Assays For Cholesterol Efflux Capacity: A Novel Biomarker of High-Density Lipoprotein (HDL) Functionality

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Abstract

Biomarkers of high-density lipoprotein (HDL) function may provide better cardiovascular risk discrimination compared to HDL-cholesterol (HDL-C) mass measurements. Cholesterol efflux from macrophages to plasma reflects the first critical step of reverse cholesterol transport (RCT) and is considered one of the key anti-atherosclerotic functions of HDL. Population-based studies in low and high-risk atherosclerotic cardiovascular disease (ASCVD) cohorts have consistently demonstrated an inverse relationship between the cholesterol efflux capacity (CEC) of human plasma and death or major adverse cardiovascular events (MACE) independent of endogenous HDL-C concentration.

Despite the importance of CEC as a biomarker of HDL function and a potential surrogate for clinical outcomes, its measurement has not been standardized to a single, reliable, and reproducible assay. The methodologies to measure CEC vary, often making comparisons between studies difficult. In this presentation, we review the pathways of cholesterol efflux, RCT, and describe the methodology of measuring CEC ex vivo and the findings linking CEC to human disease.

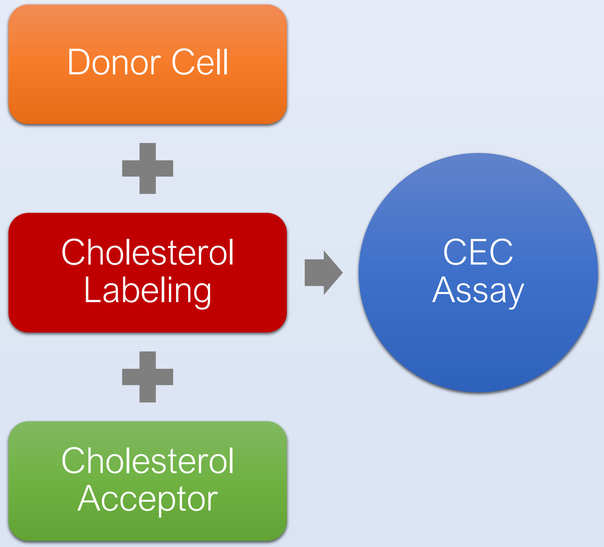
Background

- Preclinical and observational data have demonstrated an association between HDL-C and ASCVD.
- However, a number of agents developed to increase HDL-C concentration failed to improve cardiovascular outcomes, namely niacin, CETP inhibitors, as well as certain formulations of exogenous HDL.
- This discrepancy suggests that static measurements of HDL-C concentrations do not adequately quantify the physiologic functions of circulating HDL molecules.
- CEC is a dynamic measure of HDL function that has been shown to have an inverse relationship with death and MACE independent of HDL concentration.
- Despite the importance of CEC as a biomarker and a surrogate for clinical outcomes, assays that measure it have not been standardized.
- There are several ways to measure CEC, but a single, reliable, and reproducible assay, is needed to make data comparisons feasible, and for CEC to become viable assay for clinical use.

Reverse Cholesterol Transport

- RCT is the process by which cholesterol is transported from peripheral cells (e.g., macrophage foam cells within atherosclerotic plaques) to the liver for excretion in bile.
- Cholesterol efflux is the critical initial step of RCT.
- This involves rapid efflux of free cholesterol from foam cells to lipid-poor apoA-I and HDL via cell-membrane bound protein channels belonging to the ATP-binding cassette (ABC) transporter superfamily.
- ABCA1 and ABCG1 proteins redistribute intracellular cholesterol to cell-surface domains, generating a surface pool of free cholesterol that can be accessed by HDL for removal.
- They are the primary mediators of cholesterol efflux (~75% of efflux) from foam cells.

Key Considerations for Efflux Assays



Donors, Acceptors, and Labeling Methods

Donors

- **Non-transformed Human Cell Lines**
  - Pooled human umbilical vein endothelial cells (HUVECs)
  - Human aortic endothelial cells (HAECs)
  - Tangier Disease
  - Niemann-Pick Disease
  - Wolman's Disease
  - Homozygous Familial Hypercholesterolemia
- **Transformed Human Cell Lines**
  - THP-1 (human acute monocytic leukemia cells)
  - HepG2 (human hepatocarcinoma cells)
- **Animal Cell Lines**
  - J774A.1 (murine peritoneal macrophages)
  - RAW264.7/RAW2.1 (murine peritoneal macrophages)
  - Fu5AH (rat hepatoma cells)
  - BHK (baby hamster kidney cells)
  - CHO-K1 (Chinese hamster ovary cell)
  - COS7 (African green monkey kidney cells)

Labeling Methods

- **Radiolabeling**
  - Cholesterol-d7
  - 3[H]-cholesterol
  - [1,2-3H]-cholesterol
  - 14[C]- cholesterol
- **Fluorescence labeling (BODIPY)**

Acceptors

- Diluted whole serum
- ApoB-depleted plasma
- Isolated HDL
- apoA-I

Major Steps of CEC Assays

Plating of cell lines ± differentiation to macrophages (only for certain cell lines)

↓ Gentle cell wash

Cholesterol loading and radiolabeling

↓ Gentle cell wash

Equilibration of cholesterol pools

↓

Treatment with CEC modulators such as LXR agonists, cAMP, probucol or BLT1 to upregulate or downregulate cholesterol transporters

↓ Gentle cell wash

Treatment cessation

↓ Gentle cell wash

Induction of cholesterol efflux

↓ Gentle cell wash

Measurement of cholesterol efflux (labeled cholesterol in the cell culture media relative to that in the cells provides an estimation of cholesterol mobilization)

Conclusions

- Cell-based CEC assay is impacted by three key factors: expression levels of cholesterol transporters on the donor cell, the cholesterol labeling technique and the cholesterol acceptor particle.
- The CEC measurement assay is not standardized, and it is difficult to perform a comparison of results between most studies due to variations in specific protocols used.
- A single, reliable, and reproducible assay would make data comparisons feasible, and may make CEC a viable clinical assay.