STUDY POPULATION: Our analytic plan was updated based on helpful feedback from the PHASTR program. We performed a trial emulation analysis using the N3C data, comparing adult new users of metformin to controls prescribed fluvoxamine, fluticasone, ivermectin, or montelukast. The composite outcome was Long COVID or Death (LC/D) within 180 days of COVID infection. We used entropy balancing to estimate the average treatment effect with a weighted log-linear model. Productivity was enhanced by reusing code workbooks and validated codesets from related N3C projects. The team of 4 (physician, informaticist, data programmer, and statistician) and key unpaid advisors spent 10 weeks developing and analyzing the data. RESULTS/ANTICIPATED RESULTS: Totally, 9,660 patients were identified for analysis. After weighting, there were 248 in the metformin and control groups. In the metformin group, 4.0% developed LC/D vs. 8.5% in the control group, with an adjusted risk ratio (aRR) of 0.47 (95% CI 0.25 to 0.89). Results were consistent across subgroups and sensitivity analyses. The PHASTR contract structure helped produce high-quality results quickly by not only providing funding but also requiring a compressed timeline for a small team to focus on the study. The most time was spent on contract execution, enclave provisioning, and too many last-minute download requests. A project final report was submitted in March and a full manuscript was submitted in September. DISCUSSION/SIGNIFICANCE OF IMPACT: The analysis was productive because the environment made reuse easy and supported rich collaborations among clinicians, informaticists, epidemiologists, statisticians, and data developers. Advice from PHASTR advisors (Axel) and N3C diabetes domain team members was also key to a faster completion.

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## Becoming multilingual in thought languages

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OBJECTIVES/GOALS: Understanding cognitive habits and values of individuals or groups, and providing tools to apply them to collaborative, interdisciplinary endeavors to better communicate between different industries, functions, and cultures. METHODS/STUDY POPULATION: Using literary research to establish groupings of common core values in interpersonal communications, applying established 5 patterns of "thought languages" to scale to group communications. Accepted psychological personality inventories for individuals will overlay into cognitive values, primarily using the current big five OCEAN model. Demonstrating these values to find common goals among interdisciplinary collaborations can identify prospective members, cultural differences in industry, patient communication, and public messaging in STEM. Integrating these tools into research groups to establish more efficacious communication between teams, governing bodies, and patient communication can be sampled via pre and post research surveys of feeling understood. RESULTS/ANTICIPATED RESULTS: The results of feeling understood by various parties in collaborative research would be a measure of not just effective expressed communication, but received communication. Feeling understood is a current metric of communication that is correlated with satisfaction, trust, and interdependence. All of these results are integral to the successful operations of collaborative projects. Demonstrating a positive correlation between applying the 5 thought languages and better-surveyed outcomes of understanding will guide the effectiveness of this as a future collaborative tool for translational sciences. DISCUSSION/SIGNIFICANCE OF IMPACT: The significance of effective communication based on positive reception will foster future collaborations. Encouraging familiarity between differing individuals, groups, and industries, even between subjects and researchers, patients and healthcare. More satisfaction, more trust, and more interdependence will propagate between these groups.

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## Neutrophils propagate inflammation and fibrosis in primary sclerosing cholangitis

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OBJECTIVES/GOALS: Primary sclerosing cholangitis (PSC) manifests with an inflammatory milieu that leads to fibrotic scarring of the liver. Human PSC liver bile ducts are enriched with neutrophils; however, their infiltration and functional role is unexplored. Our goal is to investigate the mechanism and impact of peribiliary neutrophil infiltration observed in PSC. METHODS/STUDY POPULATION: Primary cholangiocytes (bile duct cells) isolated from WT and mouse models of PSC (3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-fed mice and Mdr2-/- mice) were analyzed by RNA-sequencing. Immunofluorescence (IF) was performed on liver tissues from PSC patients and mouse models of PSC for markers of bile ducts and neutrophils (KRT19 and MPO). Intrahepatic leukocytes (IHL) isolated from mice livers were evaluated for neutrophil abundance and activation state. Anti-Ly6G antibody-mediated neutrophil depletion in Mdr2-/- mice was analyzed by IF, histology, and cytometry by time-of-flight (CyTOF). Cholangiocytes stimulated with TNFα (to induce an inflammatory phenotype) were analyzed for neutrophil chemoattractants with genetic and pharmacological interventions. RESULTS/ANTICIPATED RESULTS: RNA-seq analysis of primary cholangiocytes from PSC mouse models revealed enrichment in inflammatory and neutrophil degranulation pathways. Flow cytometry and RT-PCR analysis revealed an increase in the neutrophil population in PSC mice with activated phenotype. Peripheral depletion of neutrophils in Mdr2-/- mice alleviated liver injury and inflammation, along with a reduction in peribiliary neutrophil infiltration and attenuated bridging fibrosis. CyTOF analysis showed a significant reduction in CD8+ T cells upon neutrophil depletion, implying neutrophils sustain CD8+ T cells in PSC liver. Mechanistically in cholangiocytes, TNFα mediates expression of neutrophil chemoattractants, CXCL1 and CXCL8, through the cyclic GMP-AMP synthase stimulator of interferon genes (cGAS-STING) pathway. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest that activation of the STING pathway in cholangiocytes in cholestatic liver disease triggers an immune response resulting in peri-portal neutrophil infiltration via CXC chemokines. The sustained presence of these activated neutrophils engages the adaptive immune system to perpetuate the inflammation and fibrosis seen in PSC.

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