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An enrichment strategy for sepsis clinical trials

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Abstract

Introduction—Selecting participants for research based on their risk is an enrichment strategy with potential for enhancing clinical trials in sepsis. Adult Septic Shock Information and Stratification (ASSIST) is a tool for estimating mortality risk that incorporates a panel of biomarkers, age, lactate, and chronic health status. We assessed the utility of ASSIST as an enrichment strategy in a clinical trial testing the efficacy of a polyclonal anti-tumor necrosis factor- α fragment antibody (AZD9773) in adults with severe sepsis or septic shock. We hypothesized that the effects of AZD9773 are dependent on baseline mortality risk, as estimated by ASSIST.

Methods—This was a *post hoc* analysis of a recently completed trial of 300 subjects, randomized to placebo, low dose AZD9773, or high dose AZD9773. The study's primary endpoint was number of ventilator free days. There were 286 subjects with available plasma samples from study entry. We measured the ASSIST biomarkers and assigned each subject to low, intermediate, and high risk strata based on their ASSIST mortality probability. To mirror the study's original statistical plan, we estimated the least squares mean ventilator free days for each study arm within risk strata.

Results—The effect of study arm within ASSIST-based risk strata was significant ($p = 0.017$). Within the low risk group, there was an increase in ventilator free days for both drug arms. Within the intermediate risk group, there was an increase in ventilator free days among those in the low dose arm, but a decrease in the high dose arm. Among high risk patients, there was a decrease of ventilator free days in both drug arms. Analogous associations were observed when modeling 28-day mortality.

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Conclusions—In this study, a beneficial effect of AZD9773 might have been observed if the trial selected low to intermediate risk patients. ASSIST has the potential to serve as an enrichment tool for sepsis clinical trials.

Keywords

sepsis; enrichment; biomarkers; clinical trial; TNF; stratification

INTRODUCTION

Experts in the field of sepsis advocate for the incorporation of biomarkers and enrichment strategies into clinical trials [1]. One form of enrichment involves stratification of study subjects according to baseline mortality risk. This is called prognostic enrichment. With this approach, the number of patients likely to have an outcome can be controlled to maximize the power of the study. For example, patients at low risk of an event might be excluded as being unlikely to benefit from a treatment, while patients at extremely high risk of an event might be excluded if their poor outcome is likely unalterable by the treatment under study. We have developed a stratification tool called Adult Septic Shock Information and Stratification Technology (ASSIST) [2, 3]. ASSIST incorporates a panel of biomarkers, age, lactate, and chronic health status to estimate a baseline 28-day mortality probability for adults with septic shock. We envision that ASSIST can serve to enrich the sample enrolled into sepsis trials.

A recent phase IIb trial tested the efficacy of a polyclonal anti-tumor necrosis factor- α fragment antibody (AZD9773) in patients with severe sepsis or septic shock [4]. The trial tested two doses of AZD9773 versus placebo. There was no difference in the primary study endpoint of ventilator free days between the three study groups, nor was there a difference in 28-day mortality.

In order to test the concept that ASSIST can serve effectively as an enrichment strategy for sepsis trials, we replicated the original analysis of the AZD9773 study but incorporated enrichment by stratifying the cohort using ASSIST. The primary hypothesis is that the potential efficacy of AZD9773 is dependent on baseline mortality risk, as estimated by ASSIST.

METHODS

Ethics, Consent, and Permissions

The original phase IIb trial was approved by the Institutional Review Boards of each participating institution, as previously published [4]. The consent process allowed for secondary analyses of biological samples and clinical data.

Study Protocol and Biomarker Measurements

Full details of the AZD9773 protocol were previously published [4]. Briefly, 300 adults with severe sepsis or septic shock were randomized 1:1:1 to placebo, a low dose of AZD9773, or a high dose of AZD9773. We used plasma samples obtained at study entry to measure the

ASSIST biomarkers. These include C-C chemokine ligand 3 (CCL3), C-C chemokine ligand 4 (CCL4), granzyme B (GZMB), heat shock protein 70 kDa 1B (HSPA1B), interleukin-8 (IL8), and interleukin 1 α (IL1A) [2]. The biomarkers were measured using a multi-plex magnetic bead platform (MILLIPLEX™ MAP) designed for this project by the EMD Millipore Corporation (Billerica, MA). Biomarker concentrations were measured in a Luminex® 100/200 System (Luminex Corporation, Austin, TX), according to the manufacturers' specifications. Technical assay performance data were previously published [5].

ASSIST was developed by combining biomarker concentrations, age, lactate concentration at study entry, and chronic disease status into a decision tree generated by Classification and Regression Tree (CART) methodology [2]. The ASSIST biomarkers were originally identified by whole genome expression studies in children with septic shock, designed to identify genes having predictive capacity for mortality [5, 6]. The biomarkers were subsequently tested in adult cohorts, leading to the development and testing of ASSIST [2, 7]. The decision tree treats chronic health status as a dichotomous variable indicating the presence or absence of any one of the following chronic conditions: New York Heart Association Class IV congestive heart failure (NYHA Class IV CHF), chronic obstructive pulmonary disease (COPD), requirement for chronic dialysis, chronic hepatic failure, hematologic or metastatic solid organ malignancy, and requirement for chronic steroids at study entry. Study subjects are allocated to the terminal nodes of the decision tree based on specific rules reflecting the biomarker concentrations, age, lactate, and chronic disease status, with each terminal node assigning a mortality probability. The mortality probabilities of ASSIST range from 0.03 to 0.75. For the current study, study subjects were grouped into one of three mortality risk strata: low risk (mortality probability <10%), intermediate risk (mortality probability 10 to 50%), and high risk (mortality probability >50%).

Data Analysis

Our statistical analysis plan was based upon the supposition that the effect of treatment would differ within each ASSIST-based risk stratum. Replicating the original primary analysis [4], we constructed a generalized linear model to estimate the least squares (LS) mean ventilator free days for each study arm, adjusted for APACHE II and age. Secondly, we constructed a logistic regression model to estimate the odds of 28-day mortality. To accommodate expected differences in the main effects of treatment within risk stratum, a nested design was used as a more powerful alternative to a stratified analysis. Analyses used SPSS v 23.0 (IBM Corp, Armonk, NY).

RESULTS

Primary Analysis

The demographic and clinical characteristics of the study cohort were previously published [4]. Among the 300 subjects in the original study, there were 286 subjects with enrollment plasma samples available. Eighty one subjects were classified low risk, 165 subjects were classified as intermediate risk, and the remaining 40 subjects were classified as high risk, based on ASSIST. The 28-day mortality rates for the three groups were 8%, 22%, and 40%,

respectively. For estimating the risk of 28-day mortality, ASSIST had an area under the receiver operating characteristic curve of 0.65 (95% C.I.: 0.58 to 0.73; $p < 0.001$). This reflects a sensitivity of 90% (95% C.I.: 79 to 96) and a specificity of 35% (95% C.I.: 29 to 42) for mortality. In comparison, APACHE II had an area under the receiver operating characteristic curve of 0.67 (95% C.I.: 0.59 to 0.74, $p < 0.001$) for estimating the risk of 28-day mortality.

Table 1 shows the impact of ASSIST, and low dose and high dose AZD9773 on the least squares (LS) mean ventilator free days, using the high dose/high risk group as the baseline because this group had the lowest number of ventilator free days. In the model, both APACHE II and the effect of study arm within risk strata were significant ($p < 0.001$ and $p = 0.017$), whereas age was not significantly associated with outcome ($p = 0.680$). Table 2 shows the mean effect of low dose AZD9773 and high dose AZD9773 on the number of ventilator free days when compared to placebo within each risk stratum. Within the low risk group, there was an increase in ventilator free days for both drug arms. Within the intermediate risk group, there was an increase in ventilator free days among those in the low dose arm, but a decrease in the high dose arm. Among high risk patients, there was a decrease of ventilator free days in both drug arms.

Secondary analysis

The logistic regression model for 28-day mortality was consistent with the model for ventilator free days. APACHE II was statistically significant ($p = 0.005$), age was marginally significant ($p = 0.058$) and study arm within ASSIST risk strata was marginally significant ($p = 0.063$). Table 3 shows the odds ratios for 28-day mortality for the low dose and high dose arms, relative to placebo within the three ASSIST risk strata. Within the low risk group, the odds for 28-day mortality were decreased for both drug arms. Within the intermediate risk group, the odds for 28-day mortality were decreased in the low dose arm, but increased in the high dose arm. Among high risk patients, the odds for 28-day mortality were increased for both drug arms.

Taken together, these analyses suggest that the potential effects of AZD9773 on outcomes are dependent on ASSIST-based mortality risk, and the dose effect differs between the three strata.

DISCUSSION

Enrichment refers to the use of any patient characteristic to select a population in which a drug or treatment effect is more likely than in an unselected population [8]. *Prognostic* enrichment refers to the selection of patients based the likelihood of a disease-related event. *Predictive* enrichment refers to the selection of patients who are more likely to respond to an intervention based on an underlying biological mechanism. Prognostic and predictive enrichment are fundamental concepts for embracing precision medicine. In addition, enrichment strategies are particularly effective in trials involving highly heterogeneous syndromes, such as septic shock [9]. We propose ASSIST as a candidate prognostic enrichment strategy for septic shock trials and test this concept in the current study.

Unlike the original analysis that adjusted for risk using APACHE II but did not consider enrichment, our models demonstrated statistically significant effects of AZD9773 within ASSIST-based risk strata. We observed an increase in the mean ventilator free days in the low and intermediate risk strata treated with a low dose of AZD, but a decrease in the high risk strata. In addition, we observed a decrease in mean ventilator free days in subjects treated with a high dose of AZD9773, in both the intermediate and high risk strata. When considering a mortality outcome, the findings were analogous.

Collectively, the data suggest that a low dose of AZD0773 may be beneficial in adults with severe sepsis or septic shock having a low to intermediate baseline risk of mortality by ASSIST, but potentially harmful in those with a high baseline risk of mortality by ASSIST. In addition, the data suggest that a high dose of AZD9773 may be harmful in subjects with an intermediate to high baseline risk of mortality. This is consistent with a previous trial in patients with septic shock, which used an anti-TNF strategy based on a TNF receptor:Fc fusion protein [10]. That study found an association between higher doses of the fusion protein and mortality, leading the investigators to speculate that excessive inhibition of TNF activity might have exacerbated systemic infection in some patients.

These observations support our primary hypothesis that the potential effects of AZD9773 are dependent on baseline mortality risk as assessed by ASSIST. Moreover, they extend the findings of the original study, which showed that in general there was a trend towards worse outcomes for high dose AZD9773 and better outcomes for low dose AZD9773 when compared to placebo [4]. We posit that had participants for the study been selected based on having a low or intermediate ASSIST mortality, AZD9773 would be more likely to have demonstrated benefit.

In the original study, adjustments for APACHE II were used to consider risk [4]. ASSIST includes biomarker information that reflects the underlying biology while APACHE II reflects symptoms and clinical risk factors [2, 3, 5, 11, 12]. As such, enrichment using ASSIST may better stratify patients according to pathophysiology. We note that the ASSIST biomarkers reflect inflammation and immune function [3, 6]. It is possible that an anti-tumor necrosis factor strategy could modify the biology reflected by these biomarkers, and hence modify outcome.

Our results must be interpreted with caution because they represent a *post hoc* analysis. We cannot reliably conclude that AZD9773 *per se* is beneficial in certain mortality risk subgroups of patients with septic shock. Rather, our data support the concept that ASSIST can potentially serve as an enrichment strategy for sepsis clinical trials. This concept requires prospective testing, which in turn requires the development of a rapid assay platform that can measure the ASSIST biomarkers in a timeframe allowing for prompt decision making in critically ill patients. We are currently working toward the development of such an assay platform.

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design, collection, analysis, interpretation of the data, writing the manuscript or the decision to submit this manuscript for publication.

Abbreviations

ASSIST	Adult Septic Shock Information and Stratification Technology
least squares	LS
APACHE	Acute Physiology and Chronic Health Evaluation

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Table 1

The effect of study arm and ASSIST-based risk strata on LS mean ventilator days with high dose, high risk as the reference.

	Low Risk	Intermediate Risk	High Risk	Un-stratified
Placebo	8.3	8.9	4.5	1.0
Low Dose	11.0	10.5	4.4	2.9
High Dose	9.9	8.0	0	0

Table 2

Difference in mean ventilator free days based on study arm and ASSIST-based risk strata.

	Low Risk	Intermediate Risk	High Risk	Un-stratified
Low Dose vs. Placebo	2.69	1.62	−0.11	1.83
High Dose vs. Placebo	1.61	−0.85	−4.50	−1.02

Table 3

Odds ratios for 28-day mortality based on study arm and ASSIST-based risk strata.

	Low Risk	Intermediate Risk	High Risk	Un-stratified
Low Dose vs. Placebo	0.17	0.80	1.64	0.72
High Dose vs. Placebo	0.89	1.53	2.58	1.62