Mechanistic Insights to Target Atherosclerosis Residual Risk

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Abstract: Current pharmacological and mechanical therapies have reduced future cardiovascular risk. Nonetheless, a significant proportion of patients remained at high risk of recurrent events despite achieving guideline-directed therapeutic targets. This residual risk poses challenges despite tackling 'traditional' risk factors. Targeting the residual risk has been the focus of numerous pharmacotherapies which were associated with variable success. Incomplete understanding of the mechanistic nature combined with the lack of tools to precisely quantify the residual risk contributed to the relatively high residual risk after 'optimal' medical therapy. The development of atherosclerotic plaque is derived from lipid retention within arterial intima that triggers an inflammatory cascade accelerating atherosclerosis progression and rendering plaque more prone to rupture. The exposed subendothelial space with activated platelets causes arterial occlusion leading to potential fatality. Therefore, a distinctive approach to characterize these features may offer the opportunity to tailor novel antiatherosclerotic to reduce the residual risk. The traditional approach of measuring risk factors is beneficial at population-level but maybe less informative upon quantifying risk at an individual-basis. This review will discuss lipid accumulation, thrombosis, and inflammation as therapeutic targets of atherosclerosis. Additionally, we

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will summarize previous challenges of antiatherosclerosis therapies and the future role to tackle the residual risk. (Curr Probl Cardiol 2021;46:100432.)

Introduction

Over the last decade, mechanical and pharmacological therapies have aggregated patients' benefits leading into reduction of future cardiovascular risk. However, this future risk, which is referred to as residual risk, is not insignificant and 1 in 5 patients return with a second event within 5 years despite being on guideline-recommended optimal medical therapy.[1] The pathology of the residual risk is widely accepted to be heterogeneous and to a large extent is viewed from the aspect of the 'traditional' risk factors such as diabetes, smoking, and hypertension. These risk factors are useful markers to estimate the future risk of a cohort but become less informative on an individual basis. In fact, the mechanistic benefits of controlling these 'traditional' risk factors are related to changes in certain features of atherosclerotic disease as will be discussed later. Healthy diet, exercise, and smoking cessation, for example, decrease systemic inflammation which will be translated into reduction of cardiovascular events.[2] Therefore, characterizing the process of atherosclerotic disease at a mechanistic level would help understanding the nature of an individual residual risk and may allow precise intervention to diminish future cardiovascular events. The intricate interactions among coagulation cascade, lipoprotein particles, and inflammatory cells form the basis of atherosclerosis-related risk. These disease characteristics could be considered as 'upstream' features that are not solely dependent on the consequence of plaque rupture leading to myocytes necrosis. In contrast, 'downstream' features such as arrhythmia risk and heart failure have more close relationship with the heterogeneity and the size of myocardial injury. Current pharmacological and mechanical management to reduce these 'downstream' features are beyond the scope of this review.

The 'upstream' characteristics tend to have significant variations among patients, even after optimal medical therapy. Therefore, recognizing the prominent features of the atherosclerotic process may help 'labeling' patients based on their disease characteristics rather than merely a category of diagnosis, that is, previous stroke or postmyocardial infarction. Such approach would help mechanistically targeting high-risk individuals using novel therapies (Fig 1). This review will discuss the rationale of using this mechanistic approach to clarify the nature of the

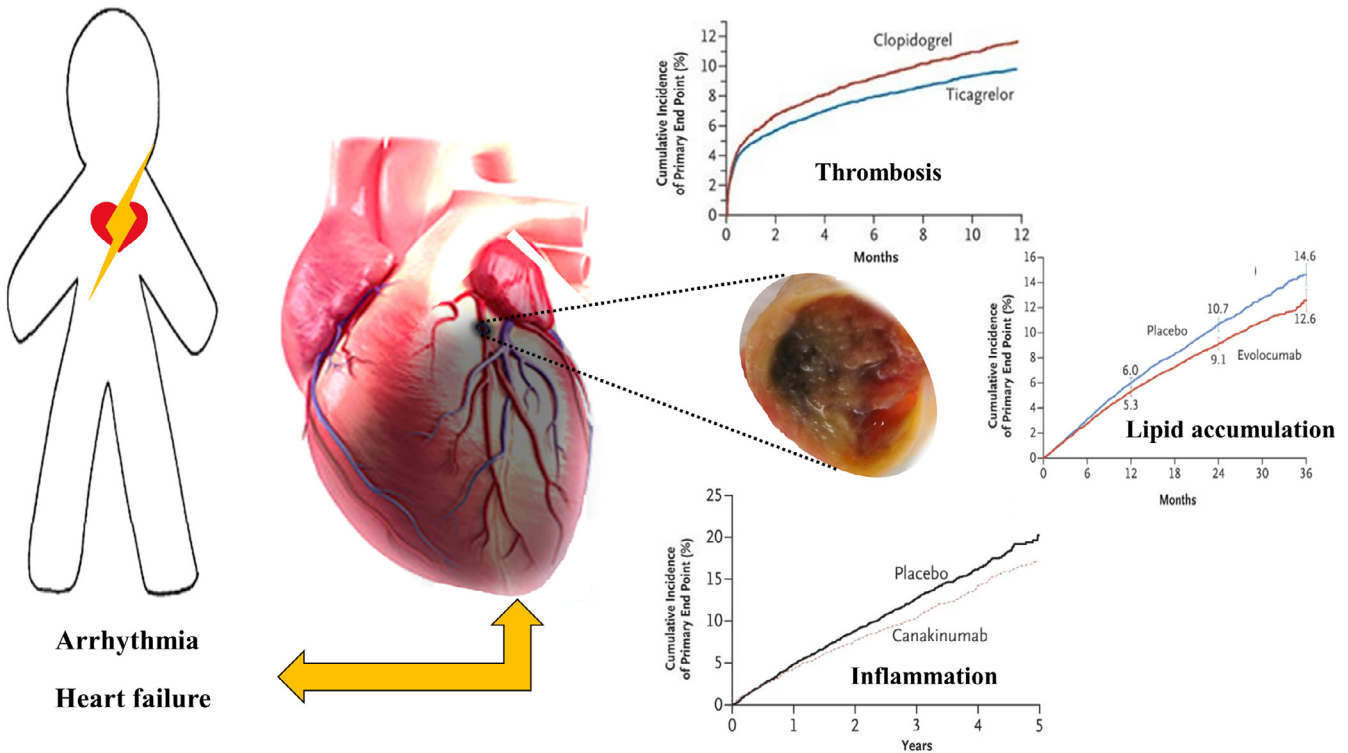

FIG 1. Schematic diagram demonstrating active features of atherosclerotic disease. Upstream disease characteristics contribute the build-up of the vulnerable plaque before its rupture. There is differential time effect upon using new antiatherosclerotic treatments. Targeting inflammation and lipid accumulation produce later effects compared to the early impact of reducing thrombosis risk. The downstream disease characteristics are more related to the size of myocardial injury.

residual risk and how such method would individualize and tailor patients' treatment.

Lipid Risk

The role of lipid accumulation, in particular LDL-c, in atherosclerosis has been established decades ago. Lowering LDL-c, mainly using statin, produced a reduction in cardiovascular events whereby the magnitude of benefits was proportional to the level of attained LDL-c.[3] Changes in atherosclerotic plaque burden were initially proposed as the mechanism of reducing cardiovascular events in response to statin treatment.[4] In fact, the decrease in plaque burden as quantified using intravascular ultrasound (IVUS) was related to the magnitude of LDL-c reduction and, subsequently, adverse cardiovascular events.[4]

Recently, the IMPROVE-IT, FOURIER, and ODYSSEY OUTCOMES trials, showed that further reduction in LDL-c was translated into better cardiovascular outcomes.[5-7] Ezetimibe in the IMPROVE-IT study was the first nonstatin treatment to show incremental cardiovascular benefits when added to statin.[5] The reduction in cardiovascular risk using ezetimibe followed a similar slope of LDL-c reduction as seen using statins. Remarkably, the PRECISE-IVUS study demonstrated that adding ezetimibe caused a significant decrease in atherosclerotic plaque volume, which was proportional to the magnitude of LDL-c reduction when compared to statins.[8] This implied that cardiovascular benefits are independent of the exerted mechanisms to reduce LDL-c using statin or nonstatin treatments.

Proprotein convertase subtilisin-kexin type 9 (PCSK9) has distinct mechanism of binding to the LDL receptor promoting its degradation.[9] Maintaining LDL receptors by inhibiting circulating PCSK9 caused unprecedented reduction in LDL-c which was translated into better cardiovascular outcomes.[6,7] The unselective nature of applying these drugs has been challenged given their cost, long-term safety and even estimated efficacy.[9,10] In fact, there were signals to suggest diminishing clinical benefits with modest risk reduction using PCSK9 inhibitors when juxtaposed to the marked attained LDL-c.[9] These signals were initially instigated from the GLAGOV trial whereby the reported decrease in plaque volume appeared modest in comparison with the projected one given the magnitude of LDL-c reduction.[9,11] This plateau effect would render LDL-c imprecise as a solitary marker of patients' response to lipid-lowering therapies at the level of atherosclerotic plaque. This should not be surprising given previous studies illustrating an over- or underestimated

coronary risk upon relying on LDL-c alone.[12] The discordant LDL-related measures using LDL-P (particle number) or apolipoprotein B highlighted the variations in long term risk beyond LDL-c and the importance of targeting non−LDL-c markers.[12]

Epidemiological studies consistently reported the inverse relationship between HDL-c and cardiovascular risk. HDL-c was subsequently proposed as a therapeutic target which could be used as a tool to identify patients with high residual risk beyond LDL-c. Nonetheless, HDL-c raising therapies failed to produce incremental benefits when added to statin.[13] Despite producing favorable lipid profile using cholesteryl ester transport protein (CETP) inhibitors, outcomes from large studies investigating CETP inhibitors showed comparable risk reduction to placebo.[10] Interestingly, changes in atherosclerosis burden were not significantly different from placebo with any of these CETP inhibitory molecules.[10]

An important clinical question is whether changes in atherosclerotic burden are the surrogate of mechanistic benefits when targeting lipid pathway. The relatively small changes in atherosclerotic plaque volume did not match and might not fully explain the decrease in the clinical risk associated with lipid-lowering treatment.[10] Therefore, changes to plaque composition with net lipid depletion and replacement with fibrous tissue were proposed as a stabilizing mechanism in response to LDL-c reduction.[10] Certainly, plaque lipid content is considered a strong discriminator in determining the vulnerability of atherosclerotic plaque.[10] Novel vascular imaging tools were able to precisely track changes in plaque composition using lipid lowering therapies.[14,15] The inherent limitations of established technologies in detecting changes in plaque composition should not deter from the proposed mechanistic benefits at the level of the atherosclerotic plaque. This may explain the lack of PCSK9-associated lipid-depletion when evaluated using IVUS virtual histology.[16]

The intricate relationship among lipoprotein particles poses challenges when targeting a single blood marker within the lipid pathway. Plaque imaging is a promising strategy that takes into considerations the integrated effects of all blood biomarkers and can accurately quantify lipoprotein-related residual risk, offering the right platform to tailor novel therapies. This becomes important to rationalize the disproportionate cardiovascular benefits that were reported in the REDUCE-IT trial when targeting triglycerides.[17] Similarly, mechanistic understanding of how targeting lipoprotein(a) may translate into cardiovascular benefits is needed before embarking on a large clinical trial targeting yet another blood biomarker.[18]

Thrombosis Risk

The formation of platelet-rich thrombi is the principal cause of coronary obstruction in segments with atherosclerotic disease. The variations in platelet hyper-reactivity among antiplatelet-naïve individuals and their association with cardiovascular events were early established.[19] Consequently targeting platelet aggregation would yield heterogeneous platelet response whereby up to one-third of patients demonstrated high platelet reactivity (HPR) despite treatment.[19] This is important as HPR was associated with increased risk of future cardiovascular events including mortality.[20] Ticagrelor and prasugrel provided faster and more consistent ADP-induced platelet inhibition that showed superior benefits in patients presenting with ACS. Moreover, both drugs dramatically reduced the prevalence of HPR when compared to clopidogrel.[21] Nonetheless, a significant proportion of patients remained with HPR in particular diabetic patients and those post-ACS which inevitably contributed to the residual risk.[22]

Thrombin-mediated platelet activation remained untargeted despite being the most potent of all platelet agonists, through cleavage of protease-activated receptors (PAR1&4).[23] Vorapaxar is an oral PAR1 antagonist that showed significant reduction in cardiovascular events following ACS when compared to placebo.[24] However, these benefits were offset by an increased risk of major bleeding including intracranial bleeding. The brisk and transient role of PAR1 in initiating platelet activation might explain the lack of success with PAR1 antagonist and promoted more studies investigating the role of PAR4 antagonist.[25] The slow and sustained PAR4 platelet activation at higher thrombin concentration may serve as an ideal target in the setting of atherosclerosis.[25] Indeed, evidence from ex vivo studies suggest that PAR4 antagonists may provide a good balance between platelet inhibition and risk of bleeding. Whether these benefits will be translated into reduction of cardiovascular outcomes are yet to be determined.

Extending the duration of antiplatelet therapies beyond 12 months after ACS was also sought to reduce thrombosis-related residual risk. The beneficial signal from the CHRISMA trial of adding clopidogrel to aspirin in patients with symptomatic atherothrombosis has instigated the approach of inhibiting platelet beyond 1 year.[26] The PEGASUS-TIMI 54 reported incremental benefits when adding reduced ticagrelor dose (60 mg) to aspirin in patients with more than 1 year of myocardial infarction.[27] Importantly, there was an increased rate of major bleeding in the combination group compared to the aspirin-only group.[27]

It remained challenging to identify those patients who may benefit maximally from prolonging antiplatelet therapies while at the same time are not counterbalanced by increased risk of bleeding. This becomes more important since bleeding events are independently linked to increased mortality.[28] Using specific clinical characteristics, certain risk models have been proposed to identify patients at low risk of bleeding who may benefit from prolonged antiplatelet treatment.[29] Importantly, these integrated clinical features were derived from cohort study that do not specifically reflect individual platelets properties and, importantly, still need to be prospectively tested in randomized trials.[28]

The concept of estimating individual thrombosis risk is appealing, however, the current tools to quantify this risk, and subsequently, utilize it are still suboptimal.[19] There is lack of consensus regarding optimal cut-off to establish HPR with most of these tools relying on a single pathway to assess residual platelet activities.[19] The complex interaction among platelets activation pathways and, importantly, their interindividual variations in contributing to thrombosis would render the assessment of a single pathway imprecise in estimating thrombotic risk.[19] This may explain the lack of success in reducing the residual risk when using these tools to escalate antiplatelet therapy.[30] Nonetheless, one promising strategy may be to de-escalate therapy in patients with low platelets activities, who are at increased risk of bleeding. Guided de-escalation platelet inhibition showed noninferiority results in the TROPICAL-ACS, and even superiority net clinical benefits in the TOPIC-VASP studies.[31,32] The rationale in de-escalating antiplatelet therapy was founded on the potential differential timing in ischemic risk following myocardial infarction. The greater reduction in ischemic events was observed within the first month with accrued bleeding risk after that.[33]

One relatively undertested approach is to target the protein arm of the coagulation cascade targeting fibrin formation. The ATLAS ACS-2 TIMI 51 study reported reduction of ischemic endpoints using rivaroxaban but at the expense of increased bleeding events.[34] The unselective approach in the ATLAS-ACS-2 alongside the significant risk of bleeding, including intracranial bleeding, have limited the use of adding anticoagulation to dual antiplatelet therapy. Recently, Sumaya et al[35] proposed a personalized strategy to identify a subgroup of patients who may benefit from additional anticoagulation therapy. Using fibrin clot properties, they demonstrated that patients with significant resistance in plasma clot lysis time are associated with adverse clinical outcomes at 1 year.[35] While appeared prognostically relevant, the significance of clot lysis time as therapeutic targets is yet to be determined.

Thrombosis risk remains one of the most challenging disease substrates in atherosclerosis in part due to the cumulative risk of bleeding with more potent treatment. In addition, the lack of optimal intermediate therapeutic target akin to LDL-c in the lipid pathway adds uncertainty into patients' response to antithrombotic therapy.

Inflammation Risk

Immune cells, noncellular components such as interleukins and circulating microparticles, and recently the perivascular adipose tissue all contribute to the inflammatory role in atherosclerosis.[36,37] Nonetheless, the central immune pathway of NLRP3-activated IL-1β, tumor necrosis factor (TNF-α), IL-6 to hs-CRP demonstrated a strong association with high-risk atherosclerotic disease.[2] The interaction with crystalline structure, in particularly the cholesterol ones, is one of several processes to activate NLRP3 inflammasome. Therefore, targeting inflammatory signaling pathways have always been an aspiration to mitigate residual atherosclerotic risk.

Aspirin was one of the earliest pharmacotherapies to illustrate incremental benefits with increasing inflammation, quantified using hs-CRP, independently of its antiplatelet properties.[38] Numerous statin trials demonstrated anti-inflammatory properties in addition to cholesterol reduction with maximal benefits in individuals achieving 'optimal' hs-CRP and LDL-c.[39] However, dedicated anti-inflammatory drugs were unsuccessful in reducing residual risk.[40] Darapladib is a lipoprotein-associated phospholipase A_2 inhibitor that did not reduce cardiovascular risk in the STABILITY trial.[40] Similarly, the inhibition of intracellular p38 mitogen-activated protein kinase using losmapimod was not translated into a significant reduction in cardiovascular risk.[41] More recently, low dose methotrexate in the CIRT trial resulted in comparable cardiovascular events when compared to placebo.[42] Importantly, these drugs did not affect the central immune pathway with no significant reduction in IL-1, IL-6, and hs-CRP.

Canakinumab, on the other hand, is a human monoclonal antibody against IL-1 which was studied in the CANTOS trial.[43] Canakinumab caused 15% reduction in cardiovascular events, without affecting mortality, when compared to placebo.[43] Nevertheless, this was associated with small but a significant increase in the rate of fatal infection.[43] The cardiovascular benefits within the CANTOS, including mortality rate, were related to the magnitude of reduction in hs-CRP.[44] Among patients who attained hs-CRP $<$ 2mg/dL there was 25% risk reduction in cardiovascular

events with almost one-third decrease in both cardiovascular and all-cause mortality.[44] In contrast, there were no cardiovascular benefits in patients with hs-CRP $\geq$ 2mg/dL following canakinumab treatment.[44] Similar results were reported when using IL-6 as an intermediate therapeutic target.[45] Achieving low IL-6 level using canakinumab halved mortality rate with one-third reduction in cardiovascular events.[45] Likewise colchicine has some NLRP3-inhibiting effects and has shown promise in reducing cardiovascular events in a relatively small randomized trial.[46] At the level of atherosclerotic plaque, colchicine stabilized plaque features as quantified using computed tomography, without affecting total burden, highlighting its anti-inflammatory properties.[47]

These studies reinforced the pivotal role of IL-1- IL-6, and hs-CRP in determining residual inflammatory risk. It is imperative to highlight that hs-CRP is not an active participant in atherothrombosis despite its significant prognostic value, alongside TNF-α, in determining vascular risk.[48] Therefore, tools to specifically reflect coronary plaque inflammation are needed to tailor anti-inflammatory therapies. 18F-fluoride positron emission tomography and perivascular fat attenuation index are promising imaging tools to identify inflamed atherosclerotic plaques.[36,49] The observed uptake of 18F-NaF was consistent with macrophage infiltration reflecting plaque-specific inflammatory status. The prognostic value of high 18F-NaF uptake in coronary arteries is currently being studied in the PREFFIR study.[50] In the CRISP-CT study, the attenuation index of perivascular adipose tissue added important insights into the long-term cardiovascular risk beyond currently-available assessments using CT.[36] This noninvasive imaging tool provided quantitative measures of coronary inflammation. Nonetheless, whether these imaging biomarkers could be used as therapeutic targets remained to be determined.

Current Challenges of Pharmacotherapy to Reduce Residual Risk

The challenges in targeting the residual risk are complex and heterogeneous. Firstly, some of the antiatherosclerotic drugs showed a degree of clinical benefits when assessed against placebo as a monotherapy but failed to demonstrate incremental benefits when added to optimal medical therapy. Advances in guideline-directed medical therapy have aggregated cardiovascular benefits making the detection of further reduction in cardiovascular risk related to any new drug more challenging. Secondly, there has been lack of mechanistic understanding in relating cardiovascular risk reduction upon targeting certain biomarkers. HDL-c raising

therapies were presumed to improve the antiatherogenic properties of HDL particles.[51] To the contrary, inhibition of CETP activity may produce dysfunctional HDL particles with impaired reverse cholesterol transfer mechanism.[51] Thirdly, identifying a biomarker as a potential risk does not always translate into reduction of cardiovascular risk by targeting this particular biomarker. In other words, prognostic markers of future cardiovascular risk should not be immediately labeled as therapeutic targets. In the GRAVITAS trial, high-dose clopidogrel reduced HPR prevalence, a prognostically significant marker of future cardiovascular risk. Nonetheless, this was not translated into reduction in cardiovascular events.[30] Fourthly, the complex interactions, individual variations and contributions among thrombotic, lipid, and inflammatory biomarkers would render a single biomarker imprecise in reflecting the future vascular risk. What is needed are tools to integrate the effects of these various biomarkers to reflect certain risk at the level of the atherosclerotic plaque. For instance, LDL-c is a direct cause of atherosclerosis and likely to be the best therapeutic target in managing atherosclerosis. Nonetheless, reduction in atherosclerotic plaque lipid content in response to statin treatment was not related to changes in LDL-c and other lipid-related biomarkers also contribute to the future cardiovascular risk (Fig 2).[14,52] The dynamic and accumulative nature of atherosclerotic plaque is unlikely to reflect the relatively narrow range of LDL-c over lifetime.[14] Fifthly, the studied cohorts in large randomized trials were rather heterogeneous and were recruited based on patient's category of disease (ie previous myocardial infarction or stroke) rather than individual disease characteristics (thrombosis, lipid accumulation, and inflammation risks). Therefore, refinement of these clinical trials is increasingly needed to detect any signal of benefits above noise. The CANTOS trial could be considered as an initial attempt in employing this approach of recruiting patients based on their disease characteristics, that is, residual elevated hs-CRP $\geq$ 2 mg/dL following myocardial infarction.[43]

It is important to highlight that the number-needed-to-treat (NNT) using antiatherosclerotic drugs, even with successful drugs such as canakinumab and PCSK9 inhibitors, remained relatively large (>1:50). This modest risk reduction poses challenges regarding the clinical applicability of these drugs in the real world, their cost effectiveness, and their safety profile.[9] Yet patients who stand to benefit greatly may miss an important therapeutic opportunity. Therefore, if the potential clinical benefits of these drugs are to be realized, risk stratification methods are needed. Importantly, these methods should reflect the nature of the risk and matches the mechanism of the tested drug to the targeted risk.

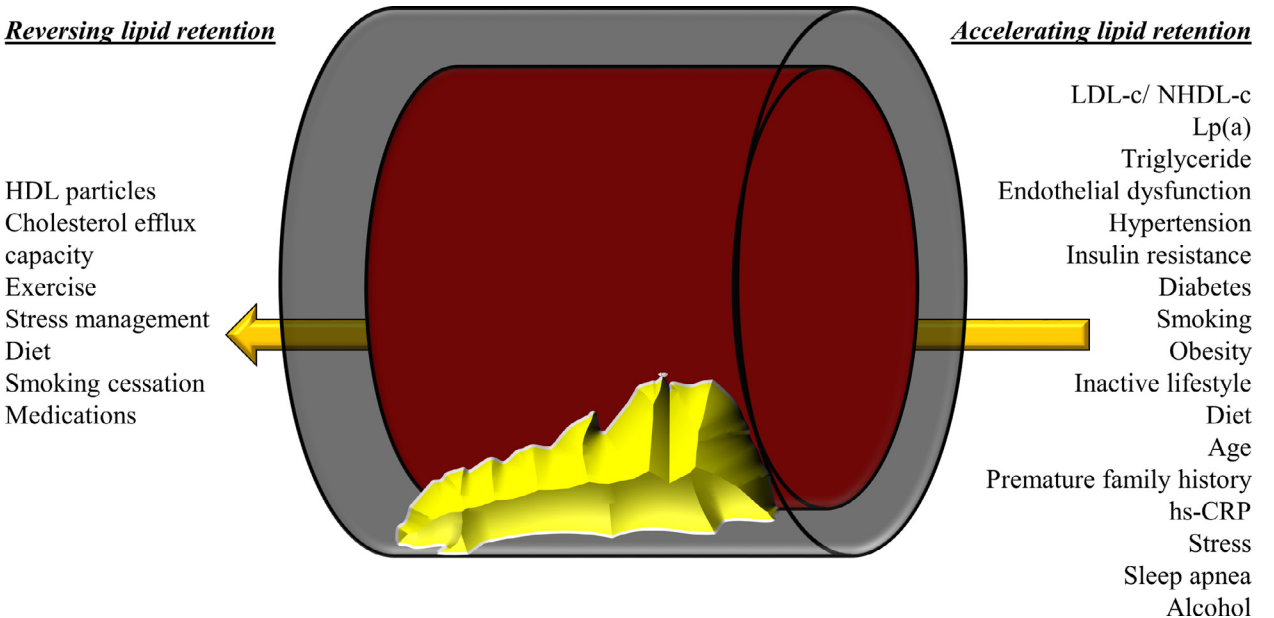

FIG 2. Factors contributing to accumulating and reversing lipid retention. The complex interaction of pro- and antiatherosclerotic biomarkers would make accurate assessment of lipid retention within arterial intima imprecise. Novel vascular imaging that can quantify atherosclerotic plaque lipid content may offer better alternative upon estimating individual risk.

Patients with propensity to develop plaques at multisites vascular territories may benefit from additional intensive lipid-lowering treatment. Such approach reduced NNT with ezetimibe from 1:50 to 1:11 when was used in patients with large burden of atherosclerosis.[53] Similarly, NNT with canakinumab was 1:16 in those considered responders compared to 57 in nonresponders.[44] Likewise, there was 71% risk reduction in the net clinical benefits (thrombosis and bleeding risks) upon de-escalating antiplatelet therapy in patients with low platelet reactivity.[32]

Overall, the heterogeneity of atherosclerotic disease features and the variations in individuals' response to currently-available therapies dictate more comprehensive approach to understand and quantify the subject residual risk. Therefore, characterizing atherosclerotic disease based on its mechanistic features and subsequently matching drugs mechanism of action to the individual amplified risk may offer the opportunity to achieve more precise intervention and greater efficacy generating more cost-effective prescribing.

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