Identifying and Validating Biomarkers for Clinical Trials

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Disclosures

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• Sepsis biomarker-related patents issued and pending.

• Scientific advisory board:
  • Inflammatix
  • Eccrine Systems
  • Endpoint Health
Biomarkers for Clinical Trials

Purpose?

Selecting a population(s) more likely to benefit from your trial intervention
Enrichment

- The use of any patient characteristic to select a population in which an intervention is more likely to be effective, compared to an unselected population.
Prognostic Enrichment

• Selection of a patient population more likely to have a disease related event (e.g. mortality).
Prognostic Enrichment

Event-based study sample size

Effect Size

Event Rate
Prognostic Enrichment

Decreased sample size ➔ Effect Size ➔ ↑ Event Rate

No change in relative risk, but an increase in absolute effect size
Prognostic Enrichment

Decreased sample size

Placebo Group
10% mortality

Intervention Group
5% mortality

Effect Size

↑ Event Rate

Placebo Group
50% mortality

Intervention Group
25% mortality

50% relative risk ↓
5% absolute risk ↓
N = 948

50% relative risk ↓
25% absolute risk ↓
N = 132
Predictive Enrichment

• Selection of a patient population in which an intervention is more likely to have an effect based on a biological or physiological mechanism.
Predictive Enrichment

Event-based study sample size

Effect Size

Event Rate
Predictive Enrichment

- Decreased sample size
- ↑ Effect Size
- Event Rate

Increase of both relative and absolute effect size

Increased feasibility and enhanced benefit-risk relationship
### Prognostic and Predictive Enrichment

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Qualifiers, Questions, and Caveats

- Primarily used prior to randomization or to inform enrollment.
- But, could also be used in an adaptive trial design.
- Generalizability and applicability of results:
  - *Can the enrichment strategy be used in practice?*
  - *Is the intervention beneficial in a non-enriched population?*
- Sensitivity and specificity of the enrichment strategy?
- Should enrichment be used primarily in initial “proof-of-concept” studies, and then broadened to a more general populations?
- **We’ve already tried this, and it hasn’t worked!**
Effect of Treatment With Low Doses of Hydrocortisone and Fludrocortisone on Mortality in Patients With Septic Shock

Djillali Annane, MD, PhD
Virginie Schalle, PhD
Clairie Chaam, MD
Pierre-Edouard Bollaert, MD, PhD
Bruno François, MD
Jean-Michel Korach, MD
Gilles Capellier, MD, PhD
Yves Cohen, MD, PhD
Elié Azoulay, MD
Gilles Tacchini, MD
Philippe Chaumet-Riffian, MD
Eric Bellissant, MD, PhD

JAMA 288:862, 2002

Predictive enrichment via ACTH stimulation test: “responders” and “non responders.”

“Non responders”: CIRCI

No efficacy for the entire cohort.

Survival benefit among “non responders” treated with hydrocortisone and fludrocortisone.

Context: Septic shock may be associated with relative adrenal insufficiency. Thus, a replacement therapy of low doses of corticosteroids has been proposed to treat septic shock.

Objective: To assess whether low doses of corticosteroids improve 28-day survival in patients with septic shock and relative adrenal insufficiency.


Patients: Three hundred adult patients who fulfilled usual criteria for septic shock were enrolled after undergoing a short corticotropin test.

Intervention: Patients were randomly assigned to receive either hydrocortisone (50-mg intravenous bolus every 6 hours) and fludrocortisone (50-μg tablet once daily) (n=151) or matching placebo (n=149) for 7 days.

Main Outcome Measure: Twenty-eight-day survival distribution in patients with relative adrenal insufficiency (nonresponders to the corticotropin test).

Results: One patient from the corticosteroid group was excluded from analyses because of consent withdrawal. There were 229 nonresponders to the corticotropin test (placebo, 115; corticosteroids, 114) and 70 responders to the corticotropin test (placebo, 34; corticosteroids, 36). In nonresponders, there were 73 deaths (63%) in the placebo group and 60 deaths (55%) in the corticosteroid group (hazard ratio, 0.67; 95% confidence interval, 0.47-0.95; P=0.02). Vasopressor therapy was withdrawn within 28 days in 46 patients (40%) in the placebo group and in 65 patients (57%) in the corticosteroid group (hazard ratio, 1.91; 95% confidence interval, 1.29-2.84; P<0.001).

There was no significant difference between groups in nonresponders. Adverse events rates were similar in the 2 groups.

Conclusion: In our trial, a 7-day treatment with low doses of hydrocortisone and fludrocortisone significantly reduced the risk of death in patients with septic shock and relative adrenal insufficiency without increasing adverse events.
Effect of Treatment With Low Doses of Hydrocortisone and Fludrocortisone on Mortality in Patients With Septic Shock

Djillali Annane, MD, PhD
Vincent de Schepper, MD
Claire Charpentier, MD
Pierre-Edouard Belliard, MD, PhD
Bruno Francois, MD
Jean-Michel Korac, MD
Gilles Capelle, MD, PhD
Yves Cotera, MD, PhD
Elie Aubry, MD
Gilles Tinotchi, MD
Philippe Chaumet-Riffault, MD
Eric Bellissant, MD, PhD

TREATMENT WITH LOW DOSES OF HYDROCORTISONE AND FLUDROCORTISONE VS. PLACEBO

Predictive enrichment via ACTH stimulation test: “responders” and “non responders.”
No efficacy for the entire cohort.
Survival benefit among “non responders” treated with hydrocortisone and fludrocortisone.
Not replicated in a subsequent study.

Hydrocortisone Therapy for Patients with Septic Shock

Charles L. Sprung, MD, Djillali Annane, MD, M.D., Ph.D., Didier Keel, M.D., Rol Moreno, M.D., Ph.D., Mervyn Singer, M.D., F.R.C.P., Klaus Frei, M.D., Ph.D., Yoram G. Weiss, M.D., Julie Berenhultz, R.N., Armin Kalenka, M.D., Helmhult Forst, M.D., Ph.D., Pierre Francois Latierre, M.D., Konrad Reinhardt, M.D., Brian H. Outhoff, M.D., Didier Payer, M.D., Ph.D., and Jöel Biege, M.D., Ph.D., for the CORTICUS Study Group

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JAMA 288:862, 2002
Guidelines for the Diagnosis and Management of Critical Illness-Related Corticosteroid Insufficiency (CIRCI) in Critically Ill Patients (Part I): Society of Critical Care Medicine (SCCM) and European Society of Intensive Care Medicine (ESICM) 2017

Djillali Annane, MD, PhD; Stephen M. Pastores, MD, FCCM; Bram Rochwerger, MD; Wiebke Arlt, MD, DSc, FRCP; Robert A. Balk, MD, MCCM; Albertus Beishuizen, MD, PhD; Josef Briegel, MD, PhD; Joseph Carcillo, MD, FCCM; Mirjam Christ-Crain, MD, PhD; Mark S. Cooper, MD; Paul E. Marik, MD, FCCM; Gianfranco Umberto Meduri, MD; Keith M. Olsen, PharmD, FCCM; Sophia C. Rodgers, RN, MSN, ACNP, FCCM; James A. Russell, MD; Greet Van den Berghe, MD, PhD

Crit Care Med 45:278, 2017
Efficacy and safety of the monoclonal anti-tumor necrosis factor antibiotic P(ab)\textsubscript{2} fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels\textsuperscript{5}

Edward A. Panacek, MD, MPH, FCCM; John C. Marshall, MD, FCCM; Timothy E. Albertson, MD, PhD, FCCM; David H. Johnson, MD, MS, MBA; Steven Johnson, MD, FCCM; Rodger D. MacArthur, MD, Mark Miller, MD; William T. Bancroft, MD; Steven Flachskoff, MD; Martin Kain, MD; Lewis Teoh, MA; Lori Van Meter, MS; Lother Duann, PhD; Stanley Lerneshow, PhD; Gregory Hickin, MD; Christopher Doug, MD, MSc; for the Monoclonal Anti-TNF: A Randomized Controlled Sepsis Study Investigators

Crit Care Med 32:2173, 2004

Objective: To evaluate whether administration of afelimomab, an anti-tumor necrosis factor (TNF), monoclonal antibody fragment, would reduce 28-day all-cause mortality in patients with severe sepsis and elevated serum levels of IL-6.

Design: Prospective, randomized, double-blinded, placebo-controlled, multicenter, phase III clinical trial.

Setting: One hundred fifty-seven intensive care units in the United States and Canada.

Patients: Subjects were 2,834 patients with severe sepsis secondary to documented infection, of whom 998 had elevated interleukin-6 levels.

Intervention: Patients were stratified into two groups by means of a rapid qualitative interleukin-6 test kit designed to identify patients with serum interleukin-6 levels above (test positive) or below (test negative) approximately 1,000 pg/ml. Of the 2,834 patients, 998 were stratified into the test-positive group, 1,836 into the test-negative group. They were then randomly assigned 1:1 to receive afelimomab 1 mg/kg or placebo for 3 days and were followed for 28 days. The a priori population for efficacy analysis was the group of patients with elevated baseline interleukin-6 levels as defined by a positive rapid interleukin-6 test result.

Sepsis has become the leading cause of mortality and mortality for patients admitted to an intensive care unit (ICU) (1), with the multiple organ dysfunction syndrome being the common final pathway leading to death (2). The pathogenesis of multiple organ dysfunction syndrome is complex and reflects the activation of an extensive cascade of host-derived mediator molecules (3).

Measurements and Main Results: In the group of patients with elevated interleukin-6 levels, the mortality rate was 243 of 510 (47.6%) in the placebo group and 213 of 498 (42.8%) in the afelimomab group. Using a logistic regression analysis, treatment with afelimomab was associated with an adjusted reduction in the risk of death of 5.0% (p = .304) and a corresponding reduction of relative risk of death of 11.9% (p = .092). Mortality rates for the placebo and afelimomab groups in the interleukin-6 test-positive population were 25 of 101 (24.7%) and 20 of 167 (15.8%), respectively. In the overall population of interleukin-6 test positive and negative patients, the placebo and afelimomab mortality rates were 477 of 1,379 (34.5%) and 421 of 1,396 (30.2%), respectively. Afelimomab resulted in a significant reduction in tumor necrosis factor and interleukin-6 levels and a more rapid improvement in organ failure scores compared with placebo. The safety profile of afelimomab was similar to that of placebo.

Conclusions: Afelimomab is safe, bioactive active, and well tolerated in patients with severe sepsis, reduces 28-day all-cause mortality, and attenuates the severity of organ dysfunction in patients with elevated interleukin-6 levels. (Crit Care Med 2004; 32:2173-2185)

Key Words: sepsis; sepsis syndrome; clinical trial; tumor necrosis factor; monoclonal antibody; interleukin-6

**Prognostic/predictive enrichment via a rapid IL6 assay.**

**Patients stratified into 2 groups:**

- IL6 > 1,000 pg/ml → “IL6 positive”
- IL6 < 1,000 pg/ml → “IL6 negative”

**No efficacy in the overall cohort and in the IL6 negative cohort.**
Research

JAMA 320:1455, 2018

Effect of Targeted Polymyxin B Hemoperfusion on 28-Day Mortality in Patients With Septic Shock and Elevated Endotoxin Level

The EUFRACT Randomized Clinical Trial

R. Philip Gilger, MD, MSc; Sayed M. Bagheri, MD, MSc; Massimo Antanelli, MD, Debora M. Yanez, BSc; Edwin J. Klune, MD, MSc; John C. Marshall, MD; Paul M. Paley, MD; Lawrence S. Metzberg, MD; Cristiana Scarpa, SNP, MSA, RN; Steven Reischoff, MD, MPH; Paul M. Miller, MD, FHS, for the EUFRACT Trial Investigators

- Predictive enrichment via a rapid endotoxin activity assay.
- Enrollment restricted to those with endotoxin activity assay level ≥ 0.6.
- No efficacy.
Qualifiers, Questions, and Caveats

• Primarily used prior to randomization or to inform enrollment.
• But, could also be used in an adaptive trial design.
• Generalizability and applicability of results:
  • Can the enrichment strategy be used in practice?
  • Is the intervention beneficial in a non-enriched population?
• Sensitivity and specificity of the enrichment strategy?
• Should enrichment be used primarily in initial “proof-of-concept” studies, and then broadened to a more general populations?
• We’ve already tried this, and it hasn’t worked!
Prognostic and Predictive Enrichment—HORRIBLE IDEAS!
Fake News!
We need to innovate and develop more robust prognostic and predictive enrichment biomarkers.

Single biomarker approaches are appealing and pragmatic, but don’t seem to capture the biological complexity of sepsis.
Development of prognostic and predictive biomarkers for critical illness

Knowledge-Based Approach

Identify a clinical phenotype, e.g. immune suppression

Search for differentiating biomarkers and characteristics

Potentially biased
Limited by current “knowledge”
Development of prognostic and predictive biomarkers for critical illness

Discovery-Based Approach

High throughput “omics” to discover differentially regulated genes, proteins, etc. “BIG DATA”

How are the data associated with phenotype and what are the biological links?
Identification of sepsis biomarkers for prognostic enrichment

Whole genome expression profiling
Transcriptomics

Bioinformatics to identify candidate prognostic genes/biomarkers
The Pediatric Sepsis Biomarker Risk Model (PERSEVERE)

- Decision tree composed of 5 biomarkers
  - CC chemokine ligand 3 (a.k.a. MIP1α)
  - Interleukin 8
  - Heat shock protein 72
  - Granzyme B
  - Matrix metalloproteinase 8

- **Serum proteins** measured at the time admission with sepsis.
- Estimates baseline mortality probability.
- Multiple validations.
- Calibrated over time: PERSEVERE II.
- AUC: 0.88 to 0.92 (sensitivity >90%).
- Potential tool for prognostic enrichment.
Identification of sepsis biomarkers for \textit{predictive} enrichment

Whole genome expression profiling \textit{Transcriptomics}

Bioinformatics to identify candidate \textit{predictive} enrichment genes

Discovery of septic shock “endotypes”
“Endotypes”: subclasses of diseases or syndromes, as defined by a biological mechanism(s).
Developing a Clinically Feasible Personalized Medicine Approach to Pediatric Septic Shock

Hector R. Wong1,2, Natalie Z. Cvijanovich3, Nick Anas4, Geoffrey L. Allen5, Neal J. Thomas6, Michael T. Bigham7, Scott L. Weiss8, Julie Fitzgerald9, Paul A. Checchia10, Keith Meyer10, Thomas P. Shanley11, Michael Quasney11, Mark Hall12, Rainer Gedest13, Robert J. Freishtat14, Jeffrey Nowak15, Raj S. Shehkar16, Shira Gertz17, Emily Dawson18, Kelli Howard1, Kelli Harmon1, Eileen Beckman1, Erin Frank1, and Christopher J. Lindsey19

• The 100 endotype-defining genes reflect:
  • Adaptive immunity
  • Glucocorticoid receptor signaling
• Repressed in endotype A.
• Endotype A subjects have greater mortality and organ failure burden.

• After accounting for illness severity, age, and co-morbidity burden:
  
  • Allocation to endotype A independently associated with increased mortality.
  • Corticosteroid prescription independently associated with increased mortality among endotype A subjects.
• The 100 endotype-defining genes reflect:
  • Adaptive immunity
  • Glucocorticoid receptor signaling
• Repressed in endotype A.
• Predictive enrichment tool?
Adaptive Immunity and Glucocorticoid Signaling

Endotype A

Endotype B

Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach

Richard J. Hutchinson, Guillaume Moreve, Yiwen Ryan

Special issue: Sepsis

The new normal: immunomodulatory agents against sepsis immune suppression

Noelle A. Hutchins1, Jacqueline Unsinger1, Richard S. Hotchkiss2, and Alfred Ayala1

1 Division of Surgical Research, Rhode Island Hospital, Providence, RI 02903, USA
2 Department of Anesthesiology, Washington University in St. Louis, St. Louis, MO 63110, USA

Invited review

Immunotherapy: A promising approach to reverse sepsis-induced immunosuppression

Naeem K. Patil1,*, Julia K. Bohannon1, Edward R. Sherwood1,2

1 Department of Pathology, Vanderbilt University Medical Center, Nashville, TN, USA
2 Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA
Adaptive Immunity and Glucocorticoid Signaling

Endotype A

Endotype B

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Adjunctive Glucocorticoid Therapy in Patients with Septic Shock

Charles L. Sprung, M.D., Djillali Annane, M.D., Ph.D., Didier Payen, M.D., Rui Moreno, M.D., Ph.D., Mervyn Singer, M.D., F.R.C.P, Klaus Fehringer, Ph.D., Yves G. Weiss, M.D., Julie Berenbruch, R.N., Armin Kalenka, M.D., Helmut Forst, M.D., Ph.D., Pierre Francois Laterre, M.D., Konrad Reichart, M.D., Brian H. Cuthbertson, M.D., Didier Payen, M.D., Ph.D., and Josef Bireg, M.D., Ph.D., for the CORTICUS Study Group

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Hydrocortisone Therapy for Patients with Septic Shock

Effect of Treatment With Low Doses of Hydrocortisone and Fludrocortisone on Mortality in Patients With Septic Shock
Proof (some) of Concept: *Can we identify patients who may benefit from corticosteroids using prognostic and predictive enrichment?*

“PERSEVERE”: A multi-biomarker stratification tool to assign a baseline mortality probability

Endotype B patients have higher expression of glucocorticoid receptor pathway genes
Proof (some) of Concept: *Can we identify patients who may benefit from corticosteroids using prognostic and predictive enrichment?*

- **Endotype B**
- **Intermediate to high baseline mortality risk**

- Increased expression of GCR genes. *Predictive enrichment*
- Higher likelihood of disease-related event. *Prognostic enrichment*

- >10 fold decreased risk for poor outcome when prescribed corticosteroids.
  - O.R. 0.07
  - 95% CI: 0.10-0.48
  - P = 0.007
Take home messages...

• “Biomarkers for Clinical Trials” can translate to “Biomarkers for Prognostic and Predictive Enrichment”

• Prognostic and predictive enrichment strategies can increase trial efficiency.

• Prognostic and predictive enrichment can potentially increase our ability to demonstrate efficacy of trial interventions.

• We’ve tried this before…and it hasn’t worked.

• This could reflect well intended, simple, and pragmatic approaches, that nonetheless failed to capture the biological complexity of critical illness.

• We need to consider alternative approaches to biomarker discovery for critically ill patients.

• Large scale collaborations are needed.