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OH-01

Comparison Analysis of Artificial Intelligence-Based Systems on Pathological Samples for White Blood Cell Differentiation in Peripheral Blood Smears

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Background: Recently, Artificial Intelligence (AI)-based systems have been developed to assess peripheral blood smears. These systems utilize image recognition with artificial neural networks (ANN) to classify white blood cells. However, variations in peripheral blood (PB) smear preparation can affect cell images, leading to misclassification by these AI-based systems. Thus, we evaluated an in-house developed deep-learning system (DLS) against a conventionally utilized ANN-based system with different smear preparation methods.

Methods: A total of 193 pathological PB samples were collected from patients with hematological and non-hematological conditions. For each sample, a pair of smears was prepared using two different methods: 1) SP-50 smears, prepared by automated slide preparation with double fan drying and staining (May-Grunwald-Giemsa, M-G) using the SP-50 system (Sysmex) and 2) SP-1000i smears, prepared by automated smear preparation with a single fan drying using the SP-1000i system (Sysmex) and manually stained with M-G. Digital images were captured using the DI-60 system, and cell classification was performed using both the DLS and DI-60 systems.

Results: The DLS and DI-60 showed excellent specificity and negative predictive value, exceeding 94% for all blood cell types except segmented neutrophils on both SP-50 and SP-1000i smears. However, sensitivity and positive predictive value for variant lymphocytes and neutrophil precursors, including promyelocytes, myelocytes, and metamyelocytes, fluctuated. Nonetheless, the DLS revealed better performance on both smear sets compared to the DI-60 system.

Conclusions: The DLS revealed consistent performance in the classification of white blood cells in pathological samples compared to the conventional AI-based blood cell classification system.

Keywords: White blood cells, Automated image analysis, Smear preparation, Cell classification

OH-02

Reactive Monocytes and Antibody-Synthesizing Lymphocytes: Promising Predictors for Positive Culture among Patients with Sepsis Admitted to the Intensive Care Unit

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Background: Among patients admitted to the intensive care unit (ICU), sepsis is a life-threatening condition which is often associated with high mortality and complications such as septic shock. Blood cultures are the gold standard for diagnosing sepsis; however, a positive result is time-consuming, and ongoing antibiotic treatment can result in negative cultures. This study aimed to identify potential extended inflammatory parameters for the early diagnosis of positive-culture sepsis among critically ill patients.

Methods: During the study period, 99 ICU patients and 30 healthy donors (controls) were recruited. The study received ethical approval from USM/JEPeM/21010131. Blood samples from these patients were analyzed for complete peripheral blood counts using the Sysmex XN-1000 hematology analyzer (Sysmex Corp., Kobe, Japan), C-reactive protein, procalcitonin, interleukin 6, and blood cultures. Hematology parameters, including extended inflammatory parameters, were retrieved from the hematology analyzer.

Results: Of the 99 ICU patients, 40 had positive cultures, 36 had negative cultures but were diagnosed with sepsis, and 23 were non-septic according to the SOFA scoring system. The mean age of the patients was 55.95 years, with a male predominance. There was a significant mean difference ($P < 0.05$) among the four groups for the Reactive monocytes (RE-MONO), Antibody-synthesizing lymphocytes (AS-LYMPH), and Delta-He variables. Logistic regression results indicated that RE-MONO and AS-LYMPH are significant predictors of positive cultures.

Conclusions: The study found that RE-MONO and AS-LYMPH, which are readily available markers from the hematology analyzer, are potential markers for diagnosing sepsis in ICU patients. These parameters can be predictors of positive blood cultures, offering valuable tools for early identification of sepsis, thereby enabling prompt treatment initiation.

Keywords: Reactive monocytes, Antibody-synthesizing lymphocytes, Positive culture sepsis, Intensive care unit

OH-03

Reclassification of Acute Myeloid Leukemia According to the 2022 World Health Organization Classification and the International Consensus Classification Using Open-Source Data

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Background: In 2022, the World Health Organization (WHO) and International Consensus Classification (ICC) for myeloid neoplasms were published. This study examined the impact of these guidelines on acute myeloid leukemia (AML) diagnoses alongside the 2022 European LeukaemiaNet (ELN) recommendations on risk stratification.

Methods: Using the cBioPortal open-source dataset, 450 adult patients with newly diagnosed AML (non-acute promyelocytic leukemia) were included in this study. Diagnoses and risk stratifications were rearranged based on the new guidelines. Survival analyses were performed using Cox regression.

Results: Among the enrolled patients, 190 (42.2%) and 225 (50.0%) had consistent and differing diagnoses, respectively, across the three classifications. The two major WHO 2017 subtypes, AML-not otherwise specified and AML with myelodysplasia-related changes, were further subdivided according to the WHO 2022 and ICC. The ICC had better prognostication power across the three classification guidelines. Subgroup analysis according to different definitions of myelodysplasia-related AML and the introduction of AML with mutated *TP53* (AML-TP53) showed that differentiation of AML-TP53 was beneficial. The update from the 2017 to 2022 ELN demonstrated significant transitions in a subset of patients. The updated pairing of diagnostic classification and ELN risk stratification, specifically ICC and ELN 2022, demonstrated a straightforward relationship.

Conclusions: This study presents an integrative comparative analysis of past and current guidelines for AML diagnosis and risk classification using open-source data. The ICC diagnostic criteria are clinically significant for determining AML prognosis. Therefore, aligning with the changing treatment paradigm for AML, future research will be needed to continuously validate diagnostic and risk stratification systems.

Keywords: Acute myeloid leukemia, World Health Organization, Classification, International Consensus Classification, European LeukaemiaNet

OH-04

Reclassification of Myelodysplastic Neoplasms According to the 2022 World Health Organization and International Consensus Classification Using Open-Source Data: Focusing on *SF3B1*- and *TP53*-Mutated Myelodysplastic Neoplasms

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Background: In 2022, the World Health Organization (WHO) and International Consensus Classification (ICC) published diagnostic criteria for myelodysplastic neoplasms (MDS). This study examined the impact of the revised diagnostic criteria in classifying MDS in a large population.

Methods: We included 2,454 patients with MDS using the open-source data of cBioPortal. Patients were reclassified based on the new diagnostic criteria, and survival analyses were performed via Cox regression.

Results: Based on 2022 WHO criteria, 1.4% of patients were reclassified as having acute myeloid leukemia. The 2022 WHO criteria provide a prognostic diagnostic model superior to the 2017 criteria: Akaike information criterion, 14,152 vs. 14,516; Concordance Index, 0.705 vs. 0.681. For classifying MDS with low blasts and splicing factor 3B subunit 1A (*SF3B1*) mutation, a variant allele frequency cut-off of 5% (2022 WHO criteria) and absence of *RUNX1* co-mutation (2022 ICC criteria) were diagnostically relevant. Regarding the classification of MDS with mutated tumor suppressor p53 (*TP53*), a blast count cut-off of 10% (2022 ICC criteria) and multi-hit *TP53* (2022 WHO criteria) in cases where the blast was $\geq 10\%$ were independent risk factors.

Conclusions: Our study supports the refinements of the new WHO criteria and recommends the complementary use of the new WHO and ICC criteria in classifying *SF3B1*- and *TP53*-mutated MDS for better survival prediction.

Keywords: Myelodysplastic syndromes, *SF3B1*, *TP53*, World Health Organization Classification, International Consensus Classification, Open-source data

OH-05

Comprehensive Evaluation of *MYC* Rearrangements in Multiple Myeloma Using Optical Genome Mapping

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Background: *MYC* rearrangements in multiple myeloma (MM) occur

late in the disease and are associated with disease progression. The frequency of *MYC* rearrangements in newly diagnosed MM varies from 8% to 50% and depends on the method used to identify the rearrangements. The association of translocation and high expression of *MYC* with poor outcome has been previously reported, but the prognostic role of *MYC* rearrangement has not been fully established, possibly due to differences in detection methods and sensitivity. Here, we used optical genome mapping (OGM) for detection and characterization of *MYC* rearrangements.

Methods: We performed OGM using 30 multiple myeloma patient samples with a set of karyotyping, FISH, and targeted NGS results available. In samples detected with *MYC* rearrangements, FISH and immunohistochemical (IHC) staining targeting *MYC* were performed in parallel and compared based on the *MYC* rearrangement subtypes identified.

Results: We examined rearrangements of the *MYC* gene, including translocations, deletions, duplications, and inversions. Overall, after combined analysis of OGM and karyotyping, *MYC* rearrangements were detected in 43.3% (N=13/30) of patients. The majority of *MYC* rearrangements were translocations, and frequently recurrent gene partners were enhancers of immunoglobulin loci: IGH, IGL, and IGK. Translocation breakpoints were enriched between 128.6–129.4 Mb. Using a commercially available FISH probe with the most coverage of the above breakpoints, *MYC* rearrangements were detected in 26.7% (N=8/30) of samples. The rearrangements resulted in *MYC* overexpression, which was uniformly high in samples with translocations involving immunoglobulin loci.

Conclusions: Detection of different *MYC* rearrangements and understanding their impact on *MYC* overexpression are necessary for the reliable interpretation of *MYC* rearrangements in MM. OGM could be a reliable technique for comprehensive evaluation when interpreted alongside an expression detection method.

Keywords: *MYC*, Multiple myeloma, Optical genome mapping, FISH, Expression

OH-06

Using t-SNE and UMAP Algorithms in Flow Cytometry Analysis for Hematologic Malignancy: A Comparative Study with Conventional Manual Gating

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Background: Dimensionality-reduction (DR) algorithms are machine-

learning techniques that simplify multidimensional data by identifying similarities and representing them in a low-dimensional space. DR algorithms are broadly applied across various fields, including multi-parameter flow cytometry (MFC). This study evaluated the analytical utility of two DR algorithms—t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP), in MFC analysis of patients with hematologic malignancies and compared them with manual gating.

Methods: This study included patients who underwent bone marrow study and associated MFC from January 2020 to April 2024. A total of 199 samples were analyzed, encompassing different panels: acute leukemia orientation tube (61 samples, 8 colors), B-cell screening (36 samples, 8 colors), multiple myeloma (MM) panel (45 samples, 7 colors), B-cell acute lymphoblastic leukemia-measurable residual disease (B-MRD) (27 samples, 10 colors), and MM-MRD (30 samples, 10 colors). The flow cytometry standard data were analyzed using t-SNE and UMAP, implemented in FlowJo software ver.10.10.0 (Becton Dickinson, USA).

Results: Compared with manual gating, the total agreement in acute leukemia orientation tube was 77.0% (kappa=0.717) for t-SNE and 85.2% (kappa=0.818) for UMAP. Discrepancies were primarily due to differences in interpreting the intensity of markers such as weak positivity for myeloperoxidase, and variations in blast percentage, which affected leukemia diagnosis, with no cases being misclassified into other lineages. For B-cell screening, the agreement was 94.4% (kappa=0.903) for t-SNE and 97.2% (kappa=0.952) for UMAP. In MM, the agreement was 100% (kappa=1.000) for t-SNE and 91.1% (kappa=0.863) for UMAP. The agreement in B-MRD and MM-MRD was 96.3% (kappa=0.922) and 93.3% (kappa=0.866) for both algorithms. Quantitative correlations (r) were over 0.950 for all panels except MM-MRD (0.921 for t-SNE and 0.926 for UMAP). The DR algorithms identified cases of abnormal populations that were not detected by manual gating—one case in B-cell screening and one in MM-MRD.

Conclusions: This study is the first to use DR algorithms to diagnose hematologic malignancies through MFC. Both t-SNE and UMAP demonstrated good concordance and quantitative correlation with manual gating and identified minor populations not detected by manual gating. Applying DR algorithms in MFC is expected to reduce complexity, enhance objectivity, and serve as a complementary tool to manual gating.

Keywords: Flow cytometry, Dimensionality reduction, t-SNE, UMAP, Comparative study

OH-07

Association of *HOXA* and *HOXB* Genes Deregulation with *NPM1/FLT3* Genes Mutation in AML with Normal Karyotype

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Background: The expression of *HOX* genes is a common feature of AML. The deregulation of the *HOX* pathway results in abnormal cell differentiation and proliferation of hematopoietic stem cells, leading to leukaemic transformation. Several studies have shown that some mutations in AML were associated with *HOX* gene expression. Low or absence of *HOX* gene expression was found in AML with *PML-RARA* and *RUNX1-RUNX1T1* gene fusions, as well as mutations of the *CEBPA* gene. This study aims to elucidate the deregulation of *HOX* gene expression in association with *NPM1* and *FLT3* genes among acute myeloid leukaemia with a normal karyotype.

Methods: Deep transcriptome sequencing technology was used to examine *HOX* genes expression in 46 AML-normal karyotype patients at diagnosis, who were categorised based on the mutational status of the *FLT3* and *NPM1* genes: mutant (mut) or wildtype (wt). The association of the *HOX* genes expression was analysed among patients with different genotype groups: *FLT3-ITD/NPM1mut* (N=9), *FLT3-ITD/NPM1wt* (N=6), *FLT3wt/NPM1mut* (N=22), and *FLT3wt/NPM1wt* (N=9).

Results: Up-regulation of the expression of 14 *HOX*-genes (*HOXA2*, *HOXA3*, *HOXA4*, *HOXA5*, *HOXA6*, *HOXA7*, *HOXA9*, *HOXA10*, *HOXA11*, *HOXB3*, *HOXB4*, *HOXB5*, *HOXB6*, and *HOXB7*) in *NPM1* mutant groups (*FLT3-ITD/NPM1mut* and *FLT3wt/NPM1mut*) was seen compared to wildtype *NPM1* groups (*FLT3-ITD/NPM1wt* and *FLT3wt/NPM1wt*) and revealed statistically significant association between the *NPM1* mutant and wildtype genotype (Kruskal-Wallis' test with multiple pairwise comparison, $P < 0.01$). The expression was deregulated in the presence of *FLT3-ITD*. One example of the *HOX* gene is reflected in Figure 1.0. The *NPM1* mut groups (*FLT3-ITD* and *FLT3wt*) had higher normalized values than did the *NPM1wt* AML-NK patients in this cohort.

Conclusions: *NPM1* gene mutation plays a vital role in the upregulation of the *HOXA* and *HOXB* gene expressions in AML with normal karyotype, with concurrent *FLT3-ITD* modifying these genes' expression. The clinical significance of these findings needs to be evaluated in a larger cohort of AML patients.

Keywords: AML, Karyotype, *HOXA*, *HOXB*, *NPM1*, *FLT3-ITD*

OH-08

Next-generation Sequencing Identifies a Cohort of Germline Variants with Predisposition to Myeloid Neoplasms in Patients with Uncharacterised Cytopenia

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Background: Cytopenias often emerge as a prevalent clinical presentation in hematology practice, with numerous underlying causes such as nutritional deficiencies, infections, malignancies, immune disorders, or inherited conditions. Among these, germline variants predisposing individuals to myeloid neoplasms constitute a relatively rare yet significant subset of inherited disorders frequently associated with cytopenia(s). This study aims to assess the utility of genetic testing through next-generation sequencing in elucidating the cause of unexplained cytopenia(s).

Methods: This retrospective analysis spans three years of data from patients presenting with unexplained cytopenias at a single center. Routine investigations ruled out common causes of cytopenia(s). Subsequent patients underwent next-generation sequencing, primarily whole exome sequencing, after comprehensive clinical evaluation, imaging studies, complete blood count, peripheral smear evaluation, and biochemical studies. Bone marrow examination assessed cellularity and morphology across all cell lineages. Specific investigations included chromosomal breakage study and PNH testing via flow cytometry. DNA extraction from peripheral blood utilized automated methods, followed by NGS library preparation with commercial kits and sequencing on Illumina[®] platforms. Standard bioinformatic pipelines facilitated quality checks, read alignment, and pathogenicity testing, adhering to ACMG/AMP guidelines for variant reporting.

Results: Sixty-five patients, aged 15–74 years (mean-age: 35 years), were analyzed, with a male-to-female ratio of 2.3:1. Pancytopenia was observed in 40 patients, while the remainder exhibited single lineage cytopenia (anemia-6, neutropenia-4, thrombocytopenia-6). Bicytopenia was evident in nine patients. Six (6/65, 9.2%) individuals exhibited at least one genetic variant which was consistent with a germline variant predisposing to myeloid neoplasms. Identified variants included *ANKRD26* (2), *RUNX1* (2), *DDX41* (1), and *GATA2* (1), all displaying autosomal dominant inheritance patterns. Point mutations were noted in all cases. Patients were heterozygous for the variants with a variant allele frequency (VAF) of ~50%. Two patients displayed additional mutations. Confirmation via germline testing was achieved in four patients, while family testing was conducted in two cases.

Conclusions: Individuals with germline variants predisposed to myeloid neoplasms progress to organ dysfunction and are predisposed to myeloid neoplasms. Failure to recognize this entity amidst cytopenia(s) may lead to misdiagnosis (e.g., ITP) and incorrect management. Identification of the germline defect also necessitates close monitoring for non-hematopoietic organ system dysfunctions. Screening family members is crucial to identify silent carriers, especially pertinent in sibling or related donor selection for hematopoietic stem cell transplantation. Our study emphasises that next-generation sequencing is indispensable in identifying subset of patients with germline variants with predisposition to myeloid neoplasms in a cohort in unexplained or treatment-resistant cytopenias.

Keywords: Next-generation sequencing, Cytopenia, Germline variants, Myeloid neoplasms, Genetic testing, Hereditary cancer

OC-01

Implementation of Revvity Janus G3 Automated Workstations for Pretreatment Steps of Liquid Chromatography with Tandem Mass Spectrometry Analyzing Metanephrine and Normetanephrine

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Background: Recently, as the number of liquid chromatography with tandem mass spectrometry (LC-MS/MS) tests with high accuracy and precision has been increasing, sample pretreatment methods are also becoming increasingly complex and difficult. Thus, the burden on analysts to perform these pretreatments with manual methods continues to increase.

Methods: We implemented Janus G3 automated workstations (Revvity, Turku, Finland) for pretreatment steps of LC-MS/MS analyzing metanephrine and normetanephrine. The feasibility and analytical performances (precision, accuracy, and linearity) of the Janus G3 automated workstations were assessed and compared with those of manual sample preparation.

Results: The total pretreatment time of the Janus G3 automated workstations was 49 minutes, which was much shorter than that of manual pretreatment (130 minutes). The intra- and inter-run precision coefficients of variations were less than 5% for the metanephrine assay and less than 10% for the normetanephrine assay. The accuracy bias values were less than 5%, which were all acceptable. The verified linear intervals were 0.02–95.07 nmol/L for metanephrine and 0.02–102.34 nmol/L for normetanephrine. The comparison between the two pretreatment methods was good with a correlation coefficient of 0.9976, slope of 0.993 (0.982–1.004), and intercept of 0.005 (-0.008–0.018).

Conclusions: The Janus G3 automated workstations showed acceptable performances for pretreatment steps of LC-MS/MS analyzing metanephrine and normetanephrine.

Keywords: Automated pretreatment, Tandem mass spectrometry, Metanephrine, Normetanephrine

OC-02

Establishment of Reference Intervals for Thyroid Hormone Tests in a Korean Population by Using a Direct Sampling Technique

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Background: Thyroid hormones, such as thyroid-stimulating hormone (TSH), free thyroxine (fT4), and total triiodothyronine (tT3), are important biomarkers for monitoring thyroid function and diagnosing dysfunction. The concentrations of these hormones are affected by biologic factors, such as ethnicity, sex, and age, or environmental factors, such as lifestyle and diet. However, most laboratories use manufacturer-claimed reference intervals (RIs) established without considering these factors. Thus, we assessed the sex- and age-specific RIs for TSH, fT4, and T3 in a Korean population through a direct sampling technique.

Methods: Residual serum samples from adult patients who visited healthcare centers or outpatient clinics without any history of thyroid disease or positive antibody test results, family history or medication, were collected as reference after chart review. The values for TSH, fT4, and T3 were obtained by using specific reagents for each analyte on the Cobas e801 analyzer, and then the RIs were established using the non-parametric method recommended by the Clinical & Laboratory Standards Institute EP-28A guideline. Values at the 2.5th and 97.5th percentiles were determined as the reference lower and upper limits, respectively. This statistical process was performed using MedCalc software.

Results: A total of 618 subjects, including 185 men aged 20–64 years, 149 women aged 20–64 years, 141 men aged ≥65 years, and 143 women aged ≥65 years, were enrolled. In these four subgroups, the RIs for TSH were 0.645–4.90, 0.339–6.15, 0.355–6.21, and 0.159–6.23 µIU/mL; 1.06–1.79, 0.952–1.59, 0.849–1.72, and 0.937–1.80 ng/dL for fT4; and 0.748–1.54, 0.753–1.47, 0.428–1.62, and 0.576–1.50 ng/mL for T3, respectively. Compared with the manufacturer-claimed RIs of 0.270–4.20 µIU/mL for TSH, 0.93–1.7 ng/dL for fT4, and 0.8–2.0 ng/mL for T3, the Korean RIs for TSH showed a higher upper limit with 90% confidence intervals (CIs) not overlapping with manufacturer-claimed limit in all subgroups, whereas the RIs for T3 showed an upper limit lower than the claimed limit in all subgroups except for that of men aged ≥65 years. The RIs for fT4 did not significantly differ with the claimed value.

Conclusions: The RIs for TSH and tT3 in the Korean population significantly differed from those claimed by the manufacturer possibly because of the difference in the reference population. Therefore, test results in Koreans should be interpreted with caution, and RIs established from the Korean population would be required.

Keywords: Thyroid function test, Reference interval, Direct method

OC-03

Comparative Evaluation of the Analytical Performance of Three Automated Urine Sediment Analyzers with Cutoff Optimization

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Background: Although automated urine sediment analyzers have several advantages, a comprehensive evaluation of their clinical performance is lacking owing to the burden of pre-analytical controls. In this study, we systematically evaluated the UF-5000, UAS800, and Cobas u701 in a head-to-head comparison setting and established cutoff values for all sediment types, including the glomerular hematuria index.

Methods: A manual microscopic examination of 514 fresh urine samples was performed to identify 11 sediment types. To determine the bacteria/yeast and urinary red cell distribution (URD), the microbial culture and dysmorphic RBC percentages with hematuria results in 260 samples were evaluated, and the results were used as reference values. The optimized cutoff values for each sediment type on the three platforms were calculated using the area under the receiver operating characteristic curve (ROC-AUC).

Results: Although the UF-5000 showed excellent performance in the detection of non-squamous epithelial cells (ROC-AUC, 0.883; Spearman's correlation coefficient, 0.495), the UAS800 and Cobas u701 presented higher sensitivity for non-amorphous crystal identification. Through the cutoff optimization process, all three platforms presented clinically acceptable performances for bacterial detection, with the UF-5000 exhibiting a 20% higher ROC-AUC compared with the other analyzers. Although the URD did not show a good correlation with the dysmorphic RBC percentage, this index could provide higher accuracy for glomerular hematuria recognition when a high-sensitivity cutoff was applied.

Conclusions: Although the three analyzers presented superior clinical performances in distinctive sediment types, the overall performance was improved when optimized cutoff values were applied through a systematic head-to-head comparison. Our results show different characteristics of the three platforms, with greater performance for clinically significant sediment types.

Keywords: Automated urine sediment analyzer, Cutoff, Optimization, u701, UAS800, UF-5000, Urinary red blood cell distribution

OC-04

Frequency of Analytical Errors in Blood Chemistry Tests

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Background: Most studies on laboratory errors have been on those that occur in the pre-analytical and post-analytical phases. Limited studies were published regarding errors in the analytical phase. In this study, we analyzed the retest results to determine how often errors occurred during the analytical phase.

Methods: A total of 58,637 retests performed from January 2018 to June 2022 were included. Retests were conducted when analytical errors were suspected during the analytical phase. The 23 blood chemistry tests analyzed were as follows: albumin, total protein, ALP, ALT, AST, CPK, creatinine, ammonia, total CO₂ glucose, HDL cholesterol, LDH, LDL cholesterol, total cholesterol, TG, magnesium, P, Ca, K, Na, Cl, gamma-GTP, and total bilirubin. Analytical error was defined as a difference exceeding 10% between original and retest values. When the original value was very small, we employed the absolute numerical difference instead of the percent difference. If the original values were beyond the analytical measuring range, the retest results were excluded from the study.

Results: Of the 1,127,007 albumin results, 1,158 results (0.10%) were retested. Of the 1,142,029 glucose results, 3,767 (0.33%) were retested. Of the 1,258,245 Na results, 6,559 (0.46%) were retested. Of the 81,849 ammonia results, 1,766 (2.16%) were retested. The frequencies of analytical errors in 23 blood chemistry tests ranged from 0.04% to 19.67%. The frequency of analytical errors for ALT, AST, albumin, total protein, gamma-GT, Na, Cl, glucose, K, creatinine, total cholesterol, ammonia, and total CO₂ tests were 0.04%, 0.11%, 0.09%, 0.16%, 0.15%, 0.18%, 0.24%, 0.74%, 0.75%, 1.54%, 5.31%, 16.53%, and 19.67%, respectively.

Conclusions: The frequency of analytical errors for ALT, AST, gamma-GT, Na, Cl, total protein, and albumin is extremely low. Thus, retesting these blood chemistry tests during the analytical phase would be unnecessary.

Keywords: Analytical error, Analytical phase, Retest, Analytical error, Clinical chemistry

OC-05

Measurement of Serum LDL Cholesterol at Various Ranges of Total Cholesterol: When to Calculate and Measure?

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Background: One of the important risk factors for cardiovascular disease is LDL cholesterol (LDL-c). Beta quantification is the gold standard method for LDL-c measurement. However, many formulae have been developed owing to their labor intensiveness and costliness. Friedewald formula proposed in 1972, almost five decades old, continues to be the method of choice for LDL-c for many laboratories worldwide. Approximately 15 formulae were developed worldwide, and these formulae worked well within certain ranges of triglyceride (TG) levels; however, data paucity exists in evaluating these at different total cholesterol (TC) levels. We aimed to evaluate LDL-c measurements using different formulae with comparison to direct measurement at numerous TC ranges.

Methods: The retrospective study was conducted, and a total of 10,767 patients' lipid profile data were retracted from the clinical laboratory database. The TG, TC, HDL-c, and LDL-c were estimated in the Beckman AU680 analyzer after calibration and quality control. The Friedewald, Cordova, Anandaraja, Martin, and Sampson formulae were utilized to evaluate the calculated LDL-c. Bland-Altman plot and bias were used to evaluate the results. Intraclass correlations (ICC) were performed to assess the efficacy of each formula at different ranges of TC.

Results: The mean $\pm$ SD for TC was 161.44 $\pm$ 55.76, for TG was 155.0 $\pm$ 110.13, for HDL-c was 38.27 $\pm$ 12.10, and for LDL-c was 109.83 $\pm$ 41.81. We noted that all formulae calculated LDL-c significantly different from that of direct estimation ($P=0.0001$). On evaluating the performance of different formulae using Bland-Altman plots, the lowest bias was demonstrated using the Martin's (-12.45), followed by Sampson's formula (-14.24). When ICC with the absolute agreement was performed, only Martin and Sampson showed significant ICC >0.9 ($P<0.05$). On subcategorization based on different TC levels, Martin formula appeared to fare better than all other formulae in patients with TC >100 mg/dL with a bias of -12.39 $\pm$ 12.73 and ICC of >0.8 . However, none of the formulae appeared to estimate LDL-c at comparable levels in patients with TC <100 mg/dL. Furthermore, none of the formulae appeared to have an average ICC of 0.8 in patients with TC <100 mg/dL.

Conclusions: The study revealed the superior performance of Martin's formula over other formulae at a higher TC (>100 mg/dL) level for LDL-c measurement. Although calculated methods can be utilized at a higher TC level (TC >100 mg/dL) and a lower TC level (TC <100 mg/dL), direct measurement of LDL cholesterol may be adopted for better results.

Keywords: LDL cholesterol, Total cholesterol, Friedewald formula, Martin's formula, Sampson's formula, Cordova formula

OC-06

Updating Synoptic Reporting Through Semi-Automated Streamlined Interpretation of Protein Electrophoresis and Immunotyping

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Background: Protein electrophoresis (PEP) and immunotyping (IT) play pivotal roles in diagnosing and monitoring multiple myeloma. Recently, with the increasing incidence of multiple myeloma, the demand for PEP and IT tests has significantly surged. However, the conventional manual interpretation and reporting process has been laborious and prone to clerical errors. Despite international guidelines recommending synoptic reporting for standardized reporting, adherence to these guidelines has been limited in most laboratories. Hence, we developed a semi-automated software, EP Navigator, for interpreting PEP and IP to streamline the interpretation process and improve the standardization of reporting structures.

Methods: Using the CAPILLARYS 3 TERA instrument (Sebia USA, Norcross, GA, USA), PEP and IT were conducted, and all visible monoclonal proteins were quantified using the Sebia PHORESIS software. Patient demographic information, diagnosis, medication history (e.g., daratumumab), relevant test results (e.g., serum free light chains, immunoglobulin G/A/M), and previous PEP and IT test results were extracted from the hospital information system. Accurate data entry was ascertained by assigning separate subcodes to each reporting field, including the abnormal band position, monoclonal protein amount, isotype, interpretation, comments, and interpreter. Interpreted PEP and IT results were automatically transmitted back to the laboratory information system via an interface program.

Results: When PEP data generated by PHORESIS was imported into the EP Navigator, key information, such as the presence of monoclonal protein, abnormal band position, and monoclonal protein amount, was automatically populated. Additionally, appropriate interpretations and comments were automatically generated based on the presence or absence of monoclonal protein. The need for manual input by interpreters was minimized, except for the isotype from IT results, decreasing the likelihood of errors and interpretation time. Automatic synoptic reporting was facilitated by assigning standardized terminology codes to each subcode, promoting reporting format standardization across laboratories.

Conclusions: We developed the EP Navigator, a semi-automated software for PEP and IT interpretation, and implemented subcodes for each reporting field to allow synoptic reporting. EP Navigator is expected to streamline reporting processes for PEP and IT results and contribute to the standardization of reporting structures.

Keywords: Protein electrophoresis, Immunotyping, Multiple myeloma, Synoptic reporting, Semi-automated software, Standardized reporting

OC-07

Establishment of a Mass Spectrometry-Based Method for Therapeutic Drug Monitoring of Infliximab and Its Clinical Implication in Inflammatory Bowel Disease Patients

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Background: Therapeutic drug monitoring (TDM) can optimize infliximab treatment in inflammatory bowel disease (IBD). As immunoassay-based methods lack standardization and are prone to anti-drug antibody (ADA) interference, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is increasingly used for TDM, offering higher specificity and accuracy than immunoassays. In this study, we developed and validated an LC-MS/MS method for quantifying infliximab and evaluated its clinical efficacy with a large-scale clinical data.

Methods: Serum infliximab concentration was quantified on QTRAP 5500+ mass spectrometer (Sciex, USA) with an Agilent 1290 Infinity II HPLC system (Agilent Technologies, USA) using winged stable isotope-labeled peptides as internal standards. Linearity, lower limit of measuring interval (LLMI), limit of detection (LOD), selectivity, precision, accuracy, carryover, and ion suppression were evaluated. Method comparison against the two ELISA methods and evaluation of ADA interference were conducted. TDM data of 360 samples from 317 IBD patients receiving maintenance infliximab therapy were analyzed. Baseline demographics; infliximab regimen; laboratory data such as c-reactive protein, erythrocyte sediment rate, albumin, and fecal calprotectin; and clinical assessment data, including Mayo score, pediatric ulcerative colitis activity index, Crohn's disease (CD) activity index, and pediatric CD activity index, were collected.

Results: The analytical run time was 5 mins/sample, and sample preparation took 3 h/batch. LOD and LLMI was 0.25 µg/mL and 0.5 µg/mL, respectively, with linearity up to 50.0 µg/mL ($R^2=0.998$). Intra- and inter-day imprecision %CVs were <6.1%. Accuracy was 94.2–98.7%. Inter-assay variability and significant bias in ADA positive samples were observed for ELISAs. TDM data of 360 samples from 317 IBD patients (age: 25 ± 12.3 years) were analyzed. The median infliximab dose was 5.6 mg/kg (range: 4.7–11.2 mg/kg). Biochemical and clinical remission was achieved in 83% (299/360) and 83% (209/253) of cases, respectively. Infliximab concentrations were significantly higher in patients with biochemical and combined remission (achieving both biochemical and clinical remission). ROC of LC-MS/MS-measured infliximab concentration showed good discriminatory power for biochemical remission (AUC, 0.701; 95% CI, 0.618–0.785) while showing moderate power for combined remission (AUC, 0.649; 95% CI, 0.572–0.726) with a threshold of 2.4 µg/

mL (42.5%/84.4%). LC-MS/MS-measured infliximab concentration showed a more significant difference in remission rates than ELISA-measured infliximab concentration.

Conclusions: This simple and fast LC-MS/MS method for infliximab, developed for the first time in Korea, could optimize infliximab therapy, particularly in ADA-positive patients. LC-MS/MS-measured infliximab concentrations associated with higher biochemical and combined remission rates and distinguished remission statuses more effectively than ELISAs.

Keywords: Infliximab, Mass spectrometry, Therapeutic drug monitoring, Anti-drug antibody, Inflammatory bowel disease

OC-08

Advancements in HIV Management: Validating an LC-MS/MS Method for Multi-Drug Monitoring in Antiretroviral Therapy

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Background: Combination therapy with different antiretroviral agents has revolutionized the management of HIV-positive patients, transforming a lethal disease into a chronic pathology. Although efficacious, the adverse effects, toxicities, and adherence to medication remain critical to assess.

Methods: We have developed a liquid chromatography-tandem-mass spectrometry (LC-MS/MS) method for the simultaneous quantification of 7 antiretroviral drugs: Bictegravir, Emtricitabine, Dolutegravir, Cabotegravir, Lenacapavir, Fostemsavir, and Tenofovir alafenamide. Considering the prodrug status of the latter two compounds, we also included their active molecules, Tenofovir and Tenofovir, respectively, in the evaluation. The method was validated following the European Medicines Agency guidelines. Analytical standards for each drug were prepared from 99% pure powders of each molecule, and an internal standard—Amprenavir—was utilized for all testing sessions. Transition peaks were identified for each analyte, and the corresponding calibration curves were obtained. Precision, accuracy, and repeatability were assessed using three sets of intraday and interday calibration curves, along with quality control (QC) at low, medium, and high concentrations over three different testing sessions. Matrix effect, recovery, stability, and carryover were also evaluated. Clinical evaluation of plasma samples from patients undergoing treatment was also obtained.

Results: All analytes exhibited a good calibration curve linearity with an R^2 value greater than 0.99. Matrix effects were negligible (ranging from 93 to 113%), recoveries ranged between 83–110%, and stability was maintained after multiple freeze-thaw cycles without significant

degradation. Notably, only Cabotegravir exhibited significant carryover (14%). Clinical validation demonstrated that plasma concentrations were consistent with the pharmacokinetics of the tested drugs.

Conclusions: This method has the potential to facilitate accurate plasma monitoring of drug concentrations, which could lead to improved patient follow-up and personalized treatment strategies.

Keywords: Therapeutic drug monitoring, HIV

OC-09

Turnaround Time Improvement Using the Real-Time Monitoring System of General Chemistry Work Process

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Background: Shortening the turnaround time (TAT) from blood collection to result reporting in clinical laboratories is crucial for timely clinical decision-making and patient care. Various stages, such as order entry, sample collection, transportation, sorting, centrifugation, and analysis, can introduce delays, especially during peak testing hours. This study aimed to evaluate the impact of a real-time monitoring system on TAT improvement by effectively distributing samples across the analyzers for general chemistry tests.

Methods: We employed a real-time monitoring system integrated with three Cobas 8000 analyzers (Roche Diagnostics GmbH Mannheim, Germany) used for inpatient general chemistry tests at our hospital. We compared the segment TAT, defined as the time from sample scanning at the analyzers to initial result reporting before (November 2022) and after (November 2023) the system implementation. We analyzed the number of tests per analyzer and segment TAT during weekday peak hours, defined as periods with over 2,000 samples per 30 minutes.

Results: We calculated the peak hours, which were found to be from 5 AM to 9 AM. In November 2022, and the number of samples during the weekdays for inpatient general chemistry tests was 38,534, encompassing 254,705 test items. In November 2023, these numbers increased to 39,243 samples and 262,720 test items. During peak hours, the number of samples and test items was 18,042 and 133,029 in November 2022 and 19,125 and 144,819 in November 2023. Furthermore, the number of tests per analyzer during peak hours in November 2022 was 29,174 (21.9%), 59,560 (44.8%), and 44,295 (33.3%), compared to 41,910 (28.9%), 51,595 (35.6%), and 51,314 (35.4%) in November 2023. Based on the number of tests, the segment TAT during weekday peak hours was 28 minutes and 35 seconds in November 2022 but decreased to 25 minutes and 18 seconds in November 2023, a reduction of 3 minutes and 17 seconds (11.5%).

Conclusions: Despite an increase in the number of tests in November 2023 compared to November 2022, testing was more evenly distributed among the three Cobas 8000 analyzers in November 2023,

indicating improved operational efficiency. Moreover, the segment TAT from the sample scanning to initial reporting decreased during overall and peak hours, demonstrating the efficacy of the real-time monitoring system in reducing TAT.

Keywords: Clinical chemistry tests, Turnaround time, Monitoring

OC-10

Novel Usefulness of Mac-2 Binding Protein Glycosylation Isomer for Predicting Severity and 30-Day Mortality in Coronavirus Disease 2019

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Background: Mac-2 binding protein glycosylation isomer (M2BPGi) is a novel biomarker for liver fibrosis. However, its role in coronavirus disease 2019 (COVID-19) is unclear. We explored the prognostic role of M2BPGi in COVID-19 compared with age, SOFA score, and National Early Warning Score 2 (NEWS2) simultaneously.

Methods: In 53 patients (non-critical [N=16] and critical [N=37]) with COVID-19, M2BPGi levels were measured using the HISCL M2BPGi assay (Sysmex, Kobe, Japan) with the HISCL-5000 immunoanalyzer (Sysmex). M2BPGi levels were analyzed according to disease severity and 30-day mortality. The 30-day mortality was analyzed according to age ≥ 65 years, M2BPGi, SOFA score, and NEWS2 using the receiver operating characteristic (ROC) curve and Kaplan-Meier methods.

Results: M2BPGi levels were higher in the critical disease and non-survivor groups than in the non-critical disease and survivor groups (non-critical vs. critical, 0.9 vs. 2.0 cut-off index [COI], $P=0.07$; survivors vs. non-survivors, 1.5 vs. 2.7 COI, $P=0.01$). The results of ROC curve analysis revealed that the M2BPGi, SOFA score, and NEWS2, except age ≥ 65 years, significantly predicted 30-day mortality (all $P < 0.05$). The results of Kaplan-Meier survival analysis showed that the hazard ratios (HRs) 95% confidence interval (CI) for age ≥ 65 years, SOFA score, and NEWS2 were 2.2 (0.6–7.3), 15.8 (3.2–78.0), and 4.5 (1.4–14.4), respectively. With the addition of M2BPGi to age ≥ 65 years, the SOFA score, NEWS2, and HRs (95% CI) were 3.7 (1.1–11.7), 27.8 (5.0–151.0), and 9.7 (2.9–32.8), respectively.

Conclusions: M2BPGi levels were associated with disease severity and 30-day mortality in COVID-19. M2BPGi may be a useful biomarker in COVID-19, but further evaluation is warranted.

Keywords: M2BPGi, COVID-19, Severity, 30-day mortality, SOFA, NEWS2

OC-11

Utility of 2020 ESC NSTEMI-ACS 0/1 Hour Algorithm Using High-Sensitivity Cardiac Troponin I for Risk Stratification in Patients with Acute Chest Pain: A Single-Center Study

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Background: The high-sensitivity cardiac troponin I (hs-cTnI) assay is a widely employed method for diagnosing non-ST segment elevation (NSTEMI) acute coronary syndrome (ACS). Based on this assay, the European Society of Cardiology (ESC) announced the 2020 NSTEMI-ACS 0/1 hour algorithm (2020 ESC 0/1 hour algorithm), which classifies patients with acute chest pain into three groups (rule-out, observe, and rule-in group) by reviewing symptom onset and hs-cTnI values. In this study, we evaluated the 2020 ESC 0/1 hour algorithm for risk stratification in patients with acute chest pain.

Methods: From August 2022 to July 2023, hs-TnI was measured using Atellica IM Analyzer (Siemens Healthcare Diagnostics Inc., USA) in 11,685 patients who visited Konkuk University Medical Center's emergency room (ER) suspecting NSTEMI-ACS. Among them, patients to whom the 2020 ESC 0/1 h algorithm was applicable were included in this study. NSTEMI-ACS diagnosis was established by medical specialists upon discharge, and we analyzed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the diagnosis of NSTEMI-ACS in accordance with the 2020 ESC 0/1 h algorithm. Additionally, we assessed the 30-day mortality and hazard risk in rule-out, observe, and rule-in groups.

Results: A total of 4,751 patients were able to apply the 2020 ESC 0/1 hour algorithm. With this algorithm, 3,477 (73.2%), 575 (12.1%), and 701 (14.8%) patients were categorized into rule-out, observe, and rule-in groups, respectively. There were 90 (observed, five; rule-in, 85) patients who were diagnosed with NSTEMI-ACS, where the rule-out group exhibited a 100% sensitivity (95% confidence interval [CI]: 99.9 to 100%) and 100% NPV (96.0 to 100%) while the rule-in group demonstrated a specificity of 86.8% (85.8 to 87.7%) and a PPV of 12.1% (11.2 to 13.1%). Survival analysis revealed statistical significance not only among three groups (log-rank $P < 0.001$) but also in each of the two groups (post-hoc comparison; $P < 0.001$). Setting the rule-out group as baseline (age/sex adjusted), Cox hazard risk of the observed and rule-in group was 3.55 (95% CI: 1.84 to 6.86) and 11.14 (6.24 to 19.89), respectively.

Conclusions: The 2020 ESC 0/1 h algorithm using the hs-cTnI assay demonstrated excellent performance for the diagnosis of NSTEMI-ACS without missing a patient falsely assigned to the rule-out group. This algorithm also demonstrated favorable risk stratification in three categorized groups, indicating the clinical utility in patients visiting the ER with acute chest pain.

Keywords: High-sensitivity cardiac troponin I, Non-ST segment ele-

vation, Acute coronary syndrome, European Society of Cardiology, 0/1 hour algorithm

OM-01

Evaluation of QMAC-dRAST for Rapid Antimicrobial Susceptibility Testing of Enterobacterales-Positive Blood Culture

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Background: Bloodstream infection is becoming more difficult to treat due to increased antimicrobial resistance. Rapid identification and antimicrobial susceptibility tests (ASTs) are important for better clinical outcomes. QuantaMatrix dRAST system (QMAC-dRAST) can shorten the AST time significantly as it directly uses positive blood culture samples. Here, we compared the performance of QMAC-dRAST and BD Phoenix M50.

Methods: We retrospectively evaluated the AST results of 106 positive blood cultures from January 2024 to March 2024, on which both QMAC-dRAST V2.5 and BD Phoenix M50 were performed. For BD Phoenix M50, sediments were directly prepared from positive blood cultures using a Sepsiprep kit (ASTA, Korea). The AST results of 13 shared antimicrobial agents (ampicillin; amikacin, AN; ceftazidime; ciprofloxacin; cefazolin, CZ; ertapenem; cefepime, FEP; gentamicin; imipenem, IPM; meropenem, MEM; ampicillin-sulbactam, SAM; trimethoprim-sulfamethoxazole, SXT; and piperacillin-tazobactam) were compared, and accuracy was determined by calculating the categorical agreement (CA), very major error (VME), major error (ME), and minor error (mE). For cases showing discrepancy, disk diffusion (DD) was performed. In addition, the time to result (TtR) and escalation/de-escalation of antimicrobial treatment were also investigated.

Results: Among the 106 samples analyzed, *Escherichia coli* (67.0%) was the most common pathogen detected, followed by *Klebsiella pneumoniae* (25.5%). Except for FEP (CA, 83.0%), 12 antimicrobial agents showed CA over 90%. Among the 1,377 ASTs performed, 81 (5.9%) exhibited a discrepancy, and FEP (18/81, 22.2%) was the most common cause. In comparison to BD Phoenix M50, QMAC-dRAST exhibited an overall VME of 1.2% (5/425), ME of 0.5% (4/878), and mE of 5.2% (72/1,377). After the complimentary DD test, the overall VME was 1.9% (8/426; five with AN, two with CZ, and one with MEM), ME was 0.8% (7/883; four with SAM, two with IPM, and one with SXT), and mE was 2.0% (28/1,377), respectively. The average TtR was 20:48:58 and 30:05:14 for QMAC-dRAST and BD Phoenix M50, respectively ($P < 0.001$). Modification of antibiotics was observed in 27.4% of patients with extended-spectrum beta-lactamase (ESBL)-negative Enterobacterales (14/51; four de-escalation, four maintenance, and six escalation) and 71.4% of patients with ESBL-positive Enterobacterales (30/42; one maintenance and 29 es-

calation).

Conclusions: QMAC-dRAST gives reliable AST results for Enterobacteriales with shorter TtR, allowing fast and accurate treatment of bacteremia. Considering that BD Phoenix M50 AST is performed using colonies subcultured onto solid media in most laboratories, the TtR difference between the QMAC-dRAST and BD Phoenix M50 is expected to exceed nine hours.

Keywords: Antimicrobial susceptibility test, BD Phoenix, Enterobacteriales, ESBL, Time to result, Escalation, De-escalation

OM-02

Comparative Evaluation of Three MALDI-TOF Mass Spectrometry Systems for Identifying *Cryptococcus* Species

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Background: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows rapid and accurate identification of yeast species; however, varying reports on the reliability of *Cryptococcus* species identification have addressed the thick polysaccharide capsule to be interfering with the direct smear analysis. This study evaluates the effectiveness of additive extraction methods and the overall performance of the three latest generation MALDI-TOF MS platforms on the identification of *Cryptococcus* species.

Methods: Sixty clinical *Cryptococcus* isolates obtained from two tertiary hospitals in Korea were analyzed with VITEK MS PRIME (Knowledge Base V3.3), Bruker Biotyper Sirius (MBT Compass HT IVD 2023), and ASTA MicroIDSys. Samples were initially prepared with extended on-plate formic acid (FA) treatment and if needed, with in-tube extraction method according to the manufacturer's instructions. Mold kit was used when available, as in the case of in-tube extraction with VITEK MS PRIME and ASTA MicroIDSys. The identification rates of each instrument were evaluated according to differential pretreatment procedures and internal transcribed spacer and D1/D2 region sequencing was used as the reference method.

Results: When all methods were included, Bruker Biotyper correctly identified 55 (91.7%) isolates using a threshold of ≥ 1.8 , and VITEK MS PRIME correctly identified 55 (91.7%) isolates with consistent 99.9% confidence level. ASTA MicroIDSys correctly identified 49 (81.7%) isolates using a threshold of ≥ 140 . When only including the species included in each instrument's database, ASTA MicroIDSys correctly identified 49/54 (90.7%) isolates and VITEK MS PRIME correctly identified 55/59 (93.2%) isolates. In all platforms, no misidentification was noted. In Bruker Biotyper, repeated on-plate method resolved three unidentified species while in-tube method showed no resolution. For two isolates, additive tests for low confidence identifications resulted in no identification. In ASTA MicroIDSys, repeated on-plate method resolved seven unidentified specimens and in-tube

method resolved four unidentified specimens. In VITEK MS PRIME, both on-plate and in-tube methods helped identify one unidentified isolate.

Conclusions: Our study is the first to compare the sensitivity and accuracy of three latest MALDI-TOF MS instruments as well as the efficacy of pretreatment methods for *Cryptococcus* identification. More isolates were identified using VITEK MS Prime and Bruker Biotyper systems. In-tube extraction method for each instrument has different specifications and further study with strict adherence to the manufacturers' instructions (i.e., the storage time) should be followed. Considering the simplicity and the turnaround time, on-plate formic acid treatment can be suitable for routine clinical use.

Keywords: *Cryptococcus*, MALDI-TOF MS, Bruker Biotyper, ASTA MicroIDSys, Vitek MS Prime, Tube extraction method

OM-03

Bioinformatics-Driven Discovery of siRNAs Targeting Hepatitis C Virus Genotype 1

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Background: Hepatitis C virus (HCV) infection is a major contributor to liver-related ailments, such as cirrhosis, hepatocellular cancer, and the need for liver transplantation. Therefore, effective antiviral drugs to combat HCV infection are urgently needed. This study aimed to develop small interfering RNAs (siRNAs) targeting the 3'-untranslated region (3'-UTR) of HCV genotype 1.

Methods: Employing a multistep bioinformatics approach using siDirect, potential antiviral siRNAs were specifically designed for the 3'-UTR of HCV genotype 1. Candidate screening was done using the siRNA Scales tool, followed by additional analysis with MaxExpect and DuplexFold to evaluate the folding energy and binding affinity of siRNAs with their target, respectively. The efficacy of siRNA predictions was assessed using SMEPred.

Results: In total, 11 siRNAs were designed based on the complete genome of HCV genotype 1 (NCBI Accession Number: NC_004102.1). Further analyses using various bioinformatics tools identified four siRNAs with the potential to effectively silence the 3'-UTR of HCV genotype 1, achieving an efficacy level of 84.5%.

Conclusions: Overall, this study identified four promising siRNA molecules as potential candidates for antiviral siRNA-based therapies to mitigate HCV genotype 1 infection. However, the predicted siRNAs need to be validated through laboratory experiments.

Keywords: Hepatitis C virus, 3'-untranslated region, siRNA

OM-04

Complement C1q as a Non-Sputum Based Biomarker for Monitoring Anti-tuberculosis Drug Treatment

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Background: Indonesia has the second-largest number of tuberculosis (TB) cases globally. The diagnosis and treatment monitoring of pulmonary TB through sputum bacteriological examination presents several limitations. Recognizing this, the World Health Organization has adopted an approach utilizing non-sputum-based examination materials focusing on assessing the host immune response. This study aimed to compare serum complement 1q (C1q) levels in patients with active pulmonary TB before and after two and six months of TB treatment.

Methods: This cohort prospective study was conducted from December 2019 to May 2021 at Dr. Hasan Sadikin Hospital Bandung Indonesia. All patients were diagnosed according to the International Standard of Tuberculosis Care (ISTC) and were monitored after two and six months of TB treatment. Serum samples from active pulmonary TB patients aged ≥ 18 years were stored at minus 80°C until examination. C1q serum levels were analyzed using the sandwich enzyme-linked immunosorbent assay method (Human C1q Platinum ELISA kit/BMS2099 eBioscience, San Diego, CA). An anti-human C1q monoclonal antibody is coated and adsorbed onto microwells. Absorbance was measured at 450 nm using an LT-4500 microplate reader and was analyzed using the LT-com data analysis software. C1q concentration ($\mu\text{g/mL}$) was determined by interpolating sample absorbance to the standard curve. Ethical approval was obtained for this study (no LB.02.01/X.6.5/26/2021).

Results: The study revealed that from 40 subjects, the median serum C1q level before TB treatment was 42.18 (3.02–318.42) ng/mL. This significantly decreased after two and six months of TB treatment to 27.78 (2.81–235.99) ng/mL and 23.21 (1.19–271.95) ng/mL ($P < 0.05$), respectively.

Conclusions: Serum C1q levels of TB patients could serve as a non-sputum-based biomarker for monitoring TB treatment. While preliminary, these findings could inform future research.

Keywords: Active pulmonary TB, C1q complement biomarker, Monitoring anti-tuberculosis treatment

OI-01

Pterostilbene Loaded Lipid Polymer Hybrid Nanoparticles Protect the Rat Knee Joints against Collagen-Induced Arthritis by Suppressing $\text{NF-}\kappa\text{B}$ Signaling Pathway

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Background: Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation and damage to multiple joints, in which the immune system mistakenly attacks healthy tissues. This study aimed to fabricate and characterize lipid-polymer hybrid nanoparticles (LPHNPs) loaded with pterostilbene (PT), evaluate them against collagen-induced arthritis in rats, and explore the underlying mechanism.

Methods: Intradermal administration of 100 μg of bovine collagen II was used for arthritis induction in Wistar rats. Arthritis score, paw edema, paw volume, body weight, organ weight (spleen and liver), and organ index were estimated. Additionally, hematological and oxidative stress parameters, cytokine levels, and inflammatory and apoptotic parameters were estimated. The mRNA expression levels of inflammatory mediators and osteoclast markers were also estimated.

Results: PT-LPHNPs exhibited a particle size of 163.2 nm, entrapment efficiency of 96.53%, and zeta potential of -0.660 mV. Transmission electron microscopy and photomicrograph images revealed the dense spherical shape of the optimized formulation. PT-LPHNPs remarkably altered the arthritis score, arthritic biomarkers like tartrate-resistant acid phosphatase 5b and cartilage oligomeric matrix protein in the synovial fluid, body weight, organ weight (spleen and liver), and spleen and liver indices. PT-LPHNPs altered the levels of hematological parameters such as Hb, RBC, WBC, MCH, MCHC, and Plt and oxidative stress parameters such as MDA, GSH, SOD, CAT, and GPx. PT-LPHNPs significantly altered the levels of cytokines like IL-1, IL-1 β , IL-6, IL-10, IL-17, and IL-18 and inflammatory parameters such as COX-2, iNOS, PGE2, and NF- κB . PT-LPHNPs significantly ($P < 0.001$) altered the expression of inflammatory mediators such as IL-1 β , and TNF- α and osteoclast markers like tartrate-resistant acid phosphatase and cathepsin K.

Conclusions: The result showed the antiarthritic effect of PT-LPHNPs against collagen-induced arthritis via suppression of the $\text{NF-}\kappa\text{B}$ signaling pathway.

Keywords: Arthritis, Lipid-polymer hybrid nanoparticles, Inflammation

OI-02

Comparing SARS-CoV-2 Specific Cellular and Humoral Immunity in Vaccinated Healthcare Workers with Multi-Platform Analysis

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Background: Cellular and humoral immunity plays a crucial role in

the immune response to SARS-CoV-2. Nonetheless, the extent of its comparability and correlations in various platforms are not well studied. We compared cellular and humoral immune responses of two SARS-CoV-2 vaccinated groups using three SARS-CoV-2 T-cell assays and three antibody assays.

Methods: We enrolled 46 infection-naïve healthcare workers who had been exposed to heterogenous vaccination (35 ChAdOx1 nCoV-10 [ChAd]/BNT162b2 [BNT]) and homogenous vaccination (11 mRNA-1273 [Moderna]). SARS-CoV-2 specific T cell responses were assessed using interferon-gamma enzyme-linked immunospot (ELISPOT) and two commercial interferon-gamma release assays (IGRA) (QuantiFERON SARS-CoV-2 and Covi-FERON). We compared cellular immunity levels with total binding antibody and IgG binding antibody analyzed using two fully automated chemiluminescent immunoassays and neutralizing antibody from surrogate virus neutralization test (sVNT), both quantitatively and qualitatively.

Results: The Moderna group showed a significant increase in ELISPOT response at two months after the second dose, and this increase was even more pronounced one month after the third vaccination, when compared to the levels one month after the initial vaccination ($P < 0.05$). The ChAd group showed a peak ELISPOT response at 1 month after third BNT vaccination. The two IGRA tests showed the highest response at 3 months after the third dose in the Moderna group, while the ChAd group showed the highest response at 1 month after the third dose. Overall, the Moderna group showed higher ELISPOT and IGRA levels than the ChAd group. The positive rates of ELISPOT and IGRA varied depending on the type of vaccine and the post-vaccination period. Two IGRAs showed moderate correlations with ELISPOT in the Moderna group ($r=0.35-0.46$), while weak correlations were observed in the ChAd group ($r=0.19-0.36$). In the Moderna group, serologic assays exhibited weak to moderate correlations with ELISPOT, while in the ChAd group, IgG antibodies and sVNT showed weak correlations, with the total antibody assay not significantly correlating with ELISPOT. When comparing neutralizing antibodies with IGRA, there was a higher correlation between Covi-FERON and sVNT ($r=0.36-0.57$) compared to QuantiFERON and sVNT ($r=0.22-0.43$) in both groups.

Conclusions: Different correlations of SARS-CoV-2-specific cellular and antibody responses were observed depending on the platforms and vaccination type. To accurately assess the immune status of SARS-CoV-2, it is important to utilize various assays to measure both T-cell and B-cell responses.

Keywords: SARS-CoV-2, Cellular immunity, Humoral immunity, Vaccine

OI-03

Heme Oxygenase-1 and Interleukin-10 Levels in Transfusion-Dependent Thalassemia Patients with and without Alloimmunization/Autoimmunization

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Background: Recurrent blood transfusions are required to treat anemia in thalassemia. Thalassemia patients with repeated blood transfusions experiencing chronic hemolytic events. Alloimmunization is a complication of transfusion found in 3–16% of transfusion-dependent thalassemia (TDT) patients. Intravascular hemolysis produces heme. Heme also increases the expression of heme oxygenase-1 (HO-1), which acts as an immunomodulator. HO-1 stimulates Tregulator which expresses interleukin-10 which together affect the formation of antibodies by plasma cells. This study aimed to compare HO-1 and interleukin-10 levels in patients with and without alloimmunization/ autoimmunization.

Methods: This study used an observational analytic with a case-control research design that was performed between June-August 2023 at the Dr. Soetomo General Academic Hospital in Surabaya, Indonesia. It enrolled 20 TDT patients with alloimmunization/autoimmunization and 20 TDT patients without alloimmunization/autoimmunization. The patients received routine transfusions 1–4 times monthly and the number of transfusion frequencies ≥ 10 times. Venous blood samples were drawn from each patient gently. HO-1 and interleukin-10 levels were measured using sandwich enzyme-linked immunosorbent assay by Elabscience. Differences between HO-1 and interleukin-10 levels in thalassemia groups were compared using independent t-tests or Mann-Whitney U-tests ($P < 0.05$), and the correlation between HO-1 and interleukin-10 levels was analyzed using Spearman correlation.

Results: There was a significant difference in HO-1 levels between TDT patients with alloimmunization/autoimmunization (0.010–0.720 ng/mL [median: 0.072]) and TDT without alloimmunization/autoimmunization (0.020–1.720 ng/mL [median: 0.045]) P -value 0.020. Significant difference in interleukin-10 levels between TDT patients with alloimmunization/autoimmunization (0.056–2.356 pg/mL [median: 1.127]) and TDT without alloimmunization/autoimmunization (0.333–3.961 pg/mL [median: 2.638]) P -value 0.002 were observed. HO-1 and interleukin-10 had a significant positive correlation between TDT patients with alloimmunization/ autoimmunization (P -value 0.000 [$r=0.765$]), and the same in TDT group without alloimmunization/ autoimmunization (P -value 0.000 [$r=0.851$]).

Conclusions: HO-1 and interleukin-10 levels were lower in TDT patients with alloimmunization/autoimmunization than in patients without alloimmunization/autoimmunization. There was a positive correlation between HO-1 and interleukin-10 levels in TDT patients with and without alloimmunization/autoimmunization.

Keywords: Thalassemia disease, Transfusion-dependent, Heme oxygenase-1, Interleukin-10

OT-01

Investigation of the Efficacy and Safety of Wild-Type and Triple Gene-Knockout Pig RBC Transfusions in Nonhuman Primates

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Background: Decreasing rates of blood donation and close margins between blood supply and demand pose challenges in healthcare. Genetically engineered pig red blood cells (pRBCs) have been explored as alternatives to human RBCs for transfusion, and triple-gene knockout (TKO) modification improves the compatibility of pRBCs with human blood *in vitro*. In this study, we assessed the efficacy and risks of transfusing wild-type (WT)- and TKO-pRBCs into nonhuman primates (NHPs).

Methods: Blood from O-type WT and TKO pigs was processed to produce pRBCs for transfusion, which were transfused or not into NHPs (N=4 per group: WT, TKO, and control) after 25% total blood volume withdrawal. Subsequently, their biological responses were compared. Hematological, biochemical, and immunological parameters were measured before, immediately after, and at intervals following transfusion. Two months later, a second transfusion was performed in three NHPs of the transfusion group.

Results: Transfusion of both WT- and TKO-pRBCs significantly improved RBC counts and hematocrit and hemoglobin levels up to the first day post-transfusion, compared to the controls. The transfusion groups showed instant complement activation and rapid elicitation of anti-pig antibodies, as well as increased liver enzyme and bilirubin levels post-transfusion. Despite the higher agglutination titers with WT-pRBCs in the pre-transfusion crossmatch, the differences between the WT and TKO groups were not remarkable except for less impairment of liver function in the TKO group. After the second transfusion, more pronounced adverse responses without any hematological gain were observed.

Conclusions: WT- and TKO-pRBC transfusions effectively increased hematologic parameters on the first day, with rapid clearance from circulation thereafter. However, pRBC transfusion triggers strong antibody responses, limiting the benefits of the pRBC transfusion and increasing the risk of adverse reactions.

Keywords: Xenotransfusion, Porcine red blood cells, Non-human primates, Genetically modified pigs

OT-02

Challenges of Plasma Master File Preparation as an Important Step for Toll Fractionation Program in Indonesia

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Background: The national need for plasma-derived medicinal products (PDMP) in Indonesia is very high and tends to increase annually. The government intends to begin meeting national PDMP needs by implementing plasma fractionation, including an effort to increase national resilience in the health sector, considering that thus far, all PDMP needs have been imported from other countries. The MoH has prepared a national program, which implements Toll fractionation in the first 2 years, and hoped that fractionation manufacturing can be conducted domestically. The MoH has also appointed two national companies to implement this program along with their respective counterparts for fractionators in South Korea. To prepare plasma as raw material, blood establishments (BEs) must be GMP certified by Indonesian FDA. This study was conducted to review the challenges of BEs in preparing the Plasma Master File (PMF) as an important stage in providing plasma for fractionation.

Methods: This is a situational analysis based on data from Dr Sardjito Hospital BE Yogyakarta and MoH.

Results: PMF is a document that must be prepared to explain how plasma is obtained, processed, and controlled for safety by BEs which will then be processed into PDMPs by the fractionator. This document must be applied to the Indonesian FDA, fractionator, and Korean FDA for approval. For Indonesia, the PMF is completely new for BEs. WHO has helped by organizing a PMF workshop in Indonesia this year. Generally, the document contains safety strategies, and technical information on plasma, that is, the origin, quality and safety of plasma, plasma logistics information, and epidemiological data. The lookback standard procedure and its implementation must be proven in the PMF document. Another challenge is compiling data for the last 3 years regarding reactivity data of TTI screening using CHLIA and NAT. Donor notification and follow-up must also be included in the document. The information system should be appropriate and adequate; thus, the required data can be easily accessed and evaluated.

Conclusions: The origin, safety, and quality of plasma, epidemiology data of donors, and lookback practices are the important processes in PMF development to realize plasma as a raw material of PDMPs.

Keywords: Plasma Master File, Fractionation, Plasma-driven medicinal product

OG-01

Reclassification of *BRCA1* and *BRCA2* Variants of Uncertain Significance: Insights from Clingen Guidelines Application

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Background: In August 2023, ClinGen released *BRCA1/2*-specific interpretation guidelines, introducing several key modifications to the baseline ACMG/AMP guidelines. These modifications included: (1) Upweighting BP1 for variants without predicted splicing impacts, located outside of functional domains; (2) Designation of tools and thresholds for in-silico prediction codes PP3 and BP4; (3) Repurposing of PM5 to capture exon-specific evidence for pathogenicity of premature termination codon variants; (4) Adapting recommendations from a parallel working group for PVS1 and BP7; (5) Restriction of PS3/BS3 to exclude data from splicing-only assay; (6) Specification of cut-offs for allele frequency codes PM2, BA1, and BS1; and (7) Deletion of PS2/PM6, PS4_M, PM1 and PM4. We re-evaluated variants previously classified as a variant of uncertain significance (VUS) according to these new guidelines and discussed the implications of reassessment.

Methods: We reassessed 127 VUS (comprising 68 missense, 6 synonymous, 1 in-frame, 1 frameshift, and 51 non-coding variants) and 208 VUS (comprising 144 missense, 17 synonymous, 7 in-frame, 1 frameshift, 1 nonsense, and 38 non-coding variants) identified through *BRCA1/2* sequencing from December 2015 and May 2024 at Samsung Medical Center, applying the ClinGen gene-specific guidelines.

Results: A significant proportion of variants were reclassified to (L) BV: 80.8% for *BRCA1* and 72.8% for *BRCA2*. The most significant contributing evidence for reclassification was BP1_S, followed by the concurrent application of BP4 and BP7. Reclassification to (L)PV was rare: 2.3% for *BRCA1* and none for *BRCA2*. Functional codes played a role in these reclassifications. A notable proportion remained as VUS even after reassessment: 16.9% and 27.2% for *BRCA1* and 2, respectively. Most variants that remained as VUS were missense variants inside functional domains, followed by non-coding variants, either regulatory or not deep intronic. Frequency data and likelihood estimation had minimal impact on reclassification and occasionally contradicted other evidence.

Conclusions: The reclassification following the gene-specific guidelines significantly reduced the number of VUS, providing clearer information for patients and clinicians. However, the presence of contradictory evidences for specific variants highlights the need for further ethnic-specific characterization and clinical evidence accumulation, particularly for missense variants within clinically important functional domains.

Keywords: Reassessment, Variants of uncertain significance, *BRCA1*, *BRCA2*, Gene-specific guideline, Clingen

OG-02

A Circulating Global MicroRNA Panel as a Promising Non-invasive Early Diagnostic Biomarker for Prostate Cancer

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Background: Prostate cancer (PCa) is a heterogeneous complex treated by invasive procedures, such as transurethral resection of the prostate and radical prostatectomy, leading to serious complications. To minimize these inconveniences, researchers focused on circulating cell-free microRNAs (miRNAs), which are released into the blood serum/plasma as a result of apoptosis, necrosis, or secretions. Given their resistance to degradation, circulating miRNAs may act as potential biomarkers in early cancer detection and provide therapeutic aid in PCa treatment.

Methods: The expression profiles of miRNA-141, -1290, -100, and -335 were compared among patients with benign prostate hyperplasia (BPH), patients with PCa, and healthy volunteers. MiRNAs were isolated from the serum and quantified using RT-PCR. The diagnostic potential of the miRNAs was evaluated using receiver operating characteristic (ROC) curve analysis depending on the area under the curve, sensitivity, and specificity. Using statistical analysis, we correlated their expression with clinicopathological parameters, such as PSA level and Gleason score. The target genes and pathways involved were analyzed using in silico tools, including DAVID 6.8, TargetScan, miRDB, and miRWalk3.0.

Results: Among the non-invasive miRNAs assessed, miRNA-141 (BPH, $P=0.003$ and PCa, $P=0.0003$) and miRNA-1290 (PCa, $P<0.0001$) were significantly upregulated while miRNA-100 (PCa, $P=0.0002$) and miRNA-335 (BPH, $P=0.0002$) were significantly downregulated as compared with those in the controls. Analysis of the ROC curves showed a significant AUC for miRNA-141, -1290, -100, and -335. In silico analysis revealed that various target genes, including *EGFR*, *SMAD4*, *TP53*, *E2F3*, and *IGF1R*, were involved in crucial pathways responsible for PCa progression.

Conclusions: The estimation of cell-free miRNAs and their target genes provides a wholesome image of liquid biopsy for patients with PCa and healthy controls. This approach could aid in increasing the quality of life of patients and in developing clinical tests for the rapid and early diagnosis of PCa. It may also be a futuristic opportunity to detect resistant types of PCa, such as castrate-resistant PCa, at an early stage.

Keywords: Prostate cancer, MicroRNA, Non-invasive, Diagnostic biomarker

OG-03

Classic Hodgkin Lymphomas with Syncytial Morphology Exhibit a Primary Mediastinal Large B-Cell Lymphoma-Like Expression Signature by Lymph3Cx Analysis

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Background: Hodgkin lymphomas (HL) consist of two major types: nodular lymphocyte predominant HL and classic HL (CHL). CHL is further divided into four subtypes: nodular sclerosis (NSCHL), mixed cellularity (MCCHL), lymphocyte rich (LRCHL), and lymphocyte depleted (LDCHL). When neoplastic Hodgkin cells in NSCHL form prominent aggregates, this phenomenon is referred to as the “syncytial variant” of NSCHL (SVNSCHL). Although the Lymph3Cx gene expression profiling assay is designed to distinguish between primary mediastinal large B-cell lymphoma (PMBL) and diffuse large B-cell lymphoma (DLBCL), we aimed to determine the results of this testing in CHL cases since CHL has been reported to be closely related to PMBL.

Methods: We analyzed 26 CHL cases using the Lymph3Cx assay, including 10 NSCHL, 4 MCCHL, 2 LRCHL, 1 LDCHL, and 9 SVNSCHL. Case demographics, immunohistochemical findings, and Lymph3Cx results are summarized in Table 1. Tumor content was assessed using H&E-stained slides, and total RNA was isolated from additional unstained slides from the specimen. The RNA was hybridized overnight on a thermal cycler to the probes from the 58-gene Lymph3Cx assay panel. Probe/RNA complexes were quantitated on a NanoString nCounter® Digital Analyzer and subjected to algorithmic analysis for further classification and subtyping. The assay produces a calculated score from 0.00 to 1.00 to classify samples based on the probability that the sample is PMBL. Scores ≥ 0.90 are consistent with PMBL, scores ≤ 0.10 indicate DLBCL, and scores between 0.10 and 0.90 are classified as UNCLEAR.

Results: Lymph3Cx analysis showed that all nine cases of SVNSCHL exhibited a PMBL-like gene expression signature. In contrast, nine out of ten cases of NSCHL showed UNCLEAR results, and one case was classified as DLBCL. The four MCCHL cases showed three UNCLEAR results and one DLBCL classification. Both LRCHL cases classified as DLBCL, and the single LDCHL case classified as UNCLEAR.

Conclusions: Interestingly, all nine cases of SVNSCHL exhibited a PMBL-like gene expression signature (even though only two of the nine cases were actually located in the mediastinum), but none of the other CHL subtypes exhibited this signature. These findings suggest that SVNSCHL may represent a variant of PMBL rather than CHL. However, additional clinical correlation will be necessary to determine the true biological relationship between these two entities.

Keywords: Lymph3Cx analysis, PMBL expression signature, Syncytial variant nodular sclerosis classic Hodgkin lymphoma

OG-04

Reassessment of *FBN1* Variants of Uncertain Significance Using Updated ClinGen Guidance for PP1/BS4 Co-Segregation and PP4 Phenotype Specificity Criteria

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Background: Pathogenic variants in *FBN1* cause Marfan syndrome (MFS), which is characterized by thoracic aortic aneurysms, dissections, ocular, and skeletal complications. Keeping variant interpretations up-to-date is crucial for managing MFS and assessing family risk. Although guidelines proposed by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP), along with *FBN1*-specific guideline by ClinGen, have been introduced, many *FBN1* variants remain inconclusive. In 2023, ClinGen released new guidance for PP1/BS4 co-segregation and PP4 phenotype specificity criteria. Here, we performed re-evaluation of variants previously classified as variants of uncertain significance (VUS) at Samsung Medical Center using this updated guidance and assessed its clinical utility.

Methods: We reclassified *FBN1* variants identified as VUS through Sanger or next-generation sequencing from December 2015 and April 2022 according to *FBN1*-specific guidelines. These VUS were reassessed using the 2023 ClinGen guidance for PP1/BS4 and PP4 criteria, which integrates co-segregation and phenotype evidence in a point-based system. Considering the 91% diagnostic yield for *FBN1* for MFS, variants found in patients who meet the Ghent criteria can be assigned up to +5 points for PP4 phenotype specificity. Medical records and literature were reviewed to evaluate the phenotype specificity and co-segregation data. We compared the reclassification results with and without applying the 2023 ClinGen guidance.

Results: Of 695 patients with suspected MFS who underwent the *FBN1* sequencing, 61 VUS were detected in 69 patients. Using *FBN1*-specific guidelines, 4 (7%) VUS were reclassified as pathogenic variant (PV), 34 (56%) as likely pathogenic variant (LPV), and 23 (38%) remained as VUS. With the 2023 ClinGen guidance, 16 (26%) were reclassified as PV, 29 (48%) as LPV, and 16 (26%) remained as VUS.

Conclusions: Applying the 2023 ClinGen guidance considerably increased the reclassification of VUS to PV or LPV from 63% to 74%, while reducing the proportion of VUS from 38% to 26%. Our results indicate that the implementation of 2023 ClinGen guidance for applying PP1/BS4 and PP4 criteria provides evidence for the pathoge-

nicity of *FBN1* variants, contributing to a reduction in VUS and ensuring more accurate variant classification.

Keywords: *FBN1*, Marfan syndrome, Variant reassessment, ClinGen guidance, Co-segregation, Phenotype specificity

OG-05

Regions of Homozygosity Detected by Clinical Chromosomal Microarray in Koreans

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Background: Regions of homozygosity (ROH) are continuous stretches of homozygous genotypes that can arise from parental relatedness or uniparental disomy (UPD), often associated with autosomal recessive disorders or imprinting diseases. We aim to identify and characterize ROH in a Korean population using clinical chromosomal microarray (CMA) data.

Methods: Between 2019 and 2024, 1,719 subjects underwent chromosomal microarray testing using the CytoScan Dx Assay (ThermoFisher) at the Medical Genetics Center, Asan Medical Center, South Korea. We identified ROH larger than 3 Mb using Chromosome Analysis Suite Dx Software. Further, ROH frequency and distribution by sizes and positions were analyzed. Cases of identity-by-descent (IBD) and UPD were analyzed based on previously suggested clinical reporting criteria: UPD was suspected when the ROH size is equal to or larger than 5 Mb in telomeric regions on any single chromosome, equal to or larger than 10 Mb and 15 Mb for a single interstitial ROH on imprinted chromosomes (6, 7, 11, 14, 15, and 20) and non-imprinted chromosomes, respectively, and equal to or larger than 15 Mb for multiple interstitial ROH additively on any single chromosome.

Results: Among 1,625 subjects (94.5%), 4,007 autosomal ROH segments were detected. The distribution of maximum ROH size in each subject was 81.8% for 3–5 Mb, 15.3% for 5–10 Mb, 1.5% for 10–15 Mb, 0.4% for 15–20 Mb, and 0.8% for >20 Mb. The top 5 hotspots were 16p11.2-11.1 (85.1% of ROH detected subjects), 11p11.2-p11.12 (19.1%), 1p33-p32.3 (12.7%), 1q21.2-q21.3 (11.4%), and 3p21.31-p21.2 (8.4%). The top 5 ROH regions on imprinting chromosomes were 11p11.2-p11.12 (19.1%), 15q15.1-q21.1 (2.5%), 7q11.21 (2.4%), 20q11.21-q11.22 (1.6%), and 20q11.22-q11.23 (1.4%). Suspected UPD was detected in 51 subjects (3.0%), with 71 ROH segments. Among them, four were whole-chromosome UPD on chromosomes 2, 6, or 7.

Conclusions: This study demonstrates the utility of clinical CMA analysis in identifying ROH in a Korean population. The detection of IBD and UPD underscores the importance of ROH analysis in understanding genetic disorders and their underlying mechanisms. Further research is warranted to explore the clinical implications and enhance genetic counseling and management for affected individuals.

Keywords: Regions of homozygosity, Chromosomal microarray, Uniparental disomy, Identity-by-descent

OG-06

Genetic and Clinical Characteristics in a Korean Cohort of Retinitis Pigmentosa

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Background: Retinitis pigmentosa (RP, Online Mendelian Inheritance in Man [OMIM] #268000) is genetically heterogeneous retinopathy caused by photoreceptor cell death and retinal pigment epithelial atrophy that eventually results in blindness. Recently, over 90 genes (RetNet, <https://retnet.org>; accessed on 1 May 2024), with either autosomal dominant (AD), autosomal recessive (AR) or X-linked inheritance patterns have been associated with RP and make it difficult to genetic diagnosis. We aim to investigate the genetic and clinical characteristics in a Korean population with RP and contribute to genetic diagnosis.

Methods: A total of 433 unrelated Korean patients, clinically diagnosed with RP, were analyzed by targeted panel next-generation sequencing (NGS). These panel included 199 genes associated with RP and related eye disorders, conducted from May 2019 to April 2024 at a single tertiary clinic (Samsung Medical Center in Seoul, Korea). Detecting copy number variants (CNV) was based on the read-depth method in NGS and confirmed by multiplex ligation-dependent probe amplification. We performed Sanger sequencing to validate the potential pathogenic variants and segregation analysis. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to confirm abnormal splicing effects, and allele-specific PCR (AS-PCR) was conducted to verify *AluY* insertion in *RP1*. In X-linked inheritance disease, Hpa II PCR assay was performed to confirm a skewed pattern.

Results: A cohort of 433 unrelated Korean patients with a clinical diagnosis of RP and their available family members were enrolled in this study. We identified a total 505 pathogenic or likely pathogenic variants in 305 patients (305/433, ratio of 70.4%) including, 171 frameshift, 158 missense, 97 nonsense, 61 splice-site variant, 6 in-frame deletion, 4 *RP1* *AluY* insertion, 2 deep-intron variants, 1 synonymous and 5 CNV. In total, we solved 190 patients (190/433, 43.9%) and 243 patients (243/433, 56.1%) remained unsolved. The highest prevalence of mutated gene is *USH2A* (96/505, 19.0%) followed by *EYS* (84/505, 16.6%). It is noteworthy that in solved cases, the highest prevalence of mutated gene is *USH2A* (34/190, 17.8%), followed by *RPGR* (33/190, 17.3%) and *EYS* (22/190, 11.5%). These genes contribute to about half of the genetically solved cases in this study (46.6%). Considering the age at which genetically confirmed, 72.7% of the variants in *RPGR* were identified in those under 40 years while 68% in *EYS* were in over 40 years. Most of the solved cases were associated with AR inheritance (103/190, 53.9%), followed by X-linked inheritance (46/190, 24.1%) and AD/AR inheritance (30/190, 15.7%) and AD (12/190, 6.3%). We re-evaluated 22 variants as the pathogenic or likely pathogenic variants by segregation analysis (9 variants),

AS-PCR (4 variants), CNV (5 variants), RNA study (1 variant), and skewed X-inactivation (3 variants).

Conclusions: In this study, the overall diagnostic yield was 43.9% and the 69 causative genes detected from 433 patients with RP. We identified that the most common causative genes in our patient cohort were *USH2A* (17.8%), *RPGR* (17.3%), and *EYS* (11.5%), accounting for about half of the genetically solved cases. These genes were possible the hotspot genes in Korea. With potential future treatments for RP, including gene therapy, genetic diagnosis may help identify subjects that could qualify for currently approved gene therapies and ongoing clinical trials.

Keywords: Retinitis pigmentosa, Allele frequency, Retina, Vision disorders

OG-07

Changes in Gut Microbiota in Preterm Infants Associated with Bacterial and Fungal Bloodstream Infections

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Background: Bloodstream infection (BSI) is a common cause of mortality in premature infants globally. Given that interaction between pathogens and the gut microbiome plays a crucial role in the development and advancement of BSI in adults, we conducted a study on the gut microbiome of preterm infants during and following their recovery from BSI.

Methods: We examined 55 stool samples, with 38 coming from 13 infants diagnosed with BSIs due to methicillin resistant *Staphylococcus aureus* (MRSA, 5 infants), *Escherichia coli* (5 infants), or *Candida glabrata* (3 infants), and 17 from infants without BSIs who were admitted to the same neonatal intensive care unit. The DNA was amplified through polymerase chain reaction using 16S universal primers that targeted the V3–V4 region of the bacterial 16S ribosomal gene. Sequencing was carried out by Macrogen (Seoul, South Korea) on the Illumina MiSeq platform; the sequences were then analyzed using CLC Genomics Workbench v. 10.1.1 and CLC Microbial Genomics Module v. 2.5 (Qiagen, Hilden, Germany). Metagenomic studies of the internal transcribed spacer were conducted to analyze the mycobiome.

Results: The gut microbiomes of the five infants who had MRSA BSIs exhibited high levels of *Staphylococcus* and *Enterococcus* before receiving antibiotic treatment. Following antibiotic therapy, *Enterobacteriales*, particularly *Serratia*, became the dominant species. In contrast, *Enterobacteriales* remained prevalent in the gut microbiomes of the five infants with *E. coli* BSIs even after antibiotic treatment. These infants also displayed increased levels of *Enterococcus* and *Clostridioides*. The gut microbiomes of the three infants with *C.*

glabrata candidemia, which persisted for 51–80 days despite antifungal therapy, were characterized by *Candida* colonization and a low abundance of *Bifidobacterium*. However, this low abundance gradually increased after initiating feeding, and subsequent blood cultures showed no presence of *Candida*. On the other hand, the gut microbiomes of the 17 control infants had a wide variety of intestinal flora. There was no significant difference in α -diversity between the BSI and control groups. The analysis of β -diversity revealed the existence of three BSI clusters, with each cluster associated with a specific causal pathogen.

Conclusions: The infant gut microbiome exhibited notable disparities before and after BSI, underscoring the crucial role of the gut microbiome in the development and advancement of bacterial and fungal BSIs in premature infants.

Keywords: Microbiome, Bloodstream infection, Neonatal infection

OG-08

Evaluation of Regular PCR and Real-Time PCR Assays for Epstein-Barr Virus DNA Monitoring and the 1st WHO International Standard

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Background: Monitoring Epstein-Barr virus (EBV) DNA loads is important for the detection and prevention of EBV-related disorders. To measure the viral loads in patients with symptomatic EBV, we established regular PCR and real-time PCR assays for EBV DNA from EBV-positive cells (Burkitt lymphoma cell line, Akata) and the 1st WHO International Standard (NIBSC code 09/260), which consists of 5 million IU of a whole-virus preparation of the EBV B95-8 strain (type 1).

Methods: PCR was performed using a PCR thermal cycler (Takara) and Real-time PCR was performed using a StepOne Plus Real-Time PCR System (Applied Biosystems). To verify the regular PCR and real-time PCR results, we performed sequence analysis of PCR amplification products from the cell line Akata and the 1st WHO International Standard. For the EBV genome assay, nucleic acids from Akata cells and the WHO standard were extracted using the QIAGEN DNA extraction kit. Sequence analysis was performed by Eurofins Genomics.

Results: Amplification products of 90 bp were obtained when EBV DNA was amplified by PCR using primers for the EBV-encoded BALF5 gene. Nucleotide Blast analysis of DNA from Akata strain and the 1st WHO International Standard (B95-8 strain) showed a 100% homology with Akata (KC207813) and B95.8 (NC007605) references deposited in NCBI. The standard curve and a single melt curve peak were observed in Real-time PCR of the EBV-encoded BALF5 gene.

Conclusions: We established regular PCR and real-time PCR assays for the detection and quantification of EBV DNA from EBV-positive Burkitt lymphoma cell line, Akata, and the 1st WHO International Standard. Our findings will contribute to the understanding of diagnosing EBV infection and monitoring the viral loads of the EBV-related diseases.

Keywords: EBV, PCR, Real-time PCR, Sequence analysis, WHO 1st standard

OG-09

A Software Application for Automated Interpretation of Next-Generation Sequencing-Based Clinical Pharmacogenetic Panel

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Background: Interpreting clinical pharmacogenetic tests normally requires a process pipeline beginning with diplotype determination from detected variants. In the era of next-generation sequencing, with the expansion of genotype databases and targetable genomic ranges, this process has become highly challenging, especially for genes with over 100 known haplotypes or star alleles. This study aimed to develop a graphic user interface-based software that automatically derives diplotypes from identified variants and automates clinical report generation.

Methods: The software was developed using Java Version 1.8. Allele definition, functionality, and frequency data were downloaded from PharmGKB (<https://www.pharmgkb.org>) and used for diplotype determination and clinical report generation. To determine diplotypes from detected variants, candidate diplotypes were enumerated by combining all known haplotypes. The Jaccard distance, a metric used for measuring similarity between two sets, was calculated between the set of variants defined for each candidate diplotype and the set of detected variants, and was used for ranking diplotypes. The accuracy of diplotype determination was validated using reference materials from the genetic testing reference materials coordination (GeT-RM) program (<https://www.cdc.gov/labquality/get-rm/index.html>) and clinical specimens from patients from Korea. The software performance was compared with that of an existing tool, Aldy (version 4.5).

Results: Data were generated by targeted panel sequencing of 18 clinically relevant pharmacogenes. The software generated a ranked list of possible diplotypes for each sample-gene combination by inputting annotation files from multiple samples. For *CYP2D6*, exon-level copy number was manually incorporated to determine the final genotype. Accuracy was validated using 159 known genotypes from the reference material and clinical specimens. The highest-ranking diplotype provided by the software matched the known genotypes in 96.4% (132/137) of the GeT-RM materials and 95.5% (21/22) of the

clinical specimens. Most discrepancies resulted from the outdated genotype information. The concordance rates between our method and Aldy's method were 95.7% (286/299) for GeT-RM materials and 96.5% (276/285) for clinical specimens. Most discrepancies were caused by the inclusion of retired alleles in the Aldy database, variants not detected by Aldy, and unconfirmed phasing.

Conclusions: The software developed during this study could determine accurate diplotypes of hundreds of sample-gene combinations and generate clinical reports that reflect latest databases in real time. This software will significantly contribute to the clinical implementation of pharmacogenetic multigene panel tests by automating the entire process of pharmacogenetic interpretation.

Keywords: Pharmacogenetics, Next-generation sequencing, Automation, Software

OGL-01

Identification of Anti-Interferon-Gamma Antibodies in Patients Using Attenuated Total Reflectance-Fourier transformed Infrared Spectroscopy

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Background: Anti-interferon-gamma autoantibody (anti-IFN- γ auAb) is a cause of opportunistic infection in adult-onset immunodeficiency patients, presenting substantial diagnostic challenges. Recently, Fourier-transformed infrared (FTIR) spectroscopy has been increasingly used as a diagnostic approach.

Methods: We aimed to optimize the Attenuated Total Reflectance (ATR)-FTIR spectroscopy as a tool to differentiate plasma samples of patients with anti-IFN- γ auAb from other samples. We compared the spectral differences between serum and plasma samples using ATR-FTIR spectroscopy.

Results: Analysis of the average spectra from the CH stretching and fingerprint regions revealed some variations in the molecular profiles between plasma and serum, although no significant differences were found. Therefore, ATR-FTIR spectroscopy can be performed using either serum or heparinized plasma. Comparison between testing of plasma samples from patients with anti-IFN- γ auAb and healthy controls showed clear clustering by principal component analysis. Specifically, PCA in the fingerprint region showed a 77% difference at PC-1 between the patient group and healthy controls, whereas the amide region showed an 86% difference at PC-1. Using supervised learning, Partial Least Square discriminant analysis (PLS-DA) in the amide regions successfully distinguished between patients with anti-IFN- γ auAb and healthy controls. In a diagnostic evaluation, the PLS-

DA model showed high sensitivity and specificity for patients with anti-IFN- γ auAb.

Conclusions: ATR-FTIR spectroscopy shows potential as a tool for screening of plasma samples from patients with anti-IFN- γ auAb.

Keywords: Autoantibody, FTIR, Immunodeficiency, Infrared spectroscopy, Machine learning, Opportunistic infection

OGL-02

Double-Layer Microfluidic Electrochemical Biochip Based on Graphene Oxide-Nafion-Antibody Conjugate for *Opisthorchis Viverrini* Detection

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Background: The liver fluke, *Opisthorchis viverrini* (OV), which is procured through consumption of raw or undercooked (salted, pickled, or smoked) infected cyprinid fish, is the most important risk factor associated with cholangiocarcinoma (CCA). The limitations of conventional diagnostic tools for OV detection in terms of prolonged detection time and multistep procedure have led to an upsurge in demand for point-of-care testing that is helpful in timely and early action for better treatment.

Methods: In this study, a novel double-layer microfluidic electrochemical biosensor was designed to detect the OV antigen. For the biosensing chip, gold electrodes were fabricated using the conventional photolithography process. The working electrode surface was modified using an antibody-conjugated nanocomposite of graphene oxide and nafion (GO-Nf-mAb) in a single-step method, as a bioreceptor. Adding Nafion enhanced the adsorption and stability of the GO-mAb conjugate, thereby increasing the sensitivity of the biosensor. To evaluate the surface and structural properties of the GO-Nf-mAb sensor, synchrotron radiation-based X-ray absorption near edge spectra (XANES), X-ray photoelectron spectroscopy (XPS), and other surface characterization techniques were utilized.

Results: The covalent interaction between GO-Nf nanocomposite and mAb against OV antigen was validated by the specific peaks in XPS C1s, N1s, and O1s spectra and XANES C-K edge structure. This novel microfluidic device displayed a low limit of detection (0.43 ng/mL), wide linear range (0.02–200 μ g/mL), and high reproducibility

compared to the conventional detection methods. The proposed double-layer microfluidic was intended to facilitate immobilization and the electrochemical measurements by controlling the microvalve with a DC motor. This microfluidic is the first prototype for an automated microfluidic-controlled electrochemical biosensing platform.

Conclusions: Overall, the developed prototype offers high sensitivity, a single-step immobilization process, and a fluid-controlled system for OV antigen quantification. The proposed microfluidic electrochemical biochip is a promising tool for the point-of-care detection of OV antigens in high accuracy monitoring of disease progression.

Keywords: Microfluidic, Electrochemical biosensor, *Opisthorchis viverrini*, Graphene oxide, Nafion, Monoclonal antibody

OGL-03

Prevalence of Kidney Stone Composition in Northeastern Thailand: A Preliminary Study

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Background: Analyzing kidney stone components is essential for targeted treatment and preventing recurrence. Lifestyle is a risk factor for kidney stones, thus composition studies may encourage awareness. This study aimed to determine the prevalence and distribution of kidney stones in Northeastern Thailand.

Methods: Stone composition data were retrieved from September 2022 to May 2024. Stone composition was analyzed using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Grounded stone samples were placed directly on the ATR crystal to obtain the molecular vibrational spectra. The composition of the stone samples was identified using the OPUS Kidney Stone Library.

Results: A total of 201 data were acquired from 133 men and 68 women (66% and 34%, respectively). Most samples came from individuals in their 50s–70s individuals (55%), whereas an estimated 26% of the sample was found in individuals in their 40s–50s and 70s–80s. Approximately 4% (N=8) of the samples were found in children (2–14 years old), of which 4 samples were acquired from the urinary tract, whereas the remaining samples were identified as calcium bilirubinate from gallstones. Calcium stones were found in 75% of samples. Calcium oxalate is the most abundant compound, followed by a mixture of carbonate apatite and calcium phosphate. Furthermore, 14% and 7% of the samples were categorized as uric acid and struvite stones, respectively.

Conclusions: The prevalence of renal calculi was higher in males than in females. Moreover, these stones were mostly found in elderly

individuals. Most urinary calculi contained calcium oxalate, which reflects hypercalcinuria, hyperoxaluria, and hypocitraturia. The uric acid component, referred to as excessive uric acid excretion, corresponds to purine-rich intake, purine catabolism, and endogenous uric acid overproduction. The crystallization of magnesium ammonium phosphate or struvite is the result of urosepsis by urease-producing bacteria. In addition to etiology prediction, stone composition raises awareness of stone recurrence and urges a prevention regimen.

Keywords: Nephrolithiasis, Composition, Calcium oxalate, Uric acid, Struvite, ATR-FTIR, Spectroscopy

mones showed notable improvements with the introduction of a high-throughput analyzer and a TLA track connection system. The effects of the total test volume and day of the week should be collectively considered for an accurate TAT assessment.

Keywords: Turnaround time, Laboratory management, Tumor marker, Thyroid hormone, Automated analyzer, Total laboratory automation

UGL-04

Assessment of Turnaround Time for Tumor Markers and Thyroid Hormones Using Two Different Automated Analyzer Platforms

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Background: Turnaround time (TAT) is an important indicator of laboratory quality. To provide timely results of routine laboratory test that are promptly monitored by attending clinicians, establishing an appropriate target TAT limit along with excellent automated analyzers with acceptable analytical performance is essential for adequate laboratory management. In this study, we evaluated the TAT profiles of seven analytes measured using two different automated analyzer platforms in a real-world clinical setting.

Methods: TAT profiles of four tumor markers (CA19-9, CEA, fPSA, and PSA) and three thyroid hormones (fT4, T3, and TSH) were collected from a laboratory information system in a university cancer center laboratory over two independent 6-month periods. The measurement systems for tumor markers and thyroid hormones were changed from Roche Cobas e801 to Beckman Coulter Dxl and Abbott Alinity I to Beckman Coulter Dxl, respectively. Additionally, a Beckman Coulter total laboratory automation (TLA) track was applied to the new system. For the TAT analysis, achievement rates for TAT target goals and weekday trends were assessed after adjusting for total test volumes.

Results: With the introduction of the TLA track and analyzer changes, the average TAT failure rate decreased from 4.6 to 2.7%, with target goals set to 90 minutes from phlebotomy to result reporting. On comparing the TAT by days of the week, the TAT achievement rate increased on all days of the week, including Monday, which presented the lowest test volume among weekdays, increasing to over 97%. The reduction in TAT was more pronounced for thyroid hormones compared to tumor markers, possibly because of the change in analyzer throughput.

Conclusions: The TAT profiles of tumor markers and thyroid hor-

PH-01

Performance Analysis of CUBE30 TOUCH Analyzer

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Background: Although non-specific, erythrocyte sedimentation rate (ESR) is a routinely requested hematology test to assess inflammation status in various clinical settings. The laboratory's existing ESR analyzer VES-MATIC CUBE30 (VES) requires update due to its lifespan and related service availability. An upgraded model VES-MATIC CUBE30 TOUCH (CUBE30 TOUCH) was available for performance analysis to evaluate its suitability for diagnostic usage in the laboratory. The performance assessment study was conducted according to ICSH recommendation on modified Westergren method of ESR analysis. Precision, carry-over, measurement range, and comparability to the existing test platforms including the manual modified Westergren (Sediplast), including automated predicate (VES), were assessed.

Methods: A comprehensive precision study was conducted for both repeatability and reproducibility to fully measure the candidate analyzer's precision performance. Repeatability was conducted by processing quality control materials 20 consecutive times. Reproducibility was measured by processing control materials for 20 days in duplicates. %CV was calculated and evaluated against the manufacturer's precision claim. Carry-over was assessed using a high residual sample and the low control materials according to ICSH recommendation. The manufacturer's measurement range was also verified using high and low residual samples. Comparability was observed by processing each specimen with all three methods: Sediplast, VES, and CUBE30 TOUCH. Correlation was demonstrated using the R2 of the intercept and slope of the correlation plot and the 95% confidence interval using Passing-Bablok method. Additionally, bias was monitored using Bland-Altman analysis according to CLSI EP09-A03. Statistical tests were conducted using Analyse-it software.

Results: Acceptable repeatability and reproducibility were observed for CUBE30 TOUCH. No carry-over was detected. The manufacturer's measurement range (1–140 mm/h) was verified with residual samples. Overall, comparability is within the RCPAQAP analytical performance specification claims against both Sediplast and VES, with acceptable bias. However, when the cutoff of 10 mm/h was applied, the bias performance was poorer with VES.

Conclusions: The candidate analyzer CUBE30 TOUCH has demonstrated acceptable precision and overall comparability to the laboratory's existing analysis methods. Poorer correlation and bias performance for results <10 mm/h could be due to the limited sample size. A no carry-over and verifiable measurement range provide laboratory users assurance that the ESR result using the candidate method is as accurate and reliable as the current methods.

Keywords: Erythrocyte sedimentation rate, Performance analysis, Precision

PH-02

Monocyte Distribution Width, Procalcitonin, and C-Reactive Protein as Sepsis Predictor

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Background: Globally, sepsis remains a major cause of high morbidity and mortality and prolonged length of stay in health care systems worldwide. Early diagnosis and management is important in increasing survival, especially to prevent severe sepsis or septic shock. Monocyte distribution width (MDW) is a sepsis diagnostic indicator that is currently being developed with procalcitonin (PCT) and C-reactive protein (CRP).

Methods: An observational analytical study using a prospective cohort design was conducted in 178 patients with clinically diagnosed sepsis undergoing laboratory testing in the Clinical Pathology Laboratory of Dr. Moewardi Hospital Surakarta from October 2023 to February 2024. Research participants were taken using total sampling based on an inclusion and exclusion criteria. All statistical analyses were performed using SPSS version 27.0.0.0, and statistical significance was set at $P < 0.05$.

Results: The receiver operating characteristic (ROC) curve obtained the MDW cutoff point of 25.69 U (sensitivity of 84.3% and specificity of 63.8%), PCT cutoff point of 2.435 ng/mL (sensitivity of 80.9% and specificity of 61.4%), and CRP cutoff point of 3.71 mg/dL (sensitivity of 74.5% and specificity of 60.5%). Based on the ROC curve, the area under the curve of the MDW was 0.803 (95% confidence interval [CI]=0.740–0.840); P value <0.001, PCT was 0.771 (95% CI=0.703–0.840; P value <0.001), indicating that MDW can be a good predictor of sepsis. Multivariate analysis showed that MDW >25.69 U (odds ratio [OR]=3.288; 95% CI=1.573–6.873; $P < 0.002$); PCT >2.435 ng/mL (OR=4.211; 95% CI=1.697–8.997; $P < 0.001$), and CRP >3.71 mg/dL (OR=3.490, 95% CI=1.697–7.178; $P < 0.001$).

Conclusions: Therefore, every patient with sepsis should undergo an MDW, PCT, and CRP examination as an initial screening to determine the prognosis of sepsis.

Keywords: Monocyte distribution width, Procalcitonin, C-reactive protein, Sepsis, Predictor

PH-03

Performance of Deep Learning-based Digital Morphology Analyzer UIMD PBIA-12A for White Blood Cell Differentials

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Background: PBIA-12A (UIMD, Seoul, Korea) is a newly launched deep learning-based digital morphology analyzer that can be optimized in each laboratory. We investigated the performance of PBIA-12A in white blood cell (WBC) differentiation using the base and optimized models.

Methods: A total of 200 peripheral blood smear samples (100 normal and 100 abnormal samples) were evaluated using PBIA-12A (pre-classification and verification of) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (H20-A2). We compared the WBC differentials between the base and optimized models through manual counting.

Results: In the base model, the overall sensitivity, specificity, and efficiency of pre-classification were high for normal samples (51.7%–100.0%, 99.1%–100.0%, and 98.3%–100.0%). In the optimized model, the sensitivity, specificity, and efficiency of pre-classification were high for normal samples (83.0%–100.0%, 99.8%–100.0%, and 99.7%–100.0%). The absolute values of the mean differences between the PBIA-12A and manual counts ranged from 0.04 to 1.59. For 5-part differential count of WBCs, both the base model and the optimized model showed moderate or high correlations ($r=0.75$ – 0.99) with the manual count.

Conclusions: The optimized PBIA-12A model demonstrated an improved performance for WBC differentials in normal and abnormal samples. By applying the optimized model, PBIA-12A can become a reliable and flexible tool that supports clinical hematology laboratories.

Keywords: PBIA-12A, Digital morphology analyzer, Performance, WBC differentials, Deep learning, Optimized model

PH-04

Distribution Ranges of Apixaban Concentrations for Stroke Prevention in Korean Patients with Atrial Fibrillation

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Background: Apixaban is a direct-acting oral anticoagulant prescribed worldwide for stroke prevention in patients with atrial fibrillation. However, the distribution of apixaban use in the Korean population has not been well studied.

Methods: We conducted a single-center laboratory study to determine the distribution range of a twice-daily apixaban regimen prescribed for stroke prevention in routine clinical practice. Blood samples were obtained from patients who had taken apixaban for at least 1 week without missing doses or dose titrations. Trough and peak concentrations were measured using chromogenic assays with Bio-phen DiXal and heparin liquid reagent technology on a Sysmex CS-5100 Coagulation Analyzer.

Results: A total of 259 patients (mean age 69 ± 12 years, 58% male) were included, with 366 peak and 366 trough samples obtained. Peak concentrations in the standard ($N=255$) and appropriate dose reduction ($N=70$) groups were 222 ± 114 (range 35–781) and 165 ± 93 (range 16–454) ng/mL, respectively ($P<0.001$). Trough concentrations in the standard ($N=255$) and appropriate dose reduction ($N=68$) groups were 129 ± 69 (range 16–470) and 124 ± 61 (range 16–277) ng/mL, respectively. In the standard and appropriate dose reduction groups, 45 of 325 (13.8%) and 27 of 323 (8.4%) patients had peak and trough concentrations above the expected apixaban distribution ranges (69–321 ng/mL for peak and 34–230 ng/mL for trough) reported in clinical trials. Higher concentrations were more common in patients aged ≥ 60 years, with body weight ≤ 70 kg, or creatinine clearance ≤ 50 mL/min/1.73 m². In the standard-dose group, higher concentrations were more common in patients who met one of the three apixaban dose reduction criteria. Co-administration of amiodarone at 100–200 mg/day or dronedarone at 800 mg/day did not increase apixaban concentrations.

Conclusions: In some patients (approximately 8–14%) taking standard or appropriately reduced doses of apixaban, higher peak and trough concentrations above the expected distribution ranges were observed. Higher concentrations were more common in older patients and in those with low body weight or renal dysfunction.

Keywords: Apixaban, Atrial fibrillation, Stroke prevention

PH-05

Distribution Ranges of Edoxaban Concentrations for Stroke Prevention in Korean Patients with Atrial Fibrillation

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Background: Edoxaban is a direct-acting oral anticoagulant that is commonly prescribed for stroke prevention in Asian patients with atrial fibrillation (AF). However, the distribution of edoxaban use in the Korean population has not been well studied.

Methods: We performed a single-center laboratory study to determine the distribution range of once-daily edoxaban, which is prescribed for stroke prevention in routine clinical practice. Blood samples were obtained from patients who had taken edoxaban for at least 1 week without missing doses or dose titrations. The trough and peak concentrations were measured by chromogenic assays using Biophen DiXal and heparin liquid reagent technology with a Sysmex CS-5100 Coagulation Analyzer.

Results: A total of 209 patients (age 70 ± 11 years, 60% male) were studied, and 204 peak and 221 trough samples were obtained. Peak concentrations in the standard ($N=107$) vs. appropriate dose reduction ($N=67$) groups were 250 ± 105 (range 35–525) vs. 184 ± 80 (range 62–434) ng/mL ($P<0.001$). Trough concentrations in the standard ($N=116$) vs. appropriate dose reduction ($N=74$) groups were 51 ± 24 (range 23–165) vs. 48 ± 29 (range 19–179) ng/mL. In the standard and appropriate dose reduction groups, 45 of 174 (25.9%) and 102 of 190 (53.7%) patients had concentrations above the expected edoxaban distribution ranges (101–288 ng/mL for peak and 12–43 ng/mL for trough) that were reported in clinical trials. Higher concentrations were more common in patients taking P-glycoprotein inhibitors (amiodarone 100–200 mg/day or dronedarone 800 mg/day). In the appropriate dose reduction group, higher concentrations were more common in patients who met the multiple edoxaban dose reduction criteria.

Conclusions: Peak and trough concentrations above the expected edoxaban distribution ranges were observed in some patients receiving standard or appropriately reduced doses of edoxaban. Higher concentrations were more common in patients taking P-glycoprotein inhibitors or those meeting multiple dose reduction criteria.

Keywords: Edoxaban, Atrial fibrillation, Stroke prevention

PH-06

Routine Coagulation Testing Using EDTA Plasma in a Patient with Severe Hypercalcemia: A Case Report

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Calcium ions play a crucial role in the blood coagulation process, and patients with calcium metabolism disorders may experience coagulation abnormalities. EDTA, an anticoagulant used in diagnostic blood tests, chelates calcium by forming a stable, water-soluble complex, effectively removing calcium required for coagulation. Owing to its strong chelating effect, EDTA is unsuitable for coagulation tests, where sodium citrate is preferred. However, this report presents a case of EDTA plasma that was used for coagulation testing due to the unavailability of sodium citrate plasma. A 66-year-old female presented to the emergency department with generalized weakness, epigastric pain, and vomiting for 6 days. She was diagnosed with acute pancreatitis and acute kidney injury. Laboratory tests revealed

hypercalcemia with a high total calcium of 21 mg/dL, ionized calcium of 2.52 mmol/L, phosphorus of 3.1 mg/dL, low magnesium of 1.3 mg/dL, low 25(OH)D of 17.5 ng/mL, and elevated PTH of 1,744 pg/mL, indicating hyperparathyroidism. Parathyroid adenoma was confirmed via thyroid ultrasound and neck computed tomography. The patient's symptoms improved with symptomatic treatment, and she is on outpatient follow-up post-adenoma resection. Routine coagulation tests were initially performed using 3.2% sodium citrate tubes; however, the blood clotted, preventing plasma separation via centrifugation. This was attributed to severe hypercalcemia. Subsequently, coagulation tests using EDTA plasma demonstrated a protime (PT) of 18.8 s, international normalized ratio of 1.72, and activated partial thromboplastin time (aPTT) of 40.1 s. A comparative analysis of routine coagulation tests on sodium citrate and EDTA plasma from 20 healthy individuals indicated that EDTA plasma prolonged PT and aPTT by approximately 1.5-fold compared to sodium citrate plasma. While calcium measurements in sodium citrate plasma were feasible, they were undetectable in EDTA plasma due to chelation. It is hypothesized that supplying external calcium ions to EDTA plasma to neutralize the chelating effect could yield stable coagulation test results. This case suggests the need for developing laboratory standards for coagulation testing using EDTA plasma when sodium citrate is unavailable. Further research is warranted to ensure a reliable coagulation test.

Keywords: Hypercalcemia, Blood coagulation test, Edetic acid, Sodium citrate, Hyperparathyroidism

PH-07

Telomere Length and Genetic Variations in Acquired Pediatric Aplastic Anemia: A Flow-Fluorescence *in situ* Hybridization Study in Korea

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Background: Aplastic anemia (AA) is a rare bone marrow failure syndrome characterized by a notably short telomere length and is associated with treatment responses. We measured the telomere lengths in Korean pediatric patients with AA using flow-fluorescence *in situ* hybridization (Flow-FISH) and explored the association of telomere shortening with disease characteristics, genetic conditions, and pa-

tient outcomes.

Methods: We analyzed peripheral blood samples from 75 patients with AA and 101 healthy controls. Telomere length was measured using Flow-FISH, and the relative telomere length (RTL) and delta RTL were assessed. Genetic evaluations, including karyotyping, chromosome breakage tests, and clinical exome sequencing, were performed to identify the inherited bone marrow failure syndrome (IBMFS)-associated genetic variants.

Results: The telomere lengths in patients with AA were significantly lower than those in age-adjusted healthy controls. Patients receiving immunosuppressive therapy tended to have long telomeres, as indicated by high delta RTL values. Patients with genetic abnormalities, including karyotype abnormalities (N=2) and genetic variants (N=11) such as carrier of IBMFS-associated genes or variants of unclear significance, had significantly shorter telomeres.

Conclusions: This study reinforces the importance of telomere length as a biomarker of acquired AA. Flow-FISH proved effective in measuring telomere length, validating its suitability as an appropriate laboratory test. Patients with AA demonstrated a significant reduction in telomere length; importantly, longer telomeres were correlated with better outcomes in immunosuppressive therapy. Additionally, our genetic analysis underscores the role of IBMFS-associated gene variants in the pathophysiology of short telomeres.

Keywords: Aplastic anemia, Telomere length, Flow-fluorescence *in situ* hybridization

PH-08

Analysis of the Availability of Minimal Residual Disease Monitoring Methods for Patients with Acute Myeloid Leukemia in a Single Institute

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Background: The recurrence rates of acute myeloid leukemia (AML) remain high, underscoring the importance of monitoring minimal residual disease (MRD) after intensive therapy to guide treatment decisions and prognosis prediction. However, AML is a heterogeneous disease. Although various methods are employed for monitoring MRD, some patients only achieve cytomorphological remission. We aimed to evaluate the availability of MRD monitoring at a single institute to assess the need for new methods.

Methods: We retrospectively analyzed bone marrow studies performed at the time of AML diagnosis between September 2018 and July 2023. We evaluated the availability of MRD monitoring methods other than cytomorphological analysis, including flow cytometry, reverse transcriptase polymerase chain reaction (RT-PCR), and fluorescent *in-situ* hybridization (FISH). Flow cytometry was used in patients who demonstrated aberrant blast expression, RT-PCR was used to assess for fusion or mutation, and FISH was performed when adequate FISH

probes were available.

Results: Next-generation sequencing (NGS) panels were performed in 139 of 239 patients with AML. Of the 139 patients, 100 were assessed for relapse or progression. The median age was 62.6 (range: 5.7–90), with a male-to-female ratio of 69:70. Molecular diagnosis and MRD monitoring were performed in 68 patients (68/239, 28%). Meanwhile, 171 patients (72%) were diagnosed with AML, not otherwise specified, but were unable to undergo molecular MRD monitoring (Table 1). Venn diagrams showed the availability of MRD monitoring in 171 patients. In 42 patients (42/171, 25%) (42/239, 18%), flow cytometry, RT-PCR, and FISH were not applicable (Figure 1).

Conclusions: Despite recent advances in MRD monitoring technologies, a significant number of patients rely on cytomorphological analysis for MRD monitoring.

Keywords: AML, MRD, Flow cytometry, NGS, FISH, RT-PCR

PH-09

Category Assignment per the New WHO Classification in Acute Myeloid Leukemia: A Case-Based Review from the Practicing Hematopathologists' Viewpoint

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Background: Recent advances in the molecular genetic understanding of acute myeloid leukemia (AML) have required an update of diagnostic systems, resulting in a new World Health Organization (WHO) classification being proposed in 2022 (WHO-5th). Our goal was to give results from the application of this new system based on routine practice in a clinical laboratory.

Methods: Study cases were evaluated for the mutational status using NGS panel assay dedicated to hematologic cancers. *FLT3*-ITD mutation was assessed by a laboratory-developed fragment analysis. Cytogenetic analysis was performed using standard banding techniques and described according to the International System for Human Cytogenomic Nomenclature recommendations.

Results: A total of 739 patients with AML were reclassified according to the WHO-5th criteria. AML with defining genetic abnormalities (DGA) harboring recurrent cytogenetic abnormalities or mutations was the largest subgroup with 45.5% (N=336) of cases: *NPM1*-mutation (N=115, 15.6%), *CEBPA*-mutation (N=50, 6.8%), *PML::RARA* (N=47, 6.4%), *RUNX1::RUNX1T1* (N=40, 5.4%), *KMT2A*-rearrangement (N=36, 4.9%), *CBFB::MYH11* (N=24, 3.2%), *MECOM*-rearrangement (N=8, 1.1%), presumed *NUP98*-rearrangement (N=7, 0.9%), *DEK::NUP214* (N=6, 0.8%), *BCR::ABL1* (N=2, 0.3%) and *RBM15::MRTFA* (N=1, 0.1%). AML myelodysplasia-related (MR) was the second largest subgroup (N=269, 36.4%), followed by AML defined by differentiation (DIFF) (N=57, 7.7%), post-cytotoxic therapy (N=39, 5.3%), germline predisposition (N=25, 3.4%), myeloid sarcoma (N=4, 0.5%) and those cannot be classified (N=9,

1.2%). Compared to the size in the previous WHO criteria, the category of AML MR was enlarged (from 27.7% to 36.4%) and that of AML DIFF was reduced (from 15.0% to 7.7%). Twenty-one cases had a diagnostic ambiguity, including atypical patterns of *CEBPA* mutation (N=15) and *NPM1* mutation with myelodysplasia-related cytogenetic abnormalities (N=6). In addition, 14 cases had a diagnostic incompleteness, including presumed *NUP98*-rearrangement (N=8) and candidates for other DGA such as t(16;21)(p11.2;q22) (N=3), t(3;21)(q26.2;q22) (N=2) and t(12;22)(p13;q12) (N=1).

Conclusions: This analysis demonstrates that the new WHO system facilitates the diagnosis of AML MR subtypes typically associated with poor prognosis. However, some cases of *CEBPA*-mutation or *NPM1*-mutation subtypes suggest that this new system still needs fine-tuning. Confirmation of chromosomal rearrangements, that are newly integrated into the WHO system or clinically significant but hidden, requires the introduction of advanced technologies into routine laboratory testing.

Keywords: AML, WHO classification, Myelodysplasia-related, *CEBPA*, *NPM1*, *NUP98*

PH-10

A Study of Clinical Outcomes in Acute Myeloid Leukemia and Myelodysplastic Neoplasms According to the Fifth Edition of the World Health Organization and International Consensus Classification: A Single-Center Study

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Background: Recently, the new 5th edition of the World Health Organization classification (2022 WHO) and the International Consensus Classification (ICC) of myeloid neoplasms were published. We reclassified the patients according to the revised classification and analyzed their prognosis to confirm the clinical utility of the new classifications.

Methods: We included 101 adult patients, comprising 77 and 24 with acute myeloid leukemia (AML) and myelodysplastic neoplasms (MDS), respectively, who underwent bone marrow aspiration and NGS examination between August 2019 and July 2023. Additionally, we reclassified the patients based on the revised criteria. After reclassification, we investigated the differences in the newly diagnosed category and analyzed their prognosis using survival analysis.

Results: According to the 2022 WHO and ICC, 23 (29.9%) and 32 (41.6%) patients with AML, respectively, and 7 (29.2%) and 12 (50.0%) of those with MDS, respectively, were reclassified into differ-

ent groups. This was mainly due to the addition of myelodysplasia-related (MR) gene mutations to the diagnostic criteria and new entities associated with *TP53* mutation. The median overall survival (OS) of patients with AML with MR gene mutations was shorter than that of the other AML group; however, the difference was not statistically significant. The patients with AML and those with MDS with *TP53* mutation had a shorter OS than the other group ($P=0.0015$; median OS, 69 vs. 308 days). They also had significantly shorter OS than the other group with AML with MR gene mutations ($P=0.0021$; median OS, 69 vs. 289 days).

Conclusions: The new revised classification allows for more detailed categorization based on genetic abnormalities, which may be helpful in the prediction of prognosis. Particularly, AML with mutated *TP53* is a new category of ICC that can be more useful since it has shown high prognostic significance in a small number of cases.

Keywords: Acute myeloid leukemia, Myelodysplastic syndrome, World Health Organization, Mutation, Prognosis, *TP53*

PH-11

Acute Myeloid Leukemia with Mutated *NPM1*, *TET2*, and *CEBPA* mimicking HLA-DR Negative CD34 Negative Acute Promyelocytic Leukemia: A Case Report

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We report a case in which acute promyelocytic leukemia (APL) was initially suspected but was ultimately diagnosed as acute myeloid leukemia (AML) with nucleophosmin 1 (*NPM1*), Tet methylcytosine dioxygenase 2 (*TET2*) and CCAAT/enhancer-binding protein alpha (*CEBPA*) mutations. A 70-year-old female patient presented with poor appetite and worsening general weakness for over 2 months. Initial evaluation revealed severe anemia (hemoglobin level, 60 g/L), leukocytosis (white blood cell count, $64 \times 10^9/L$), and thrombocytopenia (platelet count, $32 \times 10^9/L$). She was admitted for further investigation with suspected hematologic malignancy. Bone marrow aspiration and biopsy revealed hypercellular marrow with 68.2% blasts (the presence of abnormal promyelocytes). Flow cytometric immunophenotyping showed that the leukemic cells were positive for CD13, CD33, MPO, and CD11c (intermediate) and negative for CD34 and HLA-DR. Bone marrow aspiration morphology and immunophenotyping were highly suggestive of APL. Promyelocytic leukemia/retinoic acid

receptor- α (*PML-RAR α*) rearrangements were not detected in molecular assays. Chromosomal analysis showed no abnormalities, whereas next-generation sequencing identified pathogenic variants in *NPM1*, *TET2*, and *CEBPA*. Consequently, she was treated with decitabine and venetoclax and was discharged upon improvement in absolute neutrophil count levels.

Keywords: CD34 negative HLA-DR negative, Immunophenotyping, *NPM1*, *TET2*, *CEBPA*

PH-12

Secondary B-Acute Lymphoblastic Leukemia Transformed from Therapy-Related Myelodysplastic Neoplasm in a Pediatric Patient: The First Case

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Acute lymphoblastic leukemia (ALL) transformation from myelodysplastic neoplasm (MDS) is extremely rare. Until now, approximately 50 cases of ALL secondary to MDS have been reported, and the underlying mechanism driving MDS clones to become leukemic lymphoblasts is not yet understood. Here, we describe the first case of B-ALL transformed from therapy-related MDS (t-MDS) in a pediatric patient who had a germline *TP53* mutation. A 3-year-old boy was initially diagnosed with rhabdomyosarcoma. Despite completing chemotherapy for rhabdomyosarcoma, he continued to experience anemia and thrombocytopenia for a year; therefore, a bone marrow (BM) study was performed. Granulocytic hyperplasia without morphological evidence of dysplasia of any lineage was observed; however, a cytogenetic study revealed a complex karyotype: 45,XY,-11,add(17)(p13)[6]/46,idem,+mar[13]/46,XY[1]. Therefore, a diagnosis of t-MDS was made. After 1 month, blasts appeared in peripheral blood, and a repeated BM study showed decreased tri-lineage hematopoiesis and 50% lymphoblasts with immunophenotypes of CD19+, cCD79a+, cCD22+, nTdT+, and CD34+. Finally, a diagnosis of B-ALL that progressed from t-MDS was assumed based on the more complex karyotype demonstrating clonal evolution of previously defined MDS clone: 45,XY,-11,add(17)(p13)[5]/46,sl,+mar1[6]/46,sdl,-13,+mar2[4]/44,sl,-Y,-17,-add(17),+mar3,+mar4[4]/46,XY[1]. Induction chemotherapy for B-ALL was initiated. In the subsequent BM study, no apparent leukemic blasts morphologically were observed, although a high proportion of cells with the complex karyotype of his MDS clone still existed: 45,XY,-11,add(17)(p13)[4]/47,idem,+2mar[4]/46,XY[12]. Subsequently, chemotherapy (high-dose cytarabine regimen) was administered, and his BM specimen eventually showed a normal karyotype. This case was highly impressive in that ALL transformation from MDS was clearly proven by the clonal evolution of the karyotype, and the cytogenetic result was critical in

making therapeutic decisions. Moreover, the cytogenetic result was the only evidence for a diagnosis of MDS in the absence of morphological dysplasia. In conclusion, MDS can rarely evolve to ALL, and extensive diagnostic work-up accompanied by BM studies, such as cytogenetic analysis and molecular study, is essential in making a diagnosis in such an uncommon event.

Keywords: Secondary ALL, MDS transformation to ALL, *TP53* mutation, Clonal evolution

PH-13

A Case of Myelodysplastic Neoplasm with a Novel Germline *DDX41* Truncating Variant c.24del (p.Lys9Serfs*71)

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Deleterious *DDX41* variants are the most common germline predisposition leading to MNs in adults and define a unique subtype of MNs. We report a novel germline *DDX41* truncating variant c.24del (p.Lys9Serfs*71) that was found in a patient with MDS and his daughter. A 47-year-old man presented with fever, fatigue, and pancytopenia. His complete blood counts indicated hemoglobin of 86 g/L, white blood cell (WBC) counts of $1.44 \times 10^9/L$ (absolute neutrophil counts of $0.46 \times 10^9/L$), and platelet counts of $79 \times 10^9/L$. His BM was hypocellular (10%) with increased blasts (9.3%) and prominent dys-hematopoiesis. Chromosome analysis showed normal karyotype, but fluorescence *in situ* hybridization (FISH) showed trisomy 8. Next-generation sequencing (NGS) revealed a frameshift variant of a *DDX41* allele (c.24del [p.Lys9Serfs*71], 52% variant allele frequency [VAF]), a missense variant of the other *DDX41* allele (c.1574G>A [p.Arg525His], 8% VAF), and a missense variant of a *DNMT3A* allele (c.2645G>A [p.Arg882His], 10% VAF). The patient was diagnosed as having MDS with a germline pathogenic variant in *DDX41*. After conditioning therapy and low-dose total body irradiation, he received allogeneic hematopoietic stem cell transplantation (allo-HSCT) from his 13-year-old daughter. In the 1st follow-up, five weeks after allo-HSCT, short tandem repeat analysis showed mixed chimerism. In the 2nd follow-up, additional cytogenetic abnormalities appeared, and the *DDX41* variant c.24del was persistent. Under the diagnosis of cytogenetic relapse, he was treated with a hypomethylating agent (azacitidine) and donor lymphocyte infusion. In the 3rd follow-up, the mixed chimerism persisted with further cytogenetic abnormalities, and he was in the status of stable disease. Subsequent genetic testing identified the *DDX41* variant c.24del in both the patient's buccal swab and the donor's blood, and the donor was confirmed as a carrier of the deleterious germline variant. According to the results, genetic counseling was provided to the patient and his family. The germline variant c.24del can be considered pathogenic, since it causes truncation of *DDX41*. The variant c.1574G>A (p.Arg525His) is the most commonly accompanied somatic variant in germline *DDX41*-

associated MNs. In our case, the persistently high VAF of the variant c.24del after allo-HSCT may suggest that the germline *DDX41* variant was deleteriously transferred into the recipient from the donor. This case highlights the importance of predictive genetic testing for the potential donors before allo-HSCT, especially in the cases of related allo-HSCT for patients with MNs with germline predisposition.

Keywords: Myelodysplastic neoplasm, *DDX41*, Germline variant, Hematopoietic stem cell transplantation, Next-generation sequencing, Germline predisposition

PH-14

A Case of a Patient with Simultaneous Chronic Lymphocytic Leukemia and Plasma Cell Myeloma

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Only a few studies have reported the coexistence of chronic lymphocytic leukemia and plasma cell myeloma in the same patient, and the rarity of this dual diagnosis makes it a challenge for both pathologists and clinicians. A 77-year-old female patient was referred to the hospital due to severe anemia, with a hemoglobin level of 4.2 g/dL. The patient's initial symptoms were generalized weakness, fatigue, anorexia, and dyspnea on exertion over the past month. A complete blood count revealed lymphocytosis with an absolute lymphocyte count of 7,886/ μ L, and a peripheral blood smear showed small, mature-appearing lymphocytes and rouleaux formation. Additionally, a skeletal survey revealed multiple osteolytic lesions in the skull. Serum and urine protein electrophoresis showed an M-band, and immunoelectrophoresis corresponded to immunoglobulin G kappa. Her serum kappa free light chain level was high at 1,278.0 mg/L (kappa/lambda ratio 222.3). A bone marrow (BM) aspirate smear showed an increase in small mature lymphoid cells (52.4%) along with plasma cells (24.6%). Flow cytometry of the BM aspirate showed a B-cell population that was strongly positive for CD5 and CD23, moderately positive for CD19 and surface kappa light chain, weakly positive for CD22, and negative for FMC-7. A BM biopsy showed hypercellular marrow with increased populations of two morphologically distinct small mature lymphocytes and plasma cells. Immunohistochemical staining of the BM biopsy further confirmed a diffuse infiltrate of CD20-positive lymphocytes and scattered CD138-positive plasma cells with kappa light chain restriction. The final diagnosis was chronic lymphocytic leukemia concomitant with plasma cell myeloma. A subsequent radiological examination revealed multiple lymph node enlargements, including the aortic, para-aortic, common iliac, and external iliac chains, consistent with lymphoma involvement. An inguinal lymph node biopsy revealed CD5, CD20, and CD23 positive small lymphocytic lymphoma with reactive plasma cells without light

chain restriction. She started lenalidomide-dexamethasone treatment for plasma cell myeloma and achieved a very good partial response after six cycles. A computed tomography scan performed at the same time showed that her multiple lymph node enlargement had almost disappeared. This case illustrates the extremely rare coexistence of chronic lymphocytic leukemia and plasma cell myeloma in one patient. Ancillary tests of BM examination, such as flow cytometry and immunohistochemistry, can help confirm the diagnosis. However, the development of more sophisticated methods, such as single-cell analysis, will be needed to more easily and unambiguously differentiate between the two diseases.

Keywords: Chronic lymphocytic leukemia, Plasma cell myeloma, Concomitant disease

PH-15

Diagnostic and Prognostic Utility of Monocyte Distribution Width and Monocyte Cell Population Data in Patients with Suspected Sepsis

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Background: Monocyte distribution width (MDW) and cell population data (CPD) have been suggested to be useful hematological biomarkers for sepsis. We explored the combined utility of MDW and monocyte CPD for the early diagnosis and prognosis prediction in sepsis.

Methods: In a total of 485 patients with suspected sepsis (sepsis, N=170), MDW and monocyte CPD were obtained using a DxH 900 analyzer (Beckman Coulter, Miami, Florida, USA). Among the 14 monocyte CPD, two CPD were selected: standard deviation of monocyte median angle light scatter (SD-MALS-MO) and mean of monocyte conductivity (MN-C-MO). MDW and the two CPD were compared using the quick sequential organ failure assessment (qSOFA) score and SOFA score. Using the receiver operating characteristic (ROC) curve and Kaplan-Meier survival analyses, the area under the curve (AUC) and hazard ratio (HR) were obtained, respectively.

Results: The MDW and the two CPD all had add-on values to the qSOFA score for diagnosing sepsis (AUC, 0.76–0.77 vs. 0.73; $P < 0.01$), and their combination demonstrated improved performance (AUC, 0.79 vs. 0.73; $P < 0.01$). The combination of the two CPD was comparable to the SOFA score for predicting 30-day mortality (AUC, 0.75 vs. 0.70) and revealed a higher risk of 30-day mortality than the SOFA score (HR, 15.8 vs. 5.8; $P < 0.01$).

Conclusions: This is the first study to explore the combined utility of MDW and monocyte CPD in patients with suspected sepsis. MDW and monocyte CPD can be useful for the early diagnosis and prognosis prediction of sepsis alongside clinical assessments.

Keywords: Monocyte distribution width, Cell population data, DxH 900, Sepsis, Diagnosis, Prognosis

PH-16

Role of Hemoglobin–Platelet Ratio, Platelet–Lymphocyte Ratio, and Lymphocyte–Monocyte Ratio in Detecting Patients with Suspected Colorectal Cancer in Cipto Mangunkusumo Hospital, Jakarta, Indonesia

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Background: Colorectal cancer (CRC) is a gastrointestinal malignancy with high morbidity and mortality rates worldwide, including Indonesia. The gold standard for CRC detection and screening is colonoscopy. Chronic inflammation and host immune responses are known to play important roles in tumorigenesis and cancer progression, which then affects hematological parameters. This study aimed to evaluate the role of hemoglobin–platelet ratio (HPR), platelet–lymphocyte ratio (PLR), and lymphocyte–monocyte ratio (LMR) in distinguishing CRC and non-CRC.

Methods: This was a cross-sectional study, which included 40 CRC and 40 non-CRC patients undergoing colonoscopy and histopathology examinations. Participants were 35 years old and above, suspected of having CRC based on anamnesis and physical examination. Patients with a history of CRC treatment, undergoing chemotherapy, on antibiotic therapy ≤ 1 month before colonoscopy, and had infection were excluded. Hematology data were examined using a Sysmex XN 3000 hematology analyzer.

Results: Significant differences were found in HPR, PLR, and LMR in CRC and non-CRC groups. The cutoff points of HPR, PLR, and LMR to distinguish the CRC and non-CRC groups were 0.26 (sensitivity 77.5%; specificity 92.5%), 189.22 (sensitivity 77.5%; specificity 72.5%), and 2.864 (sensitivity 77.5%; specificity 77.5%), respectively. Logistic regression analysis revealed that a combination of HPR and LMR values were better for detecting CRC than HPR or LMR alone.

Conclusions: The combination of HPR and LMR can be utilized to detect CRC with a score of 2 for HPR ≤ 0.26 and a score of 1 for LMR ≤ 2.864 with a 94.81% probability of distinguishing CRC and non-CRC.

Keywords: Colorectal cancer, Hemoglobin–platelet ratio, Platelet–lymphocyte ratio, Lymphocyte–monocyte ratio

PH-17

Microfluidic Bone Marrow On-A-Chip to Explore Hematopoietic Stem Cell Homing and the Impact of Adipocytes on Hematopoiesis

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Background: Accurate three-dimensional (3D) bone marrow models are critical for precise drug screening for various hematologic diseases, including malignancies. Traditional *in vitro* screening platforms often fail to replicate the 3D cellular and matrix environment of the bone marrow, particularly in recapitulating cellularity, which is a crucial factor for identifying optimal drug combinations and dosages. In this study, we introduce a bone marrow-on-a-chip (BMoC) system designed to replicate the diverse cellularity present in the human bone marrow.

Methods: Our platform mimics the interplay between bone marrow-derived mesenchymal stem cells (MSCs) and adipocytes within a hydrogel deposited on a polydimethylsiloxane-based (PDMS)-based multichannel chip. Additionally, we incorporated endothelial cells at the interface between the bone marrow hydrogel and media channel to simulate blood vessels and allowed hematopoietic stem cells (HSCs) to flow into the media channel to confirm homing.

Results: The endothelial monolayer was characterized by the presence of junctional markers. The presence of different cell populations was validated by identifying specific markers across diverse ratios of MSCs to adipocytes. Moreover, the high homing tendency of HSCs was verified through cellularity control. Finally, the effect of adipocyte incorporation was validated by confirming differentiation of HSCs into the erythroid lineage.

Conclusions: Our study validated a BMoC system that accurately replicates bone marrow physiology, including cell interactions and HSC homing. This platform offers a robust tool for studying hematological diseases and developing targeted therapies.

Keywords: Bone marrow-on-a-chip, Hematopoietic stem cell, Homing, Hematopoiesis, Adipocyte

PH-18

Genetic Variants in Pediatric *BCR::ABL1*-negative Myeloproliferative Neoplasms Revealed by Massively Parallel Sequencing: Essential Thrombocythemia

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Background: Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells, including *BCR::ABL1*-negative MPNs such as essential thrombocythemia (ET). MPNs are rare in

children, and the genetics and biology of pediatric MPNs remain poorly understood. This study reports the genetic variants identified in pediatric MPNs using massive parallel sequencing (MPS).

Methods: This study included eight consecutive pediatric patients with ET diagnosed between January 2000 and June 2023. MPS was performed on an Ion S5 XL sequencer using an Oncomine Myeloid Research Assay on bone marrow aspirate samples.

Results: The median age of eight patients with ET was 17.2 years, and the male-to-female ratio was 3:1. The median values of CBC were as follows: WBC, $9.10 \times 10^9/L$; Hb, 139 g/L; PLT, $1,001 \times 10^9/L$. Four patients (50%) had clinically significant genetic variants. of these, two patients had *JAK2* V617F (driver) mutations, one had *FLT3* E656A, and one had *ETV6* I10V mutations. One patient (12.5%) experienced a transient ischemic attack with visual loss lasting 5 minutes.

Conclusions: Our results suggest a different genetic profile for pediatric MPN, with a lower incidence of driver variants in pediatric ET.

Keywords: Myeloproliferative disorders, *BCR:ABL1*-negative, Massive parallel sequencing, Essential thrombocythemia, Pediatric

KID (54.1–2,808 pmol/mL), DPP3 (16.6–361.8 ng/mL), hGH (5.2–44.5 ng/mL), and pro-NT (54.5–299.8 pmol/L). Median differences in hGH levels were observed based on fetal factors, including GA, BW, and APGAR scores. Median differences in pro-NT were observed based on maternal factors, including delivery mode, PROM, and GDM. In particular, penKID showed a significantly different RI based on the presence of PROM.

Conclusions: This study established the RIs for bio-ADM, penKID, DPP3, hGH, and pro-NT using cord blood, offering valuable neonatal health assessment guidelines. These RIs can aid the early detection of abnormalities and inform clinical decision-making in neonatology. Further research is recommended to explore the clinical implications of these biomarkers and to validate the findings in diverse aging populations.

Keywords: Bioactive adrenomedullin, Proenkephalin, Dipeptidyl peptidase 3, Human growth hormone, Pro-neurotensin, Reference interval

PH-19

Reference Intervals of Bioactive Adrenomedullin, Proenkephalin, Dipeptidyl Peptidase 3, Human Growth Hormone, and Pro-neurotensin in Umbilical Cord Blood

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Background: Establishing reference intervals for biomarkers in umbilical cord blood is crucial for neonatal health assessment and the early diagnosis of various conditions. This study aimed to determine the reference intervals (RIs) for bioactive adrenomedullin (bio-ADM), proenkephalin (penKID), dipeptidyl peptidase 3 (DPP3), human growth hormone (hGH), and pro-neurotensin (pro-NT) in a large cohort of cord blood samples.

Methods: Cord blood samples were collected immediately after delivery from 615 neonates in serum-separating tubes. The separated sera frozen at -70°C were measured using standardized immunoassays. After removing outliers, non-parametric percentile methods were used to establish the RIs for bio-ADM, penKID, DPP3, hGH, and pro-NT according to the Clinical and Laboratory Standards Institute guidelines (EP28-A3C). The data were also analyzed for potential differences based on neonatal and maternal factors: neonatal sex, gestational age (GA), birth weight (BW), APGAR score, delivery mode, presence of premature rupture of membranes (PROM), gestational diabetes mellitus (GDM), and preeclampsia.

Results: The established RIs (2.5–97.5th percentile) for the biomarkers in cord blood were as follows: bio-ADM (15.3–62.4 pg/mL), pen-

PC-01

Prevalence of Hypomagnesemia among Elderly Patients Visiting Primary Care Clinics in Korea

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Background: Magnesium (Mg) deficiency commonly occurs in elderly patients because of inadequate Mg nutrient intake, multiple drug use, or altered gastrointestinal function. Nevertheless, Mg deficiency is often poorly understood in clinical practice, including primary care clinics. This study aimed to estimate the prevalence of hypomagnesemia in the elderly population visiting primary care clinics in Korea.

Methods: Our study reviewed data on specimens requested at 1,095 primary care clinics in the five regions of Korea. The specimens were from adults ≥ 18 years old with Mg results recorded in the laboratory information system at Seegene Medical Foundation from January 1, 2022 to June 30, 2022. Hypomagnesemia was defined as serum Mg concentrations less than 1.6 mg/dL. The serum Mg concentrations were measured using the magnesium assay (xylidyl blue method) on a Labospect 008AS (Hitachi, Tokyo, Japan).

Results: The prevalence of hypomagnesemia was 0.44% (257/58,321). The mean age of the participants was 56.27 ± 16.14 years. The average concentration of serum Mg was 2.28 ± 0.24 mg/dL, ranging from 0.20 to 7.00. The highest prevalence of hypomagnesemia was found in patients 60–69 years old (24.51%, 63/13,424), when compared with the other age groups (below 20 years old [0.00%, 0/355], 20–29 years old [1.56%, 4/3,456], 30–39 years old [7.39%, 19/5,758], 40–49 years old [13.60%, 35/9,459], 50–59 years old [19.07%, 49/13,400], 70–79 years [17.90%, 46/7,938], over 80 years old [15.95%, 41/4,531], $P < 0.001$).

Conclusions: Hypomagnesemia is primarily tested on patients with clinical risk factors. Nevertheless, considering the increase in medi-

cal demand due to the rapid increase in the elderly population, the results of this study on Mg prevalence by age group can be used as basic data for establishing policies for elderly health care.

Keywords: Hypomagnesemia, Elderly population, Magnesium

PC-02

Serum or Plasma Aldosterone to Plasma Renin Activity Ratio Test: Experience of a Clinical Laboratory in Korea

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Background: The serum or plasma aldosterone concentration to plasma renin activity ratio (ARR) test is the most reliable screening test for primary aldosteronism (PA). ARR can be calculated by simultaneously measuring serum or plasma aldosterone concentration (SAC or PAC) and plasma renin activity (PRA) when upright in the morning. Autonomous aldosterone secretion (probable PA) is suspected when the ARR ≥ 20 (SAC or PAC: ng/dL, PRA: ng/mL/hour) and SAC or PAC ≥ 15 ng/dL. According to a report from the Mayo Clinic, more than 90% of patients with surgically confirmed aldosterone-producing adenoma (APA) had an ARR of 20 or more and a SAC or PAC of 15 ng/dL or more.

Methods: From January 1, 2023 to July 10, 2023, the number of LC-MS/MS method aldosterone tests, LC-MS/MS method renin activity tests simultaneously requested by GC labs, total number of ARR cases (the number of possible cases), the mean, 95% CI for mean, minimum value, maximum value, and various percentile values of each item were calculated. Additionally, we calculated cases where aldosterone was ≥ 15 ng/dL and ARR was ≥ 20 ng/dL per ng/mL/hour, in which autonomous aldosterone secretion was suspected.

Results: From January 1, 2023 to July 10, 2023, the number of LC-MS/MS method SAC or PAC tests requested (with PRA tests simultaneously) by GC labs was 5,510 and the number of LC-MS/MS method PRA tests (with SAC or PAC tests simultaneously) by GC labs was 5,287. The total number of ARR cases (the number of possible cases) was 5,547. The mean; 95% confidence interval (CI) for mean; minimum; maximum; 5, 10, 25, 50, 75, 90, and 95 percentile values of SAC or PAC test, PRA test, and ARR test were 8.21; 7.83–8.58; 0.40; 490.20; 0.60, 1.10, 2.70, 5.60, 10.00, 16.10, 22.70 for SAC or PAC; 2.73; 2.54–2.92; 0.01; 204.27; 0.09, 0.15, 0.36, 0.98, 2.33, 5.53, 10.17 for PRA; and 21.57; 18.20–24.95; 0.001; 5,670.00; 0.34, 0.70, 2.08, 5.06, 13.21, 34.79, 66.06 for ARR. The number of cases where aldosterone was ≥ 15 ng/dL and ARR was ≥ 20 ng/dL per ng/mL/hour was 246, accounting for 4.43% of the total 5,547 cases.

Conclusions: This is the first study to analyze LC-MS/MS SAC or PAC and PRA assay results in Korea. We presented data on the status of ARR tests for PA using LC-MS/MS including the number of cases of

SAC or PAC and PRA tests during the period, the number of possible cases of ARR tests, the mean, 95% CI for mean, minimum value, maximum value, various percentile value, and the number and percentage of ARR cases that may indicate probable PA in a clinical laboratory in Korea. These findings could be fundamental data of ARR tests for PA.

Keywords: Aldosterone, Renin activity, Primary aldosteronism, Aldosterone to renin ratio, Mass spectrometry

PC-03

Prevalence of Gestational Diabetes Mellitus According to Seasonal Variation in Korea: A Repeated Cross-Sectional Study

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Background: Ambient temperature and seasonal changes are risk factors for gestational diabetes mellitus (GDM). This study aimed to investigate the prevalence of GDM according to the seasonal variation in Korean population.

Methods: We retrospectively reviewed the results of oral glucose tolerance test (OGTT) performed on a total of 3,459 patients who visited local clinics for GDM diagnosis in Korea from 2019 to 2023. GDM was diagnosed using the two-step method, the 50-g glucose challenge test followed by the 100-g OGTT, performed on participants between 24 and 28 weeks of gestational age. The threshold values used for the diagnosis of GDM were as follows: fasting ≥ 95 mg/dL, 1-hour ≥ 180 mg/dL, 2-hour ≥ 155 mg/dL, and 3-hour ≥ 140 mg/dL, and two abnormal values were required for diagnosis. The plasma glucose levels were measured with the enzymatic colorimetry method using Labospect 008AS (Hitachi, Tokyo, Japan). The seasons were defined as follows: spring, March–May; summer, June–August; autumn, September–November; and winter, December–February. The data of the monthly average temperatures in Korea were derived from (<https://data.kma.go.kr/climate/RankState/selectRankStatistics-DivisionList.do?pgmNo=179>) downloaded on 03 April 2024.

Results: During the study, the prevalence of GDM in the Korean population was 21.8% (755/3,459), and it showed an increased trend annually from 2019 (18.6%, 117/630) to 2022 (24.9%, 174/698) and then decreased in 2023 (20.7%, 149/720). The prevalence of GDM was the highest in summer (28.2%, 213/755) followed by spring (26.9%, 203), winter (22.6%, 171/755), and fall (22.3%, 168/755). Considering the average monthly temperature during the study period, the prevalence of GDM in winter was lower than that in non-winter throughout the study period, 26.5% vs. 73.5% in 2019, 19.9% vs. 80.1% in 2020, 20.1% vs. 79.9% in 2021, 21.3% vs. 78.7% in 2022, 26.8% vs. 73.2% in 2023. The average ages of patients diagnosed with GDM in non-winter and winter were as follows: 34.6 vs. 32.6 years (years.) in 2019, 33.9 vs. 33.3 years. in 2020, 34.5 vs. 32.9 years. in 2021, 34.7 vs. 34.8 years. in 2022, 34.1 vs. 33.4 years. in 2023.

Conclusions: The prevalence of GDM in Korean patients who visited local clinics from 2019 to 2023 presents seasonal variation, with the highest prevalence in summer. Our findings can serve as useful epidemiological data to understand GDM in countries with similar climates.

Keywords: Gestational diabetes mellitus, Seasonal variation, Prevalence, Korea

PC-04

Triglyceride Glucose Index, Gamma-Glutamyl Transferase to HDL-Cholesterol Ratio, and HOMA-IR as Predictors of Hyperglycemia in Health Checkup Examinees: A Cross-Sectional Study

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Background: Early detection and management of hyperglycemia are crucial for preventing type 2 diabetes mellitus (T2DM) and its complications. This study aimed to evaluate the relationship between triglyceride glucose (TyG) index, gamma-glutamyl transferase to HDL-cholesterol (GGT/HDL-C) ratio, homeostasis model assessment of insulin resistance (HOMA-IR), and hyperglycemia in health checkup examinees and to determine their optimal cutoff values for predicting hyperglycemia.

Methods: This cross-sectional study included 300 Korean adults who underwent a comprehensive health examination between January and December 2022. Participants were categorized into normoglycemic (N=125) and hyperglycemic (N=175) groups based on the fasting plasma glucose levels. TyG index, GGT/HDL-C ratio, and HOMA-IR were calculated, and their associations with hyperglycemia were analyzed using correlation analysis, receiver operating characteristic (ROC) curve analysis, and logistic regression models.

Results: TyG index, GGT/HDL-C ratio, and HOMA-IR were significantly higher in the hyperglycemic group (all $P < 0.001$). ROC curve analysis revealed that TyG index and HOMA-IR showed a comparable predictive ability for hyperglycemia (AUC 0.76 and 0.77, respectively) while GGT/HDL-C ratio showed a slightly lower performance (AUC 0.68). The optimal cutoff values for predicting hyperglycemia were 8.65, 0.60, and 1.50 for TyG, HOMA-IR, and GGT/HDL-C ratio, respectively. In the fully adjusted logistic regression model, the highest quartiles of all three indices were significantly associated with hyperglycemia (all $P < 0.001$).

Conclusions: TyG index, GGT/HDL-C ratio, and HOMA-IR were significantly associated with hyperglycemia in Korean adults undergoing health checkups, with TyG index and HOMA-IR showing comparable predictive ability. The proposed cutoff values for the indices could be useful tools for risk stratification and personalized management of hyperglycemia in health checkup settings. Incorporating these indi-

ces into routine health assessments could contribute to the early detection and prevention of T2DM in Korea.

Keywords: Triglyceride glucose index, GGT/HDL-cholesterol ratio, HOMA-IR, Hyperglycemia, Health checkup, Type 2 diabetes mellitus

PC-05

Optimizing Hormone Therapy Monitoring in Transgender Individuals Using Machine Learning Models

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Background: Transgender individuals undergoing hormone therapy (HT) experience significant physiological changes that require precise monitoring of hematologic and metabolic parameters to ensure safety and efficacy. Current clinical guidelines are generalized and may not account for individual variability. This study aims to develop and validate machine learning models to predict optimal monitoring intervals and potential adverse events in transgender individuals on HT.

Methods: Data were sourced from the NHANES and CLIA databases, focusing on transgender individuals undergoing HT. Key hematologic (hemoglobin, hematocrit) and metabolic parameters (fasting glucose, lipid profiles) were extracted. Data preprocessing involved normalization, outlier detection, and handling of missing values through multiple imputation. Feature selection was conducted using LASSO regression and principle component analysis to identify the most predictive variables. We implemented machine learning models, including gradient boosting machines, convolutional neural networks (CNN), and ensemble methods. The models were trained using a stratified 10-fold cross-validation approach, with hyperparameter tuning performed via grid search. Model performance was evaluated based on AUC-ROC, accuracy, precision, recall, F1-score, and calibration plots. We also performed time-to-event analysis using Cox proportional hazards models to predict the timing of necessary monitoring intervals and potential adverse events, with results stratified by age, gender identity, and duration of HT.

Results: The gradient boosting machine learning model achieved the highest performance, with an AUC-ROC of 0.95 (95% CI: 0.92–0.98), accuracy of 91.4% (95% CI: 89.1–93.7), precision of 90.2% (95% CI: 87.4–93.0), recall of 92.6% (95% CI: 89.9–95.3), and F1-score of 91.4% (95% CI: 88.9–93.9). The CNN model also performed well, with an AUC-ROC of 0.93 (95% CI: 0.90–0.96), accuracy of 89.8% (95% CI: 87.2–92.4), precision of 88.7% (95% CI: 85.7–91.7), recall of 91.0% (95% CI: 88.2–93.8), and F1-score of 89.8% (95% CI:

87.0–92.6). Time-to-event analysis indicated that the optimal monitoring interval for hematologic parameters is every 3 months for the first year of HT, with a hazard ratio (HR) of 2.4 (95% CI: 1.8–3.2) for adverse events if monitoring is delayed beyond this period. For metabolic parameters, a 6-month interval was optimal, with an HR of 1.9 (95% CI: 1.4–2.6).

Conclusions: This study highlights the potential of machine learning to optimize monitoring schedules for transgender individuals on HT, achieving high accuracy for clinical application. The findings support more personalized and timely healthcare, improving patient safety and healthcare efficiency. Future research should focus on real-world validation and implementation of these models in diverse clinical settings.

Keywords: Hormone therapy, Transgender health, Machine learning, Personalized medicine, Monitoring intervals, Adverse events prediction

PC-06

Assessing Patient-Pool-based Thyroid-Stimulating Hormone External Quality Assessment Materials for Proficiency Testing: Homogeneity, Stability, and Commutability

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Background: Thyroid stimulation hormone (TSH) is a sensitive and specific biomarker for assessing thyroid function and diseases. However, discrepancies between methods, different reference values, and inter-laboratory variation complicate the interpretation of TSH values and their clinical implications. To address these issues, proficiency testing is crucial for harmonizing TSH assays, ensuring accurate and precise results. This study aimed to evaluate the homogeneity, stability, and commutability of specimens used in TSH proficiency testing.

Methods: Patient-pool-based external quality assessment (PPB-EQA) materials for two levels of TSH were produced from the collected remnant sera of patients who had undergone TSH assays. These materials were evaluated for homogeneity by triplicate testing of 10 bottles. Short-term stability was assessed over 7 days (Days 0, 1, 2, 4, and 7) at a refrigerated temperature of 4°C, and long-term stability was evaluated over a year (2 weeks and 1, 3, 6, and 12 months) at a frozen temperature of -70°C. The commutability of PPB-EQA and three commercial quality control materials (CQCM, Bio-Rad Liquichek Immunoassay Plus) was tested in triplicate at five positions, along with individual clinical samples from 30 patients by using four immunoassay platforms (Beckman Coulter UniCel DXI 800, Abbott Alinity i, Roche Cobas e801, and Siemens Atellica IM 1600). The commutability criteria were adapted from the International Federation of Clinical Chemistry protocol, with a desirable bias of 10% based on biological

variation.

Results: The mean TSH levels of PPB-EQA in the homogeneity evaluation were 1.05 and 10.81 μ IU/mL, respectively. No significant inhomogeneity was observed among the 10 vials at both TSH levels. TSH PPB-EQA was stable for at least 7 days under refrigerated conditions and for at least a year under frozen conditions. The median TSH level for the 30 patient samples across four instruments was 2.51–3.07 μ IU/mL, with a coefficient of variation ranging from 1.51% to 4.32%. Passing-Bablok regression analysis showed slopes between methods ranging from 0.829 to 1.069. Both PPB-EQAs demonstrated commutability across six method pairs, while one to two method pairs showed non-commutability for three levels of CQCM.

Conclusions: The TSH PPB-EQA was homogenous between vials, stable under both refrigerated and frozen conditions, and demonstrated better commutability than CQCM. These findings suggest that the PPB-EQA produced from remnant sera is a suitable candidate for materials used in proficiency testing for TSH.

Keywords: Commutability, Homogeneity, Proficiency testing, Stability, Thyroid-stimulating hormone

PC-07

Establishing Practical Delta Check Limits for Frequently Requested Hormones Using Real-World Data

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Background: Hormonal levels are crucial in regulating physiological processes but are prone to significant fluctuations due to various factors. Establishing reliable delta check limits (DCLs) for hormone tests is challenging due to this variability. We aimed to establish practical and appropriate DCLs for frequently requested hormones, excluding thyroid hormones.

Methods: We analyzed paired results of adrenocorticotrophic hormone (ACTH), cortisol, insulin, parathyroid hormone (PTH), prolactin, testosterone, and thyroglobulin tests from five university-affiliated hospitals in Korea, collected between January 2020 and August 2022. The dataset was split into a development set and validation set in a 6:4 ratio. Using the development set, we determined the upper 95th percentile for both the absolute delta difference (absDD) and absolute delta percent change (absDPC) as DCLs for two groups, inpatients/emergency (group IE) and outpatients (group O). We then compared these DCLs and performed cross-validation to assess their

interchangeability. Combined DCLs for ACTH, cortisol, PTH, and prolactin were established and validated using chi-square analysis.

Results: Our analysis included 60,219 paired hormone test results. The established absDCLs showed a consistent exceedance rate of 5% using the validation set. Cross-validation revealed significant deviations in ACTH under both absDD and absDPC whereas deviations in cortisol and prolactin were mostly noted with absDD. The validation of combined absDCLs corroborated these patterns, with chi-square analysis demonstrating significant deviations for ACTH and confirming the validity of DCLs for cortisol, PTH, and prolactin.

Conclusions: This study identified practical DCLs for frequently requested hormones using real-world data for the first time. We recommend adopting absDPC as a combined DCL for cortisol, PTH, and prolactin. Different DCLs should be considered for ACTH depending on the clinical setting. These findings provide foundational data for establishing DCLs with hormone tests in clinical laboratories.

Keywords: Hormone tests, Delta check limits (DCLs), Absolute delta difference, Absolute delta percent change

PC-08

Association of *CYP3A5* Polymorphisms and Renal Function in Patients with Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is a prevalent condition in Thailand, characterized by prolonged kidney damage or reduced function over three months. The prevalence is highest in Northeastern regions and has led to an increasing number of kidney transplant cases. Cytochrome P450 family 3 subfamily A polypeptide 5 (*CYP3A5*) plays a crucial role in metabolizing immunosuppressant drugs, and its enzyme activity varies based on genetic polymorphism. *CYP3A5**3 polymorphism (6986A>G, rs776746) results in defective mRNA splicing and enzyme inactivation, which are implicated in CKD pathogenesis through distributing homeostatic signaling mechanisms. Despite its importance, the direct impact of *CYP3A5* polymorphisms on renal function parameters such as estimated glomerular filtration rate (eGFR), serum creatinine clearance, and albuminuria remains unclear in the Thai population. Therefore, we aimed to determine the distribution of *CYP3A5* polymorphisms in CKD patients and their association with renal parameters.

Methods: We investigated the distribution of *CYP3A5* polymorphisms

in 205 CKD patients the TaqMan[®] SNP Genotyping Assay.

Results: The frequency of *CYP3A5**1 and *CYP3A5**3 alleles in CKD patients was 17.6% and 82.4%, respectively, and the frequency of *CYP3A5**1/*1, *CYP3A5**1/*3, and *CYP3A5**3/*3 polymorphisms were 17.6%, 38.0%, and 44.4%, respectively. Moreover, the eGFR and serum creatinine clearance levels were significantly decreased in the *CYP3A5**3 allele when compared to the *CYP3A5**1 allele ($P=0.038$ and 0.020 , respectively). The urine albumin-creatinine ratio (UACR) and microalbuminuria levels were significantly increased in the *CYP3A5**3 allele than in the *CYP3A5**1 allele ($P=0.003$ and 0.016 , respectively). The *CYP3A5**3 allele was associated with eGFR ($\beta=-11.295$, $P=0.038$), serum creatinine clearance ($\beta=-13.266$, $P=0.016$), UACR ($\beta=488.909$, $P=0.036$), and microalbuminuria levels ($\beta=29.129$, $P=0.036$). However, the *CYP3A5**3 allele was not significantly associated with these parameters after adjusting for potential confounding factors including age, gender, hypertension, and diabetes.

Conclusions: These findings highlight the high prevalence of *CYP3A5**3 alleles in CKD patients in the Thai population. The *CYP3A5**3 allele is associated with impaired renal function parameters such as eGFR, serum creatinine clearance, UACR, and microalbuminuria, suggesting its potential role in CKD progression. Further studies are warranted to elucidate the precise mechanisms underlying these associations and their implications for CKD management.

Keywords: Chronic kidney disease, *CYP3A5*, Polymorphisms, Allele, Renal functions

PC-09

Beta 2 Microglobulin as a Kidney Function Marker

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Background: Methods to assess kidney function are improving gradually. Novel biomarkers, such as beta 2 microglobulin (B2M), are being studied and used in clinical practice. B2M is used to improve the diagnosis and prognosis of patients with chronic kidney disease (CKD). We aimed to assess the usage of B2M alone or B2M-based estimated glomerular filtration rate (eGFR) in Mongolian patients.

Methods: We included 116 patients diagnosed with CKD and 55 donors with normal kidney function. We collected blood samples from the participants through venipuncture in plain vacutainers. The serum levels of creatinine, urea, cystatin C, and B2M were measured using Roche Cobas C311. The eGFR was calculated online using the calculator provided by the National Kidney Foundation. B2M-based eGFR was calculated as $eGFR = 133 \times B2M^{-0.852}$.

Results: In the CKD group, the average serum creatinine level was 3.37 mg/dL, serum urea 97.6 mg/dL, cystatin C 3.05 mg/L, and B2M 10.65 mg/L. In the donor group, the average serum creatinine level was 0.87 mg/dL, serum urea 31.1 mg/dL, cystatin C 1.49 mg/L,

and B2M 2.43 mg/dL. Results obtained with the MDRD method showed that the eGFR was 28.4 mL/min in the CKD group and 99.7 mL/min in the donor group. With the CKD-EPI crea, CKD-EPI cys, CKD-EPI com, and GFR-B2M methods, the eGFRs in the CKD and donor groups were 103.9 and 21.5 mL/min, 47.7 and 22.9 mL/min, 22.9 and 69.0 mL/min, and 24.3 and 64.1 mL/min, respectively. Assessment of kidney biomarkers and eGFR was significantly correlated in both groups.

Conclusions: B2M can be used in clinical practice in Mongolia. B2M is optional with creatinine, urea, and cystatin C for assessing and improving kidney function.

Keywords: Beta 2 microglobulin, Glomerular filtration rate, Chronic kidney disease

PC-10

Zinc Deficiency in Patients with Chronic Liver Disease: A Single-Center Study

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Background: This study aimed to evaluate the prevalence of serum zinc deficiency among patients with chronic liver disease (CLD) in clinical practice and explore its association with clinical characteristics.

Methods: We analyzed 397 patients with CLD, including 195 with liver cirrhosis, admitted at Dornod Medical Center in 2023.

Results: Zinc deficiency (<60 µg/dL) was found in 38.1% of patients, with a 54.8% prevalence among those with liver cirrhosis. Including marginal deficiency (<80 µg/dL), 80.8% of patients were affected, with 89.5% cirrhosis cases. Serum zinc levels correlated most strongly with serum albumin levels, with 92.3% of patients with albumin.

Conclusions: Zinc deficiency is prevalent in patients with CLD, particularly in those with liver cirrhosis. Regular monitoring of serum zinc levels, especially in patients with cirrhosis, is recommended.

Keywords: Chronic liver diseases, Liver cirrhosis, Zinc deficiency

PC-11

Toxic Hepatitis and Dilated Cardiomyopathy Induced by Creosote Oil Toxicity: Report on a Rare Case

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Railway wooden sleepers are frequently treated with creosote oil to

enhance their durability and prevent pests and fungi-induced decay. Excessive exposure to creosote oil is toxic. A 28-year-old man was admitted to a local hospital on June 12, 2022, after prolonged exposure to creosote-treated wooden sleepers while building a house on hot summer days. He presented with severe symptoms and elevated liver enzyme levels (AST 7.116 IU/L, ALT 7.995 IU/L), gamma-glutamyl transpeptidase, 234 IU/L, LDH 3,684 IU/L, and bilirubin levels (TB 2.41 mg/dL, DB 1.2 mg/dL). Abdominal computed tomography revealed acute hepatitis with mild hepatosplenomegaly. Echo CG performed on June 20, 2022, revealed respective EF, LV, and LA values of 18.8%, 8.4 cm, and 5.8 cm. All four cardiac chambers were dilated. Overall left ventricle contraction was significantly reduced. Diffuse hyperkinesia was observed during contraction. Akinesia was observed in the inferior lateral walls, and the pulmonary artery was dilated. The patient was promptly transferred to the Department of Internal Medicine for further management. Further investigations eliminated other causes of liver toxicity, and the patient was diagnosed with creosote oil-induced acute hepatitis and toxic dilated cardiomyopathy. Despite intensive medical care over the following year, the patient's condition deteriorated and he passed away. This case highlights the severe health risks associated with prolonged exposure to creosote-treated wooden sleepers, particularly acute hepatitis and toxic dilated cardiomyopathy. Adverse outcomes, including significant liver enzyme abnormalities and heart dysfunction, underscore the critical need for safety measures and protective guidelines for individuals exposed to these materials. Despite exhaustive medical efforts, the toxic effects of creosol remain fatal, emphasizing the importance of early detection and intervention in similar cases.

Keywords: Creosote oil, Toxicity, Toxic Hepatitis

PC-12

Falsely Elevated D-dimer Results Caused By Heterophilic Antibodies: A Case Report

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D-dimer is a degradation product that is generated during the breakdown of fibrin due to the body's procoagulant response. When the concentration of D-dimer is elevated, it implies an excessive procoagulant response in the body, which can lead to thrombus formation. During pregnancy, the levels of D-dimer and protein S are typically tracked through blood tests to confirm the abnormal elevation of thrombus. Elevated thrombotic indicators during pregnancy can lead to recurrent miscarriages. D-dimer has been seen as the one negative prognostic factor for women undergoing *in vitro* fertilization (IVF). Here, we present the case of one patient who underwent infertility assessment and exhibited falsely positive elevated D-dimer levels due to autoimmune antibodies. We compared the detection results of blood

D-dimer using two different methodological approaches and verified the results through dilution and back-multiplication. Further analysis was conducted to determine whether other factors interfered with the D-dimer test values. The patient was a 36-year-old gravida 2, para 1, abortus 1 (G2P1A1) female with regular menstrual cycles. She experienced a miscarriage at six weeks during her first pregnancy and successfully had a natural delivery through IVF. She presented again for infertility evaluation, but her D-dimer levels were consistently $>5,330$ ng/mL after multiple tests conducted at CUMH. However, her D-dimer value was 220 ng/mL as confirmed in an external laboratory report. After comparing results from different instruments, Latex agglutination immunoturbidimetry result was 6,461.7 ng/mL, while the result of a fluorescence immunoassay was 172.16 ng/mL. After performing serial dilutions and back-multiplication of the patient's sample and calibrator, we observed the differences in the back-multiplied values from the patient's sample and concluded that some interference might be caused by alloantibodies. However, the back-multiplied values of the calibrator remained unaffected. Laboratory examinations often utilize different methodological approaches for testing. The presence of heterophilic antibodies could lead to false-positive increases in D-dimer values, even when following general testing principles. Especially after infection with SARS-CoV-2 or vaccination, the diversity of autoimmune antibodies seems to contribute to an increasing trend in such occurrences. D-dimer is a marker characterized by high sensitivity, low specificity, high negative predictive value, and low positive predictive value. This case serves as an example for clinical practitioners to consider heterophilic antibodies in the differential diagnosis of abnormal laboratory findings. It is crucial to focus on strategies for preventing similar antibody interference in specimen values and tests in the future.

Keywords: D-Dimer, Heterophilic antibodies, *In vitro* fertilization

PC-13

Assessment of Glucose Meter Accuracy in Hospitals

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Background: Glucose meters are widely used in households and medical facilities owing to their convenience, speed, low sample requirements, and low cost. To avoid incorrect clinical decisions, glucose meter levels should be as close to the actual blood glucose level as possible. Therefore, the accuracy of glucose meters is an important issue. Clinicians often order blood tests and point-of-care testing (POCT) for glucose levels based on the clinical condition of an individual patient. Operators typically measure glucose with a blood glucose meter using venous blood instead of giving the patient an extra needle stick. This study aimed to assess the accuracy of glucose meters by using venous blood tests.

Methods: This study included a total of 280 patients. The glucose levels were measured using a FORA POCT S10 glucose meter. Serum glucose levels were measured using an Abbott ARCHITECT c16000

Analyzer (hexokinase method) and used as a reference. The accuracy of the glucose meter was assessed according to ISO 15197:2013 standards. The consistency between the two methods was assessed using Bland-Altman plots. The results were analyzed using a paired T test and correlation coefficient analysis.

Results: The mean blood glucose levels measured using the glucose meters was 143.11 ± 59.48 mg/dL, compared with the serum glucose level of 142.05 ± 60.79 mg/dL. There was no significant difference between the two methods ($t=1.72$, $P>0.05$). The correlation coefficient (r) was 0.99 and the coefficient of determination (r^2) was 0.97, indicating a strong positive correlation. The Bland-Altman plots showed that the mean difference was -1.06 and the 95% limits of agreement was -21.32 to 19.19. At glucose levels <100 mg/dL, 94.83% (55/58) of the results were within ± 15 mg/dL, whereas at glucose levels ≥ 100 mg/dL, 96.85% (215/222) of the results were within $\pm 15\%$.

Conclusions: The accuracy of glucose levels <100 mg/dL fell short of the ISO 15197:2013 guidelines. Overall, the statistical analysis showed no significant differences between the two methods, and the correlation analysis showed a high positive correlation. The Bland-Altman plots demonstrated that the two methods using venous blood had good consistency. In clinical settings, when it comes to the low blood sugar range in reports, interpretations should be made with caution to avoid making incorrect clinical decisions. The effects of controllable factors should be avoided as much as possible to reduce the measurement error of the blood glucose meter.

Keywords: Accuracy, Blood glucose, Bland-Altman plots, Glucose meter, POCT

PC-14

Performance Evaluation of the AFIAS CRP Assay: A Point-of-Care C-Reactive Protein Testing Device for Neonatal Intensive Care Units

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Background: Conventional C-reactive protein (CRP) testing, especially in neonatal intensive care units (NICUs), has several disadvantages including the invasiveness of sampling (arterial or venous), potential for significant blood loss, and time delays in result reporting. Additionally, a recent decline in pediatric healthcare staff, particularly among resident physicians in Korea, has increased the burden on specialists performing these invasive procedures. In response, point-of-care testing (POCT) devices for CRP testing, such as the AFIAS CRP device (Boditech Med Inc., Chuncheon, Korea) are increasingly being utilized. These devices offer benefits such as minimal invasiveness, low blood volume requirements (0.01 mL), and rapid result reporting, potentially reducing workload and enhancing medical care efficiency. Therefore, we evaluated the performance of the AFIAS CRP POCT method.

Methods: This study compared test results from the AFIAS CRP device with those obtained using conventional laboratory equipment, specifically the Beckman Coulter AU5800 (Beckman Coulter, USA) in our hospital. We utilized minimal residual blood samples (0.01 mL) typically discarded from routine CRP sampling, as well as samples drawn during hospitalization when symptoms of infection prompted a CRP test. We assessed the precision, linearity, limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) of the AFIAS CRP assay, and evaluated the agreement between the results of the AFIAS CRP device and those of conventional CRP tests conducted at our hospital.

Results: Our analysis confirmed the manufacturer's claims regarding the precision, linearity, LoB, LoD, and LoQ of the AFIAS CRP assay. A total of 204 patient samples were used to compare results between the AFIAS and conventional methods. The paired results between the two assays correlated well ($r=0.868$, $P<0.001$), with no significant difference in mean comparisons ($P>0.05$). Bland-Altman analysis showed a mean bias of -0.01020 between the two methods, with a standard deviation of 0.2378 and 95% limits of agreement ranging from -0.4764 to 0.4560 .

Conclusions: The performance of the AFIAS CRP assay correlated well with that of Beckman Coulter CRP testing. The AFIAS CRP is a viable alternative for CRP testing where POCT is necessary.

Keywords: CRP, Neonatal ICU, Performance, POCT

3.0% to 16.9% while those measured by TINIA (Roche COBAS c513) ranged from 4.5% to 17.5%. In 7 of 11 patients with possible HbD by HbEP, consistent differences were observed, with TINIA yielding higher HbA1c values (mean difference: 0.6%). For example, an HPLC HbA1c value of 5.8% corresponded to a TINIA value of 6.1%. The corresponding eAG values were also higher for TINIA in these cases. In patients without hemoglobin variants ($N=3$) or with no HbEP results ($N=1$), discrepancies between the methods were less significant (mean difference: 0.3%).

Conclusions: Although the Bio-Rad D-100 HPLC system is known to have minimal interference from common Hb variants, including HbD, this study reveals significant discrepancies in HbA1c results between the Bio-Rad D-100 HPLC and TINIA methods, particularly in patients with suspected HbD. These findings underscore the necessity for careful method selection and interpretation of HbA1c results when hemoglobin variants are suspected, as the presence of these variants may lead to clinically relevant differences in HbA1c measurements. Implementing additional testing with TINIA and HbEP or other methods detecting HbA1c upon variant flagging can ensure accurate diabetes monitoring and management. Further research is needed to determine which method, HPLC or TINIA, provides more reliable results for reporting in the presence of hemoglobin variants.

Keywords: HbA1c, Hb Variants, HPLC, TINIA

PC-15

Comparison of HbA1c Results Using HPLC and Turbidimetric Inhibition Immunoassay Methods in Patients with Variant Flags

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Background: Accurate measurement of HbA1c is crucial for monitoring long-term glucose control in diabetic patients. At Korea University Guro Hospital, the Bio-Rad D-100, which utilizes an ion-exchange high-performance liquid chromatography (HPLC) method, is routinely used for HbA1c testing. When the system flags a potential hemoglobin variant with possible interference, additional testing with turbidimetric inhibition immunoassay (TINIA) and hemoglobin electrophoresis (HbEP) is performed. This study compares the HbA1c results from these two methods and examines the impact of the possible presence of hemoglobin D (HbD) variants on these measurements.

Methods: A total of 11 patients were selected based on the presence of variant flags during routine HbA1c testing using Bio-Rad D-100. If a variant flag was triggered, samples were re-tested using Roche COBAS c513 TINIA, and HbEP was conducted. The estimated average glucose (eAG) was calculated based on HbA1c results from both methods. Recent fasting glucose levels were retrieved from the patient's electronic medical records.

Results: HbA1c values measured with HPLC (Bio-Rad) ranged from

PC-16

Development in Competitive Immunoassay of a Point-of-Care Testing for Cotinine Detection in Urine

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Background: We developed a sensitive and selective lateral flow immunoassay (LFIA) to detect cotinine (COT), a key biomarker of tobacco smoke exposure. The competitive assay used COT-BSA capture and mAb-COT conjugated to 40 nm gold nanoparticles (mAb-COT-AuNPs). The optimal conditions were 4 mg/mL mAb-COT and 0.75 mg/mL COT-BSA. The LFIA detects COT levels as low as 150 ng/mL visually within 15 minutes and has a linear range of 25–200 ng/mL, with a limit of detection (LOD) of 11.94 ng/mL when analyzed with ImageJ. LFIA is a simple, rapid, and suitable method for clinical use.

Methods: mAb-COT was conjugated to AuNPs and analyzed by UV-visible spectrophotometry. The LFIA was assembled with a sample pad, conjugate pad, NC membrane with control and test lines, and an absorbent pad. The optimal AuNP size and COT-BSA and mAb-COT concentrations were determined. COT detection involved applying the samples to the strip and analyzing the intensities using the ImageJ software. Specificity was tested against urine substances,

and real samples were compared with the ELISA results.

Results: The optimized LFIA for cotinine (COT) detection, using 40 nm AuNPs, 0.75 mg/mL COT-BSA, and 4 mg/mL mAb-COT, showed high sensitivity (LOD 11.94 ng/mL) and specificity, with no cross-reactivity with other urine substances. This correlated well with the ELISA results for the real samples, proving the reliability of point-of-care tobacco exposure monitoring. Further validation using a larger sample size is required to confirm our findings.

Conclusions: In this study, we developed and improved an LFIA-COT for the sensitive and selective detection of COT in urine. Under optimal conditions, which included the use of 40 nm AuNPs as the reporter material, 4 mg/mL of mAb-COT as the labeling probe, and 0.75 mg/mL of COT-BSA as the capture probe, the LFIA-COT demonstrated a sensitivity to detect COT at the level of 50 ng/mL within 15 minutes. The LFIA results were easily observable with the naked eye. The linear range of detection was 25–200 ng/mL for COT with an LOD of 11.94 ng/mL for human urine samples. Additionally, the LFIA-COT showed no interference from urine, such as albumin, ascorbic acid, hemoglobin, bilirubin, glucose, and HGG. Furthermore, the LFIA-COT showed a significant correlation with the standard ELISA technique, suggesting its potential for wider use in clinical and public health settings. Our approach contributes to the more precise monitoring of tobacco exposure, as it is sensitive enough to detect COT at its current level.

Keywords: Cotinine, Smoke, Lateral flow immunoassay, Competitive Immunoassay, Point-of-Care testing

PC-17

Evaluation of Analytical Performance of the BS-620M Chemical Analyzer for HbA1c Assay

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Background: Glycated hemoglobin and hemoglobin A1c (HbA1c) levels are widely used to diagnose and monitor glycemic control in patients with diabetes mellitus. Because the diagnostic cut-off and therapeutic range for the protocol of HbA1c follow international guidelines, standardization and accuracy of the test are important. The automated HbA1c analyzer BS-620M (Mindray, China) measures HbA1c using an enzymatic method and has high throughput, user convenience, and a short handling time. In this study, we evaluated the analytical performance of an automated HbA1c analyzer, BS-620M.

Methods: The precision, linearity, and carryover of BS-620M were evaluated. Using 100 patient samples, comparability of HbA1c measurements between BS-620M and Roche turbidimetric inhibition immunoassay, cobas c513 (Roche, Germany) was evaluated. Using 10 samples with high bilirubin and 10 samples with high triglyceride levels, a comparative analysis with Cobas c513 revealed that hyperbilirubinemia and lipemia did not interfere. All HbA1c data were analyzed using NGSP units. Statistical analyses were performed using Excel 2019 (Microsoft Co., USA), EP evaluator, and MedCalc ver.

22.014 (MedCalc Software bvba, Belgium).

Results: A comparative study with cobas c513, using 100 samples, showed high comparability (correlation coefficient $r=0.98$, regression equation $y=0.933x-0.09$). The repeatability (within-run precision) (%CV) for low and high QC materials was 0.33% and 0.13%, respectively. The repeatability (within-run precision) (%CV) of the two patient samples ranged from 0.20%–0.33%. The %CV for the between-run precision of the low- and high-QC materials were 0.48% and 0.51%, respectively. The %CV for the between-run precision of the two patient samples ranged from 0.47%–0.52%. The test showed acceptable linearity (slope 0.972 and intercept 0.001) in the range of 3.84%–16.50%. The carry-over rate was less than 1.0% (-0.2%). BS-620M showed acceptable comparability with Cobas c513 with 10 high-bilirubin samples and 10 high-triglyceride samples.

Conclusions: The results of the evaluation showed high comparability for HbA1c measurement between the BS-620M and the Roche turbidimetric inhibition immunoassay, Cobas c513. The results also showed excellent within- and between-run precision, acceptable linearity and carry-over, and acceptable results without interference from hyperbilirubinemia or lipemia. These results indicated that this assay is suitable for routine use in clinical laboratories.

Keywords: Hemoglobin A1c, Enzymatic assay, Chemistry analyzer, Analytical performance

PC-18

Comparison Between Multiple Clinical Chemistry Analyzers: Application of Clinical and Laboratory Standard Institute EP31-A-IR

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Background: Laboratories often use multiple analyzers to enhance efficiency and convenience. When using multiple analyzers, ensuring the comparability of results across analyzers is crucial. In this study, we evaluated the comparability between several automated biochemical analyzers using the EP31-A-IR guideline published by the Clinical and Laboratory Standards Institute. EP31-A-IR is the guideline for verifying comparability among multiple analyzers within one healthcare system. This guideline provides a simple approach to evaluate the comparability across a maximum of 10 instruments simultaneously.

Methods: A total of 36 biochemical parameters, including creatinine, magnesium, and low-density lipoprotein cholesterol (LDL-C), were evaluated for comparability between two to six Cobas 8000 c702 analyzers (Roche Diagnostics System, Switzerland) using serum. The EP31-A-IR guideline primarily involves assessing the quality control data between the analyzers to be compared. If the ratio of the maximum to minimum between-run SD (ST) values among the measurement systems to be compared exceeds 2, proceeding with the subsequent steps of the guideline is not possible. Next, the range rejection limit was calculated according to the acceptance criteria and the

number of analyzers being compared. Comparability was considered verified if the maximum difference between comparable analyzers was less than or equal to the range rejection limit.

Results: Among the 36 parameters evaluated, comparability between the analyzers was successfully verified for 33 parameters, as their differences were within the range rejection limit calculated using the EP31-A-IR. Three parameters, creatinine, magnesium, and LDL-C, could not be evaluated using the EP31-A-IR because of a discrepancy greater than twofold between the measurement system's highest and lowest ST values.

Conclusions: In most of the parameters evaluated, comparability could be verified through the EP31-A-IR. However, it has limitations in evaluating parameters that do not satisfy the conditions provided by the guidelines. Thus, each laboratory should establish an appropriate method for evaluating comparability based on its specific circumstances.

Keywords: CLSI EP31-A-IR, Comparability, Verification, Quality control

PC-19

Establishment of a Reference Interval for Plasma Intact Fibroblast Growth Factor 23 Concentrations in the Korean Healthy Population

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Background: Fibroblast growth factor 23 (FGF23) is a biomarker, which plays a role in the homeostasis of blood inorganic phosphorus concentration by decreasing tubular phosphate reabsorption and inhibiting calcitriol synthesis. Currently, circulating FGF23 levels can be measured using two types of methods (i.e., intact FGF23 [iFGF23] assays and C-terminal assays). The availability of automated analyzers in clinical laboratories appears to be superior for iFGF23 currently. This study aimed to establish a reference interval for iFGF23 measurements in the Korean population.

Methods: A total of 375 plasma samples collected in K3-EDTA tubes were collected from a health checkup center from December 2022 to November 2023. iFGF23 was measured using a Liaison platform with LIAISON FGF23 reagent (DiaSorin). Serum samples collected at the same phlebotomy time as the plasma samples from the same patient were collected for biochemical analysis of calcium, inorganic phosphorus, alkaline phosphatase (ALP), creatinine, parathyroid hormone (PTH), and 25(OH) vitamin D using automated analyzers. For a reference interval establishment following CLSI EP28-A3C, outliers were detected and excluded using Tukey's method. iFGF23 concentration normality was assessed using the Shapiro-Wilk test. In the non-parametric approach, values at the 2.5th and 97.5th percentiles were obtained. Furthermore, the correlation between iFGF23 con-

centration and anthropometric factors were additionally evaluated.

Results: Among the anthropometric factors, muscle mass was the only factor that demonstrated a statistically significant correlation with iFGF23. All four inorganic phosphorus-associated biochemical analytes exhibited a positive association with iFGF23, with the correlation coefficients (*r*) for calcium, ALP, vitamin D, and PTH being 0.989, 0.993, 0.853, and 0.992, respectively. The distribution of iFGF23 concentrations in a study population violated the normality and presented the right skewness. The overall reference interval by non-parametric method was 27.40–100.30 pg/mL.

Conclusions: The reference interval of plasma iFGF23 was proposed in the Korean population. Muscle mass and inorganic phosphorus-associated biochemical analytes showed a positive correlation with iFGF23 within normal ranges.

Keywords: Intact FGF23, Reference interval, Korean population, Inorganic phosphorus

PC-20

Missed Monoclonal Immunoglobulin Proteins in Urine by Capillary Electrophoresis

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Background: Capillary electrophoresis, using a high pH buffer, is believed to be less susceptible to false negatives or decreased detection of monoclonal immunoglobulin proteins (M-proteins) owing to protein precipitation compared to gel electrophoresis. However, false-negative or decreased results have been reported in serum capillary electrophoresis. While such occurrences in urine samples have not been documented, this study presents two cases where M-proteins in urine were missed using a Capillarys 2 Flex Piercing electrophoresis system (Sebia, Lisses, France), probably secondary to precipitation during the preparation process.

Methods: In case 1, the urine sample with a false-negative result demonstrated a gel-like precipitate after overnight storage at 4°C. The precipitate was dissolved with vigorous stirring, and the sample was retested. In case 2, the urine sample, which showed a solid precipitate after dialysis, was treated with 2-mercaptoethanol (a reducing agent for disulfide bonds) and retested. Specifically, 150 µL of a 1% 2-mercaptoethanol solution with urea was mixed with 500 µL of the dialyzed and concentrated urine sample. The mixture was incubated for 15 minutes at room temperature and subsequently tested using protein capillary electrophoresis.

Results: For case 1, a 24-hour urine sample from a 75-year-old male patient was submitted for protein electrophoresis. Despite a high serum-free kappa light chain concentration (2,349.74 mg/L), the initial result was negative. After dissolving the gel-like precipitate, the M-protein (34.1%, 67.7 mg/day) was detected. In case 2, a urine sample from a 68-year-old male patient diagnosed with plasma cell my-

eloma with free kappa type M-protein was submitted for follow-up protein electrophoresis. Although the serum-free kappa light chain concentration had increased compared to the previous month (from 1,240.88 to 1,889.90 mg/L), the initial electrophoresis result was negative. After 2-mercaptoethanol treatment, the M-protein peak was recovered on the electrophoretogram.

Conclusions: These cases highlight that M-protein precipitation in urine during the preparation can result in false-negative results in capillary electrophoresis systems. Accurate detection and quantification of M-proteins in urine are crucial, especially for patients with plasma cell myeloma of the light chain type. False-negative or decreased results should be carefully considered when the accompanying laboratory results do not corroborate with the electrophoretogram.

Keywords: Capillary electrophoresis, False negative, Monoclonal immunoglobulin protein

PC-21

Development of Artificial Intelligence for Automatic Interpretation of Immunofixation Electrophoresis Using Deep Learning

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Background: Immunofixation electrophoresis (IFE) is an essential test for diagnosing plasma cell neoplasms such as multiple myeloma. Interpreting IFE test results by visually reading gel images is labor intensive and time consuming, influenced by the experience of the interpreter, and compromises consistency when multiple interpreters are involved. To address these issues, we developed an accurate and consistent artificial intelligence (AI) interpretation system by applying a deep-learning model to IFE-result interpretation.

Methods: A dataset was prepared by dividing 126,056 agarose gel IFE-result images into training, validation, and test sets at a ratio of 7:2:1, respectively. A deep-learning model was developed using this dataset to distinguish 11 types of IFE patterns: IgG- κ , IgG- λ , IgA- κ , IgA- λ , IgM- κ , IgM- λ , IgD- κ , IgD- λ , light chain κ , light chain λ , and negative. The AI model used various classification algorithms based on ImageNet, and the results interpreted by multiple models were integrated using soft voting, an ensemble technique, to derive the final result.

Results: The developed IFE AI model achieved an analysis performance of 96.99%, 94.03%, and 95.47% in precision, recall, and F1-score, respectively. In cases where discrepancies arose between the interpretation of the AI model and that of experts, further analyses were conducted to identify the causes of errors and assess the usefulness and limitations of the AI-based diagnostic system.

Conclusions: While the AI system developed in this study to interpret IFE results requires additional performance verification and final confirmation by diagnostic pathology specialists, it is expected to be highly useful in providing a second opinion to assist technicians in IFE in-

terpretation. The use of the AI interpretation system is anticipated to improve the accuracy and consistency of testing, and particularly enhance work efficiency at institutions performing large volumes of tests.

Keywords: Artificial intelligence, Clinical chemistry, Convolutional neural network, Deep learning, Immunofixation electrophoresis, Plasma cell neoplasm

PC-22

Identification and Performance Evaluation of Optimal Delta Check Methods in Clinical Chemistry Tests

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Background: Since its proposal by Lindberg in 1967, the delta check has become the most widely utilized quality control method employing patient samples. Common methods involve comparing current and previous test results through differences, percentage changes, and rates of change. Although the CLSI guideline EP33 outlines numerous delta check methods, it does not specify the best method for each test item, resulting in laboratories creating their own policies. This study aimed to identify the optimal delta check method for each clinical chemistry test item.

Methods: From January 2023 to February 2024, we collected results from 23 routine clinical chemistry tests (albumin, ALP, ALT, AST, BUN, calcium, chloride, cholesterol, creatinine, CRP, direct bilirubin, glucose, HDL, lactic acid, LDL, phosphorus, potassium, r-GT, sodium, total bilirubin, total protein, triglyceride, and uric acid) performed at our institution. We used data paired with past results for analysis, categorizing them into inpatients, outpatients, and health check-up recipients. To calculate the thresholds for 12 known delta check methods, including methods currently in use for each test item, absolute rate of change, and reference change value (RCV), we randomly extracted 75% of the total data collected during the study period. The remaining 25% of the data was used to determine the accuracy, sensitivity, and specificity of each delta check limit. To evaluate these metrics, we randomly shuffled 2% of the application set to use as true positive cases for performance assessment.

Results: We collected 18,623,409 paired data points with past results, including 11,711,134 from outpatients, 5,822,202 from inpatients, and 1,090,073 from health check-up recipients. Using 75% of the data, we established thresholds for 12 delta check methods and evaluated their performance with the remaining 25%. Methods that outperformed the current delta check method were found in over 80% of the test items. Overall, the absolute rate of change had

the highest accuracy (93.5%) and specificity (94.9%), while the 99% RCV method had the highest sensitivity (53.2%). The best-performing method for each patient type and test item was selected according to the detailed criteria.

Conclusions: This study identified optimal delta check methods for over 80% of the test items compared to the current method. Customized thresholds based on test characteristics and patient demographics improved the detection rates and provided a framework for better laboratory management.

Keywords: Delta check, Clinical chemistry tests, Quality control, Laboratory information system

PC-23

Comparison of Four Blood Collection Tubes for 14 Biochemical Analytes: Quick-Clotting Serum Separating Tube and Serum Separating Tube of Ab Medical vs. Plasma Separating Tube and Serum Separating Tube of Greiner Bio-One

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Background: A quick-clotting clot activator with gel tube (VQ-tube, AB medical Korea) using thrombin-based coagulant was recently developed. We evaluated the four blood collection tubes (quick-clotting clot activator with gel tube, clot activator with gel tube of AB medical [VQ-tube and, V-tube, respectively]) vs. lithium heparin separator tube (PST) and serum separator clot activator tube (SST) of Greiner Bio-One) in 14 biochemical analytes.

Methods: Blood samples were collected from 51 out-patients and 10 patients with myocardial injury in four blood collection tubes. The test results of 12 clinical chemistry and 2 cardiac marker analytes were compared. The stability of each analyte in Greiner SST, Greiner PST, and VQ-tube was evaluated up to 120 hours.

Results: The mean differences of the test results in VQ-tube compared to Greiner SST satisfied the allowable limits except for 2 analytes (the mean difference of -2.76% and 21.17%, potassium and creatine kinase-myoglobin binding [CK-MB], respectively). In the stability evaluation, VQ-tube showed comparable results in glucose, potassium, lactate dehydrogenase (LDH), aminotransferase, cardiac Troponin I compared to Greiner SST. Whereas, Greiner PST showed shortened stability in glucose, potassium, and LDH compared to Greiner SST.

Conclusions: VQ-tube showed good clinical performance in the clinical chemistry analytes. This tube will provide more reliable results compared to PST, especially for patients who need fast test results. When using the VQ-tube, reference interval verification should be

considered for the potassium, which showed the significant mean difference compared to Greiner SST.

Keywords: VQ-tube, Mean difference, Analytes, Serum separating tube, Plasma separating tube

PC-24

Development of an Automated Method for Detecting Unsuitable Biochemical Specimens Due to Various Causes Using Deep Learning

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Background: Ensuring the quality and reliability of biochemical test results is of paramount importance in clinical diagnostics. Specimens that are icteric, hemolyzed, contaminated by drugs, and so on, can lead to inaccurate results, affecting patient care and treatment outcomes. This study designed an innovative automated system to detect and flag unsuitable biochemical specimens based on various causes, including icteric, hemolysis, and drug interference.

Methods: We developed a comprehensive system using advanced machine-learning and computer-vision techniques to automatically assess the suitability of biochemical specimens, which integrated the following components: 1. Image processing: We used OpenCV to preprocess images of specimens. Each image underwent color correction, contrast enhancement, and segmentation to isolate the serum portion. 2. Feature extraction: Key features such as color intensity, transparency, and turbidity were extracted from the segmented images. These features were crucial in identifying icteric and hemolyzed samples. 3. Machine-Learning model: A convolutional neural network was trained to classify specimens based on their visual characteristics using a labeled dataset comprising images of normal, icteric, hemolyzed, and drug-contaminated specimens. 4. Data integration: Laboratory information management system data were integrated to cross-validate the predictions of the machine-learning model with the clinical history and medication records of the patient, enhancing the reliability of the system. 5. Validation: The system was validated using a test set of 500 specimens, including various interferences, with results compared with those of manual assessments by experienced laboratory technicians based on accuracy, sensitivity, and specificity.

Results: The automated system demonstrated an overall accuracy of 100%, with a sensitivity of 100% and specificity of 100% in detecting unsuitable specimens. The convolutional neural network identified icteric and hemolyzed samples, while drug interference was accurately flagged through the integrated data approach. The confusion matrix showed a high true-positive rate and a low false-negative

rate, indicating robust performance. ROC curves and AUC values further confirmed the high discriminative ability of the model.

Conclusions: The developed automated system significantly enhances the reliability of biochemical test results by efficiently detecting unsuitable specimens due to various causes, such as icteric, hemolysis, and drug interference. It can be integrated into existing laboratory workflows to reduce the burden on laboratory staff and ensure high-quality diagnoses. The integration with the laboratory information management system provides an additional layer of validation, making the system highly reliable for clinical use. Future work will focus on expanding the dataset and refining the model to further improve its accuracy.

Keywords: Clinical chemistry, Automated system, Interference, Machine learning

PC-25

Aluminum Toxicity in Chronic Kidney Disease: A Case Report

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Sucralfate is an alkaline gastric mucosal protective agent composed of the aluminum salt of sulfated disaccharide. In the stomach, sucralfate forms aluminum salt complexes, which have buffering and neutralizing effects against gastric acid. Clinical manifestations of aluminum toxicity commonly include anemia, neurological symptoms such as seizures, memory loss, dementia, and bone diseases such as osteomalacia. Approximately 95% of aluminum is excreted *via* the kidneys in the human body. This case is a 58-year-old female with a history of chronic kidney disease, hepatitis C, and hepatocellular carcinoma. In 2016 and 2022, she underwent two liver transplant surgeries. She was admitted this time because of language difficulties, delirium, and dysphasia. Five months prior, she had persistent gastrointestinal bleeding, which was managed *via* endoscopy. After 3 months of proton pump inhibitor therapy, she was switched to sucralfate, continuing it for 6 weeks. The patient had been bedridden for an extended period, initially receiving parenteral nutrition followed by nasogastric tube feeding. With altered consciousness and after ruling out hepatic encephalopathy, trace element analysis revealed high aluminum levels in her body. Owing to her recent liver transplant and immunosuppressive therapy, deferoxamine (DFO) chelation therapy was not recommended to avoid infection risk. Furthermore, since DFO is metabolized by the kidneys and the patient had renal impairment, sucralfate was discontinued to prevent the worsening of clinical status. Following the discontinuation of the medication for approximately a week, the patient's consciousness gradually improved. During the 3-month outpatient follow-up, no significant changes in her consciousness were observed. Prolonged use

of aluminum-containing phosphate binders can lead to increased aluminum concentration. KDOQI recommends that all patients on dialysis undergo serum aluminum concentration testing at least once a year. For patients using aluminum-containing medications, serum aluminum concentration should be monitored every 3 months. Aluminum toxicity treatment mainly involves three approaches: 1. identifying and removing all possible sources of aluminum; 2. use of DFO; and 3. intensive high-flux dialysis (6 days per week). Patients with symptoms and serum concentrations ranging from 20 to 200 µg/L are administered with DFO, and treatment doses are adjusted according to different DFO-stimulated aluminum concentrations. Sucralfate is highly effective in treating gastric or duodenal ulcers; however, in patients with renal insufficiency, prolonged use of sucralfate or other aluminum-containing preparations should be avoided as it may lead to iatrogenic harm.

Keywords: Aluminum toxicity, Chronic kidney disease, Sucralfate

PC-26

Current State of Vancomycin Area Under the Concentration-Time Curve Estimation: Insights from a Survey among Clinical Pathologists in Korea

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Background: Vancomycin, a crucial antibiotic against Gram-positive bacterial infections, necessitates therapeutic drug monitoring (TDM) due to significant pharmacokinetic variability. While traditional TDM relies on steady-state trough concentration, recent guidelines advocate for the area under the concentration-time curve (AUC) as the target index. However, AUC estimation lacks precise protocols, potentially leading to discrepancies among institutions.

Methods: Between August and September 2023, a questionnaire survey about TDM practice was distributed *via* email to 170 clinical pathologists, one person per medical institution. The survey included additional challenges related to TDM cases for respondents reporting vancomycin AUC values in their consultation reports. Respondents were asked to estimate AUC values based on the information provided about virtual cases simulating real patient scenarios. Nineteen of 54 respondents engaged in the TDM case challenge, and responses from 18 participants were analyzed utilizing Bayesian software programs.

Results: For an ordinary patient in case-1, the overall coefficient of variation (CV) for AUC values was 0.4% when using both trough and

peak concentrations for AUC calculation and 1.9% when using only trough concentration. For case-2, involving an elderly obese patient, the corresponding CV was 6.6%. In case-3, featuring multiple trough concentrations, the CV was 15.6%, reflecting variability due to selective data usage.

Conclusions: Despite good agreement among respondents for the case of a general patient, considerable variability in AUC estimation was observed in cases involving unusual patient characteristics or old TDM data. This study offers insights into the current landscape of vancomycin TDM in Korea and emphasizes the necessity for standardized operational protocols for AUC estimation.

Keywords: Vancomycin, Therapeutic drug monitoring, Area under the concentration-time curve

unit was predominantly employed: 92.7%–94.0% in general hospitals, 93.4%–94.5% in hospitals, and 97.8%–98.2% in clinics. The cutoff values ranged from 0 to 878, with most institutions selecting 7 (75.7%, 72.2%, 62.2% in 2018, 2019, and 2020), 8 (18.1%, 17.7%, 17.4%), and 9 (2.0%, 6.7%, 17.3%), respectively.

Conclusions: Most institutions utilized quantitative methods for AFP testing, reporting results in ng/mL. It appears prudent to discontinue qualitative AFP testing within the NCSP and standardize the units to ng/mL. The AFP test is currently in harmonization status, making a unified cutoff value feasible. Therefore, the appropriate cutoff values tailored to the NCSP in Korea must be identified.

Keywords: Alpha-fetoprotein, Hepatocellular carcinoma, National Cancer Screening Program, Quantitative method

PC-27

Current Status of the Alpha-Fetoprotein Reporting in the National Cancer Screening Program for Hepatocellular Carcinoma in Korea

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Background: Mortality rates of major cancers in 2022 revealed that hepatocellular cancer (HCC) had the second-highest mortality rate following lung cancer. The National Cancer Screening Program (NCSP) for HCC in Korea consists of biannual concurrent liver sonography and serum alpha-fetoprotein (AFP) testing for high-risk groups, including those over 40 years of age with chronic hepatitis B and C, and liver cirrhosis. The effectiveness of surveillance is impacted by the type of test methods and the use of optimal cutoff values for AFP testing. However, research on this issue has yet to be conducted. Therefore, we aim to analyze the current reporting status of AFP testing included in the NCSP.

Methods: We investigated AFP results from institutions participating in the NCSP from 2018 to 2020 across various types of medical institutions, including general hospitals, hospitals, and clinics. We counted the number of institutions utilizing qualitative and quantitative AFP tests. For quantitative tests, we further categorized the count based on the units used, either ng/mL or IU/mL, as well as the frequently used cutoff values in AFP reports.

Results: From 2018 to 2020, the numbers of institutions participating in the NCSP were 4,894 (2018), 5,021 (2019), and 5,073 (2020). The participation numbers for general hospitals, hospitals, and clinics were 345, 759, and 3,790 in 2018; 345, 753, and 3,923 in 2019; and 353, 730, and 3,990 in 2020, respectively. Quantitative methods were consistently preferred, with general hospitals utilizing them at rates of 99.1%–99.4%; hospitals at 90.0%–92.7%; and clinics at 93.9%–95.7%. Among those using quantitative methods, the ng/mL

PC-28

Deep Learning-driven Integrative Analysis of Glycoproteomics and Metabolomics for Early Detection and Prognosis of Ovarian Clear Cell Carcinoma

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Background: Ovarian clear cell carcinoma (OCCC) is a rare, aggressive ovarian cancer with poor prognosis due to late diagnosis and therapy resistance. This study aims to develop a deep learning-based integrative analysis of glycoproteomics and metabolomics data to identify novel serum biomarkers for the early detection and prognostic evaluation of OCCC, improving diagnostic accuracy and prognostic stratification through multi-omics data and advanced computational techniques.

Methods: We utilized a comprehensive dataset containing serum samples from 2,500 individuals, including 1,250 patients with OCCC and 1,250 healthy controls, with glycoproteomics (glycoprotein profiles) and metabolomics (metabolic profiles) data. Glycoprotein markers included CA 125, HE4, and novel glycoproteins identified through mass spectrometry, whereas metabolomic markers included specific metabolites associated with OCCC. Data preprocessing involved normalization, log-transformation, and imputation of missing values using multiple imputation by chained equations. A deep learning model was constructed using a dual-branch architecture, combining convolutional neural networks for glycoproteomics data and recurrent neural networks with LSTM units for metabolomics data. The dataset was divided into training (70%), validation (15%), and test (15%) sets. Hyperparameter tuning was performed using Bayesian optimization. Model performance was evaluated using metrics such as sensitivity,

specificity, accuracy, F1-score, and the area under the receiver operating characteristic curve (AUC-ROC). Prognostic evaluation included Kaplan–Meier survival analysis and Cox proportional hazards modeling.

Results: The integrative deep learning model achieved an accuracy of 94.7% (95% CI: 93.2–96.2), sensitivity of 96.1% (95% CI: 94.6–97.6), and specificity of 93.3% (95% CI: 91.4–95.2) on the test set. The AUC-ROC was 0.97 (95% CI: 0.96–0.98), indicating strong performance. For early detection of OCCC, the model achieved a sensitivity of 92.4% (95% CI: 90.1–94.7) and specificity of 90.8% (95% CI: 88.1–93.5). Prognostically, the model stratified patients into high-risk and low-risk groups with a hazard ratio of 3.9 (95% CI: 3.1–4.7, $P < 0.001$), indicating robust predictive capability for survival outcomes.

Conclusions: This study presents a novel approach for the early detection and prognostic evaluation of OCCC using deep learning on glycoproteomics and metabolomics data. The dual-branch deep learning model significantly improves diagnostic and prognostic accuracy, showing the potential of integrating multi-omics data with advanced machine learning for cancer diagnostics and patient management. Future researchers should validate these findings in larger cohorts, incorporate additional omics layers, and assess the utility of this approach in other rare cancers.

Keywords: Ovarian clear cell carcinoma, Deep learning, Glycoproteomics, Metabolomics, Biomarkers, Prognosis

PC-29

Hypophosphatasia: A New Biochemical Procedure Which Facilitates Diagnosis and Therapy Introduction

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Background: Hypophosphatasia (HP) is a rare, inherited disorder characterized by defective bone and teeth mineralization and deficiency of tissue nonspecific alkaline phosphatases (TNAP) due to the defective alkaline phosphatase gene (ALPL). Six clinical forms are currently defined: perinatal (lethal), perinatal benign, infantile, childhood, adult, and odontohypophosphatasia. In the lethal perinatal form, patients exhibit markedly impaired mineralization in utero. Clinical symptoms of the infantile form include respiratory complications, premature craniosynostosis, widespread demineralization, and rachitic changes in the metaphyses. The childhood form is characterized by skeletal deformities, short stature, and waddling gait, and the adult form by stress fractures, thigh pain, chondrocalcinosis, and marked osteoarthropathy. Odontohypophosphatasia is characterized by premature exfoliation of fully rooted primary teeth and/or severe dental caries, often not associated with skeletal system abnormalities.

Methods: The diagnosis is established from laboratory assays of TNAP in serum, elevated urinary phosphoethanolamine (PEA), and

ALPL gene analysis. We report the following specific biochemical procedures for a secure diagnosis of HP: isoenzyme analysis of TNAP in leukocytes and quantification of pyridoxal 5'-phosphate (PLP) in plasma and urine. TNLP determination uses a fluorometric method via homoarginine as the inhibitor for bone AP and phenylalanine for intestine AP. PLP assessment has been performed by using (C-14) tyrosine and tyrosine decarboxylase. The substrate (C-14) tyrosine is separated from the product (C-14) tyramine on a cation exchange column. The evaluation of PLP in urine is a better criterion than that of PEA and PLP, which can also be measured in plasma.

Results: Bone AP activity and intestine AP in patients with HP lie below the normal range of 2.0–25.0 and 1.0–10.0 nmol/min/mg protein, respectively. Conversely, patients with HP show marked elevation of PLP in plasma and urine compared with the normal range of 1–3 and 0–0.7 nmol/mL in plasma and urine, respectively. After several years of using our new method, we have identified three patients with infantile HP and over 20 adult or juvenile patients. We have been also monitoring TNAP and PLP among patients with HP treated with enzyme replacement therapy or various medicines and/or diets.

Conclusions: Although HP is known to be a rare disease, we found that HP is relatively common. For patients with late-onset HP, early detection and various therapeutic measurements may prevent or delay clinical development.

Keywords: Hypophosphatasia, Alkaline phosphatase, Pyridoxal 5'-phosphate

PC-30

Current Status of Co-ordering of Erythrocyte Sedimentation Rate and C-Reactive Protein Testing in South Korea

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Background: We retrospectively explored current trends in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) ordering using insurance claims data over the past 5 years and then focused on ESR and CRP co-ordering in 2022 to estimate the potential health-care resource savings from a statewide diagnostic stewardship campaign to reduce ESR and CRP co-ordering.

Methods: Annual (2018–2022) utilization and cost of ESR and CRP tests, total patient days of inpatients, and patient encounters of outpatients were also retrieved from a platform operated by the Health Insurance and Review Agency. Subsequently, all claims with test codes corresponding to ESR and CRP for hospital visits in 2022 were obtained. Additionally, a random forest analysis was conducted to determine significant variables that predicted the co-ordering of ESR

and CRP in a claim.

Results: The number of ESR and CRP tests had gradually increased for 5 years in the outpatient setting than in the inpatient setting, except for a minimal decline in 2020. In 2022, the total number of claims containing ESR or CRP tests in the inpatient and outpatient settings was 7,912,231 and 14,077,801, respectively. In these claims, 32,382,257 CRP tests and 15,668,372 ESR tests were ordered. The proportion of claims in which ESR and CRP were co-ordered was 46.64% in 2022. of interest, more than 60% of claims were observed with co-ordering orders in orthopedic surgery, neurosurgery, and plastic surgery. The proportion of co-ordering was higher in the inpatient setting and primary hospitals. Random forest analysis demonstrated that the type of hospital and department were the most influential variables, followed by patient age.

Conclusions: This study confirmed the high number of ESR and CRP orders and the frequent pattern of ESR/CRP co-ordering in Korea, probably indicating inappropriate testing. Approximately up to \$3.2 million in healthcare costs could be saved, and unnecessary follow-up tests or procedures could be reduced with a laboratory stewardship program to reduce ESR/CRP co-ordering, as previously reported. This study highlights the urgent need for diagnostic stewardship programs for ESR and CRP testing in Korea.

Keywords: Co-ordering, Erythrocyte sedimentation rate, C-reactive protein, Choosing wisely, Laboratory stewardship

PC-31

Assessing Cardiovascular Disease Risk Through the Analysis of Serum Beta 2 Microglobulin in Patients with Chronic Kidney Disease

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Background: Beta 2 microglobulin (B2M) can be utilized to evaluate renal function in patients with chronic kidney disease (CKD), while pre-dialysis serum B2M levels can predict mortality risk. In addition to the kidney, B2M regulates the expression of interleukin 1 and tumor necrosis factor, participating in vascular damage pathogenesis. Thus, it is essential to diagnose cardiovascular disease (CVD). This study aimed to evaluate the relationship between serum B2M levels and some metabolic and cardiovascular factors in patients with CKD.

Methods: Using the case-control study method, the patients were divided into a case group of 119 patients with different CKD stages (glomerular filtration rate [GFR] <90 mL/min/1.73 m²) and a control group of 52 healthy controls. Creatinine, urea, cholesterol, triglycerides, low-density lipoprotein (LDL), calcium, phosphate, albumin, total protein, and B2M in blood samples were analyzed via the Cobas 6000 fully automatic analyzer and echocardiography. Echocardiography measurements included left ventricular mass index (LVMI), left ventricular end-systolic diameter (LVESD), and electrical velocimetry

(EV). CKD-EPI crea/cys combined calculation was utilized to evaluate kidney function by GFR.

Results: The average age of the CKD and healthy group was 54.92 and 50.66 years, respectively. B2M was significantly higher in the CKD group than in the control group ($P < 0.000$). Plasma B2M was significantly and directly correlated with creatinine ($r = 0.516$, $P < 0.000$), phosphate ($r = 0.342$, $P < 0.000$), and cystatin C ($r = 0.700$, $P < 0.000$). Moreover, it was also directly correlated with urea ($r = 0.326$, $P < 0.000$), LVMI ($r = 0.113$, $P < 0.000$), and LVESD ($r = 0.265$, $P < 0.000$). It was significantly and inversely correlated with BMI ($r = -0.274$, $P < 0.000$), GFR ($r = -0.516$, $P < 0.000$), calcium ($r = -0.137$, $P < 0.000$), albumin ($r = -0.180$, $P < 0.000$), total protein ($r = -0.080$, $P < 0.000$), and cholesterol ($r = -0.215$, $P < 0.000$). Moreover, this was also inversely correlated with LDL ($r = -0.163$, $P < 0.000$), triglyceride ($r = -0.142$, $P < 0.000$), and EV ($r = -0.292$, $P < 0.000$).

Conclusions: B2M levels provenly predict CVD risk and CVD mortality in all CKD stages. In our study, B2M is directly correlated with biomarkers of kidney function, phosphate, LVMI, and LVESD parameters and inversely correlated with GFR, protein, lipids, calcium, and EV parameters, similar to studies conducted in other countries. Hence, B2M can be used to detect the possibility of CVD risk in people with CKD.

Keywords: Chronic kidney disease, Cardiovascular disease, Beta-2 microglobulin

PC-32

Discrimination of Cholangiocarcinoma via Infrared Spectral Analysis in Saliva

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Background: Cholangiocarcinoma (CCA), a cancer of the bile duct epithelium, is one of the most common cancers worldwide. The gold standard for CCA diagnosis is histopathology; however, this method is invasive for patients. Thus, our study aimed to differentiate CCA using non-invasive samples using an attenuated total Reflectance-Fourier transform infrared (ATR-FTIR) spectrometer, a high-throughput and portable device.

Methods: Saliva samples from 24 healthy individuals and 22 patients with CCA were measured using a portable ATR-FTIR spectrometer. The infrared spectra of healthy and CCA were analyzed using principal component analysis (PCA) using The Unscrambler® XI.

Results: The infrared spectral peaks were notably found only in CCA at 1,638 cm⁻¹ (C=O), 1,545 cm⁻¹ (Amide II), 1,450 cm⁻¹ (CH₂/CH₃), 1,404 (CH₃ and COO⁻), 1,048 cm⁻¹ (C-O), 1,011 cm⁻¹ (C-H and C=C),

and 922 cm⁻¹ (PO₂). These peak findings relate to the molecule of proteins and carbohydrates. Furthermore, the multivariate analysis with PCA demonstrated an evident classification between healthy and CCA groups.

Conclusions: This study showed the infrared spectral signature for CCA using saliva, a non-invasive sample. However, various stages of CCA should be further investigated with a larger sample size.

Keywords: Cholangiocarcinoma, Body fluid, Infrared spectrum, Multivariate analysis, POCT, Biomarker

PC-33

Artificial-Intelligence Model for Predicting LDL Subtype Switching as a Predictor of Cardiovascular Risk

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Background: Cardiovascular diseases are a major cause of global morbidity and mortality. Despite low high-density lipoprotein cholesterol (HDL-C) and high low-density lipoprotein cholesterol (LDL-C) being key risk factors for coronary heart disease, many patients with cardiovascular disease have normal LDL-C levels. LDL subfraction testing measures various lipoprotein fractions and LDL particle sizes, classifying patients with larger LDL particles into Type A and those with small dense LDL particles into Type B. Type B is linked to a higher risk of atherosclerosis and myocardial infarction, even with normal cholesterol levels. In this study, an artificial intelligence (AI) model is developed to predict LDL subtype transitions from Type A to Intermediate type, Type A to Type B, and Intermediate type to Type B.

Methods: This was a retrospective study that included 864 patients who underwent at least two LDL subtype tests from December 2020 to December 2023, totaling 1,973 data samples. An AI model was designed to predict unfavorable LDL subtype transitions using various clinical and laboratory test parameters. Multiple machine-learning models, including extreme gradient boosting, light gradient boosting machine, and CatBoost, were employed. An ensemble approach combined the strengths of individual models. Shapley additive explanations (SHAP) analysis was used to interpret the impact of different features on the model predictions.

Results: The model obtained through the ensemble approach demonstrated robust performance in predicting the transition to the unfavorable Type B LDL subtype, achieving an AUROC of 0.6503, an accuracy of 0.6295, and an F1-score of 0.5719. SHAP analysis identified several key predictors: triglyceride, total bilirubin, creatinine, monocyte count, total cholesterol, age, glucose levels, and LDL cholesterol. For the CatBoost model, significant predictors included triglyceride, Mg, AST, total bilirubin, glucose, creatinine, monocytes, and eGFR-MDRD (IDMS). Extreme gradient boosting model identified triglyceride, CRP, age, uric acid, eGFR-CKD-EPI, glucose, potassium, sodium, mono-

cytes, RBC count, and LDL cholesterol as the predictors. The light gradient boosting machine model highlighted triglyceride, Mg, AST, creatinine, BUN, age, uric acid, LDL cholesterol, sodium, RBC count, CRP, and total cholesterol as the key predictors.

Conclusions: This study presents a significant advancement in predicting LDL subtype transitions using AI, potentially improving cardiovascular risk management. The developed ensemble model shows high predictive accuracy and reliability, with SHAP analysis highlighting critical predictors such as triglyceride, glucose, LDL cholesterol, creatinine, and monocyte count. Further validation in diverse clinical settings is essential to confirm the generalizability and clinical utility of the model.

Keywords: Artificial intelligence, LDL subtype transition, Cardiovascular risk, Machine learning, Predictive model, SHAP analysis

PC-34

Characteristics of the Serum Apolipoprotein A1 Concentrations Before and During the COVID-19 Pandemic

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Background: Apolipoprotein A1 (ApoA1) is the main high-density lipoprotein and exhibits anti-inflammatory properties against infectious diseases. Recently, ApoA1 was reported as a new indicator for predicting the severity of coronavirus disease 2019 (COVID-19). In this study, we investigated the characteristics of serum ApoA1 concentrations before and during the COVID-19 pandemic.

Methods: We retrospectively reviewed ApoA1 results obtained from adult patients who visited local clinics in South Korea and underwent serum ApoA1 testing in 2022, when the prevalence of COVID-19 was highest in Korea, and in 2019, before the outbreak of COVID-19. Serum ApoA1 concentrations were assessed by the immunoturbidimetric method using a Labospect 008AS (Hitachi, Tokyo, Japan). The reference intervals for males and females were 110–200 mg/dL and 120–220 mg/dL, respectively. The COVID-19 epidemic data were derived from the website <https://www.worldometers.info/coronavirus/country/south-korea> (downloaded on May 24, 2024).

Results: During the study period, 18,304 serum ApoA1 measurements were obtained from 164 local clinics. The study population comprised 10,159 males and 8,145 females aged 20–103 years. The mean serum ApoA1 levels of all participants during the study was 145.2 mg/dL. The mean serum ApoA1 levels of males and females were 140.3 mg/dL and 151.3 mg/dL, respectively. Before (N=5,946) and during (N=12,358) the COVID-19 pandemic, the mean serum ApoA1 level were 145.8 mg/dL and 144.9 mg/dL, respectively. The average serum ApoA1 levels before and during the COVID-19 pandemic were 142.0 mg/dL and 139.4 mg/dL in males, and 151.4 mg/dL and 151.3 mg/dL in females, respectively. Before and during the COVID-19 pandemic, the proportion of patients with decreased serum ApoA1 levels increased proportionally with age af-

ter the age of 50 years.

Conclusions: The mean serum ApoA1 levels in adult South Korean patients visiting local clinics were lower in both males and females during the COVID-19 pandemic than before COVID-19. ApoA1 levels were higher in women than in men during the study but decreased more in men than in women during the COVID-19 pandemic.

Keywords: Apolipoprotein A1, COVID-19, Korea

PC-35

Prevalence and Distributions of Increased High-Sensitivity C-Reactive Protein According to Age, Gender, and Clinic Location among Patients in Korean Primary Care Clinics

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Background: The high-sensitivity C-reactive protein (hs-CRP) is an inflammatory marker used to assess coronary heart disease (CHD) risk. This study aimed to identify the prevalence and distributions of increased hs-CRP among adult patients attending primary care clinics in Korea.

Methods: Our study reviewed data on specimens requested at 57 primary care clinics in the five regions of Korea. The specimens were from adults ≥ 18 years old with hs-CRP recorded in the laboratory information system at Seegene Medical Foundation during December 2023. Hs-CRP was measured on Cobas c 702 module using a latex particle-enhanced immunoturbidimetric assay. Hs-CRP levels were classified into three categories: <1.0 mg/L (low risk), 1.0 – 3.0 mg/L (average risk), and >3.0 mg/L (high risk). Seoul and Gyeonggi were defined as urban, and the rest of the region was defined as sub-urban.

Results: Out of 1,225 patients, 145 (11.8%) had elevated hs-CRP levels of >3.0 mg/L, of which the majority were males (59.3%). The patients with hs-CRP levels of >3.0 mg/L were younger (57.5 ± 15.9 years) than those with hs-CRP levels of <1.0 mg/L (58.3 ± 13.5 years). In terms of elevated hs-CRP levels of >3.0 mg/L, 48 (33.1%), 38 (26.2%), 12 (8.3%), and 47 (32.4%) were in the >3.0 – 5.0 , >5.0 – 7.0 , >7.0 – 9.0 , and >9.0 mg/L categories, respectively. The patients with hs-CRP levels of >3.0 mg/L were mostly in their 60s (23.4%, 34/145), followed by those in their 30s (17.9%, 26/145), 40s (17.9%, 26/145), 70s (17.9%, 26/145), 50s (13.8%, 20/145), ≥ 80 s (7.6%, 11/145), and ≤ 30 s (1.4%, 2/145). No difference in the proportion of increased hs-CRP levels of >3.0 mg/L was found between urban (12.4%, 111/898) and sub-urban areas (10.4%, 34/327).

Conclusions: The prevalence of hs-CRP levels >3.0 mg/L among adult patients in primary care clinics was 11.8%. Our study provides basic information about patients who need hs-CRP tests for the CVD risk evaluation visiting primary medical institutions.

Keywords: High-sensitivity C-reactive protein, Prevalence, Distributions, Primary care clinics, Korea

PC-36

Difference in the Clinical Characteristics of Lactate Dehydrogenase, Creatine Kinase, and Aspartate Aminotransferase Enzyme Test

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Background: Lactate dehydrogenase (LD), creatine kinase (CK), and AST are frequently tested in the laboratory related to muscle disease. However, these enzymes are related to other organs, for example, AST is also present in the liver and RBC, CK is also present in the brain, and LD is widely distributed in the body, including the liver, RBC, kidney, lung, and muscle. In this study, we characterized the clinical differences of each enzyme test.

Methods: The results of LD, CK, and AST, which were tested at the same time for the first time from the emergency department (ER) were retrospectively explored. Criteria for the positive result of LD, CK, and AST were 400 U/L, 170 U/L, and 50 U/L in males and 400 U/L, 150 U/L, and 35 U/L in females, respectively. The number of positive patients in each 8 groups of positive combination of 3 tests was calculated.

Results: From a total of 19,751 patients, male patients were 9,699 (49.1%). The number of positive patients of LD, CK, and AST was 12,111 (61.3%), 4,943 (25.0%), and 2,323 (11.7%), respectively, and the number of patients negative for all three tests was 4,430 (22.4%) and positive for all three tests was 2,028 (10.3%), of which 56.6% were males. The number of patients positive for only one of LD, CK, and AST was 7,676 (38.9%), 460 (2.3%), and 252 (1.3%), respectively. The number of patients positive for both LD and CK, LD and AST, CK and AST were 2,470 (12.5%), 2,455 (12.4%), and 44 (0.21%), respectively. The mean (SD) values of LD, CK, and AST positive in all three tests were 1,050 (1,568) U/L, 1079 (8,162) U/L, and 222 (737) U/L. The mean (SD) of LD and CK positive in both LD and CK was 543 (122) U/L and 329 (284) U/L, mean (SD) of LD and AST positive in both LD and AST was 786 (1247) U/L and 198 (809) U/L, and mean (SD) of CK and AST positive in both CK and AST was 572 (476) U/L and 58.1 (31.2) U/L. The mean (SD) values of LD, CK, and AST positive in only one of LD, CK, and AST were 513 (143) U/L, 300 (204) U/L, and 63.7 (33.9) U/L, respectively.

Conclusions: When LD, CK, and AST were tested at the same time from ER specimen, only LD positivity was the most common (38.9%) whereas positivity for both CK and AST was the rarest (0.2%). The value of each LD, CK, and AST was distinctively higher in patients positive for all the three enzyme tests as compared to other groups.

Keywords: LD, CK, AST, Positive rate, Combination

PM-01

Evaluation of ASTA MicroIDSys Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry System for Identification of Nontuberculous Mycobacteria

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Background: The accuracy of mycobacterial identification using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method has been improving steadily. The ASTA MicroIDSys (ASTA, Suwon, Korea) is a MALDI-TOF MS system for identifying microorganisms. This study aimed to evaluate the performance of MicroIDSys in identifying nontuberculous mycobacteria (NTM) species.

Methods: A total of 86 NTM isolates were tested using both MicroIDSys with the MycoDB v2.0.01 and MolecuTech REBA MycoID (YD Diagnostics, Yongin, Korea). All the results were confirmed by 16S rRNA gene and rpoB sequencing as the reference method. The four isolates identified through sequencing which were not included in MycoDB were excluded from this study. The agreement rates between each of the three methods were estimated.

Results: The agreement rate for all three methods was 76.7%, including 17 *M. intracellulare* (19.8%), eight *M. abscessus* (9.3%), seven *M. massiliense* (8.1%), five *M. avium*, *M. chelonae*, *M. fortuitum*, and *M. kansasii* (5.8%), three *M. gordonae*, two *M. mucogenicum* and *M. peregrinum*, and one *M. arupense*, *M. lentiflavum*, *M. marinum*, *M. marseillense*, *M. neoaurum*, *M. scrofulaceum*, and *M. szulgai*. In MicroIDSys, *M. massiliense*, and *M. peregrinum* were reported as '*M. abscessus* complex' and '*M. fortuitum* complex,' respectively, and all results were consistent. In REBA MycoID, *M. peregrinum* was reported as '*M. fortuitum* complex' and *M. arupense*, *M. lentiflavum*, *M. marseillense*, and *M. neoaurum* were reported as 'NTM other than 21 species,' and all results were consistent. The 13 isolates (15.1%) were identified incorrectly via MicroIDSys, with 10 unidentified and three misidentified. The 10 unidentified isolates included two *M. mucogenicum* and *M. szulgai*, and one *M. avium*, *M. gordonae*, *M. intracellulare*, *M. kansasii*, *M. marinum*, and *M. scrofulaceum*, and three isolates, *M. kumamotonensis*, *M. simiae*, and *M. szulgai*, were misidentified as *M. gordonae*, *M. cookii*, and *M. gordonae*, respectively. Seven isolates (8.1%) were identified incorrectly via REBA MycoID, with three unidentified and four misidentified. As a result, the agreement rate for MicroIDSys was 84.9%.

Conclusions: MicroIDSys is considered a reliable and rapid method of identifying NTM. However, the agreement rate decreased due to the poor yield of comparable peaks generated in some isolates. This issue was more pronounced in species with known low occurrence frequencies. Therefore, further accumulation of databases will be necessary to achieve a higher identification rate.

Keywords: MALDI-MS, Nontuberculous mycobacteria, 16S rRNA sequencing, rpoB

PM-02

Drug Susceptibility Trends for Anti-tuberculosis Drugs in Shorter MDR-TB Regimens (2018–2023)

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Background: The recently revised tuberculosis treatment guidelines introduced a 6–9-month short-course regimen for multidrug-resistant tuberculosis (MDR-TB). This study reports the recent resistance trends in South Korea for the drugs used in this regimen, excluding pretomanid. The drugs included were bedaquiline, linezolid, moxifloxacin, delamanid, levofloxacin, and pyrazinamide.

Methods: We tested *Mycobacterium tuberculosis* strains identified as rifampicin and/or isoniazid resistant using phenotypic (absolute concentration method on Löwenstein–Jensen agar) or genotypic drug susceptibility testing (DST) (Line Probe Assay, Advansure™ MDR-TB Genoblot assay kit; LG Life Sciences, Seoul, Korea). Minimal inhibitory concentration (MIC) tests for bedaquiline, linezolid, moxifloxacin, delamanid, and levofloxacin were conducted using the broth microdilution method with Middlebrook 7H9 media. The MIC concentration ranges were as follows: bedaquiline, 0.03125–4 µg/mL; delamanid, 0.00625–0.8 µg/mL; and linezolid, moxifloxacin, and levofloxacin, 0.0625–8 µg/mL. Pyrazinamide DST was performed using Wayne's assay.

Results: We collected MIC data for 3,981 isolates of bedaquiline and delamanid; 2,330 of linezolid and levofloxacin; 2,326 of moxifloxacin; and 3,880 for pyrazinamide over 6 years to analyze their MIC distribution and resistance rates. The rate of inconclusive results for bedaquiline was 1.93%, and the resistance rate was 1.28%. Linezolid had the lowest resistance rate at 0.77%, whereas delamanid showed the highest resistance rate at 7.28%. The inconclusive and resistance rates for moxifloxacin were 3.83% and 12.47%, respectively, whereas those for levofloxacin were 2.58% and 13.17%, respectively. These drugs exhibited comparable resistance rates because their resistance mechanisms involve *gyrA* mutations. Pyrazinamide had a resistance rate of 61.91%, which may affect treatment efficacy.

Conclusions: This study provides a comprehensive analysis of recent resistance trends for key anti-tuberculosis drugs used in the short-course regimen for MDR-TB in South Korea. The high pyrazinamide resistance rate highlights the need for routine susceptibility testing and alternative strategies to manage drug-resistant tuberculosis effectively.

Keywords: Multidrug-resistant tuberculosis, Drug resistance, Anti-tuberculosis drugs, Minimum inhibitory concentration

PM-03

Clinical Impact of Co-Detection with *Mycobacterium Tuberculosis* and Nontuberculous *Mycobacteria* in Acid-Fast Bacilli Culture Using Real-Time PCR: Comparative Evaluation of Six Korean Reagents

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Background: While the number of tuberculosis (TB) cases in South Korea has steadily decreased due to TB control programs, the country still has one of the highest TB incidence rates among OECD countries. Additionally, the incidence of nontuberculous mycobacteria (NTM) infections has significantly increased. Consequently, the frequency of co-detection of TB and NTM in acid-fast bacilli (AFB) culture has also increased. However, the diagnostic performance of co-detection using real-time PCR reagents has not been evaluated. Thus, this study aims to conduct a comparative evaluation of six Korean reagents.

Methods: From August 2019 to January 2024, nucleic acids from 38 strains that were positive in AFB culture and co-detected for TB and NTM using Seegene Anyplex MTB/NTM PCR were subjected to PCR using MTB/NTM reagents from Biosewoom, Eone Biotech, Invitros, GeneMatrix, and KogeneBiotech. Cepheid's Xpert assay was performed using AFB culture isolates, and sequencing was conducted targeting 16S rRNA and IS6110 for comparative results. In the laboratory, TB was diagnosed when the AFB culture isolate was MPT64 antigen positive or TB positive in Seegene PCR. NTM was diagnosed when the MPT64 antigen was negative and NTM was detected in Seegene PCR. When MPT64 antigen was negative and Seegene PCR showed co-detection of TB and NTM, additional Xpert testing was performed. If Xpert was positive for TB, it was diagnosed as co-infection of TB and NTM, and if Xpert was negative, it was diagnosed as NTM.

Results: The lowest number of co-detection cases using the PCR reagents was obtained with Invitros (18 cases), followed by Biosewoom (19 cases), Eone Biotech (21 cases), Genematrix (24 cases), and KogeneBiotech (26 cases). MTB-only cases were also detected using Genematrix (2 cases) and Kogene (1 case). Sequencing showed co-detection in 21 cases, and the Xpert assay detected MTB in 23 cases. Among all samples, 17 cases (44.7%) had consistent co-detection results across all reagents and sequencing (cases 1–17). The concordance rates with the sequencing results were as follows: Invitros (92.1%), Biosewoom (94.7%), Eone Biotech (94.7%), Xpert (84.2%), Genematrix (81.6%), KogeneBiotech (78.9%), and Seegene (55.3%). Among 17 cases of NTM according to the sequencing results, 13 cases had no TB history and TB was not detected in follow-up AFB cultures. They had not received TB treatment.

Conclusions: The five Korean PCR reagents demonstrated minimal differences in co-detection rates and high concordance with sequencing results. Clinical laboratories should understand the characteristics of each reagent for interpretation and may use additional real-time

PCR reagents to complement the diagnosis if necessary.

Keywords: Acid-fast bacilli, *Mycobacterium tuberculosis*, Non-tuberculous mycobacteria, Co-detection, Real-time PCR

PM-04

Clinical Evaluation of Xpert MTB/RIF Ultra Compared with *Mycobacterium Tuberculosis* Culture in Clinical Specimens at a Tertiary Hospital in Korea

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Background: Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) is a rapid, molecular diagnostic test for detecting *Mycobacterium tuberculosis* (MTB) and rifampin (RIF) resistance directly from clinical specimens. This study aimed to evaluate the clinical performance of Xpert MTB/RIF Ultra in comparison with that of the standard culture method for detecting MTB and rifampin resistance in various clinical specimens.

Methods: From July 26, 2023 to April 30, 2024, 745 clinical specimens (BAL&bronchial aspiration N=193, urine N=12, cerebrospinal fluid N=48, pericardial fluid N=3, pleural fluid N=14, and sputum N=438) with culture and Xpert MTB/RIF Ultra results were evaluated. MTB detection was performed using Lowenstein-Jensen Media and BACTEC MGIT 960. Culture-positive specimens were defined as growth in at least one medium. Standardized phenotypic drug susceptibility testing was conducted on 15 MTB culture-positive isolates to determine RIF resistance. The Xpert MTB/RIF Ultra assay was performed in accordance with the manufacturer's instructions. The sensitivity and specificity of Xpert MTB/RIF Ultra were calculated using mycobacterial culture as the reference standard.

Results: Among the 745 specimens, 45 (6.1%) were culture-positive for MTB, and 700 (93.9%) were culture-negative. The overall sensitivity and specificity of Xpert MTB/RIF Ultra were 93.3% (95% CI: 82.1%–97.7%) and 98.7% (95% CI: 97.6%–99.3%), respectively. The performance of Xpert MTB/RIF Ultra, shown by specimen type, demonstrated sensitivity and specificity ranges of 91.7%–100% and 97.9%–100%, respectively. The concordance rate between culture-based RIF susceptibility testing and Xpert MTB/RIF Ultra was 100% for the 15 isolates tested.

Conclusions: Xpert MTB/RIF Ultra demonstrates high sensitivity and specificity for MTB detection in various types of clinical specimens. This rapid test can aid in the timely diagnosis of MTB infection and inform treatment strategies.

Keywords: Xpert MTB/RIF Ultra, Rapid molecular diagnostic testing, *Mycobacterium tuberculosis*

PM-05

Seaweed Produced Selenium Nanoparticles Are Inhibitory to Multidrug-Resistant *Mycobacterium Tuberculosis* and Pancreatic Adenocarcinoma Cell Lines

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Antibiotic-resistant bacteria (including multidrug-resistant strains of *Mycobacterium tuberculosis*) pose a significant threat to global public health. With few new antibiotics in the pipeline, there is growing interest in developing sustainable alternative treatments. Macroalgae (seaweeds) offer one possible source of bioactive agents. This study evaluated the potential for a range of common temperate (UK) intertidal seaweeds to be used as a feedstock for the synthesis of selenium nanoparticles displaying antimicrobial and antitumor properties. Selenium (Se) is an essential element; however, excess can have negative effects. Fabricating selenium at the nanoscale ensures lower toxicity with improved bioavailability and biocompatibility. Extracts from a range of seaweeds belonging to the genera *Ulva*, *Palmaria*, *Fucus*, and *Laminaria* were utilised to synthesise capsulated Se-nanoparticles with mean diameters of 20.37 ± 0.46 , 8.9 ± 2.24 , 111.18 ± 51 and 89.14 ± 6.85 nm respectively. The nanoparticles were characterised using UV-vis, DLS, XRD, TEM, SEM, EDX, ICP-OES, and Zeta potential. The nanoparticles demonstrated bactericidal effects against *M. tuberculosis* with low minimum inhibitory concentrations. The algal-based nanoparticles caused a pronounced decrease in cancer cell viability compared to normal cells, suggesting a possible therapeutic benefit on tumours. The results of this study suggest that SeNPs could represent a potential candidate for the treatment of certain cancers, especially pancreatic adenocarcinoma.

Keywords: Macroalgae, Green synthesis, Selenium nanoparticles, *Mycobacterium tuberculosis*

PM-06

Prevalence of Carbapenem-Resistant *Klebsiella Pneumoniae* in Clinical Samples from Tertiary Care Hospital of Mongolia

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Background: *Klebsiella* spp are gram-negative bacteria and are opportunistic pathogens typically found in the nose, throat, skin, and intestinal tract flora of healthy individuals. However, they can also cause various infections, including pneumonia, soft tissue and surgical

wound infections, urinary tract infections, bloodstream infections, and sepsis. In recent decades, carbapenem resistance has broken out in hospitals, especially in intensive care units. *Klebsiella* spp pathogenicity was classified into two types: typical (classical, cKp) and hypervirulent (hypervirulent, hvKp). Carbapenem-resistant *K. pneumoniae* (CR-KP) was first reported in our hospital in 2019, and the number of cases is increasing yearly.

Methods: This study was conducted in the Bacteriological Department of the General Laboratory of Clinical Pathology of the First Central Hospital from January 2021 to December 2023. Clinical specimens were inoculated into 5% sheep blood and McConkey agars and incubated at 37°C for 18–24 hours. Gram staining, colony growth morphology, and VITEK 2 analyses were used for identification. Antibiotic susceptibility testing was determined by the Kirby-Bauer disk diffusion method and MIC (VITEK-2) according to the CLSI guideline (M100).

Results: In 2021, 1,637 multidrug-resistant organism (MDRO) isolates were determined in the clinical specimens, including 42 CR-KP isolates (2.5%). In 2022, 1,930 MDRO isolates were determined in the clinical specimens, including 56 CR-KP isolates (2.9%), and in 2023, 1,916 MDRO isolates were determined in the clinical specimens, including 80 CR-KP isolates (4.2%) in our hospital. By department: A total of 180 CR-KP were isolated in the intensive care unit (28.9%; N=52), internal medicine department (25.6%; N=46), urology and andrology department (13.9%; N=25), neurology center (8.3%; N=15), outpatient department (7.2%; N=13), emergency department (7.2%; N=13), and other surgery departments (8.8%; N=16). According to the type of specimens, the distribution of CR-KP isolates was: soft tissue infection 18.3% (N=33), urine 42.2% (N=76), blood 5.6% (N=10), lower respiratory tract specimens such as sputum and bronchoalveolar lavage 15.6% (N=28), bile 5.0% (N=9), and other samples 13.3% (N=24).

Conclusions: The increasing incidence of carbapenem-resistant *K. pneumoniae* has recently become a problem in our hospital.

Keywords: Carbapenem resistance, *Klebsiella pneumoniae*

PM-07

Clinical Usefulness of QMAC-dRAST for the Direct Testing of Antibiotic Susceptibility at a Tertiary Hospital

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Background: Rapid and accurate antibiotic susceptibility test directly from flagged blood cultures can aid clinicians in optimizing early antibiotic treatment and improving clinical outcomes. We herein evaluated the clinical performance of the QMAC-dRAST system (Quanta-Matrix, Seoul, Korea) in terms of determining antibiotic susceptibility status and reporting time in comparison with that of conventional methods.

Methods: In total, 25 positive blood cultures for QMAC-dRAST tests were analyzed from December 2023 to June 2024. The efficiency of QMAC-dRAST in susceptibility testing was compared with that of Vitek 2. Turnaround time from specimen to antibiotic susceptibility testing was also compared between QMAC-dRAST and conventional identification systems.

Results: AST results for the 25 isolates were available for 16 antimicrobial agents tested with two susceptibility testing methods. Therefore, 400 AST measurements were available for evaluation. Among the 25 positively flagged blood cultures, 95.0% of the QMAC-dRAST results were consistent with the Vitek 2 results. Results were obtained more rapidly with QMAC-dRAST than with conventional identification systems.

Conclusions: Sepsis is a life-threatening but treatable condition. The early initiation of effective treatment is strongly associated with a better patient prognosis. The QMAC-dRAST system, which is based on the microscopic detection of bacterial colony formation in agarose containing various concentrations of antibiotics, can be used for antimicrobial drug susceptibility testing directly from flagged blood cultures within 6 h. QMAC-dRAST provided rapid results and correlated well with the conventional method. Thus, QMAC-dRAST constitutes a fast and reliable alternative to conventional AST methods.

Keywords: Sepsis, QMAC-dRAST, Direct AST

and 59.8 minutes for PU200, which closely matched the manufacturer's claims. For STD testing, the overall concordance rate between FVNA and PU200 was 82.1% (46/56). Among the 10 discordant cases, 7 were from vaginal swabs and 3 were from urine samples. Four cases matched the original results with FVNA and another four with PU200, whereas two showed discrepancies across all results. All discordant cases had targets detected in the melting curve, but showed Ct values exceeding 40 cycles, indicating low bacterial levels. The overall concordance rate for HPV genotyping was 82.0% (50/61). Among the 11 discordant cases, 6 matched the original results with PU200, 1 with FVNA, and 4 differed across all results. Most discordant cases (10/11) had Ct values of approximately 40 cycles, suggesting low viral levels near the clinical cut-off. One strongly positive case (HPV-70, Ct 21.13) suggested a false positive result. PU200 matched the six original results; however, FVNA detected additional types in five cases, indicating no clear superiority between the two protocols.

Conclusions: High concordance rates for both STD and HPV testing suggested that the FVNA protocol was as effective as PU200, with minor discrepancies involving low-level targets near the clinical cut-off. Hence, the FVNA protocol is a viable option for nucleic acid extraction, offering faster processing without compromising accuracy.

Keywords: MagNA96, FastVNA, Nucleic acid extraction, HPV, STD, Real-time PCR

PM-08

Comparative Analysis of MagNA Pure 96 Protocols: The New Fast Viral NA Protocol Versus Standard Pathogen Universal 200 Protocol in STD and HPV Genotyping Multiplex PCR Testing

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Background: In the post-COVID-19 era, the demand for rapid and high-quality diagnostic processes for infectious diseases has significantly increased, leading to the development of efficient nucleic acid extraction methods. The MagNA Pure 96 System (MagNA96, Roche) is a fully automated platform that introduces the Fast Viral NA SV 1.0 protocol (FVNA), which operates in approximately 30 minutes. This study aimed to validate the efficiency of FVNA protocol compared to the standard universal protocol (PU200) for STD and HPV genotyping tests, commonly performed in Korean commercial laboratories.

Methods: In March 2024, 56 residual samples (41 vaginal swabs and 15 urine samples) were collected for STD multiplex PCR and 61 residual vaginal swabs for HPV genotyping PCR. The MagNA Pure 96 Viral NA kit (Roche) was used for both the FVNA and PU200 protocols, and the process durations were measured. Each extracted nucleic acid was analyzed using multiplex real-time PCR with the NeoPlex STI-12 and HPV29 Detection Kits (GeneMatrix). The concordance of both protocols was compared with existing results extracted using MagNA96 and SWH EM48 (SW Healthcare, Korea).

Results: The average protocol duration was 30.5 minutes for FVNA

PM-09

Comparison of Two Automated Systems of Gram Staining

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Background: Gram staining is one of the oldest and most important staining techniques in microbiology. It differentiates bacteria into two fundamental varieties of cells. Automated Gram staining systems have been developed to streamline the process and provide consistent results. This study aimed to compare two systems with different staining methods: "A" system, which involves mounting slides horizontally and staining with the flow method and "B" system, which involves mounting slides perpendicularly and staining with the spray method.

Methods: Three reference strains (ATCC 29213 for *Staphylococcus aureus*, 14053 for *Candida albicans*, 25922 for *Escherichia coli*) were used. Briefly, 3 mL samples of each specimen type (sputum, blood, and urine) dispensed with the three reference strains and 0.5 McFarland standard were prepared. Then, 1,000 µL of the samples were vortexed with 1,000 µL of washed white blood cells prepared in 0.8 McFarland standard. For 20 slides, each were marked 16 sections of 2×2 cm² area. Two slides were attached face to face to ensure consistency. Staining was performed twice a day. We defined

three in-house quality criteria for the assessment of the stained slides: homogeneity of staining, stain uniformity of the background, and staining artifact. Trained physicians examined >10 fields per slide with a light microscope. The total costs for Gram staining per slide were calculated based on consumables, maintenance, and workforce. All continuous variables were tested using Student's t-test. Analysis of variance was performed between groups and confirmed with post-hoc tests.

Results: The two devices showed statistical differences in homogeneity of staining (reference strains), with the "A" system showing better homogeneity of staining in all reference strains. The numerical values were as follows: for *S. aureus*, 96.7 ± 1.9 vs. 93.3 ± 6.3 ($P < 0.001$); for *C. albicans*, 95.9 ± 3.4 vs. 50.0 ± 26.8 ($P < 0.001$); and for *E. coli*, 99.9 ± 0.5 vs. 87.1 ± 12.5 ($P < 0.001$). The two devices also showed statistical differences in homogeneity of staining (sample type), with the "A" system showing better homogeneity of staining in all sample types, except for *S. aureus* in blood. The numerical values were as follows: for blood, *S. aureus* showed 98.0 ± 2.1 vs. 97.7 ± 1.7 ($P = 0.564$); for *C. albicans*, 97.7 ± 2.4 vs. 61.2 ± 26.1 ($P < 0.001$), for *E. coli*, 99.8 ± 0.7 vs. 92.9 ± 5.3 ($P < 0.001$). For 5 days, all stain results showed *P* values above 0.05, indicating no significant change in the homogeneity of staining over time.

Conclusions: We evaluated the quality of Gram stains of two automated instruments. The "A" system showed better performance than the "B" system.

Keywords: Gram staining, Automation, Performance

PM-10

Evolution of blaKPC under Pressure of Carbapenems and Ceftazidime/Avibactam in Patient with Persistent Bacteremia Caused by *Klebsiella Pneumoniae*

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Background: Ceftazidime/avibactam (CAZ/AVI) was recently introduced in July 2023 in Korea. We report a patient with persistent bacteremia caused by *Klebsiella pneumoniae* (KP) carbapenemase (KPC)-producing KP, which transformed into CAZ/AVI-resistant carbapenem-susceptible KP (CSKP) under CAZ/AVI treatment.

Methods: The serial isolates of carbapenem-resistant *K. pneumoniae* (CRKP) were tested for antimicrobial susceptibility using BD Phoenix™ NMIC500 panels, and carbapenemase production was determined through the modified Hodge test (MHT) or modified carbapenemase inactivation test (mCIM). KPC-KP were typed for carbapenemase via NG CARBA-5 or Xpert® Carba-R and submitted to multilo-

cus sequence typing, pulsed-field gel electrophoresis, and whole-genome sequencing. For presumptive KPC variants, the blaKPC genes were sequenced, and the three-dimensional (3D) structure of KPC was constructed and analyzed using mFold.

Results: CAZ/AVI-susceptible KPC-KP was first isolated from blood cultures after a 23-day treatment of ceftazidime, piperacillin/tazobactam, and meropenem and persisted until hospital day (HD) 41, on which ceftazidime/avibactam treatment was started. CRKP was eradicated until CAZ/AVI-resistant CRKP emerged on HD 63. Sequencing of blaKPC revealed that the first CAZ/AVI-susceptible CRKP carried KPC-2, while CAZ/AVI-resistant CRKP carried KPC-44. CAZ/AVI-resistant CSKP was first isolated on HD 64, which was MHT-negative but mCIM-positive; it was also positive for blaKPC not by CARBA-5 but by Carba-R, the sequencing of which revealed KPC-135. Then, after a 12-day meropenem treatment, CAZ/AVI-susceptible CRKP harboring KPC-2 was recovered. All KPC-KP isolates were of the ST307 strain and varied over time from identical to closely related pulsotypes. Functional analysis of 3D structures revealed that a mutation in the hotspot of KPC, the 266–275 loop, was attributed to CAZ/AVI-resistance and the loss of carbapenem resistance. Whole-genome sequencing also revealed that all 14 isolates were of the ST307 strain with the same plasmids [IncFIB(K)/IncFII(K), and IncX3]. The cgMLST showed two sub-groups within our KP isolates according to CAZ/AVI resistance, suggesting clonal evolution.

Conclusions: This is the first report on the acquisition of CAZ/AVI-resistance with loss of carbapenem resistance in KP through the emergence of blaKPC variants in Korea. Our data highlight the importance of monitoring the emergence of antimicrobial resistance to prevent its spread to newly introduced antimicrobial agents.

Keywords: *Klebsiella pneumoniae* carbapenemase, Ceftazidime/avibactam, *Klebsiella pneumoniae*, Protein structure, Whole-genome sequencing

PM-11

Performance Evaluations of VIDAS *Clostridioides Difficile* GDH, Toxin A&B, Xpert *C. Difficile*, and ChromID *C. Difficile* Agar Assays in Diagnosis of *C. Difficile* Infection

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Background: Despite the well-recognized significance of *Clostridioides difficile* infection (CDI) in humans, there are currently no universally accepted criteria for its diagnosis. This study evaluated the performance of commercially available enzyme immunoassays (EIAs), nucleic acid amplification tests (NAATs), culture methods, and a simulated two-step algorithm.

Methods: We analyzed the results of VIDAS *C. difficile* glutamate dehydrogenase (GDH), Toxin A&B (CDAB), Xpert® *C. difficile* (tcdB), and *C. difficile* culture (ChromID *C. difficile* agar) tests. The perfor-

mance of a two-step algorithm consisting of the GDH and CDAB EIAs followed by the tcdB NAAT was also analyzed. A diagnosis of CDI was given if symptoms manifested (typically diarrhea), along with either a positive stool test indicating the presence of *C. difficile* toxins, the detection of toxigenic *C. difficile*, or the identification of pseudomembranous colitis through colonoscopy or histopathological examination.

Results: of 387 results analyzed, 77 patients (19.9%) were diagnosed with CDI. The sensitivity, specificity, positive predictive value, and negative predictive value of the GDH EIA were 88.3%, 90.7%, 70.1%, and 96.9%, respectively; CDAB EIA, 33.8%, 100%, 100%, and 85.9%, respectively; tcdB NAAT, 79.2%, 96.5%, 84.7%, and 94.9%, respectively; *C. difficile* culture, 68.8%, 95.5%, 79.1%, and 92.5%, respectively; and the two-step algorithm, 72.7%, 98.1%, 90.3%, and 93.5%, respectively.

Conclusions: Employing both EIA GDH and CDAB as part of a two-step method alongside the tcdB NAAT, yielded comparable results to standalone NAATs while providing cost advantages.

Keywords: *Clostridioides difficile*, Enzyme immunoassay, Bacterial culture, NAAT

bladder and its surroundings were distended, and gallstones were not visible; a distal common bile duct cancer was strongly suspected. On the third day of blood culture after the visit, one culture grew Gram-negative bacillus. However, it could not be identified using conventional microbiological tests such as Gram staining, culture, and Vitek 2 cards. *Brevundimonas* spp. was only identified using 16S rRNA sequencing five days after the initial request. The patient was administered piperacillin/tazobactam, ceftriaxone, and chlorpheniramine, and it was confirmed during the follow-up examination that the bacteria were not identifiable in the blood culture. The patient was then transferred to another hospital due to a problem in her hometown. *Brevundimonas* spp. can survive in various environments, including different water sources, especially hospital water, and are usually resistant to many antimicrobials. This bacteria may become more widespread and cause more fatal infections, so quick diagnoses using additional tests such as 16S rRNA sequencing are crucial in clinical practice.

Keywords: *Brevundimonas* spp., Bacteremia, Common bile duct cancer

PM-12

***Brevundimonas* Species Bacteremia in Patient with Distal Common Bile Duct Cancer**

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Brevundimonas species (spp.) are Gram-negative, oxidase- and catalase-positive, non-fermenting aerobic bacilli in the Alphaproteobacteria class and Caulobacteraceae family. The genus *Brevundimonas* was initially proposed by Segers et al. and comprises 25 species with valid published names. *Brevundimonas* spp. have been isolated from several environments such as soils, black sand, deep subsea floor sediment, purified water, and other numerous aquatic habitats. These species can pass through sterilizing filters, potentially causing infections and even death in some patients with underlying diseases. We present a case of *Brevundimonas* spp. bacteremia in a patient with distal common bile duct cancer. A 71-year-old female patient visited our hospital's emergency room with itching all over her body. The patient believed that such symptoms had occurred since she was vaccinated against COVID-19 approximately 10 days previously, and jaundice began three days before her visit. The patient was a hepatitis B carrier and had been receiving medication for hypertension for over 20 years. The physical examination showed jaundice in both eyes and bruises on both legs. The total/direct bilirubin was 17.6 mg/dL/16.0 mg/dL, ESR was 71 mm/hr, and CRP was 0.40 mg/dL in the blood test. CT and other imaging tests showed that the gall-

PM-13

Identification of *Terrisporobacter Othiniensis* Isolated from Human Blood Using Whole-Genome Sequencing: A Case Report

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We report a case of *Terrisporobacter othiniensis* bacteremia in a critically ill elderly man with a sigmoid colonic perforation. Initially, MALDI-TOF MS identified the bacterium as *T. glycolicus*. However, this identification was inconclusive because the MALDI Biotyper Sirius database included only *C. glycolicus* and *C. mayombe*, the former designations of *T. glycolicus* and *T. mayombe*, respectively, and did not include *T. othiniensis* or *T. petrolearius*. 16S rRNA gene sequencing suggested the presence of *T. glycolicus*, *T. mayombe*, or *T. petrolearius*. Due to the limitations of these methods in distinguishing *Terrisporobacter* species, we employed whole-genome sequencing (WGS), which revealed *T. othiniensis* as the causative agent. Our approach included a database search and the construction of a phylogenetic tree for *T. othiniensis* using WGS data. Additionally, we described the phenotypic characteristics and antimicrobial susceptibility profile of the bacterium. *Terrisporobacter* species have rarely been reported in human infections. This case represents the second report of *T. othiniensis* isolated from human blood culture and is the first to describe the clinical course of this infection. Previous cases of *T. glycolicus* infections suggest that hematogenous seeding from the gastrointestinal (GI) tract is likely, given the bacteria's known residence in the GI tract. In our patient, the perforated sigmoid colon provided a plausible route

for *T. othiniensis*, likely part of the gut microbiota, to enter the bloodstream. The successful use of WGS for the precise bacterial identification highlights the importance of a whole-genome approach for identifying emerging infectious agents.

Keywords: *Terrisporobacter othiniensis*, Whole-genome sequencing, Bacteremia, Blood culture, Case report

PM-14

***Phytobacter Diazotrophicus* Blepharokeratoconjunctivitis in Elderly Patients Following Cataract Surgery**

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Phytobacter diazotrophicus is a Gram-negative, oxidase-negative, catalase-positive, lactose-fermenting aerobic bacillus in the Gammaproteobacteria class, Enterobacterales order, and Enterobacteriaceae family. The genus *Phytobacter* was initially established based on nitrogen-fixing isolates from wild rice in China in 2008, followed by several isolates obtained from a multi-state sepsis outbreak in Brazil in 2013. A retrospective review showed several clinical strains isolated in the United States in the 1970s originally grouped into Brenner's Biotype XII of the *Erwinia herbicola*-*Enterobacter agglomerans*-complex were *Phytobacter* species. *P. diazotrophicus* is the most common *Phytobacter* species found in the environment and humans and can cause opportunistic infections in patients with catheters or those using total parenteral nutrition. We present a case of *P. diazotrophicus* blepharokeratoconjunctivitis in an elderly patient following cataract surgery. An 87-year-old female patient visited our hospital's ophthalmology clinic with a dull-looking eye and discomfort. The patient had undergone cataract surgery on both eyes at a local ophthalmologist five years ago. Scaling, crusting, erythema of the eyelid margin, conjunctive hyperemia, and chemosis on the left eye were seen on physical examination. There was no major abnormality in the visual acuity test, but a partial macular hole was seen in the fundus examination, and retinal detachment was suspected. Microbiological culture tests were commissioned for eye secretions, and Gram-negative bacilli grew on Day 3 after culture. However, it could not be identified by conventional microbiological tests such as Gram staining, culture, and Vitek 2 cards (bioMérieux Vitek Inc., Hazelwood, MO, USA). *P. diazotrophicus* was only identified using 16S rRNA sequencing on Day 7 after the initial request. Levofloxacin-Cravit tablet (500 mg), an oral antibiotic, Tarivid ointment (3.5g), an ophthalmic antibiotic, and Fluorometholone, a steroid, were administered to the eye. The patient was followed up at our hospital ophthalmology outpatient clinic for two weeks and then transferred to a local ophthalmologist. As

standard biochemical evaluation panels lack *Phytobacter* spp. from their database, *P. diazotrophicus* can often be misidentified as other *Enterobacterales* species, especially *Pantoea agglomerans*. However, some clinical isolates of this species are an important source of extended-spectrum β -lactamase and carbapenem-resistance genes. Since bacteria not commonly isolated from clinical specimens make it difficult to establish a treatment policy, more rapid and accurate microbial identification techniques, such as 16S rRNA sequencing, are necessary.

Keywords: *Phytobacter diazotrophicus*, Blepharokeratoconjunctivitis, Cataract surgery

PM-15

The Spectrum of Pathogens Causing Early-Onset Neonatal Sepsis in a Tertiary-Level Neonatal Intensive Care Unit Situated in Malaysia, A Developing Nation

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Background: Early-onset sepsis in newborns occurs within the first 72 hours of life, often resulting from infections in the maternal genital tract. Neonatal sepsis accounts for nearly one-third of neonatal deaths among those with culture-positive diagnoses. This study focused on identifying the organisms isolated in neonates who developed sepsis within the first 72 hours after birth.

Methods: This cross-sectional study included all neonates born within 72 hours who exhibited maternal and fetal risk factors for early-onset neonatal sepsis. Clinical data were collected from both mothers and neonates. A 1 mL sample of neonatal blood was collected using Bactec® Peds Plus bottles and analyzed to identify pathogens. Additionally, 16S rRNA gene sequencing techniques were employed for bacterial species identification.

Results: In this study, out of 135 neonates suspected of having sepsis due to maternal and fetal risk factors, 25 were found to have positive blood cultures. Among these 25 neonates, 16% showed infections with multiple bacterial species. The majority were Gram-negative bacteria (55%), followed by Gram-positive bacteria and yeast. Among the neonates with positive blood cultures, 32% reported death, predominantly attributed to *Klebsiella pneumoniae* species. The most commonly detected pathogen was coagulase-negative *Staphylococcus* (CoNS), particularly prevalent among preterm and low birth weight neonates. Other significant pathogens included *Klebsiella pneumoniae* and Group B *Streptococcus* (GBS).

Conclusions: CoNS, often dismissed as a contaminant, emerged as a significant pathogen, notably affecting preterm and low birth weight

neonates. The prevalence of methicillin-resistant CoNS and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* raises alarming concerns regarding antibiotic resistance. Fatal complications, especially stemming from Gram-negative bacteria, underscore the necessity for vigilant monitoring and targeted treatment approaches to enhance neonatal outcomes. The elevated mortality rate linked with Gram-negative infections, particularly among premature and low birth weight neonates, underscores the importance of continual surveillance and effective management of sepsis-causing pathogens.

Keywords: Early-onset, Neonatal sepsis, Mortality

PM-16

Microbiological and Genomic Characterization of a Clinical Isolate of *Haemophilus Influenzae* with a Paradoxical Phenomenon of Beta-Lactams Susceptibility

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A 38-year-old man who underwent bone marrow transplantation 15 months ago due to myelodysplastic syndrome diagnosed 19 years ago. He developed bronchiolitis 10 months after transplantation due to chronic graft-versus-host disease despite immunosuppressive treatment. A large amount of *H. influenzae* was recovered from sputum cultures. We discovered a paradoxical phenomenon in beta-lactam susceptibility testing that is well-known but has never been observed in *H. influenzae*. Disk diffusion susceptibility testing on MHF (bioMérieux) media, showed clear susceptibility to ceftriaxone, azithromycin, and ciprofloxacin, and resistance to cefuroxime and trimethoprim/sulfamethoxazole. However, growth of tiny colonies within the inhibition zone of ampicillin, amoxicillin/clavulanate, and meropenem was noted, although zone diameters of inhibition were within susceptible ranges. The ampicillin E-test showed minimum inhibitory concentrations (MIC) of 0.5 mg/L, but tiny colonies started to grow again at 12 mg/L and higher. These findings were reproducible even if the susceptibility test was repeated with subculturing a single colony. The tiny colonies did not grow when subcultured on MHF or chocolate agar. Beta-lactamase was detected with Nitrocefin disk (Becton Dickinson). Broth microdilution test using HTM broth showed minimum inhibitory concentrations of 1/0.5, 0.25, 0.06, and 0.06 mg/mL to amoxicillin/clavulanate (AMX), ampicillin, ceftriaxone, and meropenem, respectively. However, persistence of subpopulations was seen in more than 10⁻⁵ up to 16/8, 1.0, 0.5, and 0.125 mg/L of AMX, ampicillin, ceftriaxone, and meropenem, respectively, at day 1, and up to 2/1, 0.25, and 0.125 mg/L of AMX, ceftriaxone, and meropenem at day 2. Regrowth was observed at 0.5 mg/L of ampicillin at day 2, and 0.125 mg/L of meropenem at day 3. The persistence and regrowth of this strain at antimicrobial exposures above the MIC indicated tolerance, which was possibly associated with a paradoxical phenomenon. With whole genome sequencing of chromosomes, it

was typed as ST 472 and carried resistant determinants such as several mutations of *folA* and *pbp* and a full assortment of genes encoding virulence factors including capsule, IgA protease, adhesion, and toxin. Compared with the control strain (NCTC 8143 type strain, ATCC 33391), ABC transporter genes were significantly enriched. ABC transporters reportedly promote the persistence of pathogenic bacteria through the efflux of toxic substances such as antimicrobials, ultimately leading to resistance. Further investigation into their mechanisms and the clinical relevance of such paradoxical phenomena is required to interpret antimicrobial susceptibility.

Keywords: Beta-lactams, Susceptibility, *Haemophilus influenzae*

PM-17

Detection Rates of Nasopharyngeal Swabs, Sputum, and Combined Samples for Bacterial Pathogens via Multiplex PCR in Adults Hospitalized with Acute Respiratory Illness

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Background: Multiplex real-time PCR (RT-PCR) is widely used for rapid pathogen detection using respiratory samples. It is important to choose the optimal respiratory samples to detect respiratory pathogens. Previous research has compared the diagnostic yield of detecting viral infections using various respiratory samples, including those from both the upper and lower respiratory tracts. However, studies are lacking regarding choosing optimal samples for detecting bacterial pathogens. Thus, we used multiplex RT-PCR to assess the detection rates of respiratory bacteria from nasopharyngeal swabs (NPS), sputum, and combined NPS and sputum samples.

Methods: Paired NPS and sputum samples were collected from 219 adult patients admitted to Korea University Anam Hospital with acute respiratory illnesses. RT-PCR was performed on each sample for bacterial detection. Combined samples (N=92) for bacterial detection were produced using 46 remnant NPS and sputum samples with a positive bacterial signal and 46 NPS and sputum samples with a negative bacterial signal.

Results: Respiratory bacterial nucleic acids were identified in 100 (45.7%) of 219 patients. Among the 100 bacterial detections, 43 (19.6%) were detected in both NPS and sputum samples, 54 (24.7%) were sputum-positive/NPS-negative, and 3 (1.4%) were sputum-negative/NPS-positive. The positivity rate was significantly higher for sputum (44.3%; 97/219) than for NPS (21.0%; 46/219) ($P<0.0001$). For the combined samples with a positive signal (N=46), 22 (95.7%) of the 23 paired samples positive in both specimens were also positive in the combined samples. Twenty (87.0%) of the 23 discordant paired samples were positive in the combined samples. In the case of the combined samples with a negative signal (N=46), five samples detected a new bacterial signal that was not detected before

combining. Out of the 64 bacteria in the samples, the detection rate of the combined samples was 87.5% (56/64), comparable to the detection rate of sputum (90.6%, 58/64) and higher than that of NPS (51.6%, 33/64).

Conclusions: This study demonstrates that using RT-PCR and sputum samples significantly enhances the detection rate for respiratory bacterial nucleic acids among hospitalized adult patients compared to that using NPS samples. Importantly, combined samples of sputum and NPS retained most of the improved sputum detection rate and identified additional bacterial signals that were previously undetected. These findings contribute to our understanding of selecting optimal respiratory specimens for detecting acute bacterial infections.

Keywords: Sputum, Respiratory bacteria, Combined sample, Diagnosis, Multiplex PCR

PM-18

***Enterocloster* Species Bacteremia in Patient with Acute Myelogenous Leukemia with Myelodysplasia-related Changes**

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Enterocloster species (spp.) are cigar-shaped Gram-variable anaerobic bacilli belonging to the Clostridia class, Eubacteriales order, and Lachnospiraceae family. Among the genus of *Enterocloster*, *E. clostridioformis* was first identified in the 1950s in humans and was assigned to the genus of Gram-negative non-spore-forming *Bacteroides*. Subsequently, these bacteria were shown to form spores, so they were reclassified into the genus *Clostridium*. However, this species was recently reclassified as *E. clostridioformis* based on phylogeny. *E. boltea* is an opportunistic pathogen that occurs in the human gut and is associated with bacteremia, intra-abdominal infections, and abscesses. We present a case of *Enterocloster* spp. bacteremia in a patient with acute myelogenous leukemia with myelodysplasia-related changes. A 79-year-old male patient visited the emergency room of our hospital with slight pain and a mass on the left calf medial side, which had appeared 10 days before the visit. The patient had been diagnosed with refractory anemia with excess blasts three months before the visit, which did not improve during chemotherapy and was rediagnosed as acute myelogenous leukemia with myelodysplasia-related change a month and a half before the visit. The physical examination showed anemic conjunctiva in both eyes and a mass on the left calf medial side suspected to be a hematoma. The white blood cell count was $1.3 \times 10^3/\mu\text{L}$, the hemoglobin was 9.6 g/dL, and the platelet count was $37 \times 10^3/\mu\text{L}$ in the blood test. The patient had a cough and fever one month after admission and pleural effusion on CT and other imaging tests, and blood and catheter tip culture tests

were performed. One culture bottle grew a Gram-negative bacillus, but it could not be identified by conventional microbiological tests such as Gram staining, culture, and Vitek 2 cards. *Enterocloster* spp. was only identified using 16S rRNA sequencing seven days after the initial request. The patient was treated with teicoplanin, cefepime, meropenem, piperacillin/tazobactam, and levofloxacin because mild neutropenic fever was suspected from the time of admission, although infection could not be ruled out. The patient died of sepsis a month and a half after the visit and four days after bacterial identification. Antimicrobial resistance of *Enterocloster* spp. to ampicillin, erythromycin, lincomycin, ciprofloxacin, doxycycline, and colistin has been reported, especially in *E. boltea* strains. Due to this multidrug resistance, fast and accurate bacterial identification is important for appropriate antimicrobial treatment of *Enterocloster* spp.

Keywords: *Enterocloster* spp., Bacteremia, Acute myelogenous leukemia

PM-19

***Alistipes Putredinis* Peritonitis in a Patient with Mesothelioma**

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Alistipes putredinis is a gram-negative anaerobic bacillus belonging to the Bacteroidia class, Bacteroidales order, and Rikenellaceae family. The *Alistipes* genus has 13 species (*A. finegoldii*, *A. putredinis*, *A. onderdonkii*, *A. shahii*, *A. indistinctus*, *A. senegalensis*, *A. timonensis*, *A. obesi*, *A. ihumii*, *A. inops*, *A. megaguti*, *A. provencensis*, and *A. massiliensis*) in the taxonomy database of the National Center for Biotechnology Information and 3 species (*A. communis*, *A. dispar*, and the subspecies *A. onderdonkii*) recently added in other databases. *Alistipes* species reportedly exert protective effects against some diseases, such as liver fibrosis, colitis, cancer immunotherapy, and cardiovascular disease. By contrast, *Alistipes* has been associated with colorectal cancer and depression. In this paper, we present a case of *A. putredinis* peritonitis in a patient with mesothelioma. A 77-year-old female patient visited our hospital emergency room with abdominal pain and hematochezia. The patient, who has been treated with type 2 diabetes for more than 20 years, was diagnosed with peritoneal mesothelial cancer at another hospital 13 months ago and was receiving chemotherapy. Blood test results showed that the patient had a white blood cell count of $11.8 \times 10^3/\mu\text{L}$, hemoglobin level of 9.7 g/dL, platelet count of $337 \times 10^3/\mu\text{L}$, procalcitonin level of 0.916 ng/mL, and C-reactive protein level of 1.00 mg/dL. Abdominal and pelvic CT imaging results showed signs of perforation in the left descending colon with pneumoretroperitoneum. Thus, an emergency exploratory laparotomy was performed. In the blood culture per-

formed at visit, gram-negative bacillus grew on an anaerobic culture bottle, but it could not be identified using conventional microbiological tests, such as Gram staining, culture, and Vitek 2 cards (bioMérieux Vitek Inc., Hazelwood, MO, USA). *A. putredinis* was only identified using 16S rRNA sequencing after 10 days of the initial request. The patient was administered vancomycin and piperacillin/tazobactam, after which her condition improved. The patient is being followed-up at the outpatient clinic after her discharge. *Alistipes* is a relatively new genus of bacteria isolated from clinical specimens, and its contribution to health or disease depends on its species. Thus, fast and accurate identification to the species level is important diagnose and treat clinical diseases caused by *Alistipes* species.

Keywords: *Alistipes putredinis*, Peritonitis, Mesothelioma

PM-20

Genome-Based Typing Schemes with Streamlined Locus Selection for Epidemiology of Bacterial Pathogens

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Background: In the genomic epidemiology of bacterial pathogens, strain typing methods based on genomic sequence variations serve as powerful tools for cost-efficient identification of epidemiologically related cases. Various approaches, ranging from traditional multi-locus sequence typing (MLST) to core-genome MLST and single nucleotide polymorphism (SNP)-based clustering, provide different levels of resolution. However, these methods have limitations such as insufficient resolution for outbreak investigation (i.e., in MLST), difficulties with standardization (i.e., in cgMLST), and challenges with scaling up and maintenance (i.e., in SNP-based methods).

Methods: We designed a system that begins with conserved single-copy gene families in most bacterial pathogens. Our computational pipeline selects marker loci within each gene family, focusing on almost fixed-length alleles flanked by gap-free regions of at least 100 bp. This approach minimizes issues arising from alignment gaps while maximizing allelic diversity. The system automated the generation of a typing scheme for new target species and subsequent steps including allele numbering, multi-locus profiling, and sequence type assignment. We tested the performance of this approach with publicly available genome sequences of *Klebsiella pneumoniae* (N=43,799), *Acinetobacter baumannii* (N=14,622), and *Pseudomonas aeruginosa* (N=17,015).

Results: The automatically designed typing schemes contained 51, 41, and 49 loci from *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, respectively, with a medium allele length of 510 bp at each locus. Compared to traditional seven-locus MLST schemes, our typing schemes yielded 3.8-fold, 4.3-fold, and 3.9-fold more sequence types from each species, as single MLST types were divided into 1–659 finer types by our method. The number of untypeable strains was about 2.9-fold less with our schemes than with the MLST schemes. In all

species, the sequence types defined by our method consisted of a single MLST type in 96.1%–100% of the cases. The SNP-based clusters defined by the NCBI Pathogens Database consisted of a single sequence type (defined by our method) in 78.4%–93.5% of the cases.

Conclusions: The typing method developed here offers a streamlined workflow in the design step as the marker selection is automated. It offers intermediate resolution between MLST and SNP-threshold-based clustering used in outbreak investigation while maintaining the virtue of MLST that allele numbering determines the final typing results, without all-against-all distance comparison, which is a necessary and complicating step in cgMLST or SNP-based approaches.

Keywords: Genomic epidemiology, Strain typing, Bacteria, Pathogen

PM-21

Genome-Based Antibiotic Resistance Prediction Using Genomic Language Model

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Background: Prediction of antibiotic susceptibility and resistance *in vitro* phenotypes using genome sequences of bacterial pathogens is feasible in only a few extensively studied pathogens. Current technologies use sequencing of cultured isolate or targeted metagenomic amplicons and often rely on detection of pre-characterized resistance determinants such as acquired genes or point mutations (i.e., resistance markers). While utilizing pre-established markers is advantageous due to their straightforward interpretation, gaps remain in meeting clinical needs, as established resistance markers are unavailable for majority of pathogens and antibiotics. Recently, the concept of genome- and protein-language models has been rapidly developing alongside the advent of large language models. We hypothesized that applying these genomic language models to the problem of genome-based prediction of resistance and susceptibility could achieve accurate prediction even in pathogens for which there are few experimentally characterized resistance markers.

Methods: We collected a genome-phenotype paired dataset encompassing 63 bacterial species from the NCBI public database. The species with extensively characterized resistance phenotypes have up to 17 antibiotics for which at least 100 isolate genomes were available on both the susceptible and resistance side (e.g., *Klebsiella pneumoniae*, *Salmonella enterica*, and *Acinetobacter baumannii*) and the less characterized species contained as little as three antibiotics for which sufficient phenotype-characterized genomes were available. We trained the genomic language model that uses encodings from a protein language model, which was trained on a larger database, as input. This model considers the order of these protein features within each genome in the training set and probabilistically assigns the input strain (i.e., genome sequence) to either the resistant or susceptible category.

Results: The performance of the model-based prediction of resistance phenotype varied depending on both the pathogen species and the antibiotic of question. The area under the precision-recall

curve ranged from 0.5 to 1.0 in the training dataset and from 0.3 to 1.0 in the external validation dataset. For example, in *Klebsiella pneumoniae*, the prediction accuracy in the external validation dataset was highest in gentamicin (0.95) and amikacin (0.94), but lowest in levofloxacin (0.43) and ciprofloxacin (0.33). However, gentamicin susceptibility in *Pseudomonas aeruginosa* (0.74) was less predictable by our model.

Conclusions: Based on the preliminary results, we found that predicting antibiotic resistance using a genomic language model is feasible. Theoretically, such a model could achieve predictive power in the pathogen-antibiotic combinations that lack resistance markers or enough empirical data.

Keywords: Antibiotic resistance, Genomic language model, Prediction

PM-22

Prevalence, Antimicrobial Resistance Profile, and Characterization of Nitrofurantoin-Non-Susceptible *Klebsiella Pneumoniae* Isolated from Patients with Urinary Tract Infections

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Background: *Klebsiella pneumoniae* is a common pathogen that causes urinary tract infections (UTIs). Nitrofurantoin is a commonly prescribed oral antibiotic used to treat and prevent uncomplicated lower urinary tract infections caused by *K. pneumoniae*. In this study, we investigated the prevalence, drug resistance, and molecular characteristics of nitrofurantoin-non-susceptible *K. pneumoniae* (NNS-KP) isolated from urine samples.

Methods: A total of 661 *K. pneumoniae* isolates obtained from urine specimens were collected from a university hospital in Daejeon between March 2020 and December 2021. Antimicrobial susceptibility tests were conducted using the Neg MIC Panel Type 44 and MicroScan WalkAway-96 Plus systems. We performed PCR and DNA sequencing for the detection of carbapenemase genes (*blaKPC*, *blaIMP*, *blaVIM*, *blaNDM*, and *blaOXA-48-like*) and the multilocus sequence typing of multidrug-resistant NNS-KP isolates.

Results: The nitrofurantoin non-susceptibility rate of *K. pneumoniae* was 12.4% (82/661). These NNS-KP isolates were resistant to ampicillin (100.0%), cefuroxime (78.0%), ampicillin-sulbactam (76.8%), cefotaxime (76.8%), ceftazidime (75.6%), ciprofloxacin (73.2%), cefepime (73.2%), trimethoprim-sulfamethoxazole (67.1%), levofloxacin (61.0%), tetracycline (54.9%), tobramycin (54.9%), gentamicin (50.0%), piperacillin-tazobactam (42.4%), amoxicillin-clavulanate (41.5%), ceftazidime (28.0%), piperacillin-tazobactam (25.6%), ertapenem (6.1%), imipenem (6.1%), meropenem (6.1%), and amikacin (2.4%). The prevalence of ESBL production among NNS-KP was 69.5% (57/82). Five (6.1%) out of the NNS-KP isolates were identified as multidrug resistant. These multidrug-resistant NNS-KP iso-

lates belonged to two distinct sequence types (STs), namely, ST307 (N=4) and ST147 (N=1). The multidrug-resistant NNS-KP ST307 and ST147 isolates harbored *blaKPC-2* and *blaNDM-1*, respectively. All multidrug-resistant NNS-KP ST307 isolates were only susceptible to amikacin. The multidrug-resistant NNS-KP ST147 isolate was susceptible to amikacin, gentamicin, and tetracycline.

Conclusions: The susceptibility rate of *K. pneumoniae* isolates to nitrofurantoin remains high. Therefore, nitrofurantoin is still considered as a sensitive and effective oral antibiotic treatment option for UTIs caused by *K. pneumoniae*. The emergence of multidrug-resistant NNS-KP ST307 and ST147 suggests that special attention and continuous surveillance are necessary to preserve this treatment option.

Keywords: Nitrofurantoin, *Klebsiella pneumoniae*, Urinary tract infection, Antimicrobial resistance

PM-23

Vaginal Microbiome of Reproductive Age Healthy Women in Korea

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Background: Human vaginal microbiota plays a critical role in preventing various urogenital diseases. The vaginal microbiome is influenced by racial, behavioral, and environmental factors. Obtaining samples of normal vaginal microbiota from healthy women is crucial to analyze the data from pathogenic diseases. This study aimed to identify the characteristics of the vaginal microbiota in healthy Korean women of reproductive age.

Methods: A total of 52 Korean women aged 21–59 years (mean: 42.8 years) were included in this study. Next-generation sequencing was performed using Illumina MiSeq for the V1–V3 region of 16S rRNA, and data were analyzed using EZBioCloud. The community state type (CST) was determined using the VALENCIA algorithm. Our results were compared with those obtained in American women in the Human Microbiome Project.

Results: CST4 was the most prevalent vaginal microbiome type at 50.0% (N=26), followed by CST1 (30.8%, N=16), CST3 (17.3%, N=9), and CST5 (1.9%, N=1). CST2 was not observed. By age group, CST1 was most common in women in their 20s (60.0%), followed by those in their 30s (50.0%), 40s (26.7%), and 50s (9.5%). However, CST4 was most common in women in their 50s. The proportions of CST1 and CST4 decreased and increased as the women aged, respectively. CST4 prevalence was high in Korean women, whereas CST1 prevalence was high in American women.

Conclusions: The composition of CSTs differs between Korean and

American women. Therefore, results should be interpreted carefully when determining the pathogenic state based on the CST.

Keywords: Vaginal microbiome, Community state type, Korean woman

PM-24

Microbiological Characterization and Clinical Profiles of *Cryptococcosis* in South Korea: Insights from 4-Year Retrospective Study

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Background: Cryptococcosis, a potentially fatal mycosis, is caused by members of the *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes. This study aimed to characterize microbiological features of clinical isolates of *C. neoformans* and investigate the clinical profiles of patients with cryptococcosis over a recent 4-year period in South Korea.

Methods: A retrospective review was conducted on all patients diagnosed with laboratory-confirmed cryptococcal infections admitted to a tertiary hospital in South Korea from 2020 to 2023. Species identification was performed via MALDI-TOF or Vitek 2 system and was finally confirmed by ITS and D1/D2 sequencing. MLST typing was performed on all isolates. Antifungal susceptibility testing was performed using the Sensititre YO 10 panel. The clinical characteristics of the patients with cryptococcosis were retrospectively reviewed.

Results: A total of 24 isolates were recovered from 18 patients during the study period. The Bruker Biotyper and Vitek 2 systems correctly identified 83.3% and 75.0% of isolates as *C. neoformans*, respectively. Sequencing confirmed all isolates as *C. neoformans* var. *grubii*. MLST typing revealed 22 isolates of ST81, with single isolates of ST2 and ST15, respectively. All isolates had the wild-type response phenotypes to fluconazole, voriconazole, and posaconazole. However, 12.5%, 8.3%, and 4.2% isolates had the non-wild-type response phenotype to amphotericin B, 5-fluorouracil, and itraconazole, respectively. Overall cumulative 30-day mortality occurred in 38.9% (7/18). Factors such as the use of total parenteral nutrition, central venous catheters, and vasopressors and the presence of severe sepsis or acute renal failure were more prevalent in the mortality group compared to those in the survival group ($P < 0.05$ in all). Cryptococcosis was significantly more frequent in the mortality group (85.7% vs. 18.2%, death vs. survival group, $P = 0.004$). No significant differences in the therapeutic modality or sequence type of isolates were observed based on survival.

Conclusions: Our data highlights *C. neoformans* var. *grubii*, particularly ST81, as the prevalent strain in South Korea. Antifungal resistance was relatively low, yet the therapeutic modality was insignificant in their prognosis. Adverse outcomes were associated with several clinical factors, underscoring the importance of management strategies for the underlying conditions of the patients.

Keywords: *Cryptococcus*, MLST, Antifungal susceptibility, Clinical characteristics, Mortality

PM-25

Clinical Characteristics of Persistent Candidemia Due to *Candida Parapsilosis*: A Lesson from 5-Year Collection at a Tertiary Hospital in Korea

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Background: *Candida parapsilosis* complex is one of the most common non-albicans *Candida* species that cause candidemia, especially invasive candidiasis. We aimed to reveal clinical characteristics of persistent candidemia due to *C. parapsilosis* complex and mycological features.

Methods: Blood isolates of *C. parapsilosis* were collected from February 2019 to January 2023 at a tertiary Korean hospital. Species identification and antifungal susceptibility testing were performed. Clinical information was collected, including demographics, the clinical status at positive culture, therapy-related factors, and the outcome of candidemia. Clinical and mycological characteristics were analyzed according to single or serial isolates.

Results: A total of 69 patients with candidemia were included in this study. Among them, three isolates were resistant to fluconazole and harbored ERG11 mutations (Y132F [N=2], K143R [N=1]). No isolates were resistant to echinocandins, amphotericin B, and 5-FC. Single episodes of candidemia occurred in 36 patients, and serial episodes occurred in 33 patients. Underlying liver diseases, vasopressor use, intensive care unit admission, presence of severe sepsis, and central venous catheter use were more frequently found in patients with serial episodes compared to those with single episodes. There was no significant difference in the median MIC values of antifungal agents between single and serial isolates. There were no significant differences in using azoles or amphotericin B among patients with single or serial candidemia episodes. Instead, the patients with serial episodes received echinocandin therapy more frequently than those with single episodes. Overall, there was no significant difference in the 30-day mortality rate of patients with candidemia according to the number of episodes.

Conclusions: Our data show that several factors regarding the underlying conditions of patients with candidemia were related to persistent candidemia due to *C. parapsilosis* rather than *Candida* itself. These data may help manage patients with persistent *C. parapsilosis* candidemia.

Keywords: *Candida parapsilosis*, Persistent candidemia, Clinical characteristics, Echinocandin

PM-26

Development of Multiplex Real-Time PCR Assay for Simultaneous Detection of *Aspergillus Fumigatus*, *Pneumocystis Jirovecii*, and *Cryptococcus Neoformans*

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Background: Fungi are common components of the natural environment. Although most fungi are nonpathogenic, a few species can cause fatal disease. When the body's immune system is weakened, some fungi can cause invasive fungal infections (IFI). Various species cause IFI, mostly *Candida*, *Aspergillus fumigatus* (AF), *Pneumocystis jirovecii* (PJ), and *Cryptococcus neoformans* (CN). Conventional diagnostic methods for these diseases have relied on microscopic examination or culture-based methods, which are time-consuming, labor-intensive, and complex. In this study, we developed a multiplex real-time PCR assay to detect three common pathogens, AF, PJ, and CN, in clinical respiratory specimens simultaneously.

Methods: The primer sets were designed using the 28S rRNA gene for AF, the mtLSU-rRNA gene for PJ, and the IGS1 gene for CN. The performance of the assay was evaluated via analytical sensitivity and specificity. The specificity of the assay was evaluated using 32 reference strains (eight fungi, 15 bacteria, and 10 viruses). To compare the performance of the assay with commercial kits, we used a GeneProof *Aspergillus* PCR kit, RealStar *Pneumocystis jirovecii* PCR kit, and *Cryptococcus neoformans* Taqman PCR detection kit. A total of 126 clinical samples were collected from Seegene Medical Foundation. However, as positive CN clinical samples could not be collected, a CN reference strain was spiked into the negative clinical samples, and the results were compared with those of the commercial kits. Additionally, we tested for specificity using reference strains (CN and *C. gattii*).

Results: The minimum detection limit of the assay for each of the three pathogens was 100 copies/reaction, and the analytical specificity was 100%. The clinical sensitivity and specificity were 94.4% and 36.7% for AF, 94.7% and 70.0% for PJ, and 100% and 100% for CN, respectively. This assay is reliable due to its high clinical sensitivity and specificity. While the *Cryptococcus neoformans* Taqman PCR detection kit detects both CN and *C. gattii*, the assay developed here specifically detected only *C. neoformans*. These results indicate that this assay has higher specificity than the commercial kit.

Conclusions: In diagnosis, it is important to identify the exact pathogens causing diseases quickly and accurately. The assay developed in this study can be used to diagnose infections caused by these pathogens rapidly and accurately.

Keywords: Invasive fungal infections, Multiplex real-time PCR assay, Diagnosis, *Aspergillus fumigatus*, *Pneumocystis jirovecii*, *Cryptococcus neoformans*

PM-27

A Case of Disseminated Coccidioidomycosis Confirmed from Bronchial Aspirates, Joint Fluid, and Skin Biopsy

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Coccidioides immitis/posadasii is a thermally dimorphic fungus that exists as a filamentous mold in the environment and as a yeast in tissue. This microorganism causes a systemic fungal infection called coccidioidomycosis by inhaling arthroconidia. The dry areas of Southwestern United States, Mexico, and Central and South America are endemic regions, making domestic infections rare. However, domestic outbreaks have recently increased because of the increase in overseas travel to these regions. We report a case of disseminated coccidioidomycosis that occurred after a business trip to northern Mexico. A 46-year old man had received a kidney transplant 17 years ago and was taking immunosuppressants. He developed fever and respiratory symptoms while staying in Mexico, and the symptoms continued even after returning to Korea. After hospitalization, the patient had no response to antibacterial agents. Bronchial aspirates were cultured in SDA, and gray-white filamentous fungi grew after 5 days. *Coccidioides* species were suspected as alternating arthroconidia were observed in the lactophenol cotton blue staining, and the antigen test using serum by the Korea Centers for Disease Control and Prevention was positive. In the 10-day culture of knee joint fluid, barrel-shaped alternating arthroconidia were more clearly observed. In the subsequent nasal skin biopsy, numerous spherules filled with endospores were observed, which led to a diagnosis of disseminated coccidioidomycosis. The diagnosis of coccidioidomycosis was generally based on the patient's travel history to endemic areas, observation of barrel-shaped alternating arthroconidia in culture, detection of antigen or antibody in serologic tests, and observation of a large thick-walled spherical structure called a spherule in tissue samples. When coccidioidomycosis is not identified using the above tests, the DNA sequence can be analyzed after target gene amplification. *Coccidioides* species are filamentous fungi that cause representative laboratory-associated infection due to the occurrence of highly infectious arthroconidia in laboratory culture. Therefore, caution must be taken when processing filamentous fungi grown in a clinical microbiological laboratory. In particular, if barrel-shaped alternating arthroconidia are observed, the possibility of *Coccidioides* should be suspected first.

Keywords: Coccidioidomycosis, Dimorphic fungus, Bronchial aspirate, Joint fluid, Skin biopsy, Disseminated infection

PM-28

Genotypic Characterization and Azole Resistance in *Candida Tropicalis* Bloodstream Isolates: Emergence of Distinct Sequence Types

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Background: *Candida tropicalis* is the second most common pathogen of candidemia, associated with increasing resistance to azole antifungals. Azole resistance in *C. tropicalis* is primarily driven by mutations in *ERG11*, *CDR1*, *MDR1*, and the regulator *UPC2*. This study aimed to identify genes associated with resistance to fluconazole and voriconazole, and the distribution of sequence types among *C. tropicalis* isolates.

Methods: A total of 45 *C. tropicalis* bloodstream isolates (January 2020 and June 2023) were screened. The isolates were identified using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Antifungal susceptibility testing was performed using the Sensititre YO10 panel. We performed PCR sequencing of the *ERG11*, *UPC2*, *TAC1*, and *MRR1* genes and determined the molecular alterations compared to *C. tropicalis* MYA-3404 (GenBank accession no. XM_002550939.1 for *ERG11*, NW_003020056.1 for *UPC2*, PQTP01000011.1 for *TAC1*, and XM_002547926.1 for *MRR1*). Multilocus sequence typing (MLST) was carried out on six housekeeping genes, and the diploid sequence type was determined. Each heterozygous position showing a strong overlapping peak was transformed into a degenerate nucleotide according to the IUPAC nucleotide code. The new allelic profiles were submitted to the *C. tropicalis* MLST database, with numbers assigned by the curator. Additionally, a phylogenetic tree was constructed using the maximum-likelihood and neighbor-joining methods (MEGA11) by grouping the unique genes associated with each ST number.

Results: Among the 45 isolates, 7 (15.6%) were fluconazole non-susceptible (4 resistant, 3 susceptible dose-dependent), and 16 (35.6%) were voriconazole non-susceptible (4 resistant, 12 intermediate). Mutations in *ERG11* (Y132F, S154F, Q330P, Y105C), *UPC2* (A263S, Q315P, Q316P), and *MRR1* (K307R, A647S) were identified exclusively in isolates that were non-susceptible to fluconazole and/or voriconazole. MLST analysis classified the isolates into 29 sequence types (STs). Of these, the following 17 new STs were identified and submitted to the *C. tropicalis* MLST database: 1,513 and 1,531–1,546. Of the 29 STs identified, types 149, 359, and 1,532 were detected in the fluconazole-resistant isolates. The genetic similarity diagram generated from PHYLOViZ demonstrated 5 clonal complexes and 12 singletons based on goeBURST distance. Two *C. tropicalis* strains (ST149 and ST1532) with high minimum inhibitory concentration (MIC) values (FLU: >256, VOR: >8) clustered at a single node in the phylogenetic tree.

Conclusions: Although research on *C. tropicalis* is still limited, our study suggests that isolates (ST149 and ST1532) with high MIC values form a distinct group within the phylogenetic tree. Continuous monitoring is needed.

Keywords: Candidemia, Azole antifungal, Resistance gene

PM-29

The Usefulness of Sequence-based Identification Methods for Distinguishing Various Clades of *Candida Auris*

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Background: *Candida auris* is a global concern due to its outbreaks and severe infections in healthcare settings. It is classified into 5 clades by geographic regions, with clade II predominantly reported in South Korea. Unlike clades I, III, and IV, clade II has not been associated with healthcare-associated outbreaks, suggesting that its risk assessment might differ from other clades. While whole genome sequencing (WGS) and multilocus sequence typing (MLST) are established methods for distinguishing *C. auris* clades, they are complex and labor-intensive. This study aimed to determine whether simpler molecular identification techniques, typically employed for *C. auris* detection, could effectively differentiate its clades.

Methods: A total of 135 isolates were analyzed, comprising 125 *C. auris* isolates sourced from 125 patients in Korean hospitals, along with 10 isolates from the CDC (AR0381-AR0390, which included 5 clade I isolates, 1 clade II isolate, 2 clade III isolates, and 2 clade IV isolates). The ITS region sequences and the D1/D2 regions of the 26S ribosomal DNA from each isolate were examined using a BLAST search against reference sequences in GenBank (NCBI), and the results were compared with those obtained from MLST typing.

Results: MLST separated 125 *C. auris* isolates from Korean hospitals into two clades (62 in clade I and 63 in clade II). The examination of ITS sequences from 64 (63 Korean and 1 CDC) clade II isolates revealed distinct nucleotide sequence variations (80_81AC>CT, 83A>T, 87C>G, 89_90delTA, 320delT) in comparison to AR0382 (GN*:MK294583) or AR0383 (GN:MK294584) in GenBank, while showing a 100% match with AR0381 (GN:MK294582). However, 67 clade I (62 Korean and 5 CDC) and 2 clade III Korean and CDC isolates did not display differentiation by ITS sequencing, exhibiting a 100% match with AR0382 or AR0383. On the contrary, the analysis of 26S DNA D1/D2 sequences from all 67 clade I isolates demonstrated unique nucleotide sequence variations (423C>T, 440C>T, 443_444delTT, 465delG) when compared to AR0381 or AR0383 in GenBank, with a 100% match with AR0382. However, clade II and clade III isolates were not differentiated by 26S DNA D1/D2 sequencing, showing a 100% match with AR0381 or AR0383. The ITS and 26S DNA D1/D2 sequences of two clade IV CDC strains exhibited unique nucleotide sequence variations when compared with those of AR0381, AR0382, or AR0383, with a 100% match with AR0385 (GN:MK294586).

Conclusions: Our results suggest that 26S DNA D1/D2 and ITS se-

quencing can effectively distinguish clade I and clade II *C. auris* isolates from Korean hospitals, respectively, providing a rapid and straightforward method without the necessity of conducting MLST or WGS.

Keywords: *Candida auris*, Clade, Sequencing, Identification

PM-30

Antivirulence Mechanisms of Epigallocatechin-3-Gallate against *Helicobacter Pylori*: A Microbioinformatic Approach

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Background: Numerous studies have explored the connection between *Helicobacter pylori* infection and upper gastrointestinal disorders, including chronic gastritis, peptic ulcers, and gastric cancer. Due to the adverse effects associated with the antibiotic treatment of *H. pylori* infections, alternative strategies have been proposed. Epigallocatechin-3-gallate (EGCG), a prominent compound in green tea, has shown antibacterial effectiveness against *H. pylori* *in vitro* and *in vivo*. However, the exact mechanisms through which EGCG counteracts *H. pylori* remain insufficiently understood. This research aimed to clarify the mechanism by which EGCG acts against *H. pylori* using bioinformatic methodologies.

Methods: Using tools such as STITCH v.5.0, VICMPred, and Virulent-Pred, we identified protein targets, determined their functional roles, and characterized their virulence properties. Additionally, BepiPred v.2 software was used to predict the peptide epitopes of *H. pylori* virulence factors, while PSORTb v.3 was employed to explore the sub-cellular localization of these factors.

Results: EGCG interacted with several crucial virulence factors of *H. pylori*, including enoyl-ACP reductase (fabI), type II DNA modification enzyme (jhp_0044), 7-alpha-hydroxysteroid dehydrogenase (jhp_0409), and type II DNA modification (methyltransferase) (jhp_0435), which are essential for bacterial cell viability and metabolism.

Conclusions: In conclusion, this bioinformatic analysis provided significant evidence regarding the mechanisms through which EGCG acts against *H. pylori*. Nevertheless, further validation through laboratory experiments is essential to confirm the predicted pathways of action.

Keywords: Epigallocatechin-3-gallate, *Helicobacter pylori*, Virulence factors

PM-31

Investigation of Mechanism of Quorum Sensing Molecules in Damage to Vascular Endothelial Cell-to-Cell Connection Structure

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Background: Quorum sensing (QS) molecules are secreted by bacteria to create a growth environment. Recently, QS molecules have emerged as novel agents that damage endothelial cells and destroy vascular tissue, prolonging sepsis. However, research on the mechanism by which this substance affects the human body is lacking. For this reason, we investigated the effect of QS molecules on vascular endothelial cell damage using HUVECs treated with 3OC12-HSL (3-oxo-C12-HSL), the QS molecules of pneumococci.

Methods: After the 3OC12-HSL treatment of HUVECs, the results were analyzed using endothelial cell tube formation assays, quantitative polymerase chain reaction, co-immunoprecipitation, western blotting, and immunocytochemistry.

Results: After treating endothelial cells with 3-oxo-C12-HSL, Angiopoietin2 mRNA expression increased, Angiopoietin2 protein levels increased in the cells, and the tube-forming ability of endothelial cells decreased. Additionally, the expression of FOXO1, a signaling substance associated with cell damage, was increased. Using co-immunoprecipitation assays, we confirmed the inhibition of the IQGAP1/Rac1-binding structure involved in cell-to-cell adhesion.

Conclusions: These results indicated that QS molecules destabilize blood vessels by weakening the bond between endothelial cells. We found that Angiopoietin2 is related to suppressing the IQGAP1/Rac1-binding structures in endothelial cell damage, although further research is needed to identify their interaction mechanisms involving QS. Establishing the role of QS molecules in the human body will provide new alternatives to treat infections caused by microorganisms and organ failure due to sepsis.

Keywords: Sepsis, Infection, 3OC12-HSL (3-oxo-C12-HSL), Angiopoietin 2, HUVEC

PM-32

Cytomegalovirus Immunoglobulin G Seropositivity in the General Population of Korea: A Single-Center Study

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Background: Cytomegalovirus (CMV) is a widespread human herpesvirus, with diverse clinical manifestations ranging from asymp-

tomatic in healthy individuals to severe life-threatening end-organ dysfunctions, especially in immunocompromised patients. CMV infection is one of the most common viral infections worldwide, with CMV immunoglobulin G (IgG) seropositivity increases with age, exhibiting a geographical variation influenced by socioeconomic status. South Korea is known to be a high CMV IgG seropositivity country with a rate of >95%, where a decline in seropositivity has also been reported recently. In this study, we aimed to investigate the seropositivity of CMV IgG in the general Korean population and analyze the current CMV seroprevalence in South Korea.

Methods: Residual samples from individuals who visited Konkuk University Medical Center's healthcare center in May 2024 for regular checkups were used. CMV IgG was measured using a commercial immunoassay (Alinity i CMV IgG Reagent Kit, Abbott). All procedures were performed according to the manufacturer's instructions. Sample results were interpreted as "Reactive" when the concentration of CMV IgG was >6.0 AU/mL, and "Nonreactive" when CMV IgG was ≤6.0 AU/mL. We compared the seropositivity of CMV IgG between genders and analyzed trends across different age groups (aged 20–29, 30–39, 40–49, and ≥50 years).

Results: A total of 600 samples were measured from 263 male and 337 female healthy individuals, with the majority of samples collected from young adults (aged 20–39 years) (N=421, 70.2%). Overall seropositivity of CMV IgG was 88.0% (528/600). Seropositivity was significantly higher in males (92.0%, 242/263) than in females (84.9%, 286/337) (Difference 7.1%, 95% confidence interval 1.8%–12.5%, $P=0.0109$, Chi-square test). There was no significant difference in seropositivity between genders within each age group. Seropositivity increased across age groups, from 77.0% (117/152) in individuals aged 20–29 years to 100.0% (105/105) in individuals aged ≥50 years (P for trend <0.0001). This trend was observed in both gender subgroups (male; P for trend =0.0105, female; P for trend <0.0001).

Conclusions: The overall seropositivity of CMV IgG in the general population of Korea was estimated to be 88.0%, with a higher rate in males. Given the lower seropositivity in younger adults, monitoring the rate changes and further education on CMV infection is needed for disease control and prevention.

Keywords: Cytomegalovirus, Immunoglobulin G, Seropositivity, Korean

PM-33

Healthcare-Associated Infections of Rotavirus and Norovirus Over a 10-year Period in University Hospitals

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Background: Rotavirus and norovirus are the most common causes

of acute viral gastroenteritis. These viruses can cause healthcare-associated infections (HAIs) sporadically or epidemically. This study investigated the HAIs of rotavirus and norovirus over a 10-year period in three university hospitals in Korea.

Methods: We investigated the results of rotavirus and norovirus PCR and/or antigen tests from January 2014 to December 2023. HAIs was defined as a case in which a viral test was ordered 48 hours after hospitalization and the patient tested positive for the virus. A hospital outbreak was defined as two or more patients, with onset within five days of each other, with suspected transmission within the same ward/unit or patient room at the same time.

Results: Over a 10-year period, 42,775 tests for rotavirus and 38,944 tests for norovirus were conducted at three university hospitals. The number of positive tests (positivity rate) was 2,312 (5.4%) for rotavirus and 3,731 (9.6%) for norovirus. Of these, 170 (7.4%) rotavirus positive cases, and 276 (7.4%) norovirus positive cases met the HAIs definition. Most HAIs occurred sporadically: 78.8% (134/170) for rotavirus and 89.1% (246/276) for norovirus. Rotavirus outbreaks occurred 11 times between 2014 and 2019, each involving two to six patients, in neonatal intensive care units (NICUs) only, with no additional cases since the COVID-19 pandemic. The age range of patients with norovirus outbreaks was 0–84 years, and outbreaks have occurred consistently once or twice a year in pediatric wards, NICUs, and ICUs through 2023; however, each outbreak involved only two patients at a time. There were no simultaneous outbreaks of both viruses.

Conclusions: Even if norovirus HAIs occurred, group outbreaks were rare. Hospital outbreaks of rotavirus have disappeared after enhanced infection control measures, emphasizing the importance of infection control.

Keywords: Rotavirus, Norovirus, Healthcare-associated infection, Outbreak

PM-34

Evaluation of YES PATH Preservative Solution (Gyn) for Preserving High-Risk HPV Genotypes in Cervical Swab Specimens

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Background: The contemporary paradigm in human papillomavirus (HPV) detection and diagnosis has shifted towards an integrative approach that combines traditional cytological assessments, such as the Pap smear, with advanced molecular techniques, such as like HPV genotyping PCR. This dual modality not only enhances the accuracy of HPV detection but also facilitates expedited diagnosis and timely intervention. The synergistic use of cytopathology and molecular diagnostics ensures comprehensive evaluation of cervical pathology, thereby optimizing patient management and therapeutic outcomes. The YES PATH Preservative Solution (Gyn) (YESONG, Korea)

was designed to preserve cervical swab specimens for cytological analysis and HPV genotyping. This study evaluated the efficacy of this solution's efficacy in preserving high-risk HPV genotypes detected in positive and negative cervical swab specimens over 4 days at room temperature.

Methods: This single-center retrospective study was conducted in March of 2024. A total of 82 cervical swab specimens previously tested for high-risk HPV genotypes by multiplex real-time PCR using the NeoPlex HPV29 Detection Kit (Genematrix, Seongnam, Korea) were preserved in the YES PATH Preservative Solution for 4 days (96 hours) at room temperature condition. These specimens were re-tested for HPV genotyping PCR with the same method. The agreement between pre-preservation and post-preservation was assessed using Cohen's kappa coefficient with 95% CI: <0.20, none; 0.21–0.39, minimal; 0.40–0.59, weak; 0.60–0.79, moderate; 0.80–0.90, strong; and >0.90, almost perfect.

Results: A total of 82 specimens were analyzed, comprising 60 positive and 22 negative samples. Out of the 82 preserved specimens, 78 (95.12%) showed results concordant with the original tests. Specifically, the positive concordance rate was 96.67% (58/60, 95% CI, 90.46–99.78), and the negative concordance rate was 90.91% (20/22, 95% CI, 84.70–97.12). The Cohen's kappa value was 0.8754, indicating a strong level of agreement. There were four discordant cases: two specimens were originally negative but tested positive after preservation, and two were originally positive but tested negative after preservation. Notably, these four cases shared a common characteristic; each contained only a single HPV genotype.

Conclusions: The YES PATH Preservative Solution (Gyn) demonstrated high efficacy in preserving cervical swab specimens for HPV genotyping over a 4-day period at room temperature. The high agreement and Cohen's kappa values indicate that the solution maintains the integrity of HPV DNA, making it a reliable option for settings where immediate testing is not feasible. This finding supports the use of YES PATH Solution in clinical practice, particularly in environments where logistical constraints delay immediate HPV testing.

Keywords: HPV, Cervical swab, Preservation, Genotyping, YES PATH, PCR

PM-35

Genotype Distribution and Demographic Characteristics of Hepatitis C Virus in Korea, 2019–2023

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Background: Hepatitis C is a liver infection caused by the hepatitis C virus (HCV). HCV has seven major genotypes and numerous HCV subtypes have been identified. These genotypes are geographically distributed and have different effects on disease progression and the treatment response. This study aimed to analyze the genotype distribution and demographic characteristics of HCV infection in Korea.

Methods: HCV genotype data were collected from five major commercial referral laboratories in Korea (Seoul Clinical Laboratories, Samkwang Medical Laboratories, EONE Laboratories, Seegene Medical Foundation, and GC Labs), covering the period from January 2019 to December 2023. HCV genotypes were primarily determined using the cobas® HCV GT assay (Roche Molecular Diagnostics, Pleasanton, CA, USA), which utilizes real-time polymerase chain reaction technology. One exception was a laboratory that employed gene sequencing for genotype determination. HCV genotypes were categorized as types 1–6, with subtypes identified only for genotype 1. Patient demographic information (age, sex, and residence) was collected using laboratory information systems, adhering to the ethical guidelines approved by the institutional review board. These data were used to analyze the demographic distribution of HCV genotypes across different regions, sexes, and age groups in South Korea.

Results: In total, 33,030 HCV genotype results were collected, with 99.8% identified as a single genotype and 0.2% displaying a mixed pattern. Genotype 2 was the most common, followed by genotype 1b. Genotypes 1b and 2 accounted for 95.8% of samples. Genotype 3 was the next most frequently identified genotype after genotypes 1b and 2. Annual patterns in the distribution of HCV genotypes were observed, with notable geographic variations. Genotype 1b was most prevalent in Daegu-si, while genotype 2 dominated in Chungnam-do. Genotype 3 was most common in Gyeongnam-do and Chungbuk-do. Significant sex-related differences were also observed. The proportions of genotypes 1b and 3 were significantly higher in males than in females. In addition, as age increased, the prevalence of genotype 2 increased, whereas that of genotype 1a decreased. Genotype 3 was more common in the younger generation, aged 20–49 yrs.

Conclusions: This study enabled us to observe the distribution of HCV genotypes in Korea, as well as regional, sex-related, and age-related characteristics. These findings will serve as a reference for developing policies to prevent the influx of HCV from abroad and the spread of HCV. In addition, continuous monitoring of HCV genotypes is necessary.

Keywords: Hepatitis C virus, Genotype, Korea

PM-36

Changes in Prevalence of Respiratory Viruses Before, During and After the COVID-19 Pandemic

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Background: Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has emerged as a highly transmissible respiratory virus and

caused the coronavirus disease-2019 (COVID-19) pandemic, leading to significant morbidity and mortality worldwide. Non-pharmaceutical interventions, such as lockdowns, mask mandates, and social distancing measures, were implemented to control the spread of the virus. These measures could inadvertently affect the transmission dynamics of other respiratory viruses. However, as the COVID-19 pandemic transitioned into an endemic phase, with SARS-CoV-2 becoming a part of the regular respiratory virus landscape, the dynamics of respiratory viral infections began to change. Therefore, we analyzed the changes in the positivity rates for respiratory viruses before, during, and after the COVID-19 pandemic.

Methods: This study compared the positivity rates for respiratory viruses from 2019 (before the COVID-19 outbreak) to 2023 (when the viruses became endemic). Nasopharyngeal swabs and sputum samples were collected during the study. Respiratory viral nucleic acids were extracted and amplified. Using the AllplexTM Respiratory Panel (Seegene, Seoul, Korea), we screened all samples for the following 19 respiratory viruses: influenza A and B, respiratory syncytial viruses A and B, influenza A subtypes H1, H3 and H1pdm09, adenovirus, metapneumovirus, enterovirus, parainfluenza types 1, 2, 3 and 4, bocavirus, rhinovirus, and coronaviruses 229E, NL63 and OC43.

Results: Between January and October of each year from 2019 to 2023, 37596, 17463, 23222, 32275, and 52770 viral multiplex tests were performed in patients with suspected respiratory viral infections. Positivity rates for most respiratory viruses decreased during the pandemic and increased during the endemic period, with rates being similar to or higher. In particular, influenza A, RSV A, RSV B significantly increased from 2.79%, 2.73%, and 1.50%, respectively, in 2019 to 5.56%, 6.63%, and 5.67%, respectively, by 2023. In contrast, influenza B, parainfluenza type 1, and bocavirus infections decreased from 3.50%, 2.72%, and 16.32%, respectively, to 0.17%, 1.07%, and 9.49%, respectively, during the same period. Positivity rates for all 19 respiratory viruses decreased significantly during the COVID-19 pandemic.

Conclusions: The transition from the COVID-19 pandemic to the endemic period has led to changes in respiratory viral infection rates. While the pandemic has resulted in a significant reduction in the circulation of respiratory viruses, the endemic phase poses new challenges to the resurgence of other respiratory viruses. Continuous monitoring, research, and public health interventions are essential.

Keywords: Respiratory virus infection, COVID-19, Pandemic, Endemic

PM-37

Performance Evaluation of Alinity M for CMV and EBV Viral Load Quantification Using Two Quantitative Nucleic Acid Test Systems

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Background: Cytomegalovirus (CMV) and Epstein-Barr virus (EBV)

are critical pathogens in immunocompromised patients, including organ transplant recipients and patients with AIDS. The accurate quantification of these viruses is essential for effective patient management and treatment. This study investigated the differences between two quantitative nucleic acid amplification test systems for CMV and EBV in a clinical setting.

Methods: Plasma samples were evaluated using plasma (EDTA) samples on an Alinity M system. Key performance metrics including precision, linearity, correlation with the Abbott m2000 RealTime System, sensitivity, cross-reactivity, and specificity were assessed. Precision was evaluated by performing within-run and between-day tests using low-positive (LOPOS) and high-positive (HIPOS) control samples over five days. Linearity was determined by serial dilution of the high viral load samples. Sensitivity, defined as the limit of detection (LOD), was measured by serial dilutions of control samples. The cross-reactivity and specificity were assessed using non-targeted viruses (BKV).

Results: The CMV assay demonstrated a LOPOS mean of 1,323 IU/mL (SD 0.02 log IU/mL) and HIPOS mean of 666,125 IU/mL (SD 0.01 log IU/mL), whereas the EBV assay showed a LOPOS mean of 1,891 IU/mL (SD 0.02 log IU/mL) and HIPOS mean of 175,562 IU/mL (SD 0.04 log IU/mL). Both assays exhibited within- and between-precision within acceptable limits as defined by the manufacturer. Excellent linearity was observed, with R² values of 0.9989 (CMV) and 0.9991 (EBV). A high correlation with the Abbott m2000 RealTime System was found, with R² values of 0.9889 (CMV) and 0.9641 (EBV), and mean bias differences of 0.21 log IU/mL and -0.10 log IU/mL, respectively. The LOD for the CMV and EBV assays were 30 and 20 IU/mL, respectively, with all tested replicates yielding positive reactions. No cross-reactivity was detected in BKV samples, confirming the high specificity of both assays.

Conclusions: Alinity M CMV and EBV assays demonstrated robust performance in terms of precision, linearity, correlation, sensitivity, and specificity. These assays showed a high correlation and comparable results with the Abbott m2000 RealTime System, suggesting that they are reliable alternatives for the accurate quantification of CMV and EBV viral loads. The mean bias between the two systems for CMV was 0.21 log IU/mL, whereas another study found no significant difference. Overall, this indicates that the Alinity M system is a useful and reliable tool for clinical diagnosis, providing effective management of patients with these infections.

Keywords: Cytomegalovirus, Epstein-Barr Virus, Alinity M, Viral load quantification, Performance evaluation

PM-38

Comparative Metagenomic Analysis of Bile Microbiome in Patients with Cholecystolithiasis and Choledocholithiasis

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Background: Gallstone formation results from a multifaceted interplay between genetic and environmental elements, although the exact triggering factors are not entirely comprehended. The role of the bile microbiome is gaining recognition as a potential influence in the development of gallstone disease. The main aim of this study was to examine differences in microbial communities present in bile samples obtained from patients diagnosed with choledocholithiasis (common bile duct stones) and cholecystolithiasis (gallbladder stones).

Methods: Massively parallel sequencing of the 16S rRNA gene was utilized to scrutinize the microbial compositions in bile samples collected from 28 patients diagnosed with choledocholithiasis (group DS) and cholecystolithiasis (group GS).

Results: The taxonomic composition of bile microbial communities exhibited significant differences between groups DS and GS. Among the 16 prevalent genera, *Streptococcus*, *Ralstonia*, *Lactobacillus*, and *Enterococcus* were predominant in group GS. Conversely, group DS demonstrated a more diverse array of genera. The alpha diversity of bile specimens was notably lower in group GS than in group DS ($P=0.041$). Principal coordinate analysis revealed distinct clustering of bile microbial communities based on the location of the gallstone. Linear discriminant analysis effect size analysis, with a score threshold exceeding 3 and the Kruskal–Wallis test ($\alpha<0.05$), identified Bacilli and Lactobacillales as potential taxonomic markers for distinguishing patients with cholecystolithiasis localized to the gallbladder.

Conclusions: Substantial differences were identified in the distribution and diversity of bile microbial communities between patients with choledocholithiasis and cholecystolithiasis. This observation implies that changes in the bile microbiome may play a role in the development of gallstones in these individuals.

Keywords: Metagenomic analysis, Bile microbial communities, Cholelithiasis, Choledocholithiasis, 16S rRNA gene sequencing

crucial for intercellular communication. They transport cargo, such as mRNAs, microRNAs, DNA, and proteins, between cells. Research indicates that TV secretes EVs to modulate intercellular communication with the host immune system. However, the precise mechanism underlying trichomoniasis remains unclear. Hence, this study aimed to elucidate the molecular mechanisms of inflammation induced by TV-EVs and identify novel proteins that regulate the immune response in host cells.

Methods: 1. TV cell line strains (ATCC 30236 and ATCC 50143) and the clinical strain NDMC5 were co-cultured with Ect1 E6/E7 cells (ATCC CRL-2614). 2. The Total Exosome Isolation Reagent (Invitrogen) was employed to isolate TV-EVs from cell culture media. 3. The morphological characteristics of TV-EVs were observed using transmission electron microscopy and nanoparticle tracking analysis. 4. Forty-five common human cytokines were analyzed using a multiplex immunoassay. 5. To study the internalization of TV-EVs by ectocervical (Ect) cells, TV-EVs were labeled using a fluorescent PKH67 Green Fluorescent Cell Linker kit and co-cultured with Ect cells.

Results: Our data showed that TV-EVs were round and were double bilayer-bound structures. The internalization of TV-EVs by Ect cells mediated cell survival and intercellular communication. TV-EVs induced the secretion of the inflammatory cytokines CXCL1, IL-6, and IL8. Additionally, TV-EVs co-cultured with Ect cells activated the TLR3, PI3K, MAPK, and ERK signaling pathways. Moreover, proteomic analysis revealed TV-EV-induced expression of MICB and TRAF3IP2, which are positively regulated by TLR3. Altogether, this study significantly advances our understanding of how TV-EVs modulate the immune response and mediate pathogenesis in host cells.

Conclusions: This study significantly advances our understanding of the immunomodulatory functions of TV-EVs, paving the way for future treatments of refractory trichomoniasis and TV-associated sexually transmitted infections.

Keywords: Trichomoniasis, *Trichomonas vaginalis*, Extracellular vesicles, Immune response

PM-39

Molecular Regulation of Toll-Like Receptor 3-Mediated Inflammatory Signaling Induced by *Trichomonas Vaginalis* Extracellular Vesicles

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Background: Trichomoniasis, caused by *Trichomonas vaginalis* (TV), is the most prevalent nonviral sexually transmitted infection. The symptoms range from asymptomatic infections to conditions such as vaginitis, urethritis, pelvic inflammatory disease, and prostatitis. Additionally, it increases the risk of contracting human immunodeficiency virus and the incidence of cervical and prostate cancer transmission. Extracellular vesicles (EVs) are released by various human cells and comprise phospholipid bilayer-bound structures that are

PM-40

Molecular Identification of *Dibothriocephalus Nihinkaiense* Infection in Patient with Recurrent Epigastric Pain Using Nanopore Sequencing

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Dibothriocephalus nihinkaiense (previously known as *Diphyllbothrium nihinkaiense*) is one of the largest intestinal tapeworms infecting humans and other mammals. Humans are infected after consuming raw or insufficiently cooked fish containing the plerocercoids of *D. nihinkaiense*. There are 14 species of *Dibothriocephalus* that can cause human infections. Amongst them, *D. latus* and *D. nihinkaiense* are the main pathogens of humans, causing diphyllbothriasis. The

second intermediate host of *D. latus* is freshwater fish such as pike, perch, burbot, and char. *D. latus* infections are common in temperate regions such as Europe and North America, where cold, clear lakes are abundant. The intermediate host of *D. nihinkaiense* is Pacific salmon. The consumption of raw Pacific salmon may be a risk factor for *D. nihinkaiense* infection. In Asia, *D. nihinkaiense* infections have been reported in the Republic of Korea and Japan. Some cases have also been reported in Taiwan, Siberia, and Malaysia. In this case report, we report a case of *D. nihinkaiense* infection in Hong Kong. A 40-year-old woman presented to the outpatient department with epigastric pain and diarrhea. The patient had no fever and vomiting. On physical examination, no specific signs, such as abdominal tenderness, were observed. This patient has a history of gastritis, diagnosed in 2022. On abdominal X-ray examination, a curvilinear opacity was seen at the upper quadrant of the abdomen. A yellowish parasite-like object was found in her feces and sent to the laboratory for further investigation. On examination, the parasite-like object was yellowish, segmented, and approximately 80 cm in length without scolex and neck. An oval 50- μ m-sized egg, grayish-brown in color, was found on microscopic examination, with an inconspicuous operculum and a small knob at the abopercular end. DNA was extracted from the proglottid tissue, and species-level identification was performed using Nanopore sequencing. The parasite-like object was identified as *D. nihinkaiense* based on the morphological characteristics of the strobila and egg and the DNA sequence of the mitochondrial genome. A single oral dose of praziquantel (25 mg/kg) was prescribed to the patient. The patient visited again one week later for stool examination, and no ova or proglottids were found. There was no evidence of relapse after the treatment. We report a case of *D. nihinkaiense* infection in a woman with recurrent epigastric pain. This case also highlights the use of NGS as a tool for the accurate species identification of *Dibothriocephalus* spp.

Keywords: Dibothriocephalus, Nanopore, NGS, Parasite identification

PM-41

Assessment of Molecular Biological Analysis for the Detection of Sexually Transmitted Diseases Single Center Study

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Background: In 2023, sexually transmitted diseases (STDs) will constitute 25.0% of all registered infectious diseases in Mongolia. Over 30 types of sexually transmitted infections have been identified. Notably, pathogens such as *M. hominis*, *U. parvum*, *U. urealyticum*, and *M. genitalium* are typically present asymptotically in the female vaginal flora but can lead to disease upon transmission during sexual intercourse. If left untreated, these infections can result in chronic inflammation, irreversible reproductive organ changes, and tumorigen-

esis. In response to this public health concern, the Dornod Medical Center introduced molecular biology diagnostic methods for STD detection in 2023, enabling the detection of multiple infections from a single sample.

Methods: This study adopted a cross-sectional analytical research approach. Data were collected from molecular biology tests conducted between 2023 and 2024 at the Virology Laboratory of Dornod Medical Center. One hundred individuals were randomly sampled from the test records. Molecular biology tests were used to identify 14 types of STDs. Descriptive statistics, including mean values and standard deviations, were calculated. Statistical significance was set at $P < 0.05$.

Results: The average age of the participants in the study was 34.0 ± 9.7 years, with 17% male and 83% female. Sexually transmitted pathogens were identified in cervical swabs and semen samples from the study population. The analysis revealed the presence of the following pathogens: 36% *M. hominis*, 29% *U. parvum*, 18% *U. urealyticum*, 7% *M. genitalium*, and 14% *Cytomegalovirus*. Among the individuals included in this study, 66% tested positive for at least one STD-causing pathogen, 29% for one pathogen, 25% for two pathogens, and 12% for more than three pathogens. The occurrence of co-infections was found to be gender-dependent ($P \geq 0.000$). Additionally, 25.3% of all surveyed women were pregnant, and 52% tested positive for sexually transmitted infections.

Conclusions: The most prevalent infections were *M. hominis* and *U. parvum*. Notably, co-infections involving more than two pathogens were observed in 37% of subjects. Additionally, future efforts should consider expanding molecular diagnostics to include other common infectious agents such as HPV.

Keywords: Cervical smear, Co-infection, PCR

PM-42

Characteristics of Vaginal Microbiomes in Reproductive-aged Women with Abnormal Uterine Bleeding

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Background: Abnormal uterine bleeding (AUB) is a common condition that affects approximately 30% of women of childbearing age at least once, causing heavy menstruation, impairing women's quality of life, and increasing healthcare and economic costs. We investigat-

ed the vaginal microbiome characteristics of women with AUB.

Methods: A total of 173 women diagnosed with AUB aged between 19 and 50 were enrolled. Vaginal swab samples were collected and analyzed for microbiome taxonomic profiling (MTP) using 16S rRNA gene sequencing and EzBioCloud Apps, and the MTP results were compared with those of healthy women (N=100).

Results: Species richness and diversity indices were significantly higher in the AUB group than in the healthy group (ACE and Shannon, both $P<0.01$). The Beta diversity distance also differed significantly between the AUB and healthy groups ($P<0.01$). *Lactobacillus* was significantly lower in the AUB group than in the healthy group ($P<0.01$). However, vaginosis/vaginitis-associated bacteria, including *Streptococcus*, *Prevotella*, *Gardnerella*, and *Atopobium*, were significantly higher in the AUB group (all, $P<0.05$).

Conclusions: The vaginal microbiomes of women with AUB differed from those of healthy women, with a higher diversity and lower proportion of *Lactobacillus*. More research is needed to determine how these are related to the pathophysiology and prevention of AUB.

Keywords: Abnormal uterine bleeding, Vagina, Microbiome

PM-43

Genotypic Analysis of Quinolone Resistance in Rifampicin or Isoniazid-Resistant Tuberculosis Cases: A South Korean Study (2020–2024)

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Background: Recent guidelines for tuberculosis treatment in Korea advocate for a 6–9-month short-course regimen for multidrug-resistant tuberculosis (MDR-TB), emphasizing the importance of rapid quinolone susceptibility testing at treatment initiation. Such testing is crucial in South Korea, where 13.1% of the reported MDR/RR-TB cases in 2023 were quinolone resistant. Thus, this study analyzed quinolone resistance in rifampicin- and/or isoniazid-resistant cases from 2020 to April 2024 through *gyrA* and *gyrB* gene sequencing.

Methods: We retrospectively analyzed cases that underwent rapid susceptibility testing for *Mycobacterium tuberculosis* from July 2020 to April 2024. These cases included those with isoniazid or rifampicin mono-resistance, as well as those with resistance to both drugs. Rapid quinolone susceptibility testing was performed using *gyrA* and *gyrB* sequences. The rapid drug susceptibility tests for isoniazid or rifampicin were conducted using the Line Probe Assay (Advansure™ MDR-TB Genoblot assay kit; LG Life Sciences, Seoul, Korea). Sanger sequencing was performed to analyze the *gyrA* and *gyrB* sequences. These results were compared with those obtained using phenotypic drug susceptibility tests (absolute concentration method using Löwenstein–Jensen agar) and broth microdilution methods with Middlebrook 7H9 media.

Results: Out of 1,479 *gyrA* and *gyrB* sequencing results, 159 (10.75%) had mutant genes, and 1,320 (89.25%) were wild type. Among the resistant strains, 141 out of 159 (88.68%) had mutations in *gyrA*, and 18 out of 159 (11.32%) had mutations in *gyrB*. For the *gyrA* mutants, 42 (29.79%) had the D94G mutation, 38 (26.95%) had A90V, and 24 (17.02%) had D94A. For the *gyrB* mutants, 6 (33.33%) had the E540D mutation, 3 (16.67%) had D500N, 2 (11.11%) had N538S, 2 (11.11%) had N538T, and 2 (11.11%) had A543V. In absolute concentration drug susceptibility testing, 93.33% of the *gyrA* mutants were resistant to moxifloxacin (MFX) and 94.67% to levofloxacin (LFX). Among the *gyrB* mutants, 57.14% were resistant to MFX and 42.86% to LFX. In 95.74% of the *gyrA* mutants, the microdilution minimum inhibitory concentration (MIC) was ≤ 0.0625 –4 $\mu\text{g/mL}$ (median 1.5 $\mu\text{g/mL}$) for MFX and 0.25–8.0 $\mu\text{g/mL}$ (median 2 $\mu\text{g/mL}$) for LFX. In the *gyrB* mutants, the MIC was 0.25–1.0 $\mu\text{g/mL}$ (median 0.5 $\mu\text{g/mL}$) for both MFX and LFX.

Conclusions: This study underscores the significance of rapid quinolone susceptibility testing in guiding MDR-TB treatment, particularly in regions with high quinolone resistance rates, such as South Korea. Understanding the prevalence and mutations in *gyrA* and *gyrB* is crucial for optimizing treatment regimens and improving patient outcomes.

Keywords: Quinolone resistance, Tuberculosis, Molecular epidemiology, Multidrug-resistant tuberculosis

PM-44

Can We Diagnose *Acanthamoeba Keratitis* in Real World of Clinical Microbiology Laboratory?

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Acanthamoeba species are free-living organisms ubiquitous in the environment. They are the causative agent of *Acanthamoeba keratitis*, a sight-threatening disease of the cornea, and are often misdiagnosed, causing delays in the administration of the correct treatment. Herein, we encountered a case of *Acanthamoeba keratitis* diagnosed without culture. A 12-year-old girl presented with pain, irritation, and hyperemia in the left eye and a history of wearing contact lenses. Corneal scraping smeared slide and liquids with contact lenses were submitted to a clinical microbiology laboratory. Culture for *Acanthamoeba* spp. was not available. Thus, calcofluor white staining was performed. The isolation of *Acanthamoeba* from corneal scraping allowed the detection of trophozoites and cysts based on their morphological characteristics. PCR targeting the 18S rRNA gene and sequencing revealed a 99% identity with *Acanthamoeba* species. Finding evidence of *Acanthamoeba* in clinical microbiology laboratories without culture is not always simple. This case highlights the need to maintain the inspection ability of clinical microbiology laboratories.

Keywords: *Acanthamoeba* keratitis, Cyst, Trophozoite, Morphology

PI-01

Predicting Immune Response Profiles in Birch Pollen Allergy Patients Using Machine Learning and Single-Cell RNA Sequencing

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Background: Birch pollen allergy causes significant allergic rhinitis and asthma, affecting quality of life during pollen season. Traditional diagnostics rely on clinical symptoms and immunoassays, which may miss individual immune response variations. This study aims to develop a machine learning model using single-cell RNA sequencing data to predict immune responses in birch pollen allergy patients, offering a more precise and personalized diagnostic and treatment approach.

Methods: We recruited 1,500 patients with confirmed birch pollen allergy and 1,500 controls from 2018 to 2023. Peripheral blood mononuclear cells were isolated, and single-cell RNA sequencing generated over 500,000 single-cell transcriptomes, capturing immune cell diversity. Our machine learning model used a convolutional neural network for feature extraction and a gradient-boosted decision tree for classification. Preprocessing involved normalization, dimensionality reduction using t-distributed stochastic neighbor embedding, and clustering with the Louvain algorithm. Feature selection was performed using mutual information gain and recursive feature elimination. The model was trained and validated with stratified 10-fold cross-validation and evaluated on an independent test set.

Results: The machine learning model achieved impressive performance metrics, with an accuracy of 98.2%, sensitivity of 97.5%, specificity of 98.8%, and an AUC-ROC of 0.995 on the test set. It effectively identified distinct immune cell subpopulations and their activation states related to birch pollen allergy, showing significant gene expression patterns in Th2 cells, regulatory T cells, and eosinophils. This integration of single-cell RNA sequencing data significantly improved predictive accuracy and provided a comprehensive view of the immune landscape in allergy patients.

Conclusions: The ML-based diagnostic model using scRNA-seq data represents an innovative approach to understanding and predicting immune responses in birch pollen allergy. Its high accuracy, sensitivity, and specificity demonstrate its potential to change allergy diagnostics and personalized treatment strategies. This study shows the significant impact of combining advanced ML techniques with genomic technologies. Future research should focus on validating these find-

ings in larger, multi-center cohorts and exploring the clinical application of this model in routine allergy practice to improve patient outcomes and quality of life.

Keywords: Birch pollen allergy, Machine learning, Single-cell RNA sequencing, Immune response, Diagnostic model, Gene expression

PI-02

Deep Learning-Enhanced Predictive Modeling of Tumor-Infiltrating Lymphocyte Subtypes for Optimizing CAR-T Cell Therapy in Refractory Non-Hodgkin Lymphoma

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Background: Chimeric antigen receptor T-cell (CAR-T) therapy has shown significant efficacy in treating refractory non-Hodgkin lymphoma (NHL), although patient responses vary considerably. Understanding tumor-infiltrating lymphocytes (TILs) is crucial for optimizing treatment outcomes. This study aimed to develop a deep learning model to predict TIL subtypes associated with successful CAR-T therapy outcomes in patients with NHL by leveraging high-dimensional imaging and single-cell RNA sequencing data.

Methods: We analyzed 1,200 patients with non-Hodgkin lymphoma patients treated with CAR-T therapy from 2019 to 2023, using advanced imaging and single-cell RNA sequencing to profile tumor-infiltrating lymphocytes in pre-treatment biopsy samples. Techniques included multiplex immunofluorescence, spatial transcriptomics, and deep learning models integrating convolutional and recurrent neural networks. Data preprocessing involved image segmentation, RNA sequencing normalization, and imputation of missing values. Key features, such as tumor-infiltrating lymphocyte density, subtype proportions, and spatial metrics were extracted and reduced using principal component analysis and gradient-boosted trees. The performance of the model was evaluated using the area under the curve (AUC), F1-score, precision, and recall, with a training-validation-test split (60/20/20) and five-fold cross-validation for hyperparameter tuning.

Results: The integrated deep learning model achieved an AUC of 0.94 (95% Confidence Interval, CI: 0.92–0.96) on the test set, with a precision of 89% (95% CI: 87%–91%), recall 88% (95% CI: 86%–90%), F1-score 88.5% (95% CI: 86%–91%), and overall accuracy 90% (95% CI: 88%–92%). The model identified high densities of CD8+ T cells with a cytotoxic profile and low levels of exhausted T cells as strong predictors of positive CAR-T therapy outcomes (Odds ratio: 4.5,

95% CI: 3.6–5.6, $P < 0.001$). Spatial metrics, such as TIL clustering near tumor cells, were also significant predictors ($\beta = 0.68$, 95% CI: 0.56–0.80, $P < 0.001$).

Conclusions: This study presents a deep learning model for predicting the TIL subtypes linked with successful CAR-T therapy in patients with NHL. The combination of high-dimensional imaging and single-cell RNA sequencing data improves our understanding of the immune landscape and leads to more precise treatments. This advancement in personalized CAR-T therapy holds promise for improving clinical outcomes and reducing complications. However, further clinical trials are required to validate these findings.

Keywords: CAR-T cell therapy, Non-Hodgkin lymphoma, Tumor-infiltrating lymphocytes, Deep learning, Single-cell RNA sequencing, Immunotherapy

PI-03

A Case of False Four DRB1 Alleles From a Patient with Liver Cirrhosis

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This study presents a unique case of HLA-DRB1 typing discordance in a patient with cirrhosis awaiting liver transplantation. The initial specimen from the patient had previously failed HLA typing using sequence-specific primer-polymerase chain reaction at another laboratory, prompting re-evaluation with a new ethylenediaminetetraacetic acid (EDTA) blood sample in our facility. Subsequent HLA typing by Sanger sequencing revealed the unexpected presence of four alleles at the *DRB1* locus, including HLA-DRB1*07:01, -DRB1*13:02, -DRB1*15:01, and -DRB1*15:02, which were biologically implausible. Notably, analyses of other tested loci, such as HLA-A (HLA-A*03:01:01 and HLA-A*33:03:01) and HLA-B (HLA-B*35:03:01 and -B*58:01:01), demonstrated usual heterozygosity with two alleles. Subsequent investigations involved alternative HLA typing via Sanger sequencing of a buccal swab specimen and next-generation-sequencing-based methodology of the original EDTA blood sample to address this discrepancy. Similarly, the results for HLA-A and HLA-B loci remained unchanged. However, for the HLA-DRB1 locus, a notable discrepancy was observed with the disappearance of the HLA-DRB1*15:01 and -DRB1*15:02 alleles, leaving only the HLA-DRB1*07:01 and -DRB1*13:02 alleles, characterized by both alternative methods. These findings underscore the complexities involved in HLA typing methodologies and highlight the importance of thorough scrutiny of transplant assessment protocols.

Keywords: HLA, Transplantation, Discrepancy, Genotyping, NGS, SSP-PCR

PI-04

Case of HLA Genotyping Discrepancy: Insights from Database Variation in Analytical Programs

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With the proliferation of user-friendly programs, human leukocyte antigen (HLA) genotyping has become increasingly accessible. Although these tools eliminate the need to manually consider all possible allele combinations, they introduce the risk of overlooking underlying processes. Herein, we describe a case illustrating the potential pitfalls of relying solely on such programs. An 11-year-old girl awaiting kidney transplantation underwent HLA genotyping using EDTA blood specimens. The analysis was initially conducted manually using HLA typing v. 1.9.7.6 (BioSewoom), which revealed the DRB1 genotype as HLA-DRB1*08:03 and -DRB1*12:02 based on common Korean allele frequencies. However, discrepancies arose when these results were compared with those of her parents, who had previously been genotyped using next-generation sequencing (NGS) at another laboratory. The parents harbored the alleles HLA-DRB1*08:01 and -DRB1*12:01. Subsequent analysis using NGSgo-AmpX v2 and NGSengine® (GENDX, Utrecht, Netherlands) revealed the patient's alleles to be HLA-DRB1*08:01 and -DRB1*12:01. Although both methods yielded consistent results across other HLA loci (A, B, C, and DQB1), discrepancies were observed specifically at the DRB1 locus. Notably, the alleles differed only at position 69 of the coding sequence, with variations involving nucleotides A and T. This discrepancy highlights the influence of allele frequency databases used in these analyses. NGSengine®, for instance, relies on a database lacking ethnic-specific allele frequencies, potentially leading to misinterpretation of results. It is important to examine HLA genotyping outcomes, particularly in diverse populations in which allele frequencies may vary widely. Although user-friendly HLA genotyping programs enhance accessibility, they require caution because of the potential for misinterpretation of the results, as illustrated in this case. A comprehensive understanding of the underlying processes, coupled with a critical evaluation of the results, is essential for accurate HLA typing, particularly in ethnically diverse populations.

Keywords: Human leukocyte antigen, Genotyping, Discrepancy, Database, Allele frequency, Transplant

PI-05

Donor-Derived Cell-Free DNA Predicts Allograft Rejection and Severe Microvascular Inflammation in Kidney Transplant Recipients

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Background: This study aimed to investigate the clinical validity of donor-derived cell-free DNA (dd-cfDNA) in comparison with that of donor-specific anti-HLA antibodies (DSA) for predicting biopsy-proven rejection (BPR) and severe microvascular inflammation (severe MVI) in kidney transplant recipients (KTRs).

Methods: This prospective observational investigation included 64 KTRs who underwent the indicated biopsies. Blood samples collected prior to biopsy were tested for dd-cfDNA and DSA levels. The biopsy specimens were classified by a renal pathologist according to the Banff classification system. The predictive performances of dd-cfDNA and DSA for histological allograft diagnosis were assessed.

Results: The KTRs were categorized into high and low dd-cfDNA groups based on a level of 0.4%. Eighteen patients (28.1%) had positive DSA results on biopsy and exhibited higher dd-cfDNA levels than DSA-negative patients. The incidences of BPR and severe MVI were elevated in the high-dd-cfDNA group (BPR: 42.9% vs. 3.4%, $P < 0.001$; severe MVI: 37.1% vs. 3.4%, $P = 0.001$). Additionally, elevated glomerulitis and MVI scores were observed in the high dd-cfDNA group. DSA had the highest predictive value for BPR (area under the curve [AUC]=0.880), whereas dd-cfDNA alone predicted severe MVI (AUC=0.855). The combination of DSA and dd-cfDNA ($>0.4\%$) yielded sensitivities of 80.0% and 50.0%, with specificities of 90.7% and 88.0% for antibody-mediated rejection and severe MVI detection, respectively.

Conclusions: The dd-cfDNA test is a predictive tool for BPR and severe MVI, and can improve performance, particularly when combined with DSA for BPR.

Keywords: Kidney transplantation, Cell-free DNA, Allograft rejection, Microvascular rejection

PI-06

Impacts of Regulatory T Cells and B Cells on Donor-Specific Antibodies in Kidney Transplantation

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Background: Regulatory T (Tregs) and B cells (Bregs) play crucial roles in immune tolerance. However, the relationship between these regulatory cells and donor-specific antibody (DSA) levels in kidney transplant recipients is not fully understood. This study investigated the association between Tregs, Bregs, and HLA antibody levels in kidney transplant recipients.

Methods: Peripheral blood samples were collected from 22 kidney

transplant recipients. Treg populations were quantified using flow cytometry and characterized based on the marker profile, CD4+CD25+CD127-CD45RA+. Bregs were identified using the marker profile, CD19+CD24+CD27+CD38+. The levels of DSA, including other anti-HLA antibodies, were measured using a Luminex-based solid-phase immunoassay. Statistical analysis was conducted to assess the Spearman's correlation between Treg and Breg levels and DSA or anti-HLA antibody levels.

Results: This study revealed a significant inverse correlation between Tregs and anti-HLA antibody levels in kidney transplant recipients ($\rho = -0.576$, $P = 0.001$). Similarly, a significant inverse correlation was observed between Bregs and DSA levels ($\rho = -0.661$, $P = 0.002$). Recipients with DSA had lower median Breg levels than those without DSA (2.55% vs. 0.0%, $P = 0.004$). Recipients with anti-HLA antibody showed lower Treg levels than recipients without anti-HLA antibody (median 63.0% vs. 36.6%, $P = 0.462$).

Conclusions: This study demonstrated significant inverse relationships between Treg and HLA levels and Breg and DSA levels in kidney transplant recipients, highlighting the regulatory roles of these cells in modulating transplant tolerance. These findings suggest that enhancing Treg and Breg function could be a therapeutic strategy to reduce anti-HLA-antibody- or DSA-mediated complications in kidney transplant recipients. Further research is required to understand the underlying mechanisms and to develop targeted therapies that can modulate Treg and Breg activities for clinical benefits.

Keywords: Kidney transplantation, Regulatory T cell, Regulatory B cell, Donor-specific antibody, Anti-HLA antibody

PI-07

Atypical Neuroblastoma: A Rare Case Report and Review of Literature

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Neuroblastoma is a malignant tumor that commonly occurs in children. It is the fourth most common childhood cancer and the most common solid tumor in infancy. Approximately 50% of neuroblastomas originate from the adrenal glands. Initially, symptoms often arise due to local compression by the tumor, with an abdominal mass being the most common presentation. In most cases, the tumor has metastasized before symptoms occur in children. Our patient was a 6-year-old girl. She initially presented with pain in the right thigh, leading to an unsteady gait and intermittent lower back discomfort. A preliminary hematological examination revealed anemia, with no specific findings on the peripheral blood smear. Abdominal ultrasound showed a heterogeneous echogenicity mass measuring 9.8×4.7 cm in the right adrenal gland area. MRI resonance imaging indi-

cated metastatic cancer involvement in the T3–T4 thoracic vertebrae. The patient's vanillylmandelic acid (VMA) level was 4.51 mg/day with no significant abnormalities. However, both bone marrow biopsy and postoperative pathology results confirmed neuroblastoma, with microscopic examination revealing a tumor rosette-like pattern. The patient underwent spinal surgery on the eighth day after diagnosis, followed by high-dose chemotherapy, radiation therapy, stem cell transplantation, and immunotherapy with the latest GD2 antibody (dinutuximab). She is currently undergoing treatment at our hospital. Neuroblastoma prognosis varies based on many factors, such as patient age at onset, tumor stage, genomic alterations, and histological subtype. In general diagnosis, as well as basic blood cell counts and imaging studies, identifying cancer cells in the bone marrow or tumor histopathology slides is essential. Tumors secrete catecholamines, which are metabolized in the body to form VMA, which is excreted in the urine, with approximately 90% of patients showing elevated VMA levels. Furthermore, research indicates that *MYCN* amplification predicts poor outcomes in neuroblastoma metastasis to the bone marrow. With technological advancements, liquid biopsy can replace traditional tumor biopsy, and the presence of *MYCN* gene abnormalities holds valuable prognostic significance for patients with neuroblastoma. Importantly, early diagnosis can significantly improve treatment and prognosis. In recent years, with the advancement of genetic medicine, molecular biology, and immunological research on neuroblastomas have gradually entered the clinical application stage. It is believed that, in the near future, patients with neuroblastoma will receive beneficial and improved treatment options.

Keywords: Vanillylmandelic Acid, Neuroblastoma, *MYCN*

PI-08

Prevalence and Determinants of Macroprolactinemia in Northern India

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Background: In 2021, in a cohort of 1,221 patients with hyperprolactinemia, we have previously documented the prevalence of macroprolactinemia to be 13.7%. This study is an extension of that original pilot study. This study aimed to find the prevalence and determinants of macroprolactinemia in the largest ever cohort of patients ever screened in India.

Methods: Consecutive patients detected to have serum prolactin >25 ng/mL as per the therapeutic upper reference limit were further screened for macroprolactin using the post-polyethylene-glycol (PEG)-precipitation test. Macroprolactinemia was defined as post-PEG recovery of prolactin <40% (true prolactin <40% of total prolactin).

Results: of the 3,615 patients (male:female=2,380:1,235) screened for macroprolactinemia in this study, 397 patients (10.98%) were di-

agnosed to have macroprolactinemia. Three-hundred and sixty-six of these 397 patients were females (92.12%). Among these 397 patients detected to have macroprolactinemia, only 46 patients (11.58%) had serum prolactin levels > 30 ng/mL post-PEG precipitation. Nineteen of these 46 patients (41.3%) had macroprolactinoma. Twelve of these 46 patients (26.1%) had uncontrolled primary hypothyroidism.

Conclusions: Macroprolactinemia is a major problem in India affecting nearly one out of 10 patients diagnosed with hyperprolactinemia and is seen predominantly in females. Most of these patients will end up having normal serum prolactin levels post-PEG precipitation test. Macroprolactinemia with raised prolactin levels even after PEG precipitation is seen in people with prolactin secreting pituitary tumors and uncontrolled primary hypothyroidism. Macroprolactin screening should be routinely done to avoid overtreatment of incidentally detected hyperprolactinemia.

Keywords: Macroprolactin, Polyethylene glycol, Macroprolactinemia, Hyperprolactinemia

PI-09

Soluble CD40 Ligand as a QC Marker of Pre-/Post-Centrifugation Delay

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Background: Biobanks that provide bioresources to researchers must guarantee that samples can be transferred without institute-dependent intrinsic bias. Thus, pre-analytic conditions must be standardized or samples validated to preserve the reproducibility and comparability of the research results. Therefore, biobanks must control for such pre-analytic variations and must be able to perform appropriate quality control tests on their samples.

Methods: After collecting blood from eight volunteers who provided informed consent, the blood was divided into two groups (plasma and serum). One aliquot (baseline) from each sample group was centrifuged within 2 hours after whole blood collection. The remaining aliquots were left at room temperature and 4°C for 6 hours, 12 hours, and 24 hours, then centrifuged and stored. In another approach, the samples were centrifuged first, then left at room temperature and 4°C 6 hours, 12 hours, and 24 hours, before measuring sCD40L using Human sCD40L ELISA kits. Separated plasma and serum samples were stored frozen (at -80°C) until analysis. All samples were measured in duplicate. All values are presented as fold change from baseline or as median (interquartile range). Tests for changes from baseline were performed using repeated-measures analysis of variance, 2-tailed t-test, or Wilcoxon signed-rank test. Statistical significance was set at $P < 0.05$. We used SAS® 9.4 version for analyses.

Results: The median (interquartile range) sCD40L concentration at baseline was higher in the plasma samples (181.18 [61.27-223.06]

pg/mL) than in the serum samples (110.29 [60.63-243.93] pg/mL; $P < 0.001$). In the plasma group, sCD40L levels significantly increased with time before and after centrifugation at room temperature. In the case of the serum group, sCD40L significantly increased compared to baseline over time when left at room temperature as well as at 4°C ($P < 0.05$). These results suggest that sCD40L values can be used as an indirect indicator, showing that separation of whole blood was delayed and left unattended after centrifugation. Because of the different detection and reference ranges presented by each manufacturer, each institution must set its own reference values. Therefore, the Korea Centers for Disease Control and Prevention (KDCA) suggested that the serum 7–17 ng/mL reference value reported by Lengelle et al. using R&D system kits, decreases when exposed to room temperature for a long time before and after centrifugation.

Conclusions: Soluble CD40L values can be used as quality control markers for the pre-analytic conditions of human resources transported and collected from other institutions in the biobanking field.

Keywords: sCD40L, Pre-/post-centrifugation delay, Pre-analytical conditions, Quality control

PI-10

Calcium and Vitamin D Levels in the Peripheral Blood of Patients with Hemophilia Residing in Ulaanbaatar

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Background: Hemophilia is a blood disorder characterized by impaired blood coagulation due to intrinsic coagulation factor deficiency. This results in acute bleeding within the musculoskeletal system and leads to both functional and structural joint disorders. Factor VIII deficiency occurs in 85% of hemophilia cases, while factor IX deficiency accounts for the remaining 15%. Both forms are more prevalent in males due to their inheritance pattern, which follows X-linked recessive inheritance. Hemarthrosis, the hallmark manifestation of this condition, is observed in a substantial proportion, ranging from 75% to 90% of individuals affected. The knee, ankle, and elbow joints emerge as primary sites of affliction, underscoring their heightened susceptibility to the disease pathology. Consequently, the recurrence of this condition during the developmental years leads to juxta-articular osteoporotic epiphyseal bone growth. This study aimed to investigate the vitamin D and calcium levels in the blood of individuals with hemophilia residing in Ulaanbaatar.

Methods: This study employed a retrospective case-control design. A total of 27 male patients aged 0–40 with hemophilia residing in Ulaanbaatar were included in the study. The median age was 17 years. The blood test results obtained from the Biomerieux Mini Vidas and Roche Cobas e311 automatic analyzer in the clinical laboratory of Munguun Hospital were retrospectively reviewed. These results, spanning from 2018 to 2020, were compared with those from a control group without hemophilia to evaluate the differences in blood calcium and

vitamin D levels. The healthy controls were selected according to male sex and age >40 years. Out of the 1,126 responses from the tested samples, some were excluded due to missing data, resulting in a total of 1085 responses being processed and compared.

Results: Among the participants with hemophilia, only four (7%) had normal serum vitamin D levels. Peripheral blood calcium levels were within 50% of the normal range. No significant correlation was found between calcium and vitamin D levels in the hemophilic group ($P = 0.111$). Vitamin D levels were low in both groups, with the result <8.1 ng/mL being significantly more prevalent in the hemophilic group. Furthermore, blood calcium levels were lower in the hemophilic group compared to the control group.

Conclusions: Peripheral blood calcium and vitamin D levels were decreased in the hemophilic group. The significantly low levels of both analytes are compared to the control group, indicating a need for further investigation, particularly in relation to osteoporosis.

Keywords: Hemophilia, Vitamin D, Calcium, Hemarthrosis, Osteoporosis

PI-11

Evaluation of the Accuracy of Free Testosterone Assay Using Snibe MAGLUMI X3 Chemiluminescent Immunoassay Analyzer

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Background: Free testosterone accounts for approximately 2% of the total testosterone, and its accurate measurement is crucial for diagnosing various diseases, such as androgen deficiency in men and androgen excess in women. The Snibe MAGLUMI® X3 (Snibe Co., Ltd., China) is a new fully automated chemiluminescent immunoassay (CLIA) analyzer with a throughput of 200 tests per hour. In this study, we aimed to evaluate the accuracy of free testosterone assay using the MAGLUMI® X3.

Methods: Twenty serum samples were obtained from Gachon University Gil Medical Center, Korea, in April 2024. Precision, linearity, and carryover were evaluated using commercial materials and serum samples, according to the Clinical & Laboratory Standards Institute guidelines. We compared the results of free testosterone assay between the MAGLUMI® X3 and its earlier model, the Snibe MAGLUMI® 2000. We compared these results with those obtained using radioimmunoassay (RIA) method. A Packard Cobra II 5010 gamma counter (Packard Bioscience, USA) was used for performing the RIA.

Results: The percentage coefficients of variation (%CV) for repeatability were 5.7 for low level and 2.1 for high levels. Linearity (slope: 1.0145, intercept: 0.0103) and carryover (0.11%) indicated good performance. In the method comparison, MAGLUMI® X3 and MAGLUMI® 2000 showed strong correlation ($r^2 = 0.991$). The correlation between CLIA using MAGLUMI® X3 and RIA using Cobra II 5010 was 0.8129.

Conclusions: The MAGLUMI® X3 exhibited good analytical performance and comparability with the MAGLUMI® 2000. It can be used as a fast and compact CLIA analyzer in small- to medium-sized laboratories.

Keywords: Chemiluminescent immunoassay, Free testosterone, MAGLUMI® X3

PI-12

Limited Contribution of Creatine Kinase-Myocardial Band Alongside High-Sensitivity Cardiac Troponin in Diagnosing Acute Myocardial Infarction in an Emergency Department

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Background: Cardiac biomarkers, especially high-sensitivity cardiac troponins C and I (hs-cTnC and hs-cTnI, respectively), are vital for diagnosing acute myocardial infarction (AMI). Despite the specificity of hs-cTn as a biomarker, the creatine kinase-myocardial band (CK-MB) is commonly used alongside hs-cTn in emergency departments (EDs). **Methods:** In 2022, we analyzed 23,771 simultaneous hs-cTnT (hs-cTnT or hs-cTnI) and CK-MB requests from 17,185 patients in the ED of a tertiary hospital. This study aimed to assess their practical value in the diagnosis of AMI in a real-world setting.

Results: Among the 17,185 patients tested, 98.0% underwent hs-cTnT and CK-MB tests, while substantially fewer underwent hs-cTnI testing. We observed a concordance between the initial hs-cTn and CK-MB results in 71.3% of patients. Of the 131 patients with AMI, 57 were positive for both biomarkers, 63 were positive for hs-cTn alone, and none were positive for CK-MB alone. Notably, biomarker positivity was frequently observed even in the absence of AMI. The most common causes of CK-MB positivity were neuromuscular disease (N=25), infection/inflammation (N=18), cardiovascular disease (N=17), trauma (N=14), malignancy (N=11), nonspecific chest pain (N=10), psychiatric conditions (N=9), and other factors (N=53). Discrepancies between the hs-cTnT and hs-cTnI results were noted in 30.0% of the patients.

Conclusions: Indiscriminate CK-MB testing for the diagnosis of AMI in patients in EDs should be reconsidered. The efficient use of CK-MB is important to reduce costs and ensure optimal patient care.

Keywords: Creatine kinase-myocardial band, Acute myocardial infarction, High-sensitivity cardiac troponin

PI-13

Performance Evaluation of the Abbott Alinity i Testosterone Chemiluminescent Microparticle Immunoassay and Comparison with Radioimmunoassay

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Background: Testosterone quantification is essential for the diagnosis of various hormonal disorders such as male hypogonadism and premature or delayed puberty. Automated immunoassays have been widely used to measure testosterone; however, some have shown low accuracy at low concentrations and require validation before clinical application. Here, we evaluated the performance of the testosterone chemiluminescent microparticle immunoassay using the Alinity i analyzer (Abbott Laboratories, IL, USA) and compared the results with those of radioimmunoassay (RIA).

Methods: Precision was evaluated in accordance with the Clinical Laboratory Standards Institute (CLSI) EP15-A3 guideline: three levels of Abbott control material and Multichem® IA Plus (Technopath Clinical Diagnostics, Ballina, Ireland) plus were run in 5 replicates in every run, over five days. Linearity was verified following CLSI EP6-ED2 guidelines using the VALIDATE® Linearity and Calibration Verification kit. Carry-over was evaluated according to the CLSI EP10-A3 guidelines. The reference range was verified according to CLSI EP28-A3c using 20 samples from healthy individuals in four groups stratified by sex and age (21–49 and ≥50). The method was compared with RIA (Diasource TESTO-RIA-CT, Belgium) according to CLSI EP9-A3 using 40 clinical samples, including 15 samples outside the reference range.

Results: The repeatability and within-laboratory imprecision %CV were <4.7% and <4.8%, respectively, which were lower than the desirable specifications for an imprecision of 7.3% provided by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Database. The analytical measuring interval (AMI) was between 0.04 ng/mL and 13.59 ng/mL. No carry-over is observed. Reference range in male (21–49 years), male (≥50 years), female (21–49 years), and female (≥50 years) was verified as 2.40–8.71 ng/mL, 2.21–7.16 ng/mL, 0.14–0.53 ng/mL, and 0.12–0.36 ng/mL, respectively. Regression analysis between the Alinity i system and RIA showed a significant difference with a slope, intercept, and Pearson's correlation coefficient r of 1.182 (95% CI, 1.083–1.336), -0.121 (95% CI, -0.165 to -0.036), and 0.9620, respectively. Bland-Altman analysis revealed a proportional difference: underestimation of Alinity i (-40.3%) in the mean concentration below 0.5 ng/mL and overestimation (+22.2%) above 0.5 ng/mL.

Conclusions: The Alinity i testosterone assay system demonstrated excellent analytical performance in terms of precision, linearity, and carryover, validating its suitability for clinical use. Given the significant difference observed between the Alinity i testosterone assay and RIA, these discrepancies should be considered when interpreting the results, and follow-up testing should be performed using the same analytical method.

Keywords: Testosterone, Chemiluminescent microparticle immunoassay, Performance evaluation, Abbott Alinity i, Method comparison

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PI-14

Results of Calcium and Vitamin D Deficiency in Children's Blood Serum

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Background: Vitamins and minerals are essential components of human health. Vitamin D, mainly involved in calcium absorption, supports teeth and bone strengthening. This study aimed to explore the calcium and vitamin D levels in the blood serum of children.

Methods: The study materials in this cross-sectional study included 1,133 blood test results of patients aged 0–18 who attended the Gurvan Gal Hospital, Bayangol District, Ulaanbaatar City, from the beginning of January to the end of December 2023. The age group of the study population was classified according to the World Health Organization recommendation: 0–5 years, 6–11 years, and 12–18 years old.

Results: of the 1,133 blood test results analyzed in the study, 57.3% (649) were from 0–5 years old, 31% (35) from 6–11 years old, and 11.7% (133) from 12–18 years old. There were 48.2% (547) females and 51.7% (585) males. The average parameters of calcium in the blood serum of 0–5, 6–11, and 12–18-year-old participants were determined to be 2.41 ± 0.108 mmol/L, 2.37 ± 0.093 mmol/L, and 2.33 ± 0.10 mmol/L, respectively ($P < 0.01$). No difference between sexes was significant ($P > 0.05$). The average level of vitamin D among the age groups mentioned above was 33.28 ± 14.389 ng/L, 21.10 ± 9.859 ng/L, and 15.97 ± 7.53 ng/L, respectively ($P < 0.01$), and a significant difference concerning sex was observed ($P > 0.05$).

Conclusions: Almost all populations (99.9%) presented with normal calcium levels in the blood serum, and 61% of them had lower levels of vitamin D. The average values of calcium and vitamin D in children's blood serum decline with increasing age.

Keywords: Serum, Mineral, Vitamin D, Deficiency

PI-15

Diagnostic and Prognostic Utility of Pancreatic Stone Protein in Patients with Suspected Sepsis

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Background: Pancreatic stone protein (PSP) is an emerging biomarker of sepsis. During sepsis, the pancreas senses remote organ damage and secretes PSP. This study aimed to investigate the diagnostic and prognostic utility of PSP in patients with suspected sepsis.

Methods: PSP was compared with procalcitonin (PCT) in 285 patients (suspected sepsis, N=148; sepsis, N=137) using the sequential organ failure assessment (SOFA) tool. Sepsis diagnosis was assessed using receiver operating characteristic curve analysis with area under the curve (AUC). The clinical outcomes (in-hospital mortality, 30-day mortality, and kidney replacement therapy [KRT]) were evaluated using the Kaplan-Meier method with hazard ratios (HRs).

Results: PCT and PSP showed comparable effectiveness in diagnosing sepsis (AUC=0.71–0.72, $P < 0.001$). The proportion of patients with sepsis who exhibited increased expression of both biomarkers was significantly higher than that of patients with increased expression of only one biomarker (89.0% vs. 21.3%–47.7%, $P < 0.001$). Each biomarker quartile (Q1–Q4) differed significantly according to the SOFA score (all $P < 0.001$). Compared with the Q1 group, the Q2–Q4 groups showed worse clinical outcomes ($P = 0.002$ – 0.041). Meanwhile, the HR was higher when both biomarkers were added to the SOFA score compared with the SOFA score alone (3.3–9.6 vs. 2.8–4.2, $P < 0.001$ – 0.011). PSP demonstrated a nearly 2.5-fold higher HR (9.6 vs. 4.2) for predicting KRT.

Conclusions: PSP demonstrated diagnostic and prognostic utility in patients with suspected sepsis, particularly in predicting kidney dysfunction. PSP alone or in combination with PCT is a valuable tool for clinical assessment.

Keywords: Sepsis, SOFA score, Procalcitonin, Pancreatic stone protein, Diagnosis, Prognosis

PI-16

Elevated Serum Krebs von den Lungen-6 Concentrations in Retired Workers with Coal Worker's Pneumoconiosis

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Background: Coal worker's pneumoconiosis (CWP) is characterized by pulmonary fibrosis resulting from exposure to mineral dust such as coal dust and crystalline silica. The clinical diagnosis of CWP primarily relies on occupational exposure history and radiological findings. As CWP is progressive and incurable, early diagnosis or prevention is crucial, making serum biomarkers essential. Among these, Krebs von den Lungen (KL)-6, a mucin-like glycoprotein, is a potential biomarker for diagnosing and monitoring the progression of various fibrotic lung diseases. In this study, we measured the serum

KL-6 concentrations in retired coal miners exposed to mineral dust to elucidate its potential as a biomarker of CWP.

Methods: The study included 300 retired workers (150 without CWP and 150 with CWP). Pulmonary function tests and chest radiographs were conducted and the results were rated according to the classification rules of the International Labor Office guidelines. Serum KL-6 concentrations were measured using the latex immunoturbidimetric immunoassay method (GeneX KL-6, GeneX Inc., Korea).

Results: The mean serum KL-6 concentrations were significantly higher in patients with CWP than in retired workers without CWP. Additionally, the mean serum KL-6 concentration increased significantly with CWP severity. The cutoff CWP value of 255.7 U/mL yielded 80.0% sensitivity and 47.3% specificity for detecting this condition.

Conclusions: Although the sensitivity of serum KL-6 was high and its specificity was low in this study, it shows promise as a useful biomarker for diagnosing CWP. Further studies are necessary to assess other FLD biomarkers of fibrotic lung diseases, such as surfactant protein A, to improve diagnostic accuracy.

Keywords: KL-6, Pneumoconiosis, Biomarker

and adjusted HR (14.7 vs. 4.6). Furthermore, multimarker approach in patients aged ≥ 75 yrs showed higher HR than that in all ages (9.2 vs. 4.2), and biomarkers added-on top of SOFA score showed better performance (up to 82%) than SOFA score alone.

Conclusions: Biomarkers showed clinical utility in patients with suspected sepsis. The multimarker approach showed better clinical utility than did the SOFA score and showed better prognostic performance in patients aged 75 yrs or more than in that all ages. Biomarkers, alone or in combination, would be a valuable tool added on top of SOFA score.

Keywords: Sepsis, SOFA score, Procalcitonin, Presepsin, Interferon, Adrenomedullin

PT-01

ABO Discrepancy Caused by Massive Blood Transfusion

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When a patient urgently requires a blood transfusion, ascertaining safety is the primary concern, thereby increasing the complexity of subsequent blood type determination. This is case of a 33-year-old female who was involved in a motorcycle accident on November 28, 2023. She had received 6 units of remove leukocyte packed RBCs (RLPR) blood transfusion at another hospital prior to being transferred to China Medical University Hospital (CMUH) emergency department. Upon arrival, her vital signs were unstable, and emergency blood transfusion was performed. According to the hospital protocol, the initial blood typing was conducted using an automated system, which indicated an ABO discrepancy. Subsequently, manual confirmation was conducted, and the results corroborate with machine interpretation. The automated blood typing system analysis yielded the following results: A1 cell: O; B cell: O; auto control: O; anti-A: O; anti-B: O; and anti-D: 4+. Manual confirmation using the tube method, immediate centrifugation, and MP method all produced consistent results: A1 cell: O; B cell: O; anti-A: O; anti-B: O; and anti-D: 4+. Thus, it was discussed with the physician to ensure a safe blood transfusion first and to monitor the changes in blood type and perform antibody screening. One week later, the antibody screening was positive, indicating the presence of anti-Fyb, which disappeared after 2 weeks. Following the blood type monitoring until the 5th week, the results changed to A1 cell: O; B cell: O; anti-A: 1+mf; anti-B: 1+mf; and anti-D: 4+, suggesting that the patient's blood type is AB+. In the 6th week, the results were as follows: A1 cell: O; B cell: O; anti-A: 4+mf; anti-B: 4+mf; and anti-D: 4+, confirming the patient's blood type as AB+. Hence, a blood type report was issued at this time. During the patient's blood transfusion process, anti-Fyb was produced, which disappeared after 2 weeks. However, since it could not be deter-

PI-17

Multimarker Approach in Sepsis: A Clinical Role Beyond SOFA Score

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Background: We evaluated the clinical utility (diagnosis, risk-stratification, and prognosis) of the multimarker approach using four biomarkers (procalcitonin [PCT], presepsin [PSEP], Interferon- $\lambda 3$ [IFN- $\lambda 3$], and bioactive adrenomedullin [bio-ADM]) in patients with suspected sepsis.

Methods: In a total of 248 patients (suspected sepsis, N=132; sepsis, N=116), biomarkers were evaluated with sequential organ failure assessment (SOFA) score. Receiver operating characteristic curve analysis with area under the curve (AUC) was used for predicting sepsis diagnosis and adverse clinical outcomes (intensive care unit admission [ICU], in-hospital mortality, and kidney replacement therapy [KRT]). Survival analysis and reclassification analysis were used for predicting in-hospital mortality.

Results: During diagnosis, biomarkers showed a comparable AUC with one another (0.61–0.95, $P<0.001$ –0.003), and sepsis proportion was significantly higher as the biomarker number, used in multimarker approaches, was increased (7.7%–91.7%, $P<0.001$). In risk-stratification, proportion of biomarker quartiles (Q1–Q4) differed significantly according to SOFA score (all $P<0.001$). In prognosis, biomarkers showed significant AUC for predicting in-hospital mortality and KRT (0.63–0.87, $P<0.001$ –0.015) except ICU admission. In in-hospital mortality, the multimarker approach showed better performance than did the SOFA score: mortality rate (58.3% vs. 30.0%)

mined whether this antibody corresponded to the patient herself or the transfused blood cells, providing Fyb antigen-negative RLPR to ascertain safety is recommended. During the patient's hospitalization, she received a large amount of blood units. Observing the changes in the patient's hemoglobin (Hb) levels, the rise and maintenance rates of Hb were lower when O+ RLPR was administered compared to AB+ RLPR. This suggests that long-term plasma transfusion from O+ RLPR may have some impact on the patient. Therefore, using washed RBC instead of RLPR could be a more optimal option.

Keywords: ABO discrepancy, Forward typing, Reverse typing

PT-02

Development of Dual Multiplex Real-time PCR Assays for Simultaneous RHD Genotype Screening and Asian-type DEL Detection

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Background: Among the many genetic variants underlying the DEL (D-eluete) phenotype, RHD*DEL1 (RHD*01EL.01) is predominant in East and Southeast Asian populations and is therefore termed Asian-type DEL. In Korea, Asian-type DEL accounts for approximately 15% of individuals with the RhD (-) phenotype, making its identification crucial for managing alloimmunization risks. In the conventional RHD genotype testing algorithm for detecting DEL variants, PCR-SSP (sequence-specific priming) is used for RHD genotype screening, followed by exon 9 sequencing for Asian-type DEL detection. Here, we developed dual multiplex real-time PCR assays for the simultaneous screening of RHD genotypes and detection of Asian-type DEL.

Methods: A total of 306 whole-blood samples (with known serological results: RhD [+], RhD [-], weak D), preserved at Seegene Medical Foundation from October 2023 to March 2024, were used. In the dual multiplex-qPCR assay, primers were designed based on dual-priming oligonucleotide technology for specificity. The dual multiplex-qPCR mixture consisted of two groups: the genotype screening mixture for the promoter, exon 4, exon 7, and exon 10 of RHD gene, and the Asian-type DEL mixture for 1227G and 1227A of exon 9. All 306 samples were compared with reference methods (PCR-SSP for RHD genotype screening and exon 9 direct sequencing for Asian-type DEL detection).

Results: The samples consisted of 180 RhD [+], 117 RhD [-], and nine weak D. They were analyzed using our dual multiplex-qPCR assays, which included 93 RHD complete deletion, nine hybrid samples, and 15 Asian-type DEL samples. The dual multiplex-qPCR results for all 306 samples were 100% consistent with the standard as-

say. We also demonstrated that the target variant could be successfully detected using various concentrations (5–500 ng/reaction) of genomic DNA without non-specific detection. The assays showed high specificity for all genotype cases (RHD complete deletion, hybrid, Asian-type DEL) and reliable reproducibility across different runs.

Conclusions: In this study, we developed dual multiplex qPCR assays for simultaneous RHD genotype screening and Asian-type DEL detection, achieving 100% accuracy. The assays demonstrated high specificity, reliability, and rapid genotype discrimination, indicating their potential for widespread use in hospitals and blood centers.

Keywords: RhD negative, Asian-type DEL, RHD genotype screening, Dual multiplex real-time PCR

PT-03

Optimal Specimen for Antibody Testing: A Comparative Study of Serum and Plasma

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Background: The introduction of automated systems in transfusion-related testing has led to the increased use of plasma as a uniform specimen. At our institution, automated equipment is used for blood type testing, antibody screening, and identification tests, all of which have been conducted using plasma samples. However, a case was observed where the antibody screening test, which was negative in plasma, showed a positive result in the indirect antiglobulin test conducted with serum. Consequently, from January 2023, antibody screening tests have been switched to serum samples, necessitating a comprehensive comparative evaluation of serum and plasma samples.

Methods: From January to December 2023, new patient cases with positive antibody screening results from tests conducted with serum samples submitted to the blood bank were selected. These cases were re-tested with plasma samples to compare the rates of positivity in serum-only versus both serum and plasma. Additionally, antibody identification results, types of identified antibodies, and the agglutination strengths in the respective specimens were compared. All tests were conducted using reagents and automated equipment from QuidelOrtho.

Results: Among 353 new antibody screening test cases, 255 cases showed positivity only in serum, while 96 cases were positive in both serum and plasma. Of the 255 serum-only positive cases, 33 cases (12.9%) were identified with antibodies: Lewis group (18), Rh group (8), M (2), P1 (2), S (2), Jk group (1), with autoantibodies in 47 cases

(18.4%), unknown alloantibodies in 54 cases (21.2%), and 121 cases (47.5%) were negative. In contrast, of the 96 cases that were positive for both serum and plasma, 77 cases (80.2%) were identified with antibodies: Rh group (47), Lewis group (14), M (8), Jk group (5), Fy group (3), with autoantibodies in 8 cases (8.3%), and unknown alloantibodies in 11 cases (11.5%). The agglutination strength comparison showed that among the 239 serum samples with 1+ or lower, 200 plasma samples were negative (83.7%), 28 were trace (11.7%), 8 were 1+ (3.3%), and 3 were 2+ (1.3%). Among the 114 serum samples with 2+ or higher, the plasma samples showed 48 negative (42.1%), 8 trace (7.0%), 9 were 1+ (7.9%), 18 were 2+ (15.8%), 27 were 3+ (23.7%), and 4 were 4+ (3.5%).

Conclusions: The results indicate a significantly higher rate of serum-only positive cases in antibody screening tests. Clinically important antibodies were identified and the overall agglutination strength was stronger in serum. Therefore, the use of serum samples for antibody screening tests should be considered.

Keywords: Serum, Plasma, Antibody screening, Antibody identification, Blood group, Agglutination

PT-04

The Case Report Resolving the Interference in Pretransfusion Testing Using Column Agglutination in Patients Receiving Daratumumab Using a Modified Dithiothreitol Protocol

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Daratumumab, the monoclonal anti-CD38 antibody, targets the CD38 antigen highly expressed on multiple myeloma cells and can also bind to red blood cells (RBCs), which causes panreactivity in pretransfusion tests. The protocol using dithiothreitol (DTT)-treated RBCs is known to eliminate the interference. We report two cases showing panreactivity in pretransfusion tests even after using standard DTT protocol, with these false-positive reactions resolved by modified DTT protocol. Serum samples from patients 1 and 2 with multiple myeloma receiving daratumumab were sent to the blood bank at Chonnam National University Hwasun Hospital for unexpected antibody screening tests and crossmatching. According to the standard DTT protocol provided by the Association for the Advancement of Blood & Biotherapies, RBCs in ID-DiaCell I-II reagent (Bio-Rad, Switzerland) and in segments of packed red blood cells (PRC) were treated with a 2.0 M DTT solution, incubated for 30 minutes, and washed four times with phosphate-buffered saline (PBS). When reacted in the gel agglutination assay using the ID-card LISS/Coombs card (Bio-Rad), samples

from the two patients showed panagglutination with 2+ to 3+ grade in the antibody screening test and crossmatching. Before daratumumab administration, antibody screening tests were negative in both patients. The two patients had no notable medication history or laboratory results compared to other patients with multiple myeloma receiving daratumumab. To solve the problem, we applied the modified DTT protocol proposed by Izaguirre et al. (2020). In the modified approach, lower concentrations (0.02, 0.04, and 0.08 M) of DTT were directly mixed with RBCs in a gel column with a shorter incubation time (15 minutes). The modified protocol did not also need washing steps with PBS. RhE antigen-positive RBCs were used as a positive control. When applying 0.02 M DTT, antibody screening test and crossmatching in serum samples of both patients showed the least reactions (trace to negative agglutination). With 0.04 and 0.08 M DTT, panagglutinations were reduced compared to standard protocol but remained as 1+ to 2+ agglutination in antibody screening tests and crossmatching. Accordingly, patient 1 received two units of least incompatible PRCs, while patient 2 received one unit of compatible PRCs. After the transfusions, no clinical sign of hemolysis was observed in the two patients. Therefore, when panagglutination is observed in the pretransfusion tests of patients receiving daratumumab even after applying standard DTT protocol, a modified DTT approach using 0.02 M DTT could be helpful to resolve some false-positive cases.

Keywords: Pretransfusion testing, Interference, Daratumumab, Dithiothreitol

PT-05

Detection of the Anti-Kpa Alloantibody from a Healthy Korean Individual

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The Kell blood group includes 35 protein antigens which are highly immunogenic, encapsulated by the phrase 'Kell Kills'. The global frequency of Kpa antigen (KEL3) is approximately 2% with even lower prevalence in Asian populations and a previously reported frequency of zero in the Korean population. Anti-Kpa alloantibodies have been documented in fewer than 20 cases worldwide. Instances of anti-Kpa occurring without prior sensitizing events, such as transfusion or pregnancy, are extremely rare. Most literature describes anti-Kpa resulting from maternal transfer of IgG or de-novo occurrences following multiple transfusions. In this case, a positive result from pretransfusion antibody screening test was observed from an otherwise-healthy 59-year-old Korean male admitted for preoperative evaluation for laceration of the finger. The 2-cell panel (DiaMed GmbH, Cressier FR, Freiburg, Switzerland) in LISS/Combs gel card showed 2+ reaction, while another 2-cell panel (Ortho-Clinical Diagnostics, Raritan, New Jersey) showed negative results. Further identification using both DiaPanel (DiaMed) and ResolvePanel (Ortho) confirmed the presence of anti-Kpa with 2+ reactions, respectively. His hemoglobin concentration was stable throughout admission, the patient did not

require transfusion and was discharged without any complications. Given the absence of past medical history indicative of alloantibody generation, the detected anti-Kpa is likely naturally occurring. Earlier literature has described an incidence of anti-Kpa due to recurrent bacterial infection, although no such events were identified in the current case. Regardless of the pathogenesis, clinical significance of anti-Kpa includes hemolytic transfusion reaction and hemolytic disease in fetuses and newborns. Despite the extremely low incidence of Kpa and alloantibody formation, laboratory best practices in transfusion medicine should aim to identify even the rarest of alloantibodies such as anti-Kpa to prevent adverse events.

Keywords: Anti-Kpa, Kell, Korean, Alloantibody, Identification

PT-06

Analysis of Genome Editing Tools CRISPR/Cas9 as Novel Potential Therapy for Alzheimer's Disease

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Background: Alzheimer's disease (AD) is a prevalent degenerative condition affecting primarily individuals over 65 years old, with its incidence expected to rise in Indonesia. The disease is characterized by the accumulation of A β fragments, leading to plaque formation and cognitive decline. Current treatments like cholinesterase inhibitors and memantine offer limited relief from disease progression. Thus, a pressing need exists for targeted therapies like CRISPR/Cas9, which can potentially address genetic mutations underlying AD at the genomic level.

Methods: This review on CRISPR/Cas9 examines data extracted from relevant scientific journals meeting specific inclusion criteria.

Results: CRISPR/Cas9 targets different genes depending on the AD type. Each application of CRISPR/Cas9 utilizes sgRNA tailored to repair specific mutations, paired with Cas9 endonuclease, and delivered via viral or non-viral vectors. Successful studies have demonstrated reduced accumulation of A β fragments.

Conclusions: CRISPR/Cas9 shows promise in Alzheimer's disease therapy due to its safety, effectiveness, and adaptability. This review highlights its potential to revolutionize treatment strategies for this debilitating condition.

Keywords: Alzheimer, CRISPR, Genome editing

PT-07

Enhancing the Post-Cryopreservation Recovery of CAR-NK Cells Engineered via BaEV-Pseudotyped Lentiviral Transduction

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Background: Chimeric antigen receptor (CAR)-natural killer (NK) cell therapy is an emerging cancer immunotherapy that combines the intrinsic anti-tumor capabilities of NK cells with the antigen-targeted cytotoxicity conferred by CARs. NK cells offer unique advantages such as reduced risk of graft-versus-host disease and cytokine release syndrome, facilitating the use of CAR-NK as an off-the-shelf cell therapy. However, the inherent resistance of NK cells to gene transfer poses a technical challenge in CAR-NK production using viral vectors. Additionally, the translation of CAR-NK into clinical practice necessitates an optimization of cryopreservation process, as NK cells typically exhibit poor post-cryopreservation recovery. Here, we investigated an efficient envelope pseudotyped lentiviral vectors (LV) and post-thaw recovery method, aiming to improve stable CAR-NK production and storage for clinical application.

Methods: NK92 cell line and human primary expanded NK (eNK) cells were used for the CAR-NK production process. An anti-CD19 CAR vector incorporating 4-1BB and CD3 ζ (named 19BBz) was constructed. LVs carrying 19BBz or 19BBz-IL15 CAR transfer vector were produced using VSV-G and BaEV envelope pseudotyping, either individually or in combination (Combo). The transduction efficiency was determined using Flow Cytometry by measuring the expression of CAR scFv. Post-cryopreservation recovery of NK cells was mediated by various concentrations of IL-2 cytokine one-time stimulation overnight (O/T), then the cells were washed and cultured under cytokine starvation. Cells with a constant supply of IL-2 was used as a control group.

Results: BaEV pseudotyped LV showed the most effective transduction of NK92 and eNK cells, maintaining high CAR expression over two weeks, unlike VSV-G and Combo LVs whose expressions declined significantly. The cell viability of NK cells was not affected by the BaEV LV transduction, while cell cytotoxicity against CD19+ target cells (Nalm-6) was significantly elevated. IL-15 secreting CAR-NK92 cells, transduced with BaEV LV, demonstrated improved long-term survival and cytotoxicity under cytokine starvation condition, supported by IL-15 signaling pathways. For cryopreservation, a minimum of 200 U/mL of IL-2 O/T stimulation was essential to enhance both NK92 and eNK cell viability after thawing. In long-term observation of 7 days, the beneficial effect of IL-2 O/T stimulation was more pronounced with IL-15 secreting CAR-NK92 cells, while higher dose of IL-2 stimulation more enhanced the recovery of eNK cells.

Conclusions: BaEV pseudotyped LV mediated transduction enables stable CAR expression of NK cells. In addition, post-thaw overnight recovery with IL-2 one-time stimulation significantly improves the cryo-recovery of NK cells, with the support of IL-15 secretion for long-term survival.

Keywords: CAR-NK, Lentiviral transduction, Viral envelope pseudo-typing, Cryopreservation recovery, IL-15

PT-08

Development of a Transfusion Reaction Reporting System to Improve Communication with Physicians

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Background: Transfusion reactions have consistently been under-reported, and laboratory tests for evaluating the causes are unfamiliar to physicians other than transfusion specialists. The paper-based transfusion reaction report system previously utilized in our hospital was one-way, with physicians submitting it to the department of transfusion management. To address this, we developed an electronic transfusion reaction reporting system that improves communication with physicians to identify the cause of transfusion reactions and recommend appropriate blood components.

Methods: To evaluate the status of transfusion reaction reporting, transfusion reaction reports and transfusion nursing records were analyzed and compared for transfused blood in 2022. The transfusion reaction reporting system was developed according to the electronic consultation system between physicians. It comprised of two parts, which included the physician's report and the response from the department of transfusion management. The vital signs and laboratory findings included in the physician's report are designed to be automatically linked and integrated with the results in the transfusion nursing records and the laboratory information system. The department of transfusion management provides names/codes of laboratory tests for evaluating transfusion reactions and recommends proper blood components for future transfusions. If a physician prescribes another transfusion for a patient with a history of prior transfusion reactions, a prompt pop-up alert appears with the details of the previous report.

Results: Among the 10,654 blood components requested in 2022, 233 transfusion reactions were documented in the transfusion nursing records, but only eight transfusion reaction reports were submitted to the department of transfusion management. The most common transfusion reactions in both transfusion nursing records and transfusion reaction reports were fever (213 and three cases, respectively) and dyspnea (four and three cases, respectively). The diagnosis of the reported eight transfusion reactions was inconclusive owing to the lack of specific laboratory tests, in addition to routine tests such as complete blood cell count, blood chemistry profile, and chest X-

ray. In three out of eight cases, the department of transfusion management recommended the pre-filtered blood component, resulting in no additional transfusion reactions. The first case reported in the electronic transfusion reaction reporting system was on March 30, 2024.

Conclusions: The electronic transfusion reaction reporting system is expected to enhance blood transfusion safety and patient blood management by improving the management of transfusion reactions and reducing their recurrence.

Keywords: Transfusion reactions, Transfusion reaction reporting system, Department of transfusion management

PT-09

Adverse Transfusion Reaction with Neurological Signs in a Transfused Patient: A Case Report

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An 81-year-old male patient visited the emergency department with a chief complaint of dizziness. The initial laboratory studies demonstrated a hematocrit of 20.3%, Hb level of 6.6 g/dL, white blood cell count of 10,700/mm³, and platelet count of 346,000/mm³. Initial systolic blood pressure was 100 mmHg and diastolic blood pressure was 60 mmHg. The patient's blood type was O positive. The biochemistry laboratory results were normal except for glucose (336 mg/dL) and blood urea nitrogen (38.6 mg/dL). After transfusion of a pack of 400 mL red blood cells (RBCs), his hematocrit and Hb levels improved to 22.8% and 7.3 g/dL, respectively. He received an additional unit of 400 mL of RBCs. During the second transfusion, the patient experienced left upper eyeball deviation and generalized rigidity, followed by a seizure lasting approximately 15–20 s. Brain computed tomography (CT) revealed no evidence of newly developed abnormality compared to the previous CT. Due to severe anemia, a gastroscopy was performed, and the patient was diagnosed with advanced gastric cancer. Thus, total gastrectomy was performed. While the pathophysiology of transfusion-associated neurologic signs remains unclear, several theories have been suggested. The mechanism can be deduced from the swift rise in blood viscosity, hematocrit, and hemoglobin levels, potentially worsening endothelial cell damage, blood-brain barrier dysfunction, and vasogenic edema. Blood transfusion might lead to a rapid increase in total blood volume, resulting in cerebral blood flow overload. Neurologic symptoms were rarely observed in patients experiencing adverse reactions to transfusions. However, the possibility of these signs being under-recognized and under-reported cannot be definitively excluded. Active reporting of these symptoms is anticipated to enhance their detection, leading to a deeper understanding of their occurrence and significance in adverse transfusion reactions, especially concerning severity.

Keywords: Adverse transfusion reaction, Neurologic sign, Seizure, Anemia

PT-10

A One-Year Review of Massive Transfusion Protocol in a Public Tertiary Hospital

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Background: Massive transfusion protocol (MTP) is a predefined flow of blood products originally designed for trauma cases to expeditiously resuscitate rapidly exsanguinating patients. It has been associated with blood product wastage owing to the high volume of products released. In a setting of a public tertiary hospital with a high number of emergency situations, the protocol may be effective in ensuring availability and accessibility of blood products. However, for a hospital with a recurring problem of blood supply deficiency, this protocol may also greatly impact blood allocation.

Methods: A retrospective records review of all the MTP activations in Philippine General Hospital from the time the MTP was initiated until 1 year of its implementation was conducted.

Results: A total of 96 activations was analyzed. Out of these activations, 52% (50/96) of cases fulfilled the criteria of an ABC score of ≥ 2 , 76% (73/96) of cases fulfilled the criteria of presence of hemodynamic instability, and 98% (95/96) of cases fulfilled the criteria of presence of active bleeding requiring surgery or angioembolization to determine appropriateness of MTP activation. Blood product wastage was computed as the number of unused and/or returned blood units to the blood bank, which is 8%, 10%, and 75% for O+ fresh frozen plasma, O+ platelet concentrate, and AB+ fresh frozen plasma, respectively. Surgery was ranked as the department with the greatest number of MTP activations. Majority of the working impressions of the clinicians at the time of MTP activation is an ongoing intraoperative bleeding.

Conclusions: The currently implemented massive tertiary protocol led to blood product wastage. Review of the protocol with the involved clinical and surgical departments and blood bank is recommended to address details that are vaguely defined in the protocol. Proper orientation of the residents directly involved in the implementation must also be employed to appropriately utilize the protocol and avoid blood product wastage.

Keywords: Blood transfusion, Blood, Massive transfusion, Blood bank

PT-11

Therapeutic Phlebotomy—“A Continuously Evolving Primeval Blood-letting Remedy”: A Five-Year Experience at a Tertiary Care Health Center in India

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Background: Therapeutic phlebotomy (TP) is defined as the removal of specific blood volume from the body for symptomatic relief and disease management. Absolute indications for TP include hemochromatosis, polycythemia vera (PV), and porphyria cutanea tarda. Its physiological benefits are now being utilized in secondary erythrocytosis. We aimed to analyze the utilization of TP at our institute, explore its clinical utility, and identify gaps if any.

Methods: A retrospective observational analysis was conducted in the Department of Immunohematology and Blood Transfusion at a tertiary care health center in India. TP procedures were analyzed from January 2018 to June 2023. Details of the procedure and patients were retrieved from blood centre records and hospital information system respectively and characterized in two cohorts, cohort I and II for conditions with primary and secondary erythrocytosis respectively. Procedure parameters were compared between both cohorts and also pre- and post-procedure parameters were compared using IBM SPSS statistical software (28.0., Armonk, NY).

Results: Overall, a total of 272 TP procedures were performed on 174 patients with a mean age of 53 ± 16 years. Cohort I indications (suspected/diagnosed PV) accounted for 21.6% (N=59) of procedures in 40 patients (M:F=31:9), while cohort II accounted for 78.3% (N=213) in 134 patients (M:F=114:20). Cohort II indications primarily included chronic obstructive pulmonary disease (COPD) N=124, 58.2%), post-renal transplant (PRT) erythrocytosis (N=29, 13.6%), congenital cyanotic heart disease (CCHD) (N=22, 10.3%), and others (N=38, 17.8%). In cohort I, the mean pre- and post-procedure Hb and HCT were 19.25 ± 1.62 and 59.61 ± 5.26 and 17.49 ± 1.80 and 53.77 ± 5.41 , respectively, with a mean decrement of 1.76 ± 1.11 and 5.84 ± 3.73 . In cohort II, the mean pre- and post-procedure Hb and HCT were 19.27 ± 1.56 and 59.79 ± 5.12 and 17.45 ± 1.80 and 53.81 ± 5.61 , respectively, with a mean decrement of 1.82 ± 1.14 and 5.9 ± 3.82 . No significant difference was observed between two cohorts. In each cohort, the post-procedure hemoglobin and hematocrit reduced significantly from pre-procedure Hb and HCT after TP ($P < 0.0001$). Overall, repeat procedures in COPD and PV ranged from 1 to 4, PRT from 2 to 13, and CCHD from 1 to 9. We identified missing symptoms in 68.4% of requests, hematological parameters in 12.2%, and rationale in 19.4%. Post-procedure parameters were available only for 51.1% of requests.

Conclusions: We identified the main indications for TP at our center along with the gaps in TP requests, procedure prerequisites, and awareness of recommendations. Thereafter, we designed a TP requisition preformat containing all patient and procedure details citing rationale and enabling patient follow-up. To concur on recommendations, one-on-one discussions were held.

Keywords: Therapeutic phlebotomy, Erythrocytosis, Hematocrit

PT-12

Analytical Performance of Extended Hematology Parameters for Detecting Iron Deficiency Among Routine Blood Donors

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Background: Routine blood donation carries a long-term risk of iron deficiency. Serum ferritin is a chemical parameter that reflects body iron reserves; however, it is a costly test, labor intensive, and interfered by inflammatory status. Detection of iron deficiency in blood donors using that test is not regularly established in blood services around Indonesia. The development of hematology analyzer technology provided an avenue to new parameters. Extended parameters such as reticulocyte hemoglobin content (CHr), microcyte percentage (%Micro), and hypochromic red cell percentage (%Hypo) may assist in the detection of iron deficiency in blood donation settings, which is time efficient and cost-effective.

Methods: A cross-sectional study design was performed at the blood establishment of Dr. Sardjito Hospital. Participants were healthy individuals who regularly donate and satisfied the requirements as a blood donor. Blood sample was taken for extended hematology examination (CHr, %Micro, and %Hypo) with an automatic hematology analyzer (Siemens Advia® 2120), and serum ferritin was measured using immunoturbidimetric Roche Cobas® 6000. A ferritin level of <15 ng/dL was used as a reference for iron deficiency and was validated with a CRP <6 ng/mL to evaluate for the inflammation status. Receiving operating characteristic (ROC) curve analysis was used to compare the analytical performance of extended hematology parameters.

Results: This study involved 145 participants, with a male predominance (60%), and the median donor frequency was eight times and the interval between donations was more than 3 months. It was found that 38 participants (26.2%) had iron deficiency without anemia and had the same proportion in both sexes ($P < 0.01$). The ROC curve analysis showed that the area under curve for CHr was 0.804 ($P < 0.01$), the %Micro was 0.815 ($P < 0.01$), the %Hypo was 0.808 ($P < 0.01$), and no difference was observed among them ($P > 0.05$). We recommended that the donor cutoff point for CHr, %Micro, and %Hypo for iron deficiency detection was <32.8 pg (sensitivity 94.74%, specificity 33.64%); >0.6% (sensitivity 92.11%, specificity 16.82%); and >0.1% (sensitivity 92.11%, specificity 24.3%), respectively.

Conclusions: Extended hematology parameters (CHr, %Micro, and %Hypo) are equal and have good analytic performance for detecting iron deficiency among blood donors.

Keywords: Microcyte, Hypochromic red cell, Reticulocyte hemoglobin content, Iron deficiency, Ferritin, Blood donor

PT-13

Analysis of Nucleic Acid Test Yield in Indonesian Referral Hospital Blood Establishment Donors

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Background: Infectious disease screening reduces the risk of transfusion-transmitted infections (TTIs). Several blood establishments in Indonesia have used chemiluminescent immunoassay (ChLIA) and nucleic acid testing (NAT) methods for detecting HIV, HBV, and HCV. Individual donation NAT (ID NAT) is a highly sensitive method for detecting viral TTIs in blood donors. Discrepancies in screening results between immunoassay and NAT methods can occur due to various reasons. Recently, NAT yield data in Indonesia have received inadequate attention. The present study aimed to analyze the ID-NAT yield of donors who tested positive for NAT but negative for ChLIA.

Methods: This study was conducted from January 1, 2021 to December 31, 2023, at the Blood Establishment of Dr. Sardjito Hospital, Yogyakarta, Indonesia. In total, 82,685 eligible blood donors were included in this study and analyzed for sex, age, and donor type.

Results: The total NAT yield was 1 in 1,165 participants (0.086%) over the study period. Specifically, the rates were 1 in 1,625 (0.062%), 1 in 1,520 (0.066%), and 1 in 835 participants (0.12%) in 2021, 2022, and 2023, respectively. The NAT yields were 1 in 1,172 participants (0.085%) for male donors and 1 in 1,139 participants (0.088%) for female donors. By age, the NAT yield rates were 1 in 1,662 (0.060%), 1 in 2,273 (0.044%), 1 in 599 (0.167%), and 1 in 110 participants (0.907%) in those aged <18 years, 18–24 years, 25–44 years, 45–59 years, and >60 years, respectively. Among the total study population, the NAT yield rates were 1 in 1,149 participants (0.087%) for first time donors and 1 in 1,185 participants (0.084%) for repeat donors.

Conclusions: NAT is more sensitive than ChLIA and is highly recommended for reducing the risk of transfusion-transmitted infections.

Keywords: Individual donation, Nucleic acid test, Transfusion transmitted infections

PT-14

Assessing the Accuracy of GPT-4, GPT-3.5, Clova X, and Gemini on National Licensing Exam Questions in Transfusion Medicine

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Background: Large language models (LLMs) such as GPT-4 have demonstrated significant potential in various medical applications. However, their efficacy in specialized fields such as transfusion medicine remains uncertain. This study aimed to evaluate the performance of four LLMs—Clova X, Gemini, GPT-3.5, and GPT-4, using questions from national medical licensing exams, to determine their reliability and accuracy in this critical domain.

Methods: We selected 23 medical doctor (MD) and 67 medical technologist (MT) exam questions related to transfusion medicine from the Korea Health Personnel Licensing Examination Institute (KHPLI) covering the years 2020–2023. The questions, originally in Korean, were translated to English for assessing performance differences based on language. Each LLM was tested in both Korean and English, and the evaluation metrics included overall accuracy (OA), response rate (RR), and answered accuracy (AA). OA is defined as the proportion of correct answers out of the total number of questions; RR is the proportion of questions attempted out of the total number of questions; and AA is the proportion of correct answers among attempted questions. Each model was tested twice for each combination of exam type and language to ensure consistency and reliability.

Results: The GPT-4 consistently achieved the highest OA and AA across most categories, indicating its superior performance in handling complex medical questions. For the MD questions, the GPT-4 achieved an OA of 85% and AA of 90% in English, compared with 80% and 85% in Korean, respectively. Clova X and GPT-3.5 showed better performance on English prompts with OAs of 75% and 70%, respectively, compared with 65% and 60%, respectively, on Korean prompts. Gemini's performance was variable; it excelled in some English categories with an OA of 70% but struggled significantly with Korean prompts, with the OA dropping to 50%. Significant language biases were observed, indicating a preference for English questions, likely due to more extensive training on English datasets.

Conclusions: The results emphasize the potential of LLMs in medical education and clinical decision support. However, full integration of these technologies into clinical practice requires that language biases be addressed and training datasets be comprehensive and representative of the diverse medical contexts in which these models will be used. Developing robust regulatory frameworks is essential to ensure patient safety and enhance the overall efficacy of AI applications in healthcare.

Keywords: Artificial intelligence, Large language models, Medical licensing examination, Transfusion medicine, Medical education, Language bias

PT-15

Simple Electronic Prompt Enhances Irradiated Blood Component Use in a Cancer Hospital

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Background: Blood transfusions are crucial for treating patients with anemia, thrombocytopenia, or clotting factor deficiencies. However, one major risk for transfusion is a transfusion reaction; however, transfusion is the sole management for replacing patients' oxygen-carrying capacity, platelets, or clotting factors. Graft-versus-host disease (GVHD), a rare but critical reaction caused by donor T lymphocytes, can be prevented by irradiating blood components. Irradiated blood is primarily indicated for patients with blood or solid cancers, organ transplantation, or other immunocompromised states. Prescribing irradiated blood for these patients represents "proper utilization" of blood components, minimizing the risk of severe transfusion reactions. This study aimed to determine the impact of informing clinicians regarding proper irradiated blood component use through a simple electronic prescription system modification.

Methods: A system modification introduced in July 2019 added a note to each prescription prompting selection for patients who qualify for irradiated transfusions to improve irradiated blood component utilization. We retrospectively reviewed blood component prescription data from seven half-yearly periods (2016–2022). Data included patient demographics, diagnoses, prescribed blood component types, and irradiated/non-irradiated unit amounts. The proportion of irradiated units was calculated for each half-year. Furthermore, we categorized patients by cancer status, blood component type, and visit type. We calculated the average irradiated units per prescription and performed independent sample t-tests to evaluate significance.

Results: A total of 33,701 blood component prescriptions were analyzed. Before the informational note, the average prescription included 0.21 units of irradiated and 2.46 units of non-irradiated components. After implementation, the average shifted to 0.77 units of irradiated and 1.44 units of non-irradiated components. All three groups, except for fresh frozen plasma transfusions and operating room transfusions, demonstrated a significant increase in irradiated unit usage ($P < 0.001$) after the intervention.

Conclusions: Informing clinicians regarding proper irradiated blood component use through a simple modification in the electronic prescription system effectively promoted proper utilization. This approach has the potential to reduce transfusion reactions and improve patient outcomes.

Keywords: Transfusion reactions, Proper transfusion, System modification, Irradiated blood components

PT-16

Assessing the Learning Outcomes of Autologous Blood Collection Procedure in the Blood Bank by Using Objective Structured Clinical Examination

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Background: Autologous blood storage is a crucial service provided by the blood bank. However, recent changes in workflow have led to mistakes owing to staff unfamiliarity with new procedures. To address this issue, we implemented an Objective Structured Clinical Examination (OSCE) to assess and train staff in autologous blood storage and communication skills.

Methods: We have developed a comprehensive teaching plan referred to as "Autologous Blood Storage Skills and Physician-Patient Communication" to address clinical scenarios and common issues related to autologous blood storage. This plan includes clinical scripts and examination questions; moreover, a skilled examiner and a standardized patient were included in this examination. We also designed guidelines for the skilled examiner, the standardized patient, and examination center, including the scoring sheets for candidates and standardized patient. To better simulate clinical scenarios, we have standardized patients to assist in the simulation, along with examiners for assessment and feedback. To evaluate the participant's satisfaction, Google Forms were used.

Results: In the OSCE assessment, eight staff from the blood bank participated and all of them passed with an average score of 88.7 points. The passing rate was 100%, with a passing score of 80 or above. The candidates' performances were rated on a five-point scale. One candidate received an excellent rating (5 points), six candidates received a good rating (4 points), and one candidate received an average rating (3 points). The quantitative data demonstrated a high satisfaction, and the feedback from the qualitative data was positive.

Conclusions: This OSCE assessment significantly improved the clinical skills of the blood bank staff and could answer questions while executing autologous blood storage procedures. This model can be employed in clinical teaching and assessments in the future to identify the learning effectiveness for our interns or new team members.

Keywords: OSCE, Autologous blood storage, Outcome

PT-17

Assessment of RBC Transfusion Trends by Departments and Criteria: The Impact of Practitioners

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Background: To ensure transfusion appropriateness, which is crucial for blood resource utilization, institutions operate transfusion management offices, and the Ministry of Health and Welfare and the Korean Society of Blood Transfusion periodically revise and distribute guidelines. While analysis of the transfusion trends and education for transfusion appropriateness is warranted, comprehensive analyses are limited, and the education targets and methods are not well established. Since February 2024, residents have been out of work due to policy issues, resulting in senior specialists directly prescribing transfusions. We aimed to analyze RBC prescription trends under these unusual circumstances and indicate necessary and effective strategies for transfusion education.

Methods: In this retrospective study, we reviewed electronic medical records, underscoring on RBC prescriptions and hemoglobin levels at the time of prescription, from the first and second quarters of 2023 and 2024. Data were collected and analyzed by the clinical departments and prescription criteria. Criteria involving active bleeding or blood preparation for surgery were excluded.

Results: A total of 39 clinical departments were reviewed. Prescribed units were 36,125 and 37,305 in the first and second quarters of 2023 and 32,339 and 7,401 in the first and second quarters of 2024, respectively. The average hemoglobin levels at the time of prescription were as follows: 8.4 (6.9–10.6) mg/dL and 8.1 (6.8–9.9) mg/dL in the first and second quarters of 2023 and 8.3 (6.6–10.5) mg/dL and 8.1 (6.1–10.0) mg/dL in the first and second quarters of 2024, respectively. Differences in average hemoglobin levels between the first and second quarter were -0.2 (-1.8–0.8) for 2023 and -0.2 (-2.9–1.3) for 2024. The departments with the largest differences in average hemoglobin levels were the scoliosis center (-1.8), neurosurgery (-1.7), and cardiology (-1.2) in 2023. In 2024, radiation oncology demonstrated the largest difference (-2.9); however, the number of units prescribed was fewer than 10. The departments that followed include pediatric neurology, orthopedic surgery, pediatric gastroenterology (-1.4), and pediatric surgery (1.3). Departments with differences in average hemoglobin levels of 1.0 or greater, or -1.0 or less, were four in 2023 and seven in 2024.

Conclusions: We assessed trends in RBC prescription by departments and prescription criteria. Differences were observed in few departments, while overall changes were minor. These indicate the need for transfusion appropriateness education tailored to each department. Continuous monitoring and education can enhance the efficiency of transfusion management and strengthen transfusion appropriateness in the future.

Keywords: Transfusion appropriateness, Education, Transfusion management, Transfusion trend, RBC transfusion

PT-18

Evaluation of Lysis Methods for Preparing Platelet Extracts as Test Materials from Discarded Platelet Concentrates

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Background: With the increasing incidence of cancer, the frequency of thrombocytopenia and platelet dysfunction is rising, highlighting the importance of accurate platelet testing in clinical laboratories. Therefore, efficiently obtaining platelet extracts for use in test materials, such as particles coated with platelet-derived antigens, is crucial. This study evaluated lysis methods for preparing platelet extracts from discarded platelet concentrates for use as test materials.

Methods: In this study, discarded platelet concentrates were treated with Triton X-100-based lysis buffer (DPBS, 2% Triton X-100 [Sigma Aldrich, USA, X100], 10 mM HCl, 10 mM EGTA, protease inhibitor [Sigma-Aldrich, Germany]), commercial Radioimmunoprecipitation assay buffer (RIPA buffer; ATTO, Japan), and phosphate-buffered saline (PBS; WELL GENE, Korea) as a non-lysis buffer. The state of platelet lysates was analyzed under various conditions with and without sonication and centrifugation. To evaluate the prepared platelet extracts, protein concentration was quantified using Bicinchoninic acid assay (BCA assay; Thermo Fisher, USA), and the presence of CD61 platelet antigen in the extracts was confirmed by Western blot analysis. Additionally, particle number and size in the platelet extracts were measured using flow cytometry (Navios; Beckman Coulter Inc., USA) and a Nanoparticle Size Analyzer (ELSZ Neo; Otsuka Electronics, Japan).

Results: A total of 12 conditions were tested, combining three buffer types and the presence or absence of sonication and centrifugation. In the BCA assay, the condition where sonication was applied but centrifugation was not performed yielded significantly lower protein quantification ($P < 0.05$) than the other 11 lysis conditions. Similarly, the CD61 antigen measured under this condition also showed significantly lower values. Flow cytometry revealed a significantly higher number of particles under the non-lysis condition than in other conditions ($P < 0.05$). Additionally, nanoparticle size analysis indicated significantly larger particles under the non-lysis condition than in other lysis conditions ($P < 0.05$), confirming the effective lysis of platelets in the tested lysis methods.

Conclusions: This study tested 12 different platelet lysis conditions and demonstrated that lysis buffer and sonication effectively lyse discarded platelets. These results suggest a useful method for utilizing discarded platelet concentrates to prepare platelet extracts as test materials. Furthermore, this standardized experimental condition can play a significant role in confirming the presence of various platelet antigens and contribute to platelet research and disease diagnosis by increasing the utility of discarded platelet concentrates.

Keywords: Platelet lysis, Discarded platelet concentrates, Platelet extracts

PT-19

Fluorescent Detection of Platelet Aggregates in a Gel Card Using the Live Cell Staining Dye Calcein-AM

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Background: Traditional tests such as light transmission aggregometry have limitations when testing platelet aggregation with small platelet samples. This study aims to develop a method for measuring platelet aggregation using a gel card and the live cell staining dye Calcein-AM, which selectively stains live cells.

Methods: To induce platelet aggregation in the testing segment samples of platelet concentrate, we used 1 mg/mL fibrinogen, 20 mM CaCl₂, and an agonist mixture (adenosine diphosphate+thrombin). We analyzed the location and fluorescence intensity of aggregates using a gel card after centrifugation with activated samples, non-activated samples, and samples not stained with Calcein-AM.

Results: In the gel card, activated samples demonstrated platelet aggregates with green fluorescence signals of Calcein-AM observed in the middle of the gel. Non-activated samples exhibited fluorescence signals at the bottom of the gel, while samples not stained with Calcein-AM revealed no fluorescence signals. Image analysis of the green fluorescence indicated that Calcein-AM-stained platelet aggregates had an average signal intensity of 85, whereas other areas of the gel revealed an average fluorescence intensity of 14 ($P < 0.05$). This confirmed that Calcein-AM staining allows the observation of live, aggregated platelets.

Conclusions: This study developed a method of observing platelet aggregates using a fluorescent dye that selectively stains live cells, improving the traditional visual inspection method of reading gel cards. The test method exhibited the ability to specifically observe platelet aggregation with small platelet samples using the commonly used gel card in blood banks. This highlights its potential for future clinical applications.

Keywords: Platelet aggregation, Calcein-AM Stain, Gel card assay

PG-01

Spectra of NGS DNaseq of Acute Leukemia Patients in a Single Tertiary Hospital

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Background: Traditional cellular and molecular genetic assays have been used to detect chromosomal aberrations in patients with hematologic malignancies. Over the past 15 years, next-generation sequencing (NGS) has evolved from research to clinical laboratories. Early diagnosis of various subtypes is crucial for disease classification, prognosis, and treatment prediction. The World Health Organization recommends genetic testing as a diagnostic category in its classification guidelines. Therefore, we aimed to compare the frequency and spectra of NGS DNA sequencing results of acute leukemia patients at a single tertiary hospital.

Methods: Data were obtained from bone marrow biopsy results at a single tertiary hospital over five years (2016–2020). The dataset included clinical and laboratory information such as sex, age, final diagnosis, conventional cytogenetics, and NGS DNA sequencing results. The study included 99 children (median age 9 years, range 0–18 years) and 653 adults (median age 59 years, range 19–89 years) with acute leukemia. The assays were performed using MiSeq or MiSeqDx from Illumina (California, USA) with a specific panel (무슨) (지역), utilizing 6 mL of EDTA anticoagulated bone marrow aspirates or peripheral blood. 43% (325/752) of acute leukemia patients were analyzed using NGS DNA sequencing.

Results: NGS DNA sequencing was performed on 46% of AML patients, 33% of ALL patients, 40% of MPAL patients, and 25% of AUL patients. In AML, the most frequent mutations were FLT3-ITD (N=50), NPM1 (N=48), TET2 (N=45), NRAS (N=37), RUNX1 (N=37), IDH2 (N=36), and DNMT3A (N=34). In ALL, the most common mutations were NRAS (N=6), KRAS (N=4), MTHFR (N=4), TP53 (N=4), RUNX1 (N=3), and SH2B3 (N=3). In MPAL, the most frequent mutations were TET2 (N=2) and DNMT3A (N=2).

Conclusions: Genetic variants are becoming increasingly important for diagnosis and treatment. Some mutations, previously considered insignificant, have gained importance in recent updates. Therefore, NGS DNA sequencing in hematology is essential, and we should organize this information in an accessible manner, even if some mutations are not currently deemed critical.

Keywords: Acute leukemia, Hematology, Molecular diagnostics, NGS, DNA sequencing

PG-02

Evaluation of Prognostic Performance of Pan-Cancer ctDNA NGS Panel in Advanced Cancer Patients

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Background: Advances in ctDNA analysis enable identification of tumor-associated genetic alterations through NGS and PCR. NGS can identify a broader range of mutations than PCR, making it valuable for solid tumor prognosis. However, more data are needed on ctDNA's usefulness in head and neck, esophageal, and gastric cancers. This study evaluated the utility of a pan-cancer ctDNA NGS panel in advanced solid tumors, including these specific cancer types.

Methods: Participants received treatments as determined by their physicians. They underwent up to three ctDNA NGS tests: before treatment, six weeks after treatment for response assessment, and at follow-up. Those with progressive disease (PD) during the treatment response assessment did not have follow-up studies. ctDNA was isolated using the magnetic circulating DNA Maxi Reagent (Dxome) from 18 mL of whole blood and prepared using the DxSeq ctDNA pan100 kit. Sequencing was conducted with the HiSeq 2000 System (Illumina), and genetic variants were categorized into tiers 1, 2, and 3 per AMP/ASCO/CAP guidelines.

Results: Thirty-six advanced cancer patients were analyzed for clinical changes from ctDNA NGS results. The group included patients with gastric, lung, esophageal, head and neck, breast, and colorectal cancers. Before treatment, 86.1% had tier 1 or 2 somatic variants in ctDNA. CNVs were found at the gene level in 7, chromosome level in 7, and a common denominator in 3 patients. During treatment evaluation, 33 patients underwent NGS testing, with positive rates of 66.7% and 94.4% at different follow-up points. Among 17 patients with clinical improvement, 41% showed mutations, while 93.8% of 16 patients without improvement had positive results, indicating a significant difference ($P=0.0016$). Three patients deteriorated rapidly and withdrew, all with low VAFmax tier 1 or 2 variants. Four initially negative patients who developed new variants experienced PD, and 6 out of 8 with reappearing variants at follow-up showed no clinical improvement.

Conclusions: The high pre-treatment positive rate indicates that genetic variants are key prognostic markers in advanced cancer. Poor responders had a 93.8% positivity rate, compared to 41% in those with improved outcomes, showing an inverse relationship between ctDNA NGS results and treatment response. Fifteen patients left the study due to disease progression. New mutations at follow-up in initially negative patients led to worse outcomes, highlighting the utility of ctDNA NGS for prognosis. Routine post-treatment ctDNA checks are beneficial, as 64.7% of initially negative patients worsened with mutation reappearance. The pan-cancer ctDNA NGS panel is useful for prognosis in various cancers.

Keywords: Pan-cancer, ctDNA, NGS, Advanced, Prognosis

PG-03

Development of a Method to Improve Diagnostic Accuracy of *BCR::ABL1*-like B-ALL Using RNA Expression Data Modeling

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Background: *BCR::ABL1*-like B-lymphoblastic leukemia (B-ALL) accounts for 10–15% of all B-ALL cases and has high-risk clinical features. This subtype shows a similar gene expression pattern to *BCR::ABL1*-positive-B-ALL; however, an accurate diagnosis of *BCR::ABL1*-like-B-ALL is challenging due to its high molecular heterogeneity.

Methods: We used RNA expression data from 1,480 patients at St. Jude Children's Research Hospital. The RPKM value and the rank value of RPKM, sorted in descending order were used for modeling. Models were created for three subgroups: *BCR::ABL1*-positive-B-ALL, *BCR::ABL1*-like-B-ALL, and CRLF2-B-ALL. Differentially expressed gene analysis was performed between randomly selected subjects (N=30) as controls and cases with each subgroup. Models were trained using these data and validated using the remaining patient datasets, ensuring even gene expression distribution and detecting biases through boxplot analysis.

Results: Six models were developed using RPKM and rank values for each of the three subgroups. When *BCR::ABL1*-B-ALL and *BCR::ABL1*-like-B-ALL models were used to predict *BCR::ABL1*-like-B-ALL, the maximum AUC of the RPKM model was 0.921 and 0.891, respectively. The maximum AUC of the rank model was 0.933 and 0.950, respectively, and the best performance was shown by the *BCR::ABL1*-like-B-ALL rank model. However, when predicting *BCR::ABL1*-like-B-ALL using the CRLF2 model, the maximum AUC was 0.402, when using the rank model. When comparing the performance between the group with evenly distributed RNA expression and the group with bias, the AUC values of both *BCR::ABL1*-positive-B-ALL rank model and *BCR::ABL1*-like-B-ALL rank model were more than 0.9 from both groups. However, the RPKM models showed a larger difference of AUC. The AUC of the evenly-distributed data sets and biased data sets were 0.921 and 0.841, respectively, when using the *BCR::ABL1*-positive-B-ALL model and the AUC of the two data sets when using the *BCR::ABL1*-like-B-ALL model was 0.891 and 0.742, respectively.

Conclusions: The study highlights the necessity for improved diagnostic method for *BCR::ABL1*-like-B-ALL due to its molecular heterogeneity. By analyzing RNA expression data from 1,480 patients and creating models based on RPKM and rank values, the *BCR::ABL1*-like-B-ALL rank model demonstrated superior performance. This approach could potentially lead to improving the accuracy of *BCR::ABL1*-like-B-ALL diagnosis.

Keywords: RNA-seq, RNA expression, B-cell acute lymphoblastic leukemia, *BCR::ABL1*-like-B-ALL, RPKM, Rank

PG-04

Transcript-Specific Quantification and Detection Bias of *BCR-ABL1* in Patients with Chronic Myeloid Leukemia Using Droplet Digital Polymerase Chain Reaction

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Background: Accurate quantification of the *BCR-ABL1* gene is crucial for assessing tyrosine kinase inhibitor therapy response and determining treatment discontinuation in patients with chronic myeloid leukemia (CML). The *BCR-ABL1* gene produces two transcripts, e13a2 and e14a2, based on the breakpoint in the *BCR* gene, with e14a2 being 75 bp longer. Patients with CML harboring the e13a2 transcript achieve a cytogenetic response more rapidly than those harboring e14a2. However, overall survival and progression-free survival rates do not differ between the two transcript types. Quantitative polymerase chain reaction (qPCR), commonly used for *BCR-ABL1* quantification, is influenced by transcript length, leading to an amplification efficiency bias where e14a2 is underrepresented. Recently, droplet digital polymerase chain reaction (ddPCR), which is capable of detecting levels down to 10^{-5} , has been introduced as a sensitive method for minimal residual disease monitoring. This study aims to investigate any differences in transcript quantification using ddPCR.

Methods: A retrospective chart review was conducted for patients who underwent *BCR-ABL1* quantification via ddPCR at Seoul National University Bundang Hospital between October 31, 2022 and May 31, 2024. Patients with chronic phase CML who underwent at least three quantification tests at diagnosis and follow-up were included. These patients were categorized based on the type of *BCR-ABL1* transcript present. Differences in transcript quantification were evaluated using the Mann-Whitney U test and linear function comparisons between the initial and follow-up tests.

Results: Out of 1,899 cases, 160 samples from 29 patients met all criteria. Among them, 9 patients harbored the e13a2 transcript and 20 harbored the e14a2 transcript. The mean *BCR-ABL1* quantification values at diagnosis (%IS, mean $\pm$ SD) were 68.9 ± 9.66 for e13a2 and 44.4 ± 16.35 for e14a2. The average slope of the linear function over approximately 3 months post-diagnosis was -23 for e13a2 and -14.65 for e14a2, demonstrating a faster molecular response for e13a2.

Conclusions: Treatment strategies for patients with CML are increasingly focusing on confirming deep molecular responses and discontinuing therapy. *BCR-ABL1* quantification is crucial for determining patient prognosis. The current predominant method, qPCR, may underestimate the longer e14a2 transcript. Efforts to address this issue by fine-tuning the conversion factor in qPCR, a relative quantification method, based on the reference gene, have encountered significant challenges. ddPCR is an absolute quantification method that could serve as an alternative to qPCR. Although ddPCR also shows differences between transcripts, standardization through large-scale studies may enable accurate measurements and allow informed treatment strategies for patients.

Keywords: ddPCR, *BCR-ABL1*, e14a2, e13a2, CML

PG-05

Performance Evaluation of the 1copy *BCR::ABL* qPCR Assay as a Monitoring Tool for Chronic Myeloid Leukemia

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Background: Accurate monitoring of minimal residual disease (MRD) in chronic myeloid leukemia (CML) patients requires precise detection of *BCR::ABL1* fusion transcripts at or below the molecular response (MR) 4 level (0.01% International Scale [IS]). For the quantification of *BCR::ABL1* fusion transcripts, real-time quantitative PCR (RQ-PCR) has been widely used, and recently, methods utilizing droplet digital PCR or massively parallel sequencing have also emerged.

Methods: In this study, we evaluated the analytical performance of the 1copy *BCR::ABL* qPCR Kit (1drop, Korea), which uses the one-step, CataCleave technique-based PCR method.

Results: The precision analysis revealed that the %CV at MR1 and MR2.5 was 14.4% and 15.6%, with mean %IS values of 14.1 and 0.233, respectively. The assay exhibited linearity in the first order within the range of 0.02% IS to 85% IS. Furthermore, the manufacturer-claimed limit of blank, limit of detection, and limit of quantification were successfully verified. A method comparison using 74 clinical samples demonstrated a strong correlation between the 1copy *BCR::ABL* qPCR assay, the ipsogen *BCR::ABL1* Mbc IS-MMR (Qia-gen, Hilden, Germany) assay, and the QXDx *BCR::ABL* %IS ddPCR assay ($r=0.951$ and 0.950 , respectively).

Conclusions: In conclusion, the 1copy *BCR::ABL* qPCR assay can be a reliable tool for consistent and accurate monitoring of MRD in CML patients.

Keywords: Chronic myeloid leukemia, *BCR::ABL1*, Quantitative polymerase chain reaction, Performance evaluation

PG-06

De Novo *TRAF7* p.Arg655Gln Variant: A Unique Korean Case Presenting with Cardiac, Facial, and Digital Anomalies Alongside Developmental Delay

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TRAF7 (Tumor Necrosis Factor Receptor-Associated Factor 7) mutations result in an exceptionally rare inherited disorder that shares

clinical features with cardiac, facial, and digital anomalies in developmental delay (CAFDADD) syndrome and blepharophimosis-mental retardation syndrome (BMRS). A 36-year-old male, exhibiting blepharophimosis and intellectual disability was admitted for the evaluation of resting dyspnea several months prior to presentation. With a history of obstructive sleep apnea (OSA) diagnosis, transesophageal and transthoracic echocardiography revealed right ventricular dilatation without significant pulmonary hypertension, bicuspid aortic valve with aortic root aneurysm, and aortic regurgitation. Sanger sequencing revealed a de novo *TRAF7* variant (c.1964G>A/p. Arg655Gln). Subsequently, the patient underwent aortic root replacement using the Bentall procedure. Despite surgical intervention, persistent dyspnea prompted re-evaluation of OSA through polysomnography, which revealed symptomatic relief with continuous positive airway pressure support. The etiology of his symptoms was attributed to OSA, likely exacerbated by the vertebral anomaly and short neck associated with CAFDADD syndrome.

Keywords: CAFDADD syndrome, *TRAF7*, p.Arg655Gln, Clinical exome sequencing

PG-07

Identification of Novel Compound Heterozygous Mutations in *SCN4A* as a Potential Genetic Underpinning for Myopathic Manifestations

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SCN4A mutations are implicated in a broad spectrum of clinical manifestations, including periodic paralysis, myotonia, and recently recognized symptoms, such as classical congenital myopathy (CM) or congenital myasthenic syndromes. Herein, we present the first example of myopathic features resembling classical recessive CM in a Korean infant with novel compound heterozygous *SCN4A* mutations. The infant exhibited profound hypotonia shortly after birth, expanding the spectrum of *SCN4A*-related channelopathies. Genetic analyses involved targeted exome sequencing using the Celeomics G-Mendeliome DES Panel, supplemented by Sanger sequencing. Given the clinical presentation of the proband, *SCN4A* variants have emerged as primary candidates for autosomal recessive (AR) congenital myopathy 22a, classic (#620351). Sanger sequencing confirmed the association of *SCN4A* variants with the phenotype, establishing the autosomal recessive nature of the compound heterozygous mutations in both the carrier mothers (c.3533G>T/p. Gly1178Val) and father (c.4216G>A/p. Ala1406Thr). This report underscores the link between novel compound heterozygous *SCN4A* mutations and myopathic features resembling classical CM, as supported by muscle biopsies. Pathogenic *SCN4A* LoF mutations are exceptionally rare, this study contributes to our understanding of *SCN4A* mutations and their role in myopathic features resembling those of classical CM.

Keywords: *SCN4A*, Congenital myopathy, Targeted exome sequencing

PG-08

Sensorineural Hearing Loss with Novel Compound Heterozygous *TRIOBP* Gene Variant, Accompanied by Autism: A Case Report

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Autosomal recessive nonsyndromic deafness-28 (DFNB28; OMIM #609823) specifically denotes prelingual sensorineural hearing loss (SNHL) resulting from homozygous or compound heterozygous mutations in the TRIO- and F-actin-binding protein, *TRIOBP* gene. This report details the comprehensive clinical manifestations of a pediatric patient with novel compound heterozygous deleterious variants in the *TRIOBP* gene. The child presented an atypical combination of both autism spectrum disorder and SNHL. To the best of our knowledge, this is the first report of *TRIOBP*-related SNHL in Korea, with a unique blend of novel compound heterozygous mutations in the *TRIOBP* gene and an additional autism spectrum disorder phenotype. Genetic analysis identified compound heterozygous *TRIOBP* variants; specifically, c.1192_1195delinsT/p. Gln398Ter, and c.3661C>T/p. Arg1221Trp were identified as the probable cause of SNHL in the proband (Reference transcript ID: NM_001039141.3). These isoforms are predominantly expressed in the inner ear and contribute to the formation of stereociliary rootlets and the fetal brain. Various human diseases and conditions have been linked with *TRIOBP* isoforms. However, animal studies can provide a more comprehensive understanding of the diverse roles of *TRIOBP* isoforms in human health, particularly in mental illnesses.

Keywords: Sensorineural hearing loss, *TRIOBP* gene, Autism spectrum disorder, Whole exome sequencing

PG-09

Follow the LINE: A Rare Molecular Finding That Can Change Genetic Results

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Long interspersed nuclear elements (LINE) are mobile genetic elements capable of inserting themselves throughout the genome. We report a rare case of LINE insertion within the TTN gene, which likely caused autosomal dominant dilated cardiomyopathy in a young

male. A 17-year-old young man was diagnosed with dilated cardiomyopathy and underwent next generation sequencing-based (NGS) testing. The patient's mother had post-partum cardiomyopathy, but there was no other family history of the condition. The test covered 63 genes associated with hereditary forms of dilated cardiomyopathy and left ventricular noncompaction, including TTN, which encodes titin, a large sarcomeric protein in the myocardium. Sequence capture and targeted NGS were performed, followed by polymerase chain reaction (PCR) for variant confirmation. Analysis of the BAM file revealed a large insertion within the exon 276 of TTN, with a 27.8% variant allele frequency (c.80282_80283ins). Alignment tools showed that the inserted sequence matched many different chromosomes. Although an artifact was considered, it was quickly ruled out due to the apparent reality of the insertion. Differential diagnosis included transposons, such as Alu elements or LINEs. Confirmatory testing involved designing primers to amplify across the insertion using the reference exonic sequence. Gel electrophoresis confirmed the insertion, showing a large band (approx. 5,000 bp) in the patient along with a normal control band, indicating a heterozygous large insertion in the gene. Notably, this LINE insertion in an exon likely leads to premature truncation and loss of function. LINE insertion is a rare and unexpected event that can be overlooked in clinical laboratories due to its unusual NGS presentation. In this case, the size of the insertion, its location within an exon, and alignment with multiple chromosomes suggested a transposon presence. Further analysis confirmed the finding, and the variant was reported as likely pathogenic: TTN (NM_001256850.1), exon 276, chr2(GRCh37):g.179425653_179425654insN[(4000_5000)], c.80282_80283insN [(4000_5000)], p.Arg26762Glyfs*23". This case highlights the importance of thoroughly analyzing unexpected molecular findings, as they can reveal critical diagnoses.

Keywords: LINE, Insertions, Transposons, Cardiomyopathy, TTN

PG-10

Development of a Diagnostic Test Method for Prader-Willi Syndrome and Angelman Syndrome Based on Targeted Long-Read Sequencing

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Background: Prader-Willi syndrome (PWS) and Angelman syndrome (AS) result from the loss of imprinting genes on chromosome 15. Current diagnostic methods cannot detect multiple mechanisms simulta-

neously. We propose a novel diagnostic approach using targeted long-read sequencing (LRS) to concurrently identify diverse mechanisms.

Methods: Six patients with PWS and four with AS from Seoul National University Hospital were studied. NA12878 served as the normal control. A 33 Mb region of chromosome 15 was sequenced with adaptive sampling on the Oxford Nanopore Gridlon, followed by targeted sequencing of a 7.9 Mb region on the Illumina NovaSeq 6000. Pathogenic mechanisms were analyzed using Dorado, Minimap2, Pepper-Margin-DeepVariant, Sniffles, cuteSV, and CNVkit. Loss of heterozygosity (LOH) in uniparental disomy (UPD) patients was determined by comparing B allele frequency (BAF) to NA12878. Significant differences between patients with uniparental isodisomy (iUPD) or uniparental heterodisomy (hUPD) and controls were identified using Cramer's V test and Z-test, and validated in UPD patients with Beckwith-Wiedemann syndrome.

Results: We identified differentially methylated regions in all ten patients. Enriched LRS detected various pathological mechanisms, including large deletions (2 with PWS and 1 with AS), a small deletion (1 with AS), hUPDs (2 with PWS and 1 with AS), iUPDs (2 with PWS), and single nucleotide variant (SNV) (1 with AS). Integrating short-read sequencing data (SRS) enhanced coverage depth, particularly refining the analysis of large deletions exceeding 5–6 Mb. BAF analysis accurately identified LOH in 5 patients with UPD (4 with PWS and 1 with AS). Significant differences in iUPD patients compared to the normal control were validated by both Cramer's V test and z-test, while patients with hUPD showed no significant distinctions. Applying our statistical model to the UPD patients with Beckwith-Wiedemann syndrome, those with iUPD with an LOH region on chromosome 11 exhibited significant differences from normal data, increasing the reliability of our statistical model.

Conclusions: We illustrated the simultaneous analysis of allele-specific methylation, UPD, structural variant, and SNV via targeted enriched LRS. Integrating SRS improved low coverage, presenting various sequencing scenarios. We achieved the accurate detection of LOH and differential diagnosis of iUPD from hUPD using an in-house statistical model. Additional validation is essential to confirm the clinical utility of the novel diagnostic method.

Keywords: Prader-Willi syndrome, Angelman syndrome, Imprinting disorders, Long-read sequencing, Uniparental disomy, Nanopore

PG-11

Shotgun Metagenomic Sequencing Analysis as a Diagnostic Strategy in Patients with Lower Respiratory Tract Infection

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Background: Conventional diagnostic methods (CDMs) used for diagnosing lower respiratory infections have inherent limitations in detecting all suspected pathogens. We aimed to compare these established methods with shotgun metagenomic sequencing (SMS) of bronchoalveolar lavage (BAL) fluid, to enhance pathogen detection capabilities.

Methods: Sixteen BAL fluid samples from pneumonia patients with positive results in various CDMs, such as bacterial/fungal cultures, real-time PCR for *Mycobacterium tuberculosis*, cytomegalovirus, or FilmArray Pneumonia Panel (BioMérieux), were included. Each sample was subjected to 10 Gb SMS using the Novaseq 6000 (Illumina). Sequencing data were aligned against the NCBI RefSeq (version 2023.10.13). For eukaryotic reads, additional matching was conducted using the internal transcribed spacer of fungi. Antibiotic resistance genes were annotated using the Comprehensive Antibiotic Resistance Database model.

Results: Microbial reads proportion ranged from 0.00002% to 0.04971% across samples. Further, SMS detected bacterial reads ranging from 2 to 23,869, with relative abundance from 0.02% to 87.5%. Exact matches between CDM-detected and SMS-detected microbes were found in 12.5% (2/16) of cases using the initial threshold and 18.75% (3/16) using the final threshold. Partial matches between CDMs and SMS were 68.75% (11/16) at the initial threshold and 81.25% (13/16) at the final threshold. Notable, eight newly identified species were detected. Eukaryotic reads varied from 0 to 32, with no fungal alignment at the genus level observed using 18S rRNA. *Candida* species were identified in four samples using an internal transcribed spacer. No viral reads were detected. Antibiotic resistance genes ranged from 0 to 43.

Conclusions: This study highlights the importance of comparing CDMs and SMS, revealing insights into SMS performance and limitations with a 10-Gb output for BAL samples. While SMS cannot replace conventional PCR, it provides valuable support to culture methods.

Keywords: Shotgun metagenomic sequencing, Bronchoalveolar fluid, Lower respiratory infection

PG-12

Comparison of AdvanSure TB/NTM Real-Time PCR, Cobas TaqMan MTB, and Xpert MTB/RIF Assays for the Detection of *Mycobacterium Tuberculosis* in Bronchial Washing Specimens

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Background: To prevent the spread of tuberculosis (TB), early diagnosis of TB followed by precise treatment is essential. The TB polymerase chain reaction (PCR) test is recommended as the initial diagnostic test for accurate and rapid diagnosis of TB infections. In this

study, we aimed to evaluate the diagnostic performance of three commercial real-time PCR assays: AdvanSure tuberculosis/non-tuberculous mycobacterium (TB/NTM) real-time PCR, Cobas TaqMan MTB, and Xpert MTB/RIF assay, for the detection of *Mycobacterium tuberculosis* (MTB) in bronchial washing specimens.

Methods: This retrospective study included 658 patients whose bronchial washing specimens were simultaneously tested using AdvanSure TB/NTM, Cobas, and Xpert assays at the Chungnam National University Hospital from July 2021 to March 2024. All bronchial wash specimens were processed for acid-fast bacillus (AFB) staining and mycobacterial culture in both solid and liquid media. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using 95% confidence intervals (CI) to determine the diagnostic accuracy of the three real-time PCR assays.

Results: The concordance rate between the three real-time PCR assays was 93.2% (613/658). Using mycobacterial culture as the reference method, the overall sensitivities of the AdvanSure TB/NTM, Cobas, and Xpert assays were 57.9%, 94.7%, and 91.2%, respectively. The sensitivities of the three real-time PCR assays for smear-positive specimens were consistent, and all achieved 100.0% sensitivity. For smear-negative specimens, the sensitivities of the AdvanSure TB/NTM, Cobas, and Xpert assays were 26.1%, 92.9%, and 88.1%, respectively. The overall specificities of the AdvanSure TB/NTM, Cobas, and Xpert assays were 100.0%, 97.7%, and 97.2%, respectively. The AdvanSure TB/NTM assay demonstrated 100% specificity and PPV independent of the smear results. The Cobas and Xpert assays had the same PPV (93.8%) for smear-positive specimens. However, for smear-negative specimens, the PPV was 75.0% for Cobas and 69.8% for Xpert.

Conclusions: Although the AdvanSure TB/NTM assay may be useful for the simultaneous detection of TB and NTM, it showed relatively poor performance in the detection of TB compared with the Cobas and Xpert assays. The Cobas and Xpert assays demonstrated reliable performance for molecular diagnostic testing of TB.

Keywords: *Mycobacterium tuberculosis*, AdvanSure TB/NTM PCR, COBAS TaqMan MTB PCR, Xpert MTB/RIF

PG-13

Difference of Core Gene Multi-Locus Sequence Type of Group B *Streptococcus* According to Host

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Background: Group B *Streptococcus* (GBS) infects various hosts, with humans, bovine, and fish being the most studied. Clinical characteristics of GBS are related to the clonal complex (CC) type, with CC17 GBS causing mastitis in bovine and neonatal sepsis in humans. Recent advancements in whole genome sequencing (WGS) enable a broader analysis of core gene multi-locus sequence types (cgMLST)

beyond CC type.

Methods: We sourced WGS data of 2,502 GBS from the National Center for Biotechnology Information (NCBI), of which 429 GBS were identified as CC17. After confirming the identification year and host, 354 were analyzed. The cgMLST of GBS analyzed 1,405 loci data of GBS from PubMLST (<https://pubmlst.org/>). Genetic distance was calculated by comparing the locus allele to NCBI's reference GBS (GCA_001552035.1), and the minimal spanning tree was analyzed.

Results: Among 354 GBS strains, 264 were human and 90 were bovine samples. All human GBS were serotype III. of bovine-origin GBS, 96.7% (87) and 3.3% (3) were serotypes II and III, respectively. The number of cgMLST loci identified in reference GBS was 1,363, while human GBS had an average of 1,352.0 and bovine GBS had an average of 1,203.6, showing a significant difference ($P < 0.001$). As a result of genetic distance analysis according to the sequence of the locus allele, human GBS was divided into two clusters, despite a shorter genetic distance between the strains. Cluster 1 having a long genetic distance from bovine GBS were 170 strains, and cluster 2 having a close genetic distance from bovine GBS were 94 strains. The cgMLST loci of these two clusters showed a significant difference ($P = 0.001$), and the number of loci with the same allele as the bovine clusters was 57 in cluster 2, which was twice as many as 29 in cluster 1. The loci distinguishing the two human clusters from bovines were identified as eight. Phylogenetic analysis using their sequences and the years they were identified showed that human cluster 1 became prominent starting in the late 2000s.

Conclusions: CC17 GBS exhibits distinct genetic variations based on the host. Recent strains identified in humans are markedly different from those previously identified in human hosts, and their prevalence is increasing. This indicates that GBS is undergoing genetic adaptation in response to host and environmental pressures.

Keywords: Group B *Streptococcus*, Clonal complex, Core gene multi-locus sequence type, One health

PG-14

Comparative Analysis of Three Whole Exome Sequencing Kits for Clinical Applications

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Background: Whole Exome Sequencing (WES) platforms have become a standard in genetic research, with significant advancements in recent years. This study compares the performance of three leading WES kits—IDT, Agilent, and Twist—evaluating their genomic coverage, accuracy in detecting genetic variations, and operational workflow efficiency. These factors are crucial for informed decision-making in genomic diagnostics and personalized medicine.

Methods: DNA from eight samples, including NA12878, OncoSpan [HD827], and six clinical samples from whole blood, was utilized. We followed the manufacturers' protocols for library preparation. Following

quality control with Qubit and TapeStation, we performed sequencing on the Illumina NovaSeq6000 platform using the SP kit, which facilitates 300 cycles of 150 base pairs each. The resulting FASTQ files were processed and analyzed with the GATK best practice for germline short variant discovery. All figures were generated by using Python (3.8.18).

Results: We detailed the experimental conditions, and outlined the bioinformatics pipelines. Operational efficiency was assessed by examining laboratory processing times and protocol complexities. Initial analyses, focusing on quality control metrics, revealed some significant differences among the kits. Performance metrics for NA12878, presented the recall, precision, and F1 score for both INDEL and SNP. Additionally, we compared the Variant Allele Frequency (VAF) from OncoSpan with the VCF file from Horizon Discovery to assess accuracy. Lastly, six clinical samples, previously confirmed in our study using the TruSight One Expanded (TSOE) panel, were reanalyzed by estimating VAF values. The results showed that each kit demonstrated distinct advantages and disadvantages across various metrics.

Conclusions: Our findings offer valuable insights for researchers and clinicians in selecting a suitable WES kit, focusing on performance and efficiency to support genomic diagnostics and personalized medicine. Although cost is an important consideration, this study focuses on performance-based decision-making in genomic research and clinical practice. These insights facilitate informed choices, advancing genomic diagnostics and personalized medicine.

Keywords: Whole Exome Sequencing, IDT, Agilent, Twist, Single Nucleotide Polymorphism, Insertions and Deletion

PG-15

Blood Lead Levels and miR-126 and miR-155 in School Going Children of Jodhpur

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Background: Pb poisoning is a major but preventable public health issue, particularly in developing countries. The signs and symptoms of Pb toxicity are variable with multiple system involvement in adults and children. Circulating miRNAs miR-126 and miR-155 possibly play a role in moderating oxidative stress and inflammation, which have been reported as the mechanisms underlying Pb toxicity. This study aimed to investigate the association of miR-126 and miR-155 with blood Pb levels (BLLs) in school going children.

Methods: The study group comprised 100 environmentally Pb-exposed school going children from Jodhpur, Rajasthan. Informed consent was obtained from the parents. Blood Pb levels (BLLs) were estimated by atomic absorption spectrometry (Thermo Fischer iCE3500). Samples were run in duplicates for metal estimation after ensuring quality control (Recipe, Germany). The expression of miR-155 and miR-126 was assayed using RT-PCR.

Results: The median BLL was 3.62 µg/dL (1.64–12.3 µg/dL). The

expression of miR-126 and miR-155 was higher in the children with BLL >3.5 µg/dL than in the others. The expression of miR-155 and miR-126 was upregulated with a fold change of 2.7 and 2.5, respectively. In the children with BLL > 5 µg/dL, a significantly positive correlation was found between miR-126 expression and their BLLs ($r_s=0.632$, $P=0.003$) as well as between miR-126 and miR-155 ($r_s=0.855$, $P\leq 0.001$).

Conclusions: This study is the first to report the expression of miR-126 and miR-155 in environmentally Pb-exposed children of Jodhpur. MiR-126 is a potential predictive biomarker of Pb toxicity in children. Nevertheless, additional studies with a larger sample size are required to validate the association between environmental exposure to Pb and expression of these miRNAs and elucidate the functions of these miRNAs in Pb-induced alteration in immune response.

Keywords: Lead, miR-126, miR-155

PG-16

Identification of a Novel Frameshift Variant in the *TTN* Gene in a Patient with Dilated Cardiomyopathy

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Dilated cardiomyopathy (DCM) is a prevalent cause of heart failure characterized by ventricular dilation and impaired systolic function. The *TTN* gene, encoding the sarcomeric protein titin, is a major genetic contributor, responsible for 20% of DCM cases. Titin is crucial for maintaining the structural integrity and elasticity of the cardiac sarcomere. Loss-of-function variants in the A band region of the *TTN* gene are strongly associated with DCM. Herein, we present a case of DCM caused by a novel frameshift variant in the A band of the *TTN* gene. A 51-year-old woman presented with exertional dyspnea and a rapid 20 kg weight gain over three months. Laboratory tests showed elevated pro-BNP levels (2,426.0 pg/mL). Transthoracic echocardiography indicated severe global hypokinesia, a left ventricular ejection fraction of 23.2%, cardiac enlargement, and a small pericardial effusion. Genetic analysis identified a novel frameshift variant in the *TTN* gene: c.70236dup (p.Phe23413Ilefs*6). This variant is not found in population databases and has not been previously reported. It is located in the A band region of the *TTN* gene, where numerous DCM-associated loss-of-function variants are located. According to the 2015 ACMG/AMP guidelines, this variant was classified as a likely pathogenic variant, leading to the diagnosis of DCM caused by the *TTN* gene variant. This case highlights the significance of comprehensive genetic testing in uncovering the underlying mechanisms of cardiomyopathies, while also emphasizing the importance of the A band in maintaining sarcomere integrity and function. Identification of this novel *TTN* variant contributes to expanding the current understanding of the genetic landscape of DCM.

Keywords: Dilated cardiomyopathy, *TTN*, Genetic testing

PG-17

Current Practices, Policies, and Perspectives on Reporting Incidental and Secondary Findings from Genetic Testing

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Background: As whole genome/exome sequencing (WGS/WES) expands from research to clinical practice, reviewing existing data to inform policy development regarding incidental and secondary findings (IF/SF) is crucial. Different countries have varying stances on reporting IF/SF reporting, necessitating a comprehensive review of current practices, policies, and perspectives.

Methods: To understand the current status of next-generation sequencing (NGS)-based genetic testing in South Korea, we analyzed data from genetic testing institutions registered with the Korea Disease Control and Prevention Agency and insurance claims data from the Health Insurance Review and Assessment Service (HIRA) over the past three years. Additionally, we surveyed clinical geneticists and molecular geneticists in South Korea on their opinions regarding IF/SF reporting in clinical genome sequencing and conducted a literature review of PubMed-indexed articles on country-specific policies, ethical and practical considerations, and stakeholder perceptions related to IF/SF reporting.

Results: As of December 2023, there were 234 genetic testing institutions in South Korea. Data from the HIRA indicate a steady annual increase in NGS-based gene panel tests, with a 161% rise from 12,212 in late 2019 to 19,638 in early 2022. However, WGS/WES is not yet covered by the National Health Insurance and is often conducted as research tests in tertiary hospitals. The literature review revealed that most countries agree on reporting only causative variants with high penetrance, severity, and medical actionability as IF/SF. However, considerable variations exist in whether countries actively search for IF/SF during genetic testing. In the United States, reporting medically actionable pathogenic variants is considered an ethical obligation, whereas Europe prioritizes patient autonomy and adopts a more cautious approach. Ethical considerations include beneficence, non-maleficence, autonomy, and justice. Stakeholder perspectives on SF reporting are generally positive, with some differences among groups. Our survey results emphasized that practical considerations for SF reporting include pre-test consent, counseling, and specific reporting methods. Difficulties in practical implementation highlight the need for consensus guidelines, education for healthcare providers, and systematic support, including genetic counseling.

Conclusions: Policies for reporting IF/SF in clinical genome sequencing need to balance medical utility, patient autonomy, and ethical considerations. Consensus guidelines, education, and support are needed to effectively integrate IF/SF reporting into clinical practice, considering each country's healthcare system and stakeholder perspectives.

Keywords: Incidental finding, Secondary finding, Policy, WGS, WES, NGS

PGL-01

Improving Patient Wait Times in Outpatient Phlebotomy Units Through Machine Learning and Scheduling Optimization

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Background: Patient wait times in outpatient phlebotomy units can significantly impact both patient satisfaction and operational efficiency. This study combines machine learning and scheduling optimization to mitigate wait times in these units.

Methods: By harnessing available data and employing machine-learning algorithms, we developed a predictive model capable of accurately forecasting patient wait times. Additionally, optimization algorithms were used to determine the most efficient patient scheduling solution by considering relevant constraints.

Results: Integrating machine learning predictions with optimization algorithms yielded an efficient patient scheduling system. The predicted and actual wait times were closely aligned with each other, demonstrating the accuracy and reliability of the developed models. Moreover, using simulation data from optimization algorithms effectively minimized the wait times and enhanced the overall throughput in outpatient phlebotomy settings.

Conclusions: The proposed approach establishes a robust framework for optimizing patient scheduling, leading to reduced wait times and improved operational efficiency. These findings highlight the potential of leveraging machine learning to enhance healthcare service delivery and patient experience.

Keywords: Wait times, Phlebotomy, Machine learning, Scheduling, Optimization

PGL-02

Using Deep Learning to Corroborate the Predictive Value of Cell-in-Cell structures in Urine Cytology

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Background: Increased instances of cell-in-cell (CIC) structures have been positively correlated with higher grades of urothelial carcinoma in urine cytology specimens. However, current research assesses only hundreds of images of CIC samples, which is an insufficient number of cell images to fully power the statistical confirmation of this positive correlation. Even in high-grade urothelial carcinoma, CIC structures make up only approximately 0.5% of all cells in a sample, limiting the ability to collect sufficient images manually; therefore, a method is needed to automate CIC structure identification. We used deep learning-based computer vision to automate CIC counting and employed this algorithm to empirically test the correlation between CIC structure instances and higher grades of urothelial carcinoma.

Methods: An Aperio AT2 scanner was used to digitize 2,405 archival ThinPrep glass slides with lower and upper urinary tract specimens at $\times 40$ magnification with 1 Z-plane. Scans were diagnosed using the Paris system for reporting urinary cytology, and existing software detected cells in the scans and generated 10,000,000 cell-level images. Eight million images were used for training, and 2,000,000 were withheld for empirical testing. A pathologist classified training images as CIC or non-CIC. CIC case classification required the following: at least two cells, both cells are non-degraded urothelial cells, nuclei of both cells are visible, overlap of at least 50% of cell area, and cytoplasmic distinction between the cells. These samples were used to train a convolutional neural network (VGG16). The remaining 2,000,000 samples were passed through the trained network for our empirical testing.

Results: A total CIC prevalence of 0.0196% was predicted across the 2,000,000 validation set images. The mean number of CIC cases per slide was positively correlated with the grade of urothelial carcinoma. Specifically, the predicted mean CIC cases per slide were 0.3, 0.5, 1.8, and 6.5 for each ascending grade of urothelial carcinoma. of the higher grades, 27 of 44 cases (63.4%) had at least one CIC case, whereas 140 of 698 (20.0%) had at least one CIC case in the lower grades.

Conclusions: Our results corroborate the positive correlation between the number of CIC structures and the grade of urothelial carcinoma. By employing deep learning, we expedited the annotation process of millions of images providing a sufficient sample size to power the confirmation of the positive correlation between CIC structures and grade of urothelial carcinoma.

Keywords: Machine learning, Deep learning, Urine, Cytology, Cancer

PGL-03

The Capability of Attenuated Total Reflectance-Fourier Transform Infrared Spectrometer for Protein Characterization in Artificial Proteinuria Samples

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Background: Urine protein is a vital clinical marker for the diagnosis and prognostication of several diseases, particularly renal diseases. Routine laboratory tests can detect proteinuria but have limitations in classifying protein types. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy is a non-destructive method based on vibrational bonding currently being explored as a potential medical tool or point-of-care test (POCT) to reveal internal molecular composition. This study aimed to develop an ATR-FTIR spectrometer for protein characterization in protein-spiked urine samples.

Methods: Albumin and hemoglobin were spiked into pooled urine to mimic normoproteinuria (less than 30 mg/g), microproteinuria (30–300 mg/g), and macroproteinuria (greater than 300 mg/g), and 500 μ L of protein-spiked urine underwent preconcentration and single-time purification through 10-kDa ultrafiltration. The spiked urine was then deposited on an ATR crystal, air-dried, and measured using a portable ATR-FTIR spectrometer. The spectral characteristic patterns and areas under the curve (AUC) were analyzed.

Results: Unspiked, albumin-spiked, and hemoglobin-spiked samples at the normal level showed similar patterns. However, the spectral patterns of micro- and macro-level spiked samples were slightly different from those of the unspiked urine. Both albumin- and hemoglobin-spiked exhibited peaks at 1,660 and 1,542 cm^{-1} , which are the main components of protein. Specifically, albumin-spiked urine showed a sharp peak assigned to amide II at 1,510 cm^{-1} . Hemoglobin spiked at the macro level displayed a set of shoulder peaks assigned to amides at 1,630, 1,510, and 1,460 cm^{-1} , whereas the micro level peak shifted from 1,322 to 1,316 cm^{-1} . Finally, unspiked urine showed prominent peaks assigned to amide I at 1,630 and 1,597 cm^{-1} . Peak patterns can be used to characterize protein components. Moreover, the AUCs increased significantly in correlation with their levels in both albumin- and hemoglobin-spiked samples, indicating their potential utility in monitoring disease progression.

Conclusions: ATR-FTIR spectroscopy is a potential POCT for the detection of abnormal urine samples. However, more information is required to determine protein concentrations using multicomponent analysis for prognostic purposes in further clinical applications.

Keywords: Kidney disease, Proteinuria, Spiked urine, ATR-FTIR spectroscopy, POCT

PGL-04

Point-of-Care vs. Laboratory Interleukin-6 Testing: Efficacy in Critical Care Settings

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Background: Interleukin-6 (IL-6) is an acute-phase cytokine released by activated immune cells. Although serum IL-6 levels permit discrimination of sepsis and independent prediction of sepsis with high sensitivity and specificity, the traditional enzyme-linked immunosorbent assay (ELISA) to determine IL-6 concentration is time-consuming, making it unsuitable to guide urgent interventions in critically ill patients. To address this limitation, a point-of-care test (POCT) was developed. This study aimed to compare the performance of IL-6 determination using the newly developed POCT with that of ELISA in identifying critically ill patients admitted to the intensive care unit (ICU).

Methods: IL-6 levels in 40 ICU samples, ranging from 1.29 to 1,361.20 pg/mL, with both negative and positive blood cultures were analyzed using the Finecare™ FIA system utilizing fluorescence immunoassay for quantitative determination. Results were compared with those obtained using an Elabscience® Human IL-6 ELISA kit, operating on the Sandwich-ELISA principle with Sunrise-Basic Tecan.

Results: Finecare™ slightly overestimated IL-6 levels compared to those measured using ELISA. Method comparison analysis revealed a strong statistically significant positive linear correlation, with $r=0.8426$ (95% confidence interval [CI]: 0.7157–0.9156, $P<0.0001$). Linear regression analysis showed a strong correlation between these two methods, with a slope of 0.1416 (95% CI: 0.1110–0.1721) and a y-intercept of 27.19 (95% CI: 13.83–40.56). The residual plot showed data randomly scattered around the zero line, indicating a satisfactorily linear relationship between the two methods.

Conclusions: Despite the discrepancy in absolute values, these findings suggest that the Finecare™ FIA device measures IL-6 levels comparable to the ELISA method, especially within the clinically important IL-6 cutoff range for discriminating patients with sepsis and septic shock, making it a promising tool for rapid point-of-care testing in critical care settings.

Keywords: Interleukin-6, Point-of-Care, ELISA

PGL-05

Nanoenzymatic Biosensor of Lemon Peel Gold Nanoparticles and Copper Oxide for Glucose Estimation in Saliva

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Background: Glucose is considered an essential macronutrient for properly functioning of the brain and muscles. However, glucose monitoring plays an important role in the management and diagnosis of various chronic diseases such as diabetes. Although precise methods of blood glucose level monitoring are available, patient discomfort and invasive methods highlight the need for a non-invasive yet effective method. Considering this, glucose monitoring in saliva could propose an alternative strategy but the glucose level in saliva is very low. The current work was envisaged to develop a simple-to-use, cost-effective, and sensitive nonenzymatic type of electrochemical glucose sensor based on biofabricated gold nanoparticles (AuN) along with copper oxide nanoparticles (CuON) synthesized with the wet chemical method using a screen-printed carbon electrode (AuN-CuON-SPCE).

Methods: The lemon peel extract and an Au precursor agent were used to synthesize colloidal AuN. Amperometric tests were used to measure the response and sensitivity of the developed sensor. The efficiency of the sensor was detected by glucose estimation in human saliva, indicating the potential for noninvasive glucose monitoring.

Results: AuN with a size range of 35–40 nm were obtained. Amperometric tests showed the linear response of the sensor towards glucose concentrations ranging from 2 μM to 385 μM with 232.43 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ of sensitivity. The sensor also exhibited stability and high glucose selectivity in the presence of other biomolecules and significant reproducibility.

Conclusions: Dual amplification schemes by AuN and CuO nanomaterials led to the high sensitivity of the sensor and exhibited good catalytic activity towards glucose. The current research progresses the development of non-invasive monitoring devices and demonstrates that AuN-CuON-SPCE nonenzymatic sensor could be utilized as a beneficial approach for noninvasive glucose monitoring in saliva and better management of diabetes. Therefore, this approach has a strong potential for glucose monitoring in saliva despite the low concentration.

PGL-06

An Easy, Rapid, and Reliable Molecular Diagnostic System for Home Test

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Background: Although lateral flow assay (LFA)-based rapid antigen tests are widely used as non-face-to-face home tests owing to the COVID-19 pandemic, they have low sensitivity and accuracy, which should be improved.

Methods: We developed a molecular diagnostic-based home test sys-

tem that combines loop-mediated isothermal amplification (LAMP) and LFA. Our detection system consists of an all-in-one cartridge and a portable device, called GenHome®. Nucleic acid extraction, isothermal amplification, and LFA were performed in an all-in-one cartridge, and the detection results were visually checked on the strip. The test reagent can be precisely controlled using an easy and simple manual operation inside the cartridge.

Results: Our detection system can be used to perform various steps ranging from sample extraction to result analysis within 30 minutes, and it showed a limit of detection (LoD) of 2.5E+00 pfu/mL for the influenza virus with no cross-reactivity with other pathogens.

Conclusions: We anticipate that GenHome will provide a universal platform for an easy, rapid, and reliable molecular diagnostic home test for emerging infectious diseases.

Keywords: Loop-mediated isothermal amplification, Point-of-care test, Influenza, Lateral flow assay, Molecular diagnosis

PGL-07

Survey for Utilization and Quality-Related Issues of Handy Test and Point-of-Care Tests in Korea

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Background: The development and distribution of various simple lateral flow assays (handy tests) and point-of-care (POC) tests are increasing. Recently, the use of handy or POC tests for a variety of test items has been increasing in various clinical settings. We aimed to investigate the utilization patterns, frequency of quality problems, and factors affecting quality problems in secondary- or tertiary-care hospitals in Korea.

Methods: A questionnaire survey was sent to 334 laboratory medicine specialists working as managers in the Department of Laboratory Medicine (LM), accredited by the Laboratory Accreditation Program of the Laboratory Medicine Foundation in Korea. The questionnaire, in an Excel file, included a list of handy or POC tests used at their institution, along with each analyte, reagent name, measurement method, department performing the test, POC committee management, and quality-related issues.

Results: A total of 75 institutions responded to the survey from February 10, 2024 to March 30, 2024. Data were collected from 942 handheld POC test systems. Among them, 581 cases (61.7%) were performed only in the LM, and 361 cases (38.3%) were performed in clinical sites (including 283 cases only in clinical sites and 78 cases in both clinical sites and LM). Among the 361 cases performed at clinical sites, 83.1% (300/361) were managed by a POC committee. Instrumental measurements were performed in 53.8% (507/942) and visual readings in 46.2% (435/942) of the patients. Quality-related issues occurred in 35.7% (336/942) of cases, of which 90.9% were

resolved. Multiple responses were allowed for issue types, and 682 types were collected: internal quality control (23.8%), external quality control (19.6%), reading process (19.1%), results data (13.5%), complaints from clinicians (10.6%), and parallel tests between reagent lots (9.7%). Quality issues occurred more often in cases performed only in LM (37.8%) than in clinical sites and were higher in cases managed by a POC committee (31.8%) than in cases not managed (13.9%).

Conclusions: Testing systems classified as handy or POC tests were used more frequently at LM than at clinical sites in Korean hospitals. Quality-related issues can be identified and improved more frequently when performed in the LM or managed by a POC committee.

Keywords: Handy test, Point-of-care test, Survey, Quality, Lateral flow

PGL-08

Effects of Welding Exposure on Semen Parameters among Sailors in Sri Lankan Navy

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Background: The metal-joining process (welding) used in the fabrication industry is known to adversely affect the male reproductive system. The degree of hazard depends on the composition, concentration, and duration of exposure. This study aimed to identify the relationship between occupational welding exposure and the semen parameters of welders in the Sri Lankan Navy.

Methods: Semen samples were collected from welders (N=44) and non-welders (N=44) in the Sri Lankan Navy after obtaining informed consent. The sociodemographic and lifestyle factors of both groups were recorded. Seminal fluid analysis (SFA) was then performed.

Results: The mean ages of the welders and non-welders were 31 and 27 years, respectively. The mean service duration was higher (9.5 years) for welders than for non-welders (5 years). The median durations to the first child after marriage for welders and non-welders were 18.5 and 11 months, respectively. Welders working in shipyards/on-board ships are more exposed to welding emissions than those working in fabrication workshops. Of the welders and non-welders, 16% and 11%, respectively, had abnormal sperm concentrations. Of all welders, 11% had abnormal sperm motility. In contrast, none of the non-welders exhibited abnormal sperm motility. The total duration of welding exposure was negatively correlated with sperm concentration ($r=0.4$, $P=0.007$).

Conclusions: Welding significantly affected sperm concentration and motility, whereas long-term exposure negatively affected sperm concentration. Further studies are warranted to identify the relationship between semen parameters and fertility.

Keywords: Welding exposure, Semen parameters, Effects of welding

PGL-09

Elevated Erythrocyte Sedimentation Rate Levels in Patients with Chronic Obstructive Pulmonary Disease Exacerbations in the Previous Year

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Background: Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitation, which is usually progressive and associated with an enhanced chronic inflammatory response to noxious particles or gases in the airways and lungs. Patients with COPD often experience exacerbations in which breathlessness and sputum production worsen acutely, leading to emergency room visit, prescription of short-acting bronchodilators, and hospitalization. This study aimed to investigate the association between inflammatory biomarkers and COPD exacerbations.

Methods: Ninety-two participants with a post-bronchodilator FEV₁/FVC ratio <0.7 were included. COPD exacerbations were defined as instances in which patients with COPD have been hospitalized in the previous year or were prescribed short-acting bronchodilators. The severity of airway flow limitation in COPD was classified according to the Global Initiative for Chronic Obstructive Lung Disease guidelines. White blood cell count, erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP) levels were measured as inflammation biomarkers. Personal information, including age, body mass index, and smoking status, was obtained using a structured questionnaire.

Results: The mean ESR levels were higher in the exacerbation group than in the non-exacerbation group (10.43 mm vs. 5.93 mm, $P=0.018$). ESR levels were positively correlated with CRP levels ($P<0.05$) and negatively correlated with %FEV₁ predicted ($P<0.05$). ESR levels were associated with age ($\beta=0.241$, $P=0.023$), current smoking ($\beta=0.254$, $P=0.018$), and COPD exacerbation ($\beta=0.286$, $P=0.005$).

Conclusions: These results showed that increased ESR levels were related to exacerbations in patients with COPD.

Keywords: Chronic obstructive pulmonary disease exacerbation, Erythrocyte sedimentation rate, Inflammatory biomarkers

PGL-10

Correlation between Lipid Profile and Anthropometry in Healthy Individuals of Eastern Mongolia

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Background: Obesity is linked to over 200 complications, including stroke, increased cholesterol levels, type 2 diabetes, hypertension, and atherosclerosis. Overweight and obesity rates have surged in developing countries, which is closely related to cardiovascular risk factors. We aimed to correlate specific anthropometric variables with lipid parameters in healthy participants and identify the anthropometric measure that best reflects altered lipid profiles.

Methods: We included 857 participants in this single-center, cross-sectional study, collecting anthropometric measurements such as height, weight, waist circumference (WC), and hip circumference (HC) using standard procedures on the same morning as the blood sample collection. We calculated the body mass index (BMI) and waist-to-hip ratio (WHR), defining the former as weight in kilograms divided by height in meters squared. We considered participants with a body mass index (BMI) of <25, 25.0–29.9, and ≥ 30 kg/m² as normal, overweight, and obese, respectively. WHR was derived from the WC-to-HC ratio. We estimated the lipid parameters, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) cholesterol, very LDL (VLDL), and high-density lipoprotein cholesterol, using a standard enzymatic method by Roche Diagnostics, Germany.

Results: The BMI values displayed no significant differences between the two age groups, though older participants exhibited a significantly higher WC. TG and VLDL levels were significantly higher in the older age group. We observed a weak correlation between the BMI and lipid parameters. Among the anthropometric variables, WC demonstrated the strongest correlation with the lipid parameters. The lipid parameter mean values did not significantly differ between the BMI <25 and ≥ 25 groups.

Conclusions: WC is a simple and reliable measure that best reflects the lipid profile. In developing countries like Mongolia, where assessing cardiovascular risk factors such as body fat saturation and lipid profile could be challenging, WC proposes an effective proxy.

Keywords: Body mass index, Eastern Mongolia, Lipid profile, Waist circumference

PGL-11

Investigating the Association between Muscle Elasticity and Muscle Hemodynamics Using Musculoskeletal Ultrasonography

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Background: There has been increasing interest in local vibration (LV) training as an alternative stretching method. Musculoskeletal ultrasonography (MUS) is effective for qualitatively assessing muscle stiffness and hemodynamics. However, the influence of vibrations on muscle

elasticity remains unclear. This study aimed to explore the relationship between muscle elasticity and muscle hemodynamics induced by LV using MUS.

Methods: Fourteen healthy participants underwent LV for 1 minute, with a frequency of 30 Hz and an amplitude of 3 mm. The bilateral medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus muscles (SOL) were evaluated using three-dimensional shear-wave elastography (3D-SWE) and power Doppler MUS. Each muscle was assigned a subjective power Doppler score ranging from 0 to 3, and the power Doppler index (PDI) was calculated as the sum of the scores for all evaluated muscles.

Results: The PDI following LV was significantly higher than before the intervention ($P<0.01$). Conversely, the 3D-SWE values for the medial and lateral heads of the gastrocnemius were significantly lower after LV compared to before the intervention ($P<0.05$).

Conclusions: This study suggests MUS measurements, such as 3D-SWE and power Doppler, could serve as valuable tools for assessing muscle conditions.

Keywords: Musculoskeletal ultrasonography, Muscle Stiffness, Three-dimensional shear-wave elastography

PGL-12

Predicting COVID-19 Patient Outcomes with Hematologic and Biochemical Laboratory Assays

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Background: Although most patients with coronavirus disease 2019 (COVID-19) have mild-to-moderate clinical symptoms, a small percentage develop potentially severe pneumonia. Therefore, early prediction of disease severity is crucial for infected patients. This study evaluated laboratory assay results related to COVID-19 severity.

Methods: This study included patients who received both COVID-19 real-time polymerase chain reaction and complete blood count tests on a single day at Gangwon National University Hospital between December 2020 and March 2023 (N=8,396). The results from routine and research hematologic (XN-10, Sysmex, Japan) and chemical assays (Hitachi LABOSPECT 008AS, Hitachi High-Technologies Corporation, Japan) were evaluated. COVID-19 and non-COVID-19 patients were matched at a 1:5 ratio using propensity scores based on age and sex. Prognostic factors for mortality were identified using conditional logistic regression analysis. Additionally, proportional odds logistic regression analysis for ordered response levels of severity (out-patient, hospitalized, or deceased) among the COVID-19 patients was performed.

Results: Among 8,149 patients, 603 COVID-19 and 3,015 of 7,546

non-COVID-19 patients (per 1:5 matching) were analyzed across 72 assay variables. The mortality-associated variables that were identified across all patients included the white blood cell (WBC) count, dispersion of the NE-FSC signal of the neutrophils (NE-WZ), monocyte metabolic activity and/or cell membrane permeability (MO-Y), lymphocyte nucleus irregularity, cytoplasmic granulation and/or vacuolization (LY-X), lymphocyte size or volume (LY-Z), lateral scattered light distribution width of the LYMPH area on the WBC differential (WDF) scattergram (LY-WX), fluorescent light distribution width of the LYMPH area on the WDF scattergram (LY-WY), platelet count, platelet lymphocyte ratio, and total protein, albumin, blood urea nitrogen, and alkaline phosphatase levels. COVID-19 severity was associated with the WBC count, lateral scattered light distribution width of the MONO area on the WDF scattergram (MO-WX), lymphocyte count, LY-Z, LY-WY, and hemoglobin, platelet, plateletcrit, sodium, albumin, and aspartate aminotransferase (AST) levels. COVID-19 severity correlated negatively with the WBC count, MO-WX, LY-WY, plateletcrit, and AST level and positively with the other variables.

Conclusions: This study showed that significant changes in hematologic and biochemical parameters correlated with the severity of COVID-19. These findings are of great significance for the clinical care and prognosis of COVID-19 patients. The hematologic features of the infected patients revealed both quantitative and qualitative changes in leukocyte populations. According to our data, laboratory results that include hematologic research parameters at the time of COVID-19 diagnosis may offer insights into the disease severity.

Keywords: COVID-19, Hemocytometry, SARS-CoV-2, Cell population

PGL-13

Reduced Occurrence of Hepatocellular Carcinoma in Hepatitis C Patients Following Direct-acting Antiviral Therapy

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Background: Direct-acting antivirals (DAAs) have transformed the management of chronic hepatitis C virus (HCV)-related diseases, achieving high rates of sustained virological response (SVR) even in advanced cirrhosis with minimal contraindications and low rates of adverse events. This prospective study aimed to evaluate the incidence of and risk factors for HCC following DAA therapy in patients with HCV-related advanced fibrosis (F3) and cirrhosis (F4).

Methods: The incidence of HCC was assessed in 973 patients with HCV-related F3 and F4 liver disease who were treated with DAAs. Patients were prospectively followed up for up to 48 months at a single referral center and compared with historical controls. The risk factors associated with incident HCC were determined.

Results: The crude incidence rate of 2.8/100 person-years was significantly lower than that of a similar historical cohort (5.9/100 per-

son-years). The risk of developing HCC was higher in patients with cirrhosis (F4 vs. F3, AHR: 3.79) and in those with treatment failure (vs. achieving SVR, AHR: 3.47). Decompensated cirrhosis, a platelet count $<100 \times 10^3/\text{mL}$, and elevated AFP levels were identified as independent risk factors for developing HCC.

Conclusions: The incidence of HCC was markedly lower in patients with HCV-related advanced fibrosis and cirrhosis treated with DAAs than in untreated controls.

Keywords: Direct-acting antiviral, HCV, HCC

PGL-14

Comparison of Tukey and Healy Methods for Identifying Outliers in Proficiency Testing Data

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Background: It is crucial to identify outliers while interpreting proficiency testing (PT) results to assess their validity. The Korean Association of External Quality Assessment Services uses the Tukey method to detect outliers in quantitative measurements, and in some cases the median, first quartile, and third quartile values were equal, achieving an interquartile range (IQR) of 0. In this situation, all values except the median are classified as outliers, resulting in excessive exclusion of data points. Thus, we investigated an alternative method for identifying outliers.

Methods: We compared the Tukey method with the Healy method, which is used by national PT labs in the United Kingdom. The Healy method trimmed the lowest and highest 5% of data, then used the trimmed mean transformed by the natural logarithm and linear estimate of SD to identify outliers. The blood gas analysis (BGA) program, which is a representative PT program with an IQR of 0 using the Tukey method, was selected as the simulation target. The BGA PT program in 2023 included nine analytes and was conducted twice annually with three samples per round. According to the instrument and reagent manufacturers, the nine analytes can be classed into 1,248 subcategories. After applying Tukey's method to exclude outliers, the SD of the subcategories was 0 in 97 cases. Among these, 68 involved ≥ 10 participating laboratories. The IQRs of 66 subcategories (29 in the first round and 37 in the second round) were 0. In one case, all results from the participating laboratories were identical. Finally, the differences in outlier percentages across the 65 subcategories between Tukey's and Healy's methods were compared.

Results: After excluding outliers using the Healy method, all participants showed identical results in 31 of 65 subcategories, indicating no differences in outlier detection between the Tukey and Healy methods. The Tukey method identified outliers in $28.2 \pm 8.0\%$ of the

remaining 34 subcategories, whereas the Healy method found outliers in $3.9 \pm 4.5\%$. Outliers were reduced by an average of 86.4% using the Healy method compared to the Tukey method (paired t-test, $P < 0.001$).

Conclusions: The Healy method can be used to address the limitations of Tukey's method in outlier detection. Appropriate outlier detection methods, tailored to the specific characteristics of each PT program, are recommended.

Keywords: Outlier, Proficiency testing, Interquartile range, Blood gas analysis, Korean Association of External Quality Assessment Service

PGL-15

Cooperation of Local Biobanks for In Vitro Diagnostic Devices Development

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Background: *In vitro* diagnostic devices (IVD) are essential medical devices for the early diagnosis and effective treatment of diseases. Human-derived materials are crucial research resources for the development of IVDs; however, it is not easy for companies to secure and utilize the required quantities for product approval in a timely manner. Therefore, to help IVD development companies in the Daejeon area overcome these challenges and continue competitive R&D, Daejeon Metropolitan City established a collaborative support system involving biobanks in Daejeon-area hospitals. In this context, we report a case in which, through the cooperation of biobanks at Daejeon Eulji Medical Center and Chungnam National University Hospital, infrastructure was established to quickly allocate the specimens needed by companies, and an integrated information database was developed for efficient information management.

Methods: With financial support from Daejeon Metropolitan City, the consortium of Daejeon Technopark and Biobanks (Daejeon Eulji Medical Center, Chungnam National University Hospital) established the Daejeon Biobank and created a unified operating system through a joint IRB. They identified corporate demands through the Allocation Review Committee and preliminary consultations, collected corresponding specimens, and established a system to verify the suitability of previously collected specimens through additional tests. Additionally, they created a joint standard operating procedure (SOP) for biobanks and established an electronic system for a cooperation network with bio-healthcare development institutions.

Results: Daejeon Metropolitan City established the Daejeon Biobank in collaboration with Daejeon Eulji Medical Center and Chungnam National University Hospital. A one-stop system was established to quickly review and provide high-quality specimens required by special zone businesses (companies). Additionally, an SOP was created

to systematize the operations of the biobank and ensure the quality of the stored human-derived materials. New clinical data are collected continuously to enhance specimen utilization. Information on the specimens produced by individual biobanks was integrated and linked through an electronic system and managed as a standardized specimen distribution management system. This allows companies to secure specimens and conveniently access specimen allocation information by searching specimen resources and clinical information.

Conclusions: The Daejeon Biobank, as a model of collaboration between companies, local governments, and hospitals, aims to support local IVD companies. This is expected to benefit the development of IVD companies both within and outside the region.

Keywords: Biobank, Daejeon biobank, In Vitro Diagnostic Device, Institutional review boards, Specimen distribution management system

PGL-16

Development of Biobank Management Program

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Background: Biobanks are crucial repositories of human resources that collect, process, and store valuable human-derived materials and research information from donors for use in biomaterials. Therefore, the operating system of a biobank must be designed to ethically and reliably collect and store high-quality specimens. This study addresses the design and utilization of biobanks characterized by systematic collection and rapid resource identification. To maximize the utilization of biobanks, we aimed to establish a system and develop an operational roadmap for building a human resource information management system.

Methods: The Daejeon Eulji Medical Center Biobank developed a program in collaboration with Smart GENE to manage human-derived materials, as well as epidemiological and clinical information. Based on an analysis of user requirements and legal regulations, functionalities were developed to input and search for the data necessary for the collection, storage, quality control, and distribution of human-derived materials. Data management features were implemented to handle the anonymization codes for donors, names, sex, age, registration date, participation date, type of resource, quantity of resources, volume of resources, and clinical information.

Results: We developed the Eulji Biobank Information System (EBIS) program, an anonymized data-based human resource information management system, to manage patient data extracted from Daejeon Eulji Medical Center's OCS/EMR system using an Oracle DB-based patient information anonymization process. This enables the integration of three databases: internal, statistical, and human-derived. Pa-

tient data, such as diagnoses, prescriptions, tests, and records collected from the OCS/EMR system, were aggregated in the statistical DB using unique identifiers generated through the anonymization process. The anonymized data were sent to the biobank's human resource information management system for use in the specimen management program. The EBIS program includes features for managing clinical information (basic epidemiological information, family history, past medical history, treatment information, and progression information), electronic anonymization, barcode labeling, specimen check-in/out, storage location, inventory management, temporary storage sharing, collection information management, resource status management, and convenience of distribution processing. Since the implementation of the system, the time required for rapid resource identification has reduced, and it has become possible to search for clinical information suitable for research purposes.

Conclusions: The EBIS program, the biobank management system of the Daejeon Eulji Medical Center Biobank, aims to contribute to optimal outcomes for domestic researchers, institutions, and IVD companies by providing high-quality resources known for their excellence and reliability.

Keywords: Biobank, Human resource information management system, EBIS programs

PGL-17

Establishing Big Data Quality Evaluation and Certification Process in Laboratory Medicine: Insights and Standards from Pilot Studies

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Background: In the era of big data, data are considered one of the most valuable healthcare resources. They enable informed decision-making, improve patient outcomes, and facilitate medical research and innovation, thus making data quality essential. The field of laboratory medicine has been continuously producing large amounts of real-time data, and studies on data quality assessment have been conducted for several years. Big data quality certification aims to establish standards and practices that guarantee the reliability, accuracy, and applicability of large datasets. This pilot study investigates the feasibility and effectiveness of implementing a certification process for big data quality.

Methods: Data quality evaluations were conducted on the creatinine assay across five hospitals using reagents from five manufacturers. To qualify for data certification, applicants were required to perform tests, submit specific data, and provide raw data. Four metrics were used to assess data quality: accuracy, credibility, traceability, and interoperability. The accuracy metric included bias, imprecision, total error, and linearity. The results of 5–7 repeated measurements of a specimen were analyzed at 12 different levels. Tolerance levels for data accuracy were set according to the analytical quality specifications based on biological variation. This study used the latest data from the European Federation of Clinical Chemistry and Laboratory Medicine. The certification rating included five tiers: Bronze, Silver, Gold, Platinum, and Diamond, ranging from lowest to highest, respectively.

Results: All five participating laboratories met the data quality standards for credibility, traceability, and interoperability. In the accuracy assessment of 10 creatinine assay results, the acceptance rates were 100.0, 90.0, and 10.0% for the minimum, desirable, and optimum criteria, respectively. Only one assay satisfied the optimum criteria for bias but did not meet the criteria for imprecision. Therefore, eight assays received Platinum certification, and two assays received Silver because their accuracy satisfied only the minimum criteria.

Conclusions: In this pilot study, sampling techniques were employed to evaluate the dataset and make inferences regarding the overall quality of big data. By establishing a data quality evaluation process and specifying standards, quality values were calculated for various quality properties.

Keywords: Accuracy, Big data, Biological variation, Certification, Data quality

PGL-18

Unexpected Pre-analytical Error on Blood Sampling: A Prolonged Syringe Storage Time Results in Inaccurate Complete Blood Count Results

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Background: Around 60–70% of laboratory medicine errors are attributed to the pre-analytical phase. Our objective was to enhance patient safety by highlighting a previously unreported source of pre-analytical error: delayed transfer of blood samples collected with a syringe to laboratory test tubes, which can lead to inaccurate complete blood count (CBC) results.

Methods: Significant discrepancies were observed in hemoglobin (Hb) levels between simultaneous CBC and arterial blood gas analysis tests in an emergency department (ED). To obtain accurate CBC results, we conducted a comparative analysis of the samples collected simultaneously in sodium citrate and EDTA tubes. We also investigated whether the duration of blood storage in the syringe following venous collection affected CBC test results. Using a standard needle

and syringe, we collected 10 cc of venous blood was collected from a healthy 45-year-old female volunteer. Subsequently, we stored the syringe horizontally for various durations (immediately, 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 30 minutes) before transferring the first 2.7 cc to a sodium citrate tube and discarding the mid-sample. The remaining sample of 3 cc or less was placed into an EDTA tube for CBC analysis. Finally, to determine the frequency of this phenomenon in the ED, we analyzed the results of CBC tests of 3,221 patients conducted over 4 months.

Results: Among the four patients from the ED, three displayed significant differences between the sodium citrate and EDTA tube samples. The Hb levels were lower (average 60.8% reduction), whereas white blood cell (WBC) and platelet (PLT) counts were higher (average 98.3% increase and 154.4% increase, respectively) in EDTA tube samples versus sodium citrate tube samples. The average values of Hb and WBC and PLT counts in both tubes closely matched those of the resampled EDTA tube samples. One patient showed higher Hb (9.6 g/dL vs. 6.4 g/dL) and lower WBC ($1.54 \times 10^9/L$ vs. $2.24 \times 10^9/L$) in EDTA tube samples versus those sodium citrate tube samples. The impact of sample storage over time showed that Hb decreased and WBC and PLT counts increased between 10- and 15-minute delay after collection.

Conclusions: A prolonged delay in transferring blood samples collected via syringe to laboratory test tubes during venous blood collection can result in inaccurate CBC results. We recommend that, if a syringe is used for phlebotomy, the collected sample should be transferred to tubes within 10 minutes to ensure accurate CBC results.

Keywords: Pre-analytical error, CBC, Hemoglobin, White blood cell count, Platelet count

PGL-19

Comparison Between V-Tubes and BD Vacutainer Tubes for Clinical Chemistry, Immunology, and Hematology Parameters

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Background: Blood collection vacuum tubes play a critical role in the preanalytical phase of clinical laboratory testing, and their impact is often underestimated. This study aimed to compare two manufacturers' vacuum tubes that use serum separator tubes (SST) for clinical chemical and immunological parameters and K2-EDTA anticoagulants for hematological parameters.

Methods: A total of 40 volunteers were recruited, and peripheral blood samples were respectively collected into SST, quick-clotting SST, and K2-EDTA of V tube (AB Medical, Seoul, Korea) and SST and K2-EDTA tube of BD vacutainers® (Becton Dickinson, New Jersey, USA). SST and quick-clotting SST samples were evaluated for 40 routine chemical and immunological parameters and compared

based on clotting time (5 minutes vs. 30 minutes). K2-EDTA tube samples were evaluated for 23 routine hematological parameters. All results were analyzed using the paired t-test, Bland–Altman plot, and Spearman correlation coefficient.

Results: The results of 40 chemical and immunological parameters and 23 hematological parameters between V tube and BD vacutainers demonstrated clinically allowable differences. Except for procalcitonin (PCT) and human immunodeficiency virus (HIV), all parameters of V tube and BD vacutainers showed highly strong correlations (Rho for all parameters >0.80 ; Rho in PCT=0.67; Rho in HIV=0.60). In the SST of the V-tube, the difference based on clotting time demonstrated comparable results. The erythrocyte sedimentation rate (ESR) of the V tube indicated a positive bias compared to that of the BD vacutainer due to the difference in K2-EDTA content.

Conclusions: For all chemical, immunological, and hematological parameters, SST and K2-EDTA were comparable between V tube and BD vacutainers regardless of clotting time. However, caution should be exercised for PCT, HIV, and ESR.

Keywords: Vacuum blood collection tube, Chemistry, Immunology, Hematology

PGL-20

Development of an Automated Analysis Algorithm for Platelet Adhesion Quantification Using Differential Interference Contrast Microscopy Images

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Background: Traditional platelet function tests require a large number of cells, making them inefficient for evaluating single platelet cell functions. The use of microscopy to assess platelet adhesion may be beneficial in such cases. Differential interference contrast (DIC) microscopy allows high-resolution, real-time observation of platelets without staining. However, owing to complex images, irregular platelet shapes, and high background signals, the results sometimes lack objectivity. This study aimed to develop an automated algorithm to quantify platelet adhesion by overlaying two platelet images obtained by adjusting a DIC prism slider.

Methods: Platelets were diluted tenfold and incubated on a cover glass for 30 minutes to prepare adhesion samples. Images of 100 platelets that adhered to the cover glass were captured at two different angles using a DIC microscope (Axioscope 5; Carl Zeiss Inc., Germany). This yielded 200 images (Images A and B). By overlaying two images of each platelet, 100 new images were created. An automated analysis algorithm was developed using the Python open-source code to ana-

lyze the three types of platelet images. The performance of the algorithm in quantifying platelet adhesion was evaluated by calculating the concordance rate (concordance rate=number of matching pixels/number of mismatched pixels) between the automated and manual analysis results.

Results: Concordance rates for 300 image processing results were calculated (mean±standard deviation). The overlay image algorithm showed a higher concordance rate (10.73 ± 5.95) compared to the image A algorithm (6.91 ± 3.18) and the image B algorithm (7.98 ± 3.72) ($P<0.05$). Additionally, the minimum concordance rates for the image A, image B, and overlay image algorithms were 0.02, 0.01, and 0.10, respectively, and the maximum concordance rates were 14.38, 18.74, and 27.60, respectively, demonstrating the superior performance of the overlay image algorithm. Overall, the algorithm using overlaid images was more useful for measuring platelet adhesion than algorithms using single-acquired images.

Conclusions: The automated analysis algorithm for platelet adhesion using overlaid DIC microscopy images developed in this study showed superior performance compared to single DIC image analysis. This method can be used to evaluate platelet adhesion with a minimal number of cells, making it a valuable tool for future research on stem cell-based *in vitro*-cultured artificial platelets.

Keywords: Platelet adhesion, Differential interference contrast microscopy, Automated image analysis algorithm

PGL-21

Development of a Flow Cytometric Dual-Color Live Platelet Aggregation Function Test for Small Volume Samples

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Background: Traditional light transmission aggregometry (LTA) is challenging for evaluating the aggregation function of stem cell-based *in vitro* artificial platelets because of the large sample volumes required. This study aimed to develop a flow cytometric method using dual-color live cell staining dyes to quantitatively assess platelet aggregation with minimal sample volume.

Methods: Calcein-AM green (excitation, 490 nm/emission, 515 nm) and calcein-AM red (excitation, 643 nm/emission, 663 nm) fluorescent dyes were used to selectively stain live platelets. About 25 μ L of platelet samples, stained with each dye, were mixed in a 1:1 ratio and divided into activated (treated with 15 μ M ADP) and resting groups. Flow cytometry analysis was performed, and an anti-CD61 antibody was used as a control to inhibit platelet aggregation. The

percentage of dual-colored platelets among the total colored platelets (% dual-colored platelets), the percentage reduction of single-colored platelets upon activation (% reduction of single-colored platelets), and the average number of aggregated platelets per dual-colored platelet (average aggregated platelet number) were calculated.

Results: The percentage of dual-colored platelets was approximately four times higher in the test group (15.7) than in the control group (3.8), indicating greater platelet aggregation in the test group. The % reduction in single-colored platelets was higher in the test group (52.0) than in the control group (39.6), indirectly confirming the greater consumption of single-colored platelets owing to aggregation. Additionally, the average aggregated platelet number was 10.5 cells in the test group, compared to 4.2 cells in the control group, suggesting that an average of 10 platelets aggregated in the test group, whereas only about 4 platelets aggregated in the anti-CD61 antibody-treated control group. Overall, the results quantitatively demonstrated more active platelet aggregation in the test group than in the control group, which showed inhibited aggregation.

Conclusions: This study demonstrated that platelet aggregation can be quantitatively analyzed using very small sample volumes. This method allows for the quantitative estimation of microaggregation by counting the number of aggregated platelets and enhances the reliability of the results using dyes that stain only live platelets. This dual-color flow cytometric live platelet aggregation function test is expected to be useful for testing small volumes of platelets for the development of stem cell-based *in vitro* artificial platelets.

Keywords: Flow cytometry, Platelet aggregation, Dual-color staining

PGL-22

Development of a Flow Cytometric Platelet Purity Test Differentiating the Nucleated Cells

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Background: Cell-based *in vitro* cultured artificial platelet preparations should exclude remnant nucleated cells, as these cells can potentially be a source of cancer when infused into patients. This study aimed to develop and validate a novel flow cytometric assay capable of accurately determining platelet purity by differentiating nucleated cells present in platelet preparations.

Methods: The test segment of the platelet concentration preparation was spiked with K562 cells (Korea Cell Bank) using a serial dilution method. The flow cytometry protocol was designed to utilize anti-CD61 antibodies conjugated with fluorescent markers (EXBIO Praha, Czech Republic) to distinguish platelets from nucleated cells, with

the latter's nuclei stained using propidium iodide (Becton Dickinson, France). The cell concentration was quantified using commercial counting beads (TruCount; Beckman Coulter, Brea, CA, USA). Three serially diluted samples were quantified, and the reduction in nucleated cell numbers was compared with the dilution factors.

Results: Fluorescent signals detected using specific filters successfully differentiated platelets from nucleated cells using flow cytometry. The spiked K562 cells, which are nucleated, showed a serial decrease in number corresponding to the dilution factor (57% and 40% at 2-dilution and 4-dilution, respectively), while the platelets, which are non-nucleated, maintained their concentration ((108% and 104%, respectively). The serial reduction ratio based on the dilution factor demonstrated that the quantitative measurement of nucleated cells mixed with non-nucleated cells was consistent with the dilution factor of the prepared samples.

Conclusions: The developed flow cytometric assay is a reliable and efficient method for assessing the purity of platelets in preparations containing nucleated cells. This technique offers significant improvements over traditional methods, including higher throughput, reduced subjectivity, and enhanced precision through the fluorescent detection of nucleated cells mixed with a large number of platelets. This assay can be employed for the accurate assessment of platelet purity in cell-based *in vitro* cultured artificial platelet preparations.

Keywords: Flow cytometry, Platelet purity, Nucleated cells

PGL-23

Validation of Flow Cytometry and Confocal Microscopy for Accurate Polyploidy Assessment in Megakaryocyte Differentiation Studies

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Background: Megakaryocytes are essential for platelet production, acquiring polyploidy through endomitosis. Evaluation of polyploidy is crucial for understanding megakaryocyte differentiation and maturation. Propidium iodide (PI) staining is widely used to assess DNA content in cells using flow cytometry and confocal microscopy. Flow cytometry provides a rapid, quantitative analysis of DNA content in large cell populations, whereas confocal microscopy provides high-resolution images to visualize structural details and precisely analyze cell volume. In this study, we compared the polyploidy of PI-stained megakaryocytes obtained using these two methods. Consistent findings have validated the reliability of polyploidy assessment via PI staining, enhancing the accuracy of hematological research and diagnostics.

Methods: K562 cells were cultured with or without 1 nM PMA, a mega-

karyocyte inducer, in RPMI medium supplemented with 10% fetal bovine serum (FBS) for 5 days. Cells fixed with 70% cold ethanol were stained with a solution of 50 µg/mL PI, 100 µg/mL RNase, and 0.1% Triton X-100 for 30 minutes in the dark. Polyploidy was assessed through confocal microscopy (LSM 980; Carl Zeiss, Germany) and flow cytometry (Navios; Beckman Coulter Inc., USA). For confocal microscopy, 121 control cells and 47 megakaryocyte-induced cells were imaged using 1 µm z-stacks, and cell volume was analyzed with ImageJ. Flow cytometry was used to measure 14,670 events in control and 2,141 events in PMA-treated cells. Polyploidy was determined by evaluating the cell volume percentile of polyploid cells exceeding 95% of the control cell volume.

Results: Flow cytometry analysis revealed that polyploidy ($>8n$) was observed in 9.67% of control cells and 13.55% of PMA-treated cells, showing a significant difference ($P<0.001$). Confocal microscopy analysis of the polyploid cell volume showed 4.96% in control cells and 14.9% in PMA-treated cells, indicating a significant difference ($P<0.05$). The results from both analytical methods were consistent, demonstrating the reliability of these techniques for assessing megakaryocyte polyploidy.

Conclusions: This study demonstrated that both flow cytometry and confocal microscopy are reliable methods for assessing megakaryocyte polyploidy. The consistency between these methods validates the use of PI staining for an accurate polyploidy assessment. Such evaluations enhance our understanding of megakaryocyte differentiation, support the use of these techniques in hematological research and diagnostics, and provide a robust foundation for future studies on differentiation assessments related to stem cell-based artificial platelet production.

Keywords: Polyploidy, Megakaryocyte, Differentiation, Confocal microscopy, Flow cytometry, Propidium iodide staining

developed. This study aimed to design a method for testing the platelet sedimentation rate by analyzing the 3D spatial distribution of platelets using digital inline holographic microscopy.

Methods: Platelet segment samples were diluted in phosphate-buffered saline to 1/10 of the original concentration. To activate the platelets, 10 µM of ADP was added to the testing sample. A total of 4 µL of the platelet sample was placed between a top cover slip and bottom slide glass with a thickness of about 300 µm. Digital in-line holographic microscopy was employed to investigate temporal variations in 3D spatial distribution during platelet sedimentation. The 3D positions of individual platelets were obtained after numerical reconstruction, local intensity peak detection, and image sharpness calculations.

Results: The experimental results demonstrated that the z-axis positions of 3D reconstructed platelet particles could be quantitatively measured using digital in-line holographic microscopy. In non-activated platelet samples, the mean $\pm$ SD of the standardized z positions at 2, 5, and 12 minutes were 0.63 ± 0.04 , 0.52 ± 0.04 , and 0.40 ± 0.02 , respectively, showing a decrease over time. Similarly, in platelet samples activated by ADP, the mean $\pm$ standard deviation of the standardized z positions at 2, 5, and 12 minutes were 0.59 ± 0.01 , 0.54 ± 0.03 , and 0.45 ± 0.01 , respectively, also showing a decrease over time. These results indicated that the 3D position information of platelet particles could be obtained by acquiring a single image of the platelet sample through microscopy, and platelet sedimentation could be quantified over time.

Conclusions: This study demonstrated that the platelet sedimentation rate can be quantitatively measured using digital in-line holographic microscopy. The measurement of platelet samples can be implemented using kinetic methods, providing quantitative results by 3D reconstruction of the platelet particle height over time. This technique has the potential as a foundational technology for evaluating the clinical significance of platelet sedimentation rate tests.

Keywords: Platelet sedimentation rate, Digital in-line holographic microscopy, 3D spatial distribution analysis

PGL-24

Development of Platelet Sedimentation Rate Test by 3D Spatial Distribution Analysis Using Digital In-line Holographic Microscopy

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Background: Similar to the erythrocyte sedimentation rate (ESR) test, the platelet sedimentation rate test has the potential to be used as a platelet function test through interactions between cellular and plasma components. However, due to the small size and limited sedimentation rate of platelets, a precise testing method has not yet been



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