also negative, and JAK-2 Mutation comes out to be positive. Again, on analysis of radiological findings, complete blood count (CBC) and bone marrow (BM) findings that is raised hemoglobin, raised hematocrit, thrombosis of splenic vein and coeliac artery, positive JAK-2 mutation and negative BCR-ABL translocation, the final diagnosis of polycythemia vera was given.

Conclusion: Myeloproliferative disorders are a group of disease entities which have got overlapping clinical, blood, and bone marrow findings. Therefore, a thorough examination with clinical correlation and molecular diagnosis is necessary. For polycythemia vera, early diagnosis is important to reduce the rate of mortality and morbidity associated with disease.

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Molecular detection of KRAS and BRAF mutation in colorectal cancer by real-time polymerase chain reaction

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Introduction: Colorectal carcinoma is the second most common cause of mortality associated with cancers worldwide, with a total number of new cases of 1.93 million cases in 2021. Development of most colorectal cancer (CRC) is related to CIN pathway, in which alteration occurs in RAS/RAF/MEK/ERK cascade.

Aim and Objectives: This cross-sectional observational study aimed at establishing the prevalence of KRAS and BRAF mutation in colorectal adenocarcinomas.

Material and Methods: Our study involved 40 treatment-naïve primary colorectal adenocarcinoma patients. After histopathological diagnosis, tissue sections were cut into 6-10 µm thickness from FFPE tissue blocks, and DNA extraction was done from these sections using the FFPE Tissue DNA Extraction Kit and was quantified by spectrophotometer. DNA samples were run by real-time polymerase chain reaction using a TRUPCR-KRAS and TRUPCR-BRAF kit which could qualitatively detect 11 somatic mutations on exon 2 and 3 of KRAS gene and 7 mutations on exon 15 of BRAF gene, respectively, from tumor tissue DNA. Mutation analysis was performed from multicomponent plots by calculating cycle threshold values.

Results: We detected a total of 37.5% mutation rate in this study cohort involving 31 males and 09 females with a median age of 59 years. KRAS mutation was present in 14/40 cases, with G12S being the most common mutation followed by G12V. Only 1/40 case showed mutation in BRAF i.e. V600E.

Conclusion: The prevalence of KRAS mutation came out to be almost equal to other similar studies conducted; however, the most common mutation was G12S as compared to G12V, which was the most common mutation seen in other studies compared and also carries worst prognosis. The BRAF mutation rate was lower and most common being V600E. KRAS- and BRAF-mutated colorectal carcinomas do not respond to conventional treatment, i.e., anti-EGFR and tyrosine kinase inhibitors. Furthermore, different mutations carry different prognoses. A paucity of literature in the Indian population warrants a definitive screening for these mutations.

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Role of metal ion transporters in the pathogenesis of malaria parasite

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Emerging drug resistance to currently available antimalarials demands the development of novel drug targets to combat malaria infection. Upon invasion, the intracellular parasite exploits host machinery for its benefit through acquisition of essential nutrients and ions from human plasma. Metal ion transport is a complex process which is attributed to epigenetic regulation of multigene family. Three functional homologs of metal ion transporters consisting a conserved GMN motif have been identified in Plasmodium falciparum. One of the homologs displayed dual targeting to both organelles (apicoplast and mitochondria) in P. falciparum. Furthermore, the transport of characteristic metal ions through these transporter proteins was assessed by yeast complementation assay. The intracellular localization and functional role of these homologs was tried to reveal by generating transgenic parasites. The inhibitors targeting these transporters exhibited promising antimalarial activities and the molecular insight into their action was revealed through molecular docking. The findings provide a novel example of gene family expansion to allow ion transport and insight into pharmacology relevant to drug development against malaria parasite.

Keywords: Metal ion transporter, *Plasmodium falciparum*, Malaria, Apicoplast, Mitochondria.

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Integrated clinicopathological and molecular analysis of Endometrioid carcinoma: A study in Indian patients

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Introduction: Endometrial carcinomas have been classified into 4 molecular subgroups as per The Cancer genome Atlas (TCGA): polymerase epsilon (POLE) ultramutated, mismatch repair gene (MMR)-deficient, p53 mutant, and no specific molecular profile. The classification is independent of the histological subtype and has prognostic and therapeutic implication. Data on the relative prevalence of these molecular subtypes and their clinicopathological relevance in the Indian population are limited. This study was taken up to analyze the same in a cohort of endometrioid carcinoma patients.

Aims and Objectives: To categorize 46 endometrioid endometrial carcinomas by the proposed molecular classification and analyze the histopathological and clinical parameters in the various subgroups.

Methods: In 46 endometrioid carcinoma cases, routine grossing as per the CAP protocol was done and an initial immunohistochemical (IHC) panel comprising but not limited to p53, p16, ER, and PR was performed. Further testing for polymerase epsilon (POLE) mutation and IHCs for MMR proteins (MLH1, PMS2, MSH2, and MSH6) was done. Immunomolecular status was integrated with histological parameters for further analysis.

Results: On histological examination, 11 tumors were FIGO Grade 1 (23.9%), 27 were FIGO Grade 2 (58.7%), and three were FIGO Grade 3 (6.5%). Four cases were diagnosed as dedifferentiated endometrioid carcinoma (8.7%) and one was diagnosed as mixed endometrioid and serous carcinoma (2.2%). 14 patients were MMR deficient (30.4%) and six harbored p53 mutation (13%). None of the patients tested positive for POLE mutation (0%), leaving 26 patients in the no specific molecular profile group (56.6%). MMR-mutated tumors were mostly low-grade (Grade 1 [3/14] and Grade 2 [8/14]) with one case each of dedifferentiated endometrial carcinoma and mixed serous and endometrioid carcinoma. p53-mutated tumors comprised three Grade 2, one Grade 3, and two dedifferentiated endometrioid carcinoma.

Conclusion: There can be geographical variations in the molecular expression patterns of tumor. Prevalence studies and correlation with clinicopathological features is important to determine their therapeutic and prognostic significance.

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An association of DNA mismatch repair protein expression status with histological prognostic indicators in an institutional colorectal cancer cohort from North India

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Aims and Objectives: The microsatellite instability (MSI) is implicated in the pathogenesis of approximately 15% of sporadic colorectal cancers (CRCs) and >95% of HNPCC syndrome (overall 3–5% of all CRCs).[1] DNA mismatch repair (MMR) status helps in prognostication and deciding targeted therapy in CRCs. To study the association of DNA mismatch repair protein expression status with histological prognostic indicators in CRCs.

Methods: We analyzed 100 CRC patients who underwent upfront surgery without neoadjuvant therapy. The MMR protein expression status was analyzed on formalin-fixed tumor tissue using immunohistochemistry for MLH1, PMS2, MSH2, and MSH6 proteins. Clinical and histological variables were correlated with MMR status using nonparametric tests.

Results: In this cohort, a total of 35% of cases showed MMR-deficient (MMRd) expression pattern characterized by loss of one or more MMR proteins. About 41.18% of MMRd CRCs (n = 7/17) had metastasis in <4 lymph nodes, whereas 7.14% (n = 1/14) of MMRd patients had metastasis in ≥ 4 lymph nodes (P = 0.04). In MMRd colorectal cancers, 47.50% (n = 19/40) exhibited extracellular mucin in less than 50% of the tumor area, while 25.53% (n = 12/47) did not show any extracellular mucin within the tumor (P = 0.03). Among the MMRd cases, 30 were negative for <4 MMR proteins and more commonly identified in men than in women (39.34% vs. 15.38%, P = 0.01%). There was no definite correlation of MMRd status with other histological prognostic parameters or overall outcomes in this cohort.

The prevalence of KRAS gene mutation noted in the MMRd group versus the MMR proficient cases was 36.59% versus 34.48%, however statistically insignificant ($P = 0.83\%$).

Conclusions: In our study cohort, approximately one-third of colorectal cancer (CRC) patients demonstrated MMRd status. However, the association with histological prognostic indicators remains unclear. Therefore, further analysis on a larger cohort is warranted to elucidate this relationship.

Keywords: Mismatch repair protein, Colorectal cancer, Histological prognostic indicators.

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A study of bone marrow morphology and molecular profile in myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms (MPNs) are clonal-origin hematopoietic stem cell disorders. Molecular studies in case of BCR-ABL negative MPN (JAK2v617F, CALR, and MPL) have revolutionized the diagnostic approach of MPNs. PV is expected to be almost always accompanied by a JAK2 mutation, whereas the specific driver mutation cannot otherwise distinguish one MPN from another; however, in distinguishing essential thrombocythaemia (ET) from pre-primary myelofibrosis (PMF) or polycythaemia vera (PV), a higher JAK2V617F allele burden favors the diagnosis of the latter rather than the former.

Method: This was a cross-sectional study, for 18 months which included clinical presentation, peripheral blood picture, bone marrow aspiration (BMA), and bone marrow biopsy (BMB) features in cases with BCR-ABL and JAK2V617F CALR and MPL mutation analysis.

Result: There was 100% level of agreement between bone marrow morphology and molecular studies in case of 16 chronic myeloid leukemia patients diagnosed on bone marrow morphology (BCR-ABL positive). However, in case of BCR-ABL negative MPNs out of 16 cases, only 13 cases showed genetic mutation, which included JAK2V617F and CALR gene mutation.

Conclusion: Multimodal approach is needed for classifying MPNs. Increased knowledge about molecular alteration has led to drastic shift in classification of MPNs, with molecular features gaining importance and resulting in entities defined in part or even exclusively, by recurrent genetic alterations.

Keywords: Bone marrow, Molecular, Mutations, Myeloproliferative neoplasms.

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Myeloid neoplasm with ETV6:ABL1 fusion - Clinical, laboratory, and genomic correlates

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Introduction: ETV6:ABL1 rearrangements have been described as the underlying cause of various hematologic malignancies. Due to the opposite orientation of genes, it is a biologically implausible fusion and is thus rare. Its characteristic in myeloid neoplasms (MNs) is reported anecdotally.

Aim and Objectives: We sought to evaluate clinical presentation, diagnostic features, treatment, and outcome of MN with ETV6:ABL1 fusion.

Methods: In this retrospective observational study (2018–2023), nine cases of MN with ETV6:ABL1 were assessed. Clinical and hematological details were noted from electronic medical records (EMR). Next-generation RNA sequencing "NARASIMHA" was performed on BMA samples. Sequencing was done on Illumina NextSeq 1000 (P1 300-cycle) and the data were analyzed using customized pipeline. Real-time polymerase chain reaction and targeted DNA next-generation sequencing was conducted wherever applicable.

Results: We report 9 cases of MN with ETV6:ABL1 fusion. Seven were male and two were female (M: F = 3.5:1), with a median age of 37 years (range: 22–64 years). Organomegaly was seen in 6 (66.67%) patients. Eight cases had leukocytosis with eosinophilia. The median TLC of $86.4*10^9$/L (range: 7.82–$206*10^9$/L) and AEC of $1.89*10^9$/L (range: 0.16–$41.31*10^9$/L). Eight patients presented in chronic phase (CP) with hypercellular bone marrow and increased M: E ratio (range1.56:131.7:1). Increase in megakaryocytes was seen in 3 cases, while 2 cases harbored fibrosis. One case was in blast-crisis on presentation. This case had 67% MPO-positive blast. These blasts expressed moderate CD34 and CD33; variable HLA-DR, CD13 and CD15 and partial CD117. Cytogenetics detected the fusion in two cases. Pathogenic variants detected include ETV6, U2AF1, EZH2, NRAS, BCOR, and HBB, among others. Six patients of CP were started on hydroxyurea and tyrosine kinase inhibitor. Two cases received palliation. BC patient was managed with intensive chemotherapy with dasatinib and succumbed to illness within 6.61 months. On follow-up, 2 patients of CP were deceased. The median overall survival for CP was 38.65 months (range: 11.48–65.63 months).

Conclusion: In the first series from India, we highlight the clinicopathological connotations in MN with ETV6:ABL1 rearrangements and their therapeutic implications.

Keywords: ETV6:ABL1, RNA sequencing, Myeloid neoplasms, Tyrosine kinase inhibitors.

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Prevalence and coexistence of KRAS and EGFR gene mutations in Indian colorectal cancer patients: Next-generation sequencing-based cohort study

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Introduction: Molecular testing of predictive biomarkers has become essential in selection of patients for targeted therapies. Epidermal growth factor receptor (EGFR)/RAS/RAF/MEK/MAPK pathway is an important pathway in carcinogenesis, invasion, and metastasis of colorectal cancers (CRCs).

Aims and Objectives: To analyze mutation frequencies of KRAS and EGFR in CRC patients.

Material and Methods: Next-generation sequencing (NGS) was performed to identify mutations in KRAS and EGFR genes using formalin-fixed paraffin-embedded (FFPE) tissue blocks of 88 colorectal cancer patients. Clinicopathological characteristics of all patients were recorded. The DNA was isolated using Qiagen DNA FFPE Kit (Qiagen, USA) as per the manufacturer's instructions.

Results: The mean age was 49.85 ± 10.56. CRC was more in males (64.77%) than females (35.33%) and more in the distal colon (64.77%) than in the proximal colon (35.33%). CRC was more in stage III+IV (44.31%) than in I+II (39.77%). Stages of lymph node invasion were N0 (39.77%), N1 (26.13%), and N2 (18.18%). KRAS and EGFR gene was mutated in 46.86% and 25.0% of cases, respectively. Cases with necrosis (P = 0.006), high tumor budding score (P = 0.015), Desmoplasia (DPA) of 3+ (P = 0.037), and perinuclear invasion (P = 0.027) showed significantly higher KRAS gene mutations. EGFR gene mutation was significantly higher in cases with necrosis (P = 0.006). Cases with well-differentiated histology, lymph node metastasis, lymphovascular invasion evident, T4 stage, N2 stage, and stage III+IV showed higher frequency of KRAS gene mutation. The EGFR gene mutation frequency was higher in well-differentiated histology, DPA score of 3+, and in cases with tumor budding. EGFR gene mutation coexisted with KRAS gene mutation in 11/22 cases. Mutation frequency of EGFR was almost similar in distal and proximal colon. KRAS gene mutation was higher in proximal colon.

Conclusion: CRCs showed mutations higher in KRAS gene. NGS is a way to detect amplifications associated with resistance to therapies and low-prevalence mutations in actionable genes, providing patients access to innovative targeted therapies.

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ROLE of molecular diagnostics in extrapulmonary tuberculosis: A prospective study at a medical college in East India

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Aims and Objectives: To investigate the role of molecular methods in clinically suspected patients of extrapulmonary tuberculosis (EPTB).

Methods: A prospective observational institution-based study was conducted after attaining scientific and ethics committee clearance for 18 months. Inclusion criteria: All cases of clinically suspicious EPTB presenting to KPC Medical College and Hospital or referred to NTEP center undergoing molecular diagnostic tests and tuberculosis culture testing after mandatorily obtaining informed consent from each patient. Exclusion Criteria: Patients with pulmonary tuberculosis (TB) and patients currently on antituberculosis therapy. Statistical tests for diagnostic accuracies and Cohen Kappa tests were done.

Results: An anthropometric study was done of the total 212 patients. Other ancillary tests were conducted in these patients. TrueNat and TB culture had true positive of 203, false positive of 03, false negative of 03, and true negative of 05 cases. With respect to cartridge based nucleic acid amplification test (CBNAAT) and TB culture, 193 cases were true positive, 04 cases were false positive, 10 cases were false negative, and 05 cases were true negative. Keeping TB culture as the gold standard, the sensitivity of CBNAAT and TrueNat was 95.07% and 99.51%, respectively, and specificity was 55.55% and 62.05%, respectively. CBNAAT had an accuracy of 97.96% and negative predictive value of 33.33% with positive likelihood being 2.15 and negative likelihood of 0.72%. TrueNat had a positive predictive value of 98.54%, negative predictive value of 83.33%, positive likelihood ratio of 2.62, and negative likelihood ratio of 0.007. Cohen Kappa statistical evaluation was done, and it was 0.70 for TrueNat and 0.38 for CBNAAT. Rifampicin drug sensitivity was also evaluated, eventually being sent for line probe assays.

Conclusion: TrueNat is a better diagnostic modality for detecting EPTB though CBNAAT is advocated currently in most resource-limited settings. The role of ancillary testing is highlighted.

Keywords: Extrapulmonary tuberculosis, Tuberculosis, CBNAAT, TrueNat, Molecular diagnosis, Line probe assay, Rifampicin sensitivity.

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Mismatch repair protein deficiency in high-grade gliomas in pediatric, adolescents, and young adults

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Aims and objectives: DNA mismatch repair (MMR) is a system that repairs errors during DNA replication. Conditions associated with MMR deficiency, such as Lynch syndrome and constitutional mismatch repair deficiency syndrome (CMMRD), carry an elevated risk of colorectal cancer, hematological malignancy, gliomas, and other brain tumors. CMMRD is caused by biallelic variants in MMR genes (MLH1, MSH2, MSH6, and PMS2), most commonly PMS2. Recent trials suggest that tumors with defective MMR genes, such as high-grade gliomas (HGG), show a positive response to immune checkpoint inhibitors. To assess the frequency of loss of MMR protein expression in high-grade gliomas, particularly in the pediatric, adolescent, and young adult age groups.

Methods: Paraffin blocks of ambispective cases of high-grade glioma (central nervous system [CNS] World Health Organization [WHO] Grade 3, 4) were retrieved, and IHC with four MMR antibodies (MLH1, MSH2, MSH6, and PMS2) was performed. Immunohistochemical loss of MMR protein expression was recorded as partial or complete loss of at least 1 MMR protein.

Results: 60 cases of high-grade glioma (CNS WHO Grade 3, 4) with male preponderance (M: F = 1.3:1), with a mean age of 16.5 years, were included in the study. Partial or complete immunohistochemical loss of MMR proteins was found in 17/60 cases (28.3%). Alteration of PMS2 expression was found in 10/17 (58.8%), MSH2 in 5/17 (29.4%), and MSH6 in 1/17 (5.9%) cases. A single case (5.9%) showed the loss of both PMS2 and MLH1. Among the 17 MMR deficient cases, 52.94% and 47.06% were observed in the pediatric and AYA cohort, respectively.

Conclusion: Universal screening is imperative given the significant morbidity and high tumor mutational burden, compounded by the co-occurrence of other malignancies and contraindications to standard treatments such as Temozolomide. However, promising immunotherapeutic approaches offer potential solutions. Additionally, genetic testing and family counseling are essential for identifying cancer predisposition syndromes.

Keywords: Mismatch repair protein, Constitutional mismatch repair deficiency syndrome, High-grade gliomas.

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Unravelling the genetic landscape of meningiomas: Molecular insights in translational advances

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Introduction: Meningiomas, the primary adult intracranial tumor, stem from arachnoid cap cells, constituting 34% of central nervous system (CNS) tumors. With an age-related rise, they are commonly diagnosed at a median age of 65. Categorized by the World Health Organization (WHO) (CNS5) criteria into three grades and 15 subtypes based on histopathological features, this system lacks accuracy in predicting clinical behavior. Molecular advancements enable genomic and epigenomic studies.

Aim and Objectives: This study aims to analyze mutation frequencies across meningioma grades, correlate mutations with histological features and WHO grades, and assess concordance between targeted and Sanger sequencing for clinically relevant mutations.

Methods: The study employed targeted next-generation sequencing (Haloplex-HS Kit) on 88 meningioma cases and 3 controls, covering 36 recurrently affected genes. Illumina Platform (MiSeq kit V3, 600 cycles) was used for sequencing, and mutations were validated via Sanger sequencing.

Results: 88 meningiomas underwent sequencing (Grade 1: 39, Grade 2: 43, Grade 3: 6). Mutations occurred in 68.1% (60/88), mostly NF2-related (71.6%, 63/77), with 28.4% (25/88) having non-NF2 mutations. NF2 mutations increased significantly with the WHO grade. Key genes included TRAF7, KLF4, AKT1, SMO, and pTERT, displaying varied mutation frequencies. AKT1 E17K mutations spanned grades 1–3 basal meningiomas (12.5% overall). TRAF7 mutations (20.6%) included rare variants (G560D, T391A, R653Q, and A358P), predicted deleterious. pTERT mutations (4.8%) exclusive to NF2 were Grade 2, suggesting poor prognosis or recurrence. Other mutations (BAP1, SUFU, TP53, POLR2A, SMARCB1, SMARCE1, PIK3CA, and CREBBP) occurred across all grades. Sanger sequencing had 100% concordance for pTERT.

Conclusion: Rare pTERT mutations in meningiomas correlate with higher grades and poorer survival. Co-occurrence of KLF4 and TRAF7 is indicative of Grade 1 secretory meningiomas. Our research unveils key molecular traits, with robust 100% concordance in Sanger sequencing, bolstering the credibility of our findings. These insights offer a significant value for future diagnostics and treatments.

Keywords: Meningioma, Genetic profiling, Sequencing, Prognosis.

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Antinuclear antibody study in disseminated lupus erythematous patients

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Introduction: Disseminated lupus erythematous is a benign disorder of the skin, most frequently involving the face and scalp, and characterized by well-defined red, scaly patches of variable size, which heal with atrophy, scaring, and pigmentary changes. A diagnostically important feature of disseminated lupus erythematous is the presence of circulating antinuclear antibody and line immunoassay is a confirmatory test.

Aims: To study the antinuclear antibody by immunofluorescence assay in disseminated lupus erythematous patients.

Objectives: To study the correlation of antinuclear antibody in disseminated lupus erythematous patients and to do confirmatory test line immunoassay whenever possible.

Methods: Hep 2 cell immunofluorescence was used for the detection of autoantibodies in the patient sera. And, under immunofluorescence pattern of immunofluorescence was studied to see antibodies detected.

Observation and Result: Received single, gray-white to gray-brown, soft to firm skin-covered lesion bits measuring 0.4 cm. Microscopy shows sections studied reveal epidermis, dermis, and subcutis. The epidermis is lined by keratinized stratified squamous epithelium showing atrophied epidermis, hyperkeratosis, blunting of rete ridges, and vacuolar change in interface. The dermis is composed of fibrocollagenous tissue showing pigment incontinence along with mild lymphohistiocytic infiltrate and few congested and dilated blood vessels. Histomorphological features are suggestive of discoid lupus erythematous, which was confirmed by antinuclear antibody positivity.

Conclusion: Disseminated lupus erythematous is benign disorder of skin, which showed antinuclear antibody positivity and line immunoassay is a confirmatory test which is more specific, histormorphological features are suggestive of discoid lupus erythematous.

Keywords: Discoid lupus erythematous, Antinuclear antibody positivity, Immunofluroscence.

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Increase and development of immunity through the use of antirabies vaccine in India

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Introduction: In our country, antirabies vaccines have been manufactured separately for use in both humans and animals. This antirabies vaccine has proven to be almost 100% effective when taken at the right time and in full dose.

Objective: To rid both our humans and animals of rabies disease, we can successfully use antirabies vaccine on them. By taking it at the right time and in full dosage, all of us can get rid of rabies disease forever.

Method: We use this antirabies vaccine in both humans and animals through a new syringe. We can use this antirabies vaccine once a year in animals and four times in humans to get rid of diseases like rabies. This requires a trained human therapist/veterinarian.

Result: By taking regular and full dose of antirabies vaccine, human and animal body can get rid of deadly disease like rabies forever. The use of this antirabies vaccine also increases the immunity of our body to fight diseases like rabies in humans and animals. Due to this, we can easily defeat deadly diseases like rabies.

Conclusion: Human anti-rabies vaccine is manufactured by the Ministry of Health and Family Welfare, Government of India. On which approximately thousand crore rupees are spent. If we do anti-rabies vaccination in all the dogs of our country, then all of us human beings will not need human anti-rabies vaccine. With this, half the expenditure of our government treasury will be easily reduced. The Ministry of Health and Family Welfare,

Government of India will be able to save about Rs. 1000 crores very easily. Apart from this, an important goal of the Ministry of Health and Family Welfare, Government of India is to make the entire country of India free from rabies by the year 2030.

Keywords: Antirabies vaccine, Animal birth control, Rabies control, Human health protection, Rabies free India

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Comparison of Identiclone & T Cell Receptor Gamma Gene Rearrangement Assay 2.0 kit with lab standardized primer set in the evaluation of T- cell receptor Gamma gene rearrangement assay

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Introduction: Assays portraying the T-cell receptor (TCR) clonality are essential in questionable nature of lymphoid infiltrate, to delineate origin of the malignant lymphoid processes with overlapping histomorphology and immunophenotype and for minimal-residual-disease measurement in T-cell malignancies. TCR-gamma assays currently form the basis of TCR clonality detection, mainly focusing on the V and J-gene segments. Many currently available primer sets exclude the uncommon gene segments, expecting a rate of up to 33% of false negativity. The commercially available primer is not cost-effective in resource-poor setting. A custom-made primer set by Dr. Greiner was developed to combat this issue and lower the cost of resource crunch setting.

Aims and Objectives: This parallel study aims to compare the sensitivity of the lab-standardized Dr. Greiner TCR-gamma primers (University of Nebraska) and commercially available Identiclone primers to develop a cost-effective method for TCR-gene assessment.

Methods: A total of 44 formalin-fixed paraffin-embedded samples suspicious for T-cell neoplasms were subjected to TCR gamma gene rearrangement assay. This parallel study aims to compare the sensitivity of the lab standardized Dr.Greiner TCR-gamma primers (University of Nebraska) and commercially available Identiclone and T Cell Receptor Gamma Gene Rearrangement Assay 2.0 primers to develop a cost-effective method for TCR-gene assessment. The gel electrophoresis was used to confirm the amplification, and fragment analysis was performed with the help of ABI3500 using capillary electrophoresis.

Results: All FFPE samples yielded DNA products suitable for clonality assessment. Of the T-cell lymphomas, all cases showed clonal peaks with Identiclone and Dr.Greiner primers except for two cases of PTCL which showed polyclonal peaks with Dr.Greiner assay. The rest all the cases showed polyclonal peaks with either of the assays. Dr.Greiner assay showed a specificity and positive predictive value of 100% in comparison to the Identiclone with a sensitivity of 88.23% and negative predictive value of 93.1%. The final diagnosis were the peripheral T cell Lymphoma not otherwise specified (PTCLNOSCL) - 13, angioimmunoblastic T-cell lymphoma - 2, nodal T-follicular helper-cell lymphoma - 2, Hodgkin lymphoma - 8, atypical lymphoproliferation - 8, reactive process - 5 and B-cell neoplasms - 6.

Conclusion: Dr. Greiner assay is a cost-effective and specific method for detecting TCR-gene clonality. However, further validation with large sample cohort is essential.

Keywords: T cell clonality, Identiclone, T-cell receptor gene rearrangement.

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CMYC rearrangement and Immunohistochemical expression in diffuse large B-cell lymphomas NOS and high-grade B-cell lymphomas

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Introduction: Among the B-cell non-Hodgkin lymphomas, few of the lymphomas are associated with treatment refractoriness, progression, and dismal outcome, which include particularly activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (HGBL-DH – double hit lymphoma) and high-grade B-cell lymphoma-not other specified (HGBL-NOS).

Aims and Objectives: This study aims to study the clinicopathological and molecular profile of this aggressive group of lymphomas.

Methods: A total of 110 cases of DLBCL and 30 cases of HGBL-NOS were included in the study. Immunohistochemistry for CMYC and BCL2 was performed, followed by fluorescent *in situ* hybridization (FISH) in the double-positive DLBCL cases, recurrent or refractory to treatment cases and HGBL-NOS cases.

Results: The young adult male population dominated the cohort with a male-to-female ratio of 2.9 in DLBCL and 2.3 in HGBL-NOS. The age group of 20–65 years formed majority (71.2% in DLBCL and 66.7% in HGBL-NOS). Among the DLBCL, 62.8% of the cases were post-germinal center subtype and five cases transformed from follicular lymphomas. Upon FISH analysis, rearrangement for both CMYC and BCL2 was seen in two cases of DLBCL and five cases of HGBL-NOS. Four cases of DLBCL and single case of HGBL-NOS showed BCL2 rearranged and two cases of DLBCL and six cases of HGBL-NOS showed solitary CMYC rearrangement. Twelve of DLBCL and two of HGBL-NOS were negative for both; however, the remaining cases were negative for CMYC rearrangement (BCL2 rearrangement was not performed at the outset).

Conclusion: Heterogeneity in the clinical and genetics of DLBCL and HGBL-NOS presents major challenges in preparing the criteria for CMYC FISH testing. As rearrangements of BCL2 and CMYC genes act as an important biomarker and aid in patient management, all cases of DLBCL NOS and HGBL should be screened to rule our HGBL with recurrent MYC and BCL2 rearrangement.

Keywords: CMYC, High-grade B-cell lymphoma-not other specified, Diffuse large B-cell lymphoma not other specified.

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