and APOE ε4 status on total WMH volume. RESULTS/ANTICIPATED RESULTS: Older age, greater time since MRI, and larger ICV were significantly associated with increased WMH burden across cognitive groups. Use of FLAIR imaging was negatively associated with WMH volume, reflecting differences in image acquisition sensitivity. Higher educational attainment was linked to lower WMH burden, suggesting a possible neuroprotective effect. The most pronounced associations were observed within the AD subgroup. After adjusting for all covariates, race was not a statistically significant independent predictor of WMH burden. DISCUSSION/SIGNIFICANCE OF IMPACT: Although race did not independently predict WMH burden, structural and demographic factors – such as age, education, and MRI technique – significantly influenced WMH volume. Findings underscore the interplay of biological, social, and technical variables in brain aging and the need for greater African American inclusion.

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Dimensionality provides translational insights into hormone-responsive behaviors in breast cancer

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OBJECTIVES/GOALS: We aim to understand how estradiol (E2) modulates proliferation and migration in distinct breast cancer subtypes within a collagen-based 3D platform that serves to better recapitulate human tissue architecture. This work seeks to bridge in vitro hormone biology with translational modeling of hormone-driven tumor behaviors. METHODS/STUDY POPULATION: MDA-MB-231 (triple-negative) and MCF-7 (hormone receptor-positive) breast cancer cells, tagged with red fluorescent protein, were exposed to varying E2 concentrations (0 to 10-4 M) in both 2D and 3D culture. In 2D, 2,000 cells/well were seeded in 96-well plates. Wells were imaged on Day 1 using confocal fluorescent microscopy, treated with E2, and reimaged on Day 5 to assess post-treatment counts. Normalized proliferation was calculated as Day 5/Day 1 cell count. In 3D, per well, 60,000 cells were embedded in a 12 μL base layer of 0.3% type I collagen, overlaid with 40 μL plain collagen, and topped with 150 μL E2-containing media. On Day 5, microscopy and a machine learning algorithm assessed cell counts and migration, defining migratory cells as those >1 standard deviation from the mean z-position of the tumor body. RESULTS/ANTICIPATED RESULTS: In 2D, MCF-7 cells demonstrated significant dose-dependent proliferation to E2. Normalized cell counts increased progressively from 0 M to 10-10 M, at which point proliferation peaked, followed by a significant decline at higher concentrations. In contrast, MDA-MB-231 cells showed stable proliferation across the 0 to 10-6 M range, with a marked reduction in count at 10-4 M. In 3D, MDA-MB-231 cell proliferation remained stable across low and intermediate E2 doses, with a marked reduction at 10-4 M. MCF-7 cells, however, showed no significant differences in normalized proliferation across any E2 concentration in 3D. Quantitative migration analysis revealed that the percentage of migratory cells remained statistically unchanged across all E2 concentrations for both MCF-7 and MDA-MB-231 cells in the 3D matrix. DISCUSSION/SIGNIFICANCE OF IMPACT: Dimensionality influences hormone responsiveness in breast cancer, highlighting limitations of 2D assays in modeling clinical behaviors and predicting outcomes. Advancing biomimetic 3D platforms like these may ultimately enable patient-specific screening of endocrine response and guide personalized therapy for hormone-sensitive cancers.

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Mercury exposure in companion animals: A sentinel study for environmental contamination

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OBJECTIVES/GOALS: This study assesses mercury exposure in dogs and cats from Heredia, Costa Rica, near artisanal gold mining areas. As companion animals share environments with humans, they may serve as sentinel species for mercury contamination in mining-affected regions. METHODS/STUDY POPULATION: Noninvasive hair samples are being obtained by trained veterinary professionals from each animal through opportunistic collection or with owner's consent, ensuring minimal stress. One hundred and fourteen samples were collected from Costa Rica, and matched samples are being collected from the Colorado sites. Mercury concentrations will be measured using inductively coupled plasma mass spectrometry (ICP-MS). RESULTS/ANTICIPATED RESULTS: Results will be reported in parts per billion and assessed for statistical significance between groups. The results of this feasibility study will help determine whether companion animals living near ASGM sites exhibit higher mercury accumulation compared to those in less contaminated or historically impacted regions. DISCUSSION/SIGNIFICANCE OF IMPACT: Findings may inform future public health surveillance, environmental monitoring, and policy decisions related to mining practices and community exposure risk.

Other

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Discovery and evaluation of a methylpyrazolopyrimidine antibacterial active against methicillin-resistant Staphylococcus aureus

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OBJECTIVES/GOALS: Methicillin-resistant Staphylococcus aureus is a nefarious bacterial pathogen and is classified as a serious threat. MRSA is resistant to most B-lactam antibiotics (penicillins, cephalosporins, etc.). The goal of this study is to identify an antibiotic

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adjuvant capable of resurrecting B-lactam antibiotics for treatment of MRSA infections. METHODS/STUDY POPULATION: A fluorescence-reporter assay was used to screen a compound library. Minimum-inhibitory concentrations were assessed for the compounds against various MSSA and MRSA strains. A common resistance mechanism to B-lactams by MRSA is by the function of the bla operon. One gene in this operon encodes for a B-lactam sensor/signal transducer protein BlaR, the primary target of this study. Inhibition of BlaR by compound 1 (best potentiator of oxacillin) was studied by nano-differential scanning fluorimetry (nanoDSF), surface plasmon resonance (SPR), scanning electron microscopy (SEM), and *in vivo* assays. RESULTS/ANTICIPATED RESULTS: We identified 80 compound hits from a 1,974-compound NCI library. Twenty-four compounds showed potentiating ability (2- to 4,096-fold decrease in MIC for oxacillin). Seven compounds exhibited melting temperature shifts by nanoDSF of BlaR, indicating binding. SPR determined compound 1 has a binding affinity of 31 mM to BlaR-SD. SEM images showed disruption in the S. aureuscell wall on exposure to compound 1and oxacillin. S. aureusN315 showed 3-log reduction in bacterial count treated with a mixture of compound 1 and oxacillin. DISCUSSION/SIGNIFICANCE OF IMPACT: Compound 1 targets BlaR-SD, which restores S. aureus-susceptibility to treatment by oxacillin. There are currently few antibiotics available in the clinic capable of treating MRSA infections. The combination hold promise of a treatment option for MRSA.

AI and Data Science

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Learning health systems and digital twins: Advancing translational science with MS as a use case

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OBJECTIVES/GOALS: We advance multimodal modeling, including digital twins (DTs), within an AI-enabled Learning Health System (LHS) to turn healthcare data into insights for precision care and translational science. DTs are dynamic patient models integrating EHRs, neuroimaging, biomarkers, wearables, and other real-world data (RWD) tools. METHODS/STUDY POPULATION: Multiple sclerosis (MS) provides a compelling use case of heterogeneous manifestations including fatigue, mobility deficits, cognitive deficits, and sleep disturbance, requiring multimodal integration to capture complexity. DTs integrate EHR, neuroimaging, biomarkers, wearable, and other RWD via ETL pipelines using standards like FHIR, OMOP, and DICOM. Modeling uses ensemble learning, LSTM/DBNs, dimensionality reduction, and bias auditing. Explainable AI (XAI) provides transparency through counterfactual "what if" analyses, such as testing whether risk changes if a patient improves gait speed, adjusts therapy, increases sleep duration, or lowers biomarker levels. Continuous monitoring detects model drift, ensuring reliability per FDA V3: verification, validation, vigilance. RESULTS/ANTICIPATED RESULTS: A robust extract–transform–load (ETL) pipeline maps these inputs into standards such as Fast Healthcare Interoperability Resources (FHIR), the Observational Medical Outcomes Partnership (OMOP) common data model, and the Digital Imaging and Communications in Medicine (DICOM) format, ensuring interoperability and reuse across sites. Development emphasizes robustness through parameter optimization, ensemble learning, prevention of data leakage, preprocessing, dimensionality reduction, and regularization. Key AI applications include MRI lesion and atrophy segmentation with nnU-Net, temporal deep learning for longitudinal forecasting with deep belief networks (DBNs) and long short-term memory (LSTM) models, ensemble modeling to improve generalizability, and bias auditing to promote fairness. DISCUSSION/SIGNIFICANCE OF IMPACT: This initiative advances digital health, neuroinformatics, and precision medicine for MS and other complex diseases. Aligned with NIH ODSS Data COUNTS and FDA V3, the framework supports discovery, regulatory science, and generalization to other variable conditions.

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Emergency medical services (EMS) 12-lead electrocardiographic-based clinical and transport decision support in acute heart failure patients with reduced HFrEF

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OBJECTIVES/GOALS: Early identification of heart failure with reduced ejection fraction (rEF) by emergency medical service (EMS) providers is challenging, particularly in rural settings. We developed a rEF decision aid that addresses barriers to adoption using demographics, measurements, and computerized findings from ubiquitous EMS 12-lead defibrillators. METHODS/STUDY POPULATION: We identified all patients at Tufts Medical Center between Jan 1, 2012 and Apr 16, 2024 who had a 12-lead ECG taken within 30 days of an echocardiogram. Using the echocardiogram left ventricular ejection fraction (LVEF), each ECG-echo pair was classified as reduced EF (rEF – LVEF < 40) or non-rEF (LVEF >= 40%). The rEF is the outcome in a step-wise logistic regression model (LRM) to predict rEF. Variables of interest in the model were selected based on literature review in clinical assessment for heart failure with reduced ejection fraction (HFrEF) and what can be provided by a 12-lead ECG. We also included left ventricular damage score (e.g., Selvester Score) due to previous myocardial infarction (MI). Prior MI is a known risk factor in HFrEF. We used R 4.5 to perform the analysis and logistic regression modeling. RESULTS/ANTICIPATED RESULTS: 2,289 patients were identified with a 12-lead ECG taken within 30-days of an echocardiogram. Our final regression model included demographic and ECG features: age, sex, myocardial scarring score, computerized interpretation of conduction defects (bundle branch block, early repolarization, and intraventricular conduction defect), and acute myocardial infarction findings. The rEF prediction model had an area under the curve

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