from patients with non-PH IPF in a cohort study (8). This may be a result of the absence of severe PH in these patients, or because of differences in the mechanisms driving PH in IPF versus PAH.

We conclude that nintedanib shows disadvantageous effects in PAH, and future investigations are needed to reveal the precise underlying mechanisms. Moreover, the effect of this agent on pulmonary hemodynamics in patients with IPF and severe accompanying PH should be investigated in future studies. ■

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Heterogeneity of Treatment Effect by Baseline Risk in a Trial of Balanced Crystalloids versus Saline

To the Editor:

Heterogeneity of treatment effect (HTE) is nonrandom variability in the direction and magnitude of an intervention's effect on outcomes for individuals within a population. HTE has important implications for the design, analysis, and interpretation of clinical trials in critical care (1). Traditional, explanatory clinical trials typically attempt to understand the efficacy of an intervention by enrolling a carefully selected, homogeneous population and deliberately excluding patients with characteristics that might contribute to variation in treatment response. In contrast, pragmatic trials enroll a more diverse population to enhance generalizability. This increases the opportunity for HTE, and the risk/benefit balance for an intervention may differ dramatically for subpopulations within a pragmatic trial. HTE within a trial may arise as a result of variation among patients, providers, treatment delivery, or study environments. Among these, HTE by patients' baseline risk of the study outcome is increasingly being proposed as a key factor in the reporting and interpretation of critical-care clinical trials (1). To better understand the analysis and interpretation of HTE by baseline risk of the primary outcome in pragmatic critical-care trials, we examined this relationship in a large pragmatic trial comparing the use of balanced crystalloids with the use of saline in critically ill adults.

SMART (Isotonic Solutions and Major Adverse Renal Events Trial) compared balanced crystalloids with saline for intravenous fluid administration in 15,802 critically ill adults (2). The primary outcome was major adverse kidney events within 30 days (MAKE30)—a composite of in-hospital death, new receipt of renal-replacement therapy, and persistent renal dysfunction (defined as a final inpatient creatinine value $\geq 200\%$ of the baseline value) (3). While the SMART trial was ongoing, we developed and published a multivariable model with high predictive accuracy for MAKE30 (area under the curve = 0.903) using variables available at ICU admission from a separate observational cohort of critically ill adults (4), with which we calculated the baseline risk of MAKE30 for participants in the SMART trial. To assess for HTE by patients' baseline risk of the primary outcome, we fit a logistic regression model for MAKE30 with independent covariates of fluid group assignment, the

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Figure 1. Observed incidence of major adverse kidney events within 30 days (MAKE30) by baseline risk of MAKE30. For each quartile of baseline risk of the primary outcome of MAKE30, the observed incidence of MAKE30 is displayed for patients in the balanced crystalloid group compared with the saline group. The absolute risk, relative risk, and odds of MAKE30 are compared between the balanced crystalloid group and the saline group (referent). The absolute risk differences for quartiles 1–4 and the overall group with 95% confidence intervals (CIs) were -0.3% (-0.9% to 0.2%), -0.1% (-1.0% to 0.8%), -0.9% (-2.8% to 1.0%), -3.7% (-6.9% to -0.6%), and -1.2% (-2.3% to -0.1%), respectively. The corresponding risk ratios (95% CI) were 0.67 (0.31–1.34), 0.96 (0.62–1.50), 0.91 (0.76–1.10), 0.92 (0.86–0.99), and 0.92 (0.86–0.99), respectively. The corresponding odds ratios (95% CI) were 0.67 (0.31–1.34), 0.96 (0.61–1.51), 0.90 (0.73–1.11), 0.86 (0.76–0.98), and 0.91 (0.83–0.99), respectively.

Figure 2. Observed incidence of 30-day in-hospital mortality by baseline sequential organ failure assessment (SOFA). The observed incidence of 30-day in-hospital mortality is displayed for patients in the balanced crystalloid group compared with the saline group for each five-point span of SOFA scores. The absolute risk, relative risk, and odds of mortality are compared between the balanced crystalloid group and the saline group (referent). The absolute risk differences for SOFA 0–5, 6–10, 11–15, and >15, and the overall group with 95% confidence intervals (CIs) were -0.4% (-1.2% to 0.5%), -0.2% (-2.1% to 1.6%), -3.2% (-7.6% to 1.1%), -4.2% (-16.4% to 7.9%), and -0.8% (-1.8% to 0.2%), respectively. The corresponding risk ratios (95% CI) were 0.92 (0.75–1.13), 0.99 (0.86–1.13), 0.90 (0.77–1.03), 0.94 (0.77–1.13), and 0.93 (0.85–1.02), respectively. The corresponding odds ratios (95% CI) were 0.91 (0.74–1.13), 0.98 (0.84–1.15), 0.86 (0.69–1.05), 0.83 (0.48–1.42), and 0.92 (0.83–1.02), respectively.

baseline risk of MAKE30 as a nonlinear continuous variable, and the interaction between group assignment and baseline risk of MAKE30. To present the results visually, we displayed the incidence of MAKE30 by randomized study group within each quartile of baseline risk for MAKE30. MAKE30 is a composite endpoint, and the MAKE30 risk model is complex, which can make it difficult to understand the concept of risk in this setting. To address this issue, we performed a sensitivity analysis in which we calculated each patient's sequential organ failure assessment (SOFA) score on the day of ICU admission (5), and modeled the interaction between fluid group assignment and SOFA score with regard to 30-day in-hospital mortality. All analyses were performed with R version 3.3.2 (R Foundation for Statistical Computing).

Among 15,802 patients from five ICUs enrolled in the original trial, 126 were missing data elements required for the MAKE30 prediction model. Among the remaining 15,676 patients, the mean predicted risk of MAKE30 was 15.4% (range, 0.1–99.3%) and was similar between groups (balanced group 15.3% vs. saline group 15.5%, $P = 0.61$ by Mann-Whitney U test). The incidence of MAKE30 in the trial was 14.9% (balanced group 14.4% vs. saline group 15.5%), and the model predicting MAKE30 demonstrated excellent discrimination (area under the curve = 0.891). Adjusting for baseline risk, the odds of MAKE30 were significantly lower in the balanced crystalloid group than in the saline group (odds ratio, 0.88; 95% confidence interval, 0.79–0.98; $P = 0.02$). The patients' baseline risk of MAKE30 did not modify the effect of group assignment on the observed incidence of MAKE30 (P value for interaction = 0.66). In each quartile of baseline risk, balanced crystalloids had lower odds of MAKE30 than saline, with similar odds ratios between quartiles (Figure 1). Meanwhile, the absolute risk difference between study groups varied more than 10-fold, increasing from -0.3% in the lowest-risk quartile to -3.7% in the highest-risk quartile. Results were similar in the sensitivity analysis using baseline SOFA and mortality. Formal testing demonstrated no statistical interaction (P value for interaction = 0.61), odds ratios were stable across SOFA score intervals, and the absolute risk reduction with balanced crystalloids compared with saline increased 10-fold between the lowest- and highest-risk patients.

This preplanned secondary analysis of a large, pragmatic critical-care trial yielded two findings with important implications for reporting and interpreting HTE in clinical trials. First, the absolute difference in the incidence of the primary outcome between study groups was more than 10-fold greater for the highest-risk patients than for the lowest-risk patients. Second, the relative risk difference in the primary outcome between study groups was consistent across the spectrum of baseline risk, with no statistical evidence by formal interaction testing that the baseline risk of the primary outcome modified the effect of study group on the incidence of the primary outcome. These findings are not contradictory. Prior discussions about HTE in pragmatic trials have emphasized that HTE by baseline risk may exist on the absolute scale, relative scale, or both (6). In the SMART trial, the effect of balanced crystalloids on MAKE30 was similar to that of saline regardless of the baseline risk of MAKE30 (relative scale), but for higher-risk patients this mathematically

translated into a larger difference in events between groups (absolute scale).

One interpretation of these findings is that clinicians should selectively administer balanced crystalloids to patients at higher risk and consider the choice of crystalloid less important for patients at lower risk. However, because the number of low-risk patients admitted to the ICU dramatically exceeds the number of high-risk patients, nearly half of the total outcome events prevented by the use of balanced crystalloids rather than saline occurred among patients with a lower baseline risk (Figure 2). Coupled with the similar cost of balanced crystalloids and saline, these findings could support the preferential use of balanced crystalloids irrespective of patients' baseline risk.

Our findings are strengthened by a preplanned analysis, use of a previously published model for baseline risk that performed well in the trial population, and consistent results from a sensitivity analysis using a second, independent risk score and clinical outcome. Our analysis is limited by the fact that we focused exclusively on HTE by baseline risk for the outcome. HTE by other patient, provider, treatment delivery, and environmental factors may potentially confound analysis of baseline risk. The complexity of the MAKE30 prediction model limits its application to other centers, but the findings of the analysis using SOFA scores may be more generalizable. The trial was powered to compare the primary endpoint between groups and may have been underpowered to detect statistical interactions.

In conclusion, the relative risk reduction in MAKE30 with balanced crystalloids compared with saline was consistent across the spectrum of baseline risk of MAKE30, resulting in a numerically greater absolute risk reduction in MAKE30 with balanced crystalloids compared with saline among patients at high risk of MAKE30 compared with those at low risk. Interpretation of HTE by baseline risk of the primary outcome in future critical-care clinical trials should consider absolute effects, relative effects, and the results of formal interaction testing. ■

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Mycobacterial Lipids Induce Calcium Mobilization and Degranulation of Mast Cells

To the Editor:

Tuberculosis (TB) remains the leading cause of death from a single infectious agent, followed by HIV/AIDS (1). Emerging countries, such as Panama, have not been able to curb TB infection rates. The development of novel biomarkers for diagnosis, treatment monitoring, and vaccine development is urgently needed. The innate immune response during TB infection is a source of biomarkers. When a susceptible host is exposed to *Mycobacterium tuberculosis*, the bacteria translocate to the mucosal barrier, interacting with a variety of immune cells, including mast cells (MCs). The initial interaction between *M. tuberculosis* and first-line defense cells induces the accumulation and subsequent activation of these immune cells within lung tissue, and the release of proinflammatory mediators (2). Mycobacterial lipids appear to define the clinical fate of the infection by modulating the immune response during TB (3). However, the precise role of *M. tuberculosis* lipids in the immunopathogenic and molecular mechanisms of MC activation is still unknown.

MCs are abundant in the lung tissue, where they act as sentinels for a wide variety of invading pathogens, as well as regulatory cells during the course of acute inflammation (4). Besides specific

antigen-driven IgE crosslink stimulation, MCs can be activated by complement components, IgG, neuropeptides, pathogen-associated molecular patterns, mainly peptides, and whole *M. tuberculosis* interaction (5). MC activation promotes the secretion of stored proinflammatory mediators as well as *de novo* synthesis of leukotrienes, platelet-activating factor, and prostaglandins. Together with the transcription and release of cytokines and chemokines by MCs, the host begins to make an adaptive immune response (6).

Recently, Garcia-Rodriguez and colleagues revised our perspective of the MC strategies used in bacterial defense and reported interactions occurring between *M. tuberculosis* and MCs (7). Shortly after exposure, MCs recognize *M. tuberculosis* via the TLR2 and CD48 receptors. During this initial stage, ESAT-6 and MPT-63 induce MC degranulation, cytokine release, and the secretion of antimicrobial peptides such as β -hexosaminidase and LL-37. We hypothesized that *M. tuberculosis* lipid extracts are able to activate MCs. In this preliminary study, we aimed to elucidate the role of single phospholipids and whole-lipid extracts in MC activation. Some results from these studies have been previously reported in the form of an abstract (8).

Methods

Cells and culture conditions. Murine MCs from line C1.MC/C57.1 (C57 MC) were kindly provided by Dr. Stephen Galli (Stanford University) and cultured in Dulbecco's modified Eagle's medium according to standard methods (9).

***M. tuberculosis* lipid extracts.** The bacterial lipid fractions extracted from H37Rv *M. tuberculosis*, including total lipids (T-L, NR-14837), insoluble lipids (I-L, NR-14843), soluble lipids (S-L, NR-14842), *M. leprae*, phenolic glycolipid I (PGL-I, NR-19342), and *M. tuberculosis* phosphatidylinositol mannoside 6 (PIM6, NR-14847), were obtained from BEI Resources. Purified bovine heart L- α -phosphatidylcholine (PC, 840052C) and cardiolipin (CL, 840012C) were obtained from Avanti Polar Lipids. All *M. tuberculosis* lipid extracts were resuspended according to the manufacturer's recommendations.

β -Hexosaminidase release and calcium mobilization assays. C57 MCs were stimulated for 1 hour with T-L, S-L, I-L, PC, CL, PIM6, and PGL-I lipid fractions of *M. tuberculosis* at 20 μ g/ml each. MC degranulation was determined by measuring β -hexosaminidase release according to a previously described protocol (10). Ionomycin was used as a positive control. Similarly, C57 MCs were stimulated with the above-mentioned lipids, and intracellular calcium levels were determined using a modified fluorimetric assay with Fluo 3-AM (Molecular Probes, Invitrogen) for intracellular calcium labeling (10). Student's *t* test was performed for all experiments, and *P* values of <0.05 were considered significant.

Results

***M. tuberculosis* lipid extract fractions significantly increased MC degranulation.** β -Hexosaminidase release was measured to assess the degranulation capacity of C57 MCs after lipid

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