and carotid artery stenosis. However, it is unknown whether cFAS is similarly elevated in patients with DM and advanced peripheral artery disease. This study aims to evaluate whether cFAS content and enzyme activity are biomarkers for clinical severity in patients with DM and chronic limb-threatening ischemia (CLTI).

Methods: Serum samples were prospectively collected from patients undergoing arterial revascularization procedures and maintained in an institutional review board-approved institutional biobank. The cFAS content and enzyme activity were evaluated using colorimetric ELISA assays. Multivariable logistic regression was used to evaluate DM and CLTI outcomes while adjusting for patient clinical characteristics. Hosmer-Lemeshow tests and C-index assessed goodness of fit and classification accuracy. All tests were two sided and a *P* value of less than .05 was considered significant.

Results: A total of 86 patients underwent cFAS analysis (67 content; 63 activity). Mean age was 65.0 ± 8.5 years with 67.4% male, 46.5% had DM, and 47.7% had CLTI. Bivariable analyses demonstrated association of CLTI with cFAS content ($P < .01$), DM ($P = .01$), and insulin use ($P = .01$); DM was associated with body mass index ($P = .001$), CKD ($P = .001$), and current smokers ($P < .05$). On multivariable analysis, CLTI was associated with cFAS content (odds ratio [OR] 1.16; 95% confidence interval [CI], 1.02-1.32; $P = .02$) and closely with DM (OR, 2.72; 95% CI, 0.94-7.89; $P = .066$), whereas DM was associated with body mass index (OR, 1.13; 95% CI, 1.05-1.23; $P < .01$) and CKD (OR, 4.86; 95% CI, 1.72-13.73; $P < .01$). No interactions were observed to be significant. Both models showed good fit ($P > .15$) and classification (Fig).

Conclusions: Serum cFAS content is associated with an increased risk of CLTI but not DM. Each unit increase in cFAS content increases the odds of CLTI by 16%. Future analysis with a larger sample and statistical bootstrapping will determine whether cFAS content is a clinically relevant biomarker of CLTI severity in patients with advanced atherosclerosis.

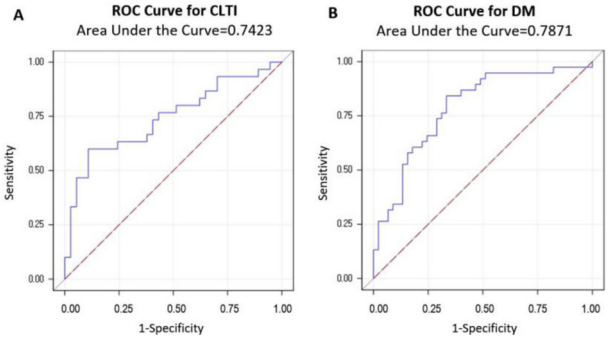

Fig. C-index and area under the receiver operating characteristic curve (AUROC) for crotocal limb-threatinging ischemia (*CLTI*) and diabetes mellitus (DM) multivariable logistic regression models. **A**, CLTI model with cFAS content and DM, AUROC = 0.7423 (95% conifdence interval, 0.6184-0.8663). **B**, DM model with BMI and chronic kidney disease (CKD); AUROC, 0.7871 (95% confidence limit, 0.8869).

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ZEB2 Regulates Activation and Exhaustion Programming of CD8 T Cells in Atherosclerosis

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Background: T cells are among the most prevalent immune cells found in human atherosclerotic lesions, yet their role remains obscure. In previous single-cell immunophenotyping studies we found that CD8$^+$ T cells of carotid atherosclerotic plaques display a spectrum of functionally heterogeneous states that vary based on differentiation, activation, and exhaustion. Furthermore, CD8$^+$ T-cell profiles varied between patients without (asymptomatic) and with (symptomatic) recent cardiovascular events (ie, transient ischemic attack and stroke), suggesting that T cells might contribute to adverse outcomes. The transcriptional regulator *ZEB2* has dual roles in T-cell differentiation and cardiovascular disease, as genome-wide association studies have reported *ZEB2* polymorphisms as independent risk alleles for coronary artery disease and myocardial infarction.

Methods: Our independent analysis identified *ZEB2* as a key driver of CD8$^+$ T-cell alterations in atherosclerotic lesions.

Results: We found that *ZEB2* was differentially regulated between patient types, with asymptomatic patients expressing higher *ZEB2* and *GZMB* levels compared with symptomatic (Fig, *A*). *ZEB2^{high}* CD8$^+$ T cells upregulated genes involved in cytotoxic functions, and conversely *ZEB2^{low}* CD8$^+$ T cells upregulated *PD-1* signaling in T-cell exhaustion (Fig, *B*). To probe the mechanistic implications of *ZEB2*, we performed in vitro chronic stimulation assays of human primary CD8$^+$ T cells using depleted of *ZEB2* using CRISPR/CAS9. *ZEB2* knockout cells had reduced cytotoxic function and had a dampened activation state upon acute stimulation.

Conclusions: Persistent stimulation-induced exhaustion in these cells showed that *ZEB2* knockout increased PD-1 expression levels, a protein marker that is critical for T-cell exhaustion (Fig, *C*). Collectively, these experiments suggest that *ZEB2* may contribute to the regulation of T-cell activation and exhaustion states, which is different between clinical phenotypes.

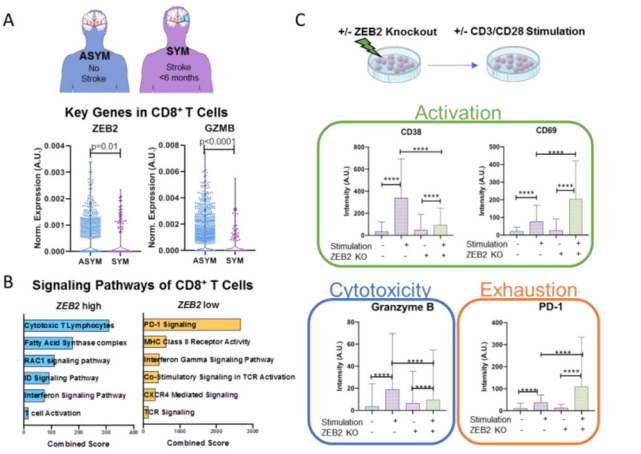

Fig. ZEB2 is a key driver of CD8* T cells in atherosclerosis. **A**, Expression of key genes from CD8* T cells stratified by patient type. Statistics by Welch's *t*-test. **B**, Signaling pathways upregulated in ZEB2^{high} and ZEB2^{low}CD8 T cells from scRnaseq data. **C**, ZEB2 knockout by CRISPR/CAS9 in human PBMCs and evaluated for expression of expression of activation (CD38, CD69), cytotoxicity (granzyme B), and exhaustion (PD-1) markers.

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The Role of Mast Cells in Atherosclerotic Plaque Calcification

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Background: Vascular calcification is a key feature of atherosclerosis and has been associated with major adverse cardiovascular events.