From Oxidative LDL Modification to Composite Inflammatory Indices: A Review of Diagnostic Biomarkers in Atherosclerosis

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Abstract

Background: Atherosclerosis remains the principal substrate for ischaemic heart disease and stroke, yet conventional lipid testing alone fails to identify a substantial proportion of at-risk individuals (1,2). Oxidative modification of LDL and downstream inflammatory activation are now recognised as central, biomarker-rich processes driving plaque initiation, progression, and rupture (3,4).

Objective: To systematically compile and appraise biomarkers used in atherosclerosis diagnosis and risk stratification — traditional lipid markers, oxidative LDL-modification products, inflammatory mediators, composite ratios, and emerging molecular markers — classifying each by structure, mechanism, estimation method, reference range, and temporal (early versus late) predictive utility, individually and in combination.

Methods: A narrative-systematic search of PubMed/MEDLINE, Scopus, Embase, Cochrane Library, and Web of Science (January 2000 – June 2026) identified original studies, meta-analyses, and reviews reporting biomarker biology, methodology, or diagnostic performance in atherosclerosis. Data were extracted and synthesised thematically across five predefined biomarker domains.

Results: Oxidative products of LDL modification (oxLDL, malondialdehyde, 4-hydroxynonenal, oxysterols, modified apoB-100) (3–6) and plaque-specific inflammatory enzymes (Lp-PLA2, myeloperoxidase, MMP-9) (7–9) consistently outperformed static lipid values in identifying biologically active and vulnerable plaque. Composite indices integrating lipid and leukocyte-derived parameters (NLR, MHR, SII, SIRI) achieved the highest reported diagnostic accuracy (AUC generally 0.75–0.85) (10,11), exceeding any single marker in isolation.

Conclusion: No single biomarker captures the full biology of atherosclerosis. A tiered strategy combining lipid, oxidative, and inflammatory markers offers the most evidence-supported approach to early detection and risk stratification, pending further assay standardisation.

Keywords

Atherosclerosis; biomarkers; oxidised LDL; malondialdehyde; 4-hydroxynonenal; lipid peroxidation; oxysterols; apolipoprotein B; C-reactive protein; lipoprotein-associated phospholipase A2; myeloperoxidase; inflammation; neutrophil-to-lymphocyte ratio; monocyte-to-HDL ratio; Lipoprotein(a); cardiovascular risk stratification; endothelial dysfunction

How to cite this article: Hivre M, Vaishnav D, Holkar S. From Oxidative LDL Modification to Composite Inflammatory Indices: A Review of Diagnostic Biomarkers in Atherosclerosis. Int J Drug Deliv Technol. 2026;16(63s):658-682. DOI: 10.25258/ijddt.16.63s.68

Introduction

Atherosclerosis is a chronic, progressive inflammatory disease of medium- and large-sized arteries characterised by the accumulation of lipids, inflammatory cells, fibrous elements, and calcified deposits within the arterial intima, culminating in the formation of atheromatous plaque (1). It constitutes the principal pathophysiological substrate for ischaemic heart disease, ischaemic stroke, and peripheral arterial disease, which together account for the largest single share of mortality worldwide (1,2).

For more than half a century, clinical risk assessment for atherosclerotic cardiovascular disease has relied predominantly on static lipid measurements, principally total cholesterol, LDL-cholesterol, and HDL-cholesterol, embedded within composite risk calculators such as the Framingham Risk Score and the Pooled Cohort Equations (2). While these tools retain clinical utility at a population level, a substantial proportion of acute coronary and cerebrovascular events occur in individuals with LDL-C concentrations within or near the therapeutic target range, indicating that the conventional lipid panel captures only one dimension of a multifactorial disease process (18). This observation has driven three decades of biomarker research aimed at characterising the biochemical processes that convert a stable, lipid-laden plaque into one that is inflamed, structurally weakened, and prone to rupture.

Central to this evolving understanding is the oxidative modification hypothesis of atherosclerosis, which proposes that native LDL particles, upon retention within the subendothelial space, undergo enzymatic and non-enzymatic oxidative modification to generate a heterogeneous family of biologically active products (3,4,20). These include lipid hydroperoxides derived from oxidised phospholipids and cholesteryl esters, reactive aldehydes such as malondialdehyde, 4-hydroxynonenal, and acrolein, oxysterols including 7-ketocholesterol and 7β-hydroxycholesterol, and structurally modified apolipoprotein B-100 bearing fragmentation, cross-linking, and aldehyde-adduct formation (5,6,19). Beyond simply disrupting the native structure and clearance of LDL, these oxidation products function as potent signalling molecules that activate endothelial adhesion molecule expression, recruit and differentiate monocytes into lipid-laden macrophages and foam cells, and sustain a self-perpetuating cycle of vascular inflammation (3,4). This mechanistic insight has positioned oxidative and inflammatory biomarkers as complementary, and in some clinical contexts superior, tools to conventional lipid measurement for capturing biologically active disease (20,21).

In parallel, the recognition that atherosclerosis is fundamentally an inflammatory disease has elevated the clinical and research importance of circulating inflammatory mediators (C-reactive protein, interleukin-6), enzymes localised to vulnerable plaque (Lp-PLA2, myeloperoxidase) (7,8,12,13), and inexpensive composite haematological indices derived from the routine complete blood count (NLR, MHR, SII, SIRI) (10,11). These markers, individually and especially in combination with lipid and oxidative indices, have demonstrated incremental value in discriminating stable from vulnerable plaque phenotypes and in predicting incident cardiovascular events independent of traditional risk factors.

The present review was undertaken to consolidate this expanding and heterogeneous body of literature into a single structured reference. It addresses four specific aims: first, to catalogue the principal biomarkers of atherosclerosis across the lipid, oxidative, inflammatory, and emerging molecular domains; second, to summarise, for each marker, its biochemical structure, site and pathway of synthesis or generation, mechanism of pathogenic action, standard laboratory method of estimation, and accepted normal reference range; third, to classify markers according to their temporal utility as early (subclinical, pre-atheroma) versus late (established plaque, vulnerable plaque, or acute event) predictors of disease; and fourth, to evaluate the comparative and combinatorial diagnostic performance of individual markers and composite indices/ratios, drawing on diagnostic accuracy data reported in the appraised literature.

Materials and Methodology

1.Review design

This article was structured as a narrative systematic review, combining the comprehensive search and transparent reporting principles of a systematic review with a thematic, mechanism-oriented narrative synthesis appropriate to the heterogeneous nature of the biomarker literature, which spans biochemistry, cell biology, and clinical diagnostics. The review followed the general reporting framework of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement where applicable to the search and selection process.

2.Search strategy

A structured electronic search was performed across five databases: PubMed/MEDLINE, Scopus, Embase, the Cochrane Library, and Web of Science Core Collection, covering the period from January 2000 to May 2026. Search terms were combined using Boolean operators and included: “atherosclerosis” AND (“biomarker*” OR “diagnos*” OR “predict*”); “oxidised LDL” OR “oxidized LDL” OR “oxLDL”; “malondialdehyde” OR “MDA”; “4-hydroxynonenal” OR “4-HNE”; “oxysterol*” OR “7-ketocholesterol”; “apolipoprotein B” OR “apoB-100”; “lipoproteinÂ (a)”; “C-reactive protein” AND “atherosclerosis”; “lipoprotein-associated phospholipase A2” OR “Lp-PLA2”; “myeloperoxidase” AND “plaque”; “neutrophil to lymphocyte ratio” OR “NLR”; “monocyte to HDL ratio” OR “MHR”; “intima-media thickness” AND “biomarker”; and “microRNA” AND “atherosclerosis”. Reference lists of retrieved articles and prior reviews were hand-searched to identify additional eligible studies.

3 Eligibility criteria

Inclusion criteria: (a) original research articles, meta-analyses, pooled analyses, and systematic reviews reporting biochemical structure, synthetic pathway, mechanism of action, laboratory estimation method, reference range, or diagnostic/prognostic performance of a biomarker in relation to atherosclerosis or its clinical sequelae; (b) studies involving adult human subjects, or foundational mechanistic studies in validated animal/cell-culture models where directly relevant to biomarker biology; (c) articles published in English between January 2000 and May 2026.

Exclusion criteria: (a) case reports and case series with fewer than 10 subjects; (b) conference abstracts without full-text data; (c) articles focused exclusively

on therapeutic intervention without biomarker-relevant diagnostic or mechanistic data; (d) articles lacking extractable data on assay methodology, reference ranges, or diagnostic performance; (e) duplicate publications or superseded preliminary reports where a later full study was available.

4 Study selection and data extraction

Titles and abstracts identified through the search strategy were screened for relevance, followed by full-text assessment of potentially eligible articles against the inclusion and exclusion criteria. Data extracted from each eligible article included: biomarker name and classification domain; site of synthesis or generation and biochemical structure; proposed mechanism of pathogenic action; standard laboratory method(s) of estimation; reported reference range or clinically applied cut-off value; and reported diagnostic, prognostic, or correlative performance metrics (sensitivity, specificity, area under the receiver operating characteristic curve, odds ratio, or correlation coefficient) where available.

5 Data synthesis

Given the marked heterogeneity in biomarker class, assay platform, study population, and outcome definition across the included literature, a quantitative meta-analysis was not undertaken. Data were instead synthesised narratively and organised thematically into five biomarker domains: traditional lipid/lipoprotein markers, oxidative LDL-modification products, inflammatory mediators, composite haematological/lipid ratios, and emerging genomic/proteomic/imaging-correlated markers. Within each domain, markers were further classified, where supported by the literature, as predominantly early (subclinical/pre-atheroma) or late (established/vulnerable plaque or acute-event) predictive markers, and their reported performance individually and in combination was tabulated for comparative appraisal.

6 Quality considerations

Where diagnostic accuracy data were extracted from primary studies or meta-analyses, the methodological quality of source studies was considered using domains consistent with the QUADAS-2 framework (patient selection, index test, reference standard, and flow/timing), and findings from studies with high inherent risk of bias were interpreted cautiously and flagged accordingly in the relevant sections of this review.

7 Search and selection summary

Stage	Outcome / criteria applied
Databases searched	PubMed/MEDLINE, Scopus, Embase, Cochrane Library, Web of Science Core Collection
Date range	January 2000 – June 2026
Records identified	Combined keyword search across five databases plus reference list (hand) searching
Screening	Title/abstract screening for relevance to biomarker structure, mechanism, estimation, or diagnostic performance in atherosclerosis
Full-text assessment	Applied inclusion/exclusion criteria detailed in Section
Final synthesis	Narrative thematic synthesis across five biomarker domains; no quantitative pooling performed due to outcome heterogeneity

Structure and Synthesis of the Atherogenic Substrate: LDL and Its Oxidative Modification

1 Native LDL and subendothelial retention

LDL is a spherical particle (18–25 nm) comprising a cholesteryl-ester/triglyceride core surrounded by a phospholipid monolayer and a single copy of apolipoprotein B-100 (apoB-100), generated through lipolytic processing of VLDL and cleared mainly via hepatic LDL-receptor-mediated endocytosis (5,19,20). Atherogenesis begins when LDL, particularly small dense subfractions, crosses a dysfunctional endothelium and becomes retained in the subendothelial matrix through ionic interaction between apoB-100 and proteoglycans. This retained LDL is removed from plasma's antioxidant milieu and becomes susceptible to sustained oxidative attack by endothelial cells, smooth muscle cells, and macrophages (5,19,20,24).

2 Molecular products of oxidative LDL modification

Oxidative modification of retained LDL is a graded continuum from minimally modified LDL to extensively oxidised LDL (ox-LDL), generating a family of biologically active molecules that disrupt LDL's structural integrity while also functioning as potent signalling mediators of vascular inflammation, endothelial dysfunction, and immune activation (3,4,5,6):

- Lipid hydroperoxides of oxidised phospholipids and cholesteryl esters — early peroxidation products of polyunsaturated fatty acyl chains, and direct precursors of reactive aldehydes and oxidised phospholipid species such as POVPC and PGPC (6).
- Reactive aldehydes — malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and acrolein — stable end-products of polyunsaturated fatty acid peroxidation that form covalent adducts with lysine residues on apoB-100, altering its structure and receptor-binding properties (5,6).
- Oxysterols — 7-ketocholesterol and 7β-hydroxycholesterol — formed by free-radical oxidation of the cholesterol moiety; cytotoxic to vascular cells and implicated in foam cell formation and necrotic core apoptosis (3,4).
- Structurally modified apoB-100 — fragmentation, cross-linking, and aldehyde-adduct formation — which abolishes native LDL-receptor recognition and generates neo-epitopes recognised by macrophage scavenger receptors (CD36, SR-A), driving unregulated foam cell formation (3,4,20).

Collectively, these products transform LDL from a passive lipid carrier into a pro-inflammatory, immunogenic structure that upregulates endothelial adhesion molecules, promotes macrophage foam cell transformation, and sustains a self-amplifying cycle of vascular inflammation underlying every subsequent stage of plaque progression and instability (3,4,19,20).

Mechanism of Atherogenesis: A Biomarker-Oriented Overview

Understanding the staged mechanism of atherosclerotic plaque development is essential to appreciating why different biomarkers carry differing predictive value at different stages of disease (3,19,20). The process can be conceptually divided into five overlapping stages, each associated with a relatively distinct biomarker signature.

1. Endothelial dysfunction and lipoprotein retention — Reduced nitric oxide bioavailability, increased endothelial permeability, and upregulated adhesion molecule expression (VCAM-1, ICAM-1, E-selectin) permit subendothelial entry and proteoglycan-mediated retention of apoB-containing lipoproteins, particularly small dense LDL and Lipoprotein(a). This stage is best captured by early markers of endothelial activation (soluble VCAM-1/ICAM-1) and by quantification of atherogenic particle number (apoB, non-HDL-C).
2. Oxidative modification and foam cell formation — Retained LDL undergoes progressive oxidative modification, generating lipid hydroperoxides, reactive aldehydes (MDA, 4-HNE, acrolein), oxysterols, and modified apoB-100, as given in detail. Macrophages recognise oxidised LDL via scavenger receptors (CD36, SR-A, LOX-1) in an unregulated, non-feedback-inhibited manner, leading to intracellular cholesteryl ester accumulation and foam cell transformation. This stage is captured by direct oxidative markers (ox-LDL, MDA, 4-HNE, oxidised LDL autoantibodies) and is considered the principal domain of early, pre-clinical biomarker detection.
3. Inflammatory amplification — Foam cells and activated vascular cells secrete pro-inflammatory cytokines (IL-1β, IL-6, TNF-alpha), chemokines (MCP-1), and acute-phase reactants (CRP via hepatic IL-6 stimulation), establishing a self-sustaining inflammatory milieu within the plaque and systemically. Plaque-resident inflammatory cells also secrete enzymes such as Lp-PLA2 and myeloperoxidase that further propagate oxidative and inflammatory injury. This stage is captured by systemic inflammatory markers (hs-CRP, IL-6, fibrinogen) and leukocyte-derived composite ratios (NLR, MHR, SII).
4. Fibrous cap formation and necrotic core expansion — Smooth muscle cell migration from the media, proliferation, and extracellular matrix (collagen) synthesis form a protective fibrous cap over the lipid-rich necrotic core, which itself expands through ongoing macrophage apoptosis and defective efferocytosis driven by oxysterol toxicity. This intermediate stage is less well

captured by circulating biomarkers and remains primarily an imaging-defined stage (intima-media thickness, plaque burden on CT/MR angiography), though emerging markers such as osteopontin and matrix metalloproteinases correlate with active remodelling.

5. Plaque destabilisation and rupture/erosion — Matrix metalloproteinases (particularly MMP-9), secreted by activated macrophages and foam cells in response to sustained inflammatory and oxidative stimulation, degrade the collagenous fibrous cap, thinning it and rendering the plaque vulnerable to mechanical rupture or superficial erosion, the proximate trigger for acute thrombus formation and clinical events (myocardial infarction, ischaemic stroke) (9). This terminal stage is best captured by late/acute markers reflecting plaque instability — Lp-PLA2, myeloperoxidase, MMP-9, and elevated NLR/PLR ratios — which together define the "vulnerable plaque" biomarker signature (7,8,9,11).

This staged framework underlies the early-versus-late biomarker classification used throughout the remainder of this review: markers reflecting endothelial activation and early oxidative modification are positioned as early/subclinical predictors, whereas markers reflecting active plaque inflammation, matrix degradation, and instability are positioned as late/acute predictors of clinical events.

I.Traditional Lipid and Lipoprotein Markers

Traditional lipid markers remain the foundation of cardiovascular risk assessment and are universally available, well standardised, and supported by decades of outcome data (2,18). They are best classified as early-to-intermediate predictive markers, reflecting the quantity and atherogenic potential of circulating lipoprotein particles rather than the active biological state of an existing plaque.

Table 1. Traditional lipid and lipoprotein markers of atherosclerosis: structure/synthesis, mechanism, estimation, and reference ranges.

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
Total chole	Sum of choles	Substrate pool for	Enzymatic (cholesterol oxidase–	Desirable:
sterol	terol carried in all lipoprotein fractions (LDL, HDL, VLDL, chylomicrons); hepatic synthesis (endogenous) plus dietary absorption (exogenous)	lipoprotein-mediated cholesterol delivery to peripheral tissues and arterial wall; non-specific marker of overall lipid burden	peroxidase) colorimetric assay on automated analyser; fasting or non-fasting sample	<200 mg/dL; Borderline high: 200–239 mg/dL; High: ≥240 mg/dL
LDL-cholesterol (LDL-C)	Cholesterol content of the LDL particle (apoB-100-containing); derived from VLDL via	Principal carrier of cholesterol delivered to and retained within the arterial subendothelial space;	Friedewald calculation (TC − HDL-C − TG/5, valid if TG <400 mg/dL) or direct homogeneous enzymatic assay	Optimal: <100 mg/dL; Near-optimal: 100–129; Borderline high:

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	lipoprotein lipase /hepatic lipase action	substrate for oxidative modification		130–159; High: 160–189; Very high: ≥190 mg/dL
HDL-cholesterol (HDL-C)	Cholesterol carried by HDL particles; synthesised via hepatic/intestinal apoA-I lipidation and reverse cholesterol transport	Mediates reverse cholesterol transport from peripheral tissue/foam cells to liver; possesses antioxidant (paraoxonase-1-mediated) and anti-inflammatory properties protective against LDL oxidation	Direct homogeneous enzymatic (immunoinhibition/PEG-modified enzyme) assay	Low (increased risk): <40 mg/dL (men), <50 mg/dL (women); High (protective): ≥60 mg/dL
Triglycerides (TG)	Glycerol esterified with three fatty acids; carried mainly in chylomicrons and VLDL; hepatic synthesis and dietary fat absorption	Marker of VLDL/remnant lipoprotein burden; elevated TG associated with small dense LDL phenotype and reduced HDL — an atherogenic dyslipidaemic triad	Enzymatic (glycerol-3-phosphate oxidase) colorimetric assay; fasting sample preferred	Normal: <150 mg/dL; Borderline high: 150–199; High: 200–499; Very high: ≥500 mg/dL
Non-HDL cholesterol	Calculated as TC minus HDL-C; represents cholesterol carried by all atherogenic apoB-containing particles	Captures total atherogenic particle cholesterol burden, including remnant particles missed by LDL-C alone; superior	Calculated (no separate assay required) from standard lipid panel	Target generally set 30 mg/dL above the corresponding LDL-C target per risk category

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	(LDL, VLDL, IDL, Lp(a), remnants)	predictor in hypertriglyceridaemia		
Apolipoprotein B (ApoB)	Single structural apolipoprotein (apoB-100) present on each LDL, IDL, VLDL, and Lp(a) particle; hepatic synthesis	Direct count of circulating atherogenic particle number, irrespective of cholesterol content per particle; superior to LDL-C in discordant or hypertriglyceridaemic states	Immunoturbidimetric or nephelometric assay; fasting sample	Desirable: <90 mg/dL (general); <80 mg/dL high risk; <65 mg/dL very high risk
Lipoprotein(a) [Lp(a)]	LDL-like particle with apoB-100 covalently linked to apolipoprotein(a), a plasminogen-homologous glycoprotein; hepatic synthesis, largely genetically determined (LPA gene)	Independently atherogenic (LDL-like particle) and prothrombotic (plasminogen-competitive, antifibrinolytic) via apo(a) homology to plasminogen; promotes oxidised phospholipid delivery to the vessel wall	Immunoturbidimetric/immunonephelometric assay reported in nmol/L (preferred) or mg/dL	Desirable: <30 mg/dL (~75 nmol/L); High risk: ≥50 mg/dL (~125 nmol/L); levels are largely genetically fixed from birth

Despite their central role in risk algorithms, conventional lipid parameters share an important limitation: they quantify the amount of circulating lipid substrate rather than the degree to which that substrate has undergone the oxidative and inflammatory transformation that drives plaque vulnerability (3,18,20). This limitation has motivated the parallel development of the oxidative and inflammatory biomarker domains discussed in the following sections, which are increasingly used in combination with, rather than in place of, traditional lipid measurement.

II. Molecular Products of Oxidative LDL Modification

As detailed mechanistically above, oxidative modification of subendothelially retained LDL generates a structurally diverse family of lipid and protein oxidation products (3,4,5,6,20). These molecules represent the most direct biochemical evidence of the oxidative modification hypothesis of atherosclerosis and are increasingly regarded as early, mechanistically specific biomarkers capable of detecting biologically active disease before it becomes

apparent on conventional lipid testing or vascular imaging (19,21).

Table 2. Molecular products of oxidative LDL modification: structure/source, mechanism, estimation, and reference ranges.

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
Oxidised LDL (ox-LDL)	LDL particle bearing oxidised phospholipids, oxidised cholesteryl esters, and modified apoB-100; generated by free-radical, lipoxygenase, or myeloperoxidase-mediated oxidation of retained LDL within the arterial intima	Recognised by macrophage scavenger receptors (CD36, SR-A, LOX-1) in unregulated fashion, driving foam cell formation; activates endothelial adhesion molecules and MCP-1 secretion; directly cytotoxic and pro-apoptotic to vascular cells at high concentration	Sandwich ELISA using monoclonal antibody (commonly clone 4E6) against oxidation-specific epitopes on apoB-100	No universal reference range (assay-dependent); commonly reported 30–60 U/L in healthy adults; elevated in proportion to plaque burden/instability
Lipid hydroperoxides (oxidised phospholipids / cholesteryl ester hydroperoxides)	Primary, unstable oxidation products formed by peroxidation of polyunsaturated fatty acyl chains within LDL phospholipids and cholesteryl esters (e.g. POVPC, PGPC species)	Earliest detectable products of LDL oxidation; serve as direct precursors of reactive aldehydes; oxidised phospholipids are potent activators of endothelial inflammatory signalling and innate immune (Toll-like receptor) pathways	Ferrous oxidation-xylenol orange (FOX2) assay; LC-MS/MS for specific oxidised phospholipid species (research/reference laboratories)	Not routinely standardised for clinical use; primarily a research biomarker reported in arbitrary or molar units specific to the assay platform
Malondialdehyde (MDA)	Three-carbon reactive aldehyde generated	Forms stable MDA-lysine adduct	Thiobarbituric acid reactive substan	TBARS assay: approximately

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	as a stable end-product of peroxidation of omega-3 and omega-6 polyunsaturated fatty acids (e.g. arachidonic acid) within oxidised LDL lipids	s on apoB-100, abolishing native LDL-receptor recognition and generating neo-epitopes recognised by scavenger receptors; widely used surrogate marker of systemic lipid peroxidation/ oxidative stress burden	ces (TBARS) colorimetric/fluorometric assay (simple, widely used but limited specificity); HPLC with fluorescence or UV detection (more specific, reference method)	1–4 µmol/L (laboratory- and method-dependent); HPLC-based values are generally lower and more specific
4-Hydroxynonenal (4-HNE)	Highly reactive α,β-unsaturated aldehyde derived from peroxidation of	Forms Michael-addition adducts with cysteine, histidine, and lysine residues on apoB-100 and other vascular proteins; disrupts protein function and cell signalling; activates Nrf2 antioxidant response at low concentration but is cytotoxic/pro-apoptotic at higher concentration	ELISA using anti-4-HNE-protein adduct monoclonal antibody; LC-MS/MS for free and protein-bound 4-HNE (reference method)	Plasma free 4-HNE approximately 0.3–0.7 µmol/L in healthy adults (assay-dependent); protein-adduct levels reported in arbitrary ELISA units
Acrolein	Smallest and most reactive α,β-unsaturated aldehyde; generated both endogenously from omega-6 polyunsaturated fatty acids, principally linoleic and arachidonic acid	Forms protein and DNA adducts; potently depletes	LC-MS/MS quantification of acrolein-protein (e.g. acrolein-lysine,	No standardised clinical reference range; predominantly a

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	usly via lipid peroxidation/myeloperoxidase-catalysed pathways and exogenously from tobacco smoke and combustion sources	cellular glutathione, amplifying oxidative stress; modifies apoB-100 lysine residues and promotes endothelial dysfunction; principal mechanistic link between smoking exposure and accelerated atherosclerosis	3-HPMA urinary metabolite) adducts; ELISA for protein-bound acrolein	research/exposure biomarker, with urinary 3-HPMA used as an exposure proxy in smokers versus non-smokers
7-Ketocholesterol	Major oxysterol formed by autoxidation (non-enzymatic, free-radical-mediated) at the C-7	Cytotoxic and pro-apoptotic to macrophages and vascular	Gas chromatography–mass spectrometry (GC-MS, reference	Plasma levels typically <200 ng/mL in healthy adults (method-dependent); elevated in advanced atherosclerotic plaque and several lysosomal/peroxisomal disorders
7β-Hydroxycholesterol	Oxysterol formed by autoxidation at the C-7 position, structurally related to 7-ketocholesterol but bearing a hydroxyl rather than a keto group; generated via free-radical attack on the cholesterol B-ring within oxidised LDL	Shares cytotoxic, pro-inflammatory, and pro-apoptotic actions with 7-ketocholesterol on vascular smooth muscle cells, contributing to necrotic core expansion; activates inflammasome (NLRP3) signalling and promotes foam cell apoptosis with defective efferocytosis	GC-MS (reference method); HPLC with UV/fluorescence detection; ELISA kits available for clinical research use	Plasma levels generally in the low ng/mL to tens of ng/mL range in healthy adults (method-dependent); elevated with

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	radical and singlet-oxygen-mediated pathways	and macrophages; used together with 7-ketocholesterol as a composite oxysterol index of LDL oxidative burden		increasing plaque burden
Modified apoB-100 (fragmentation / cross-linking / aldehyde adducts)	ApoB-100 polypeptide bearing oxidation-induced fragmentation, intra/intermolecular cross-links, and covalent aldehyde adducts (MDA-lysine, 4-HNE-lysine, malondialdehyde-acetaldehyde, MAA-adducts)	Structural modification abolishes native LDL-receptor recognition while generating neo-epitopes recognised by scavenger receptors and circulating autoantibodies	Western blot/immunoblot using adduct-specific monoclonal antibodies; competitive or sandwich ELISA for specific adduct epitopes (e.g. MDA-LDL, MAA-LDL)	No universally standardised clinical reference range; reported in arbitrary ELISA units relative to a reference standard curve
		(anti-MDA-LDL, anti-oxLDL antibodies); a structural marker integrating the cumulative effect of all upstream oxidation products		

Clinical and diagnostic significance

Circulating ox-LDL has shown consistent association with carotid intima-media thickness, coronary plaque burden, and incident cardiovascular events across multiple cohort studies and meta-analyses, with several studies reporting that ox-LDL adds incremental predictive value beyond conventional lipid measurement, particularly in populations with chronic inflammatory disease (21,22). In a recent study of patients undergoing carotid endarterectomy, serum ox-LDL above a threshold of approximately 31.4 ng/mL was associated with an estimated 82.5% probability of unstable plaque morphology, underscoring its utility as a marker of late-stage plaque vulnerability rather than purely early disease (4).

MDA and 4-HNE, owing to their relative assay simplicity (TBARS and ELISA-based methods respectively), remain the most widely used surrogate markers of systemic lipid peroxidation burden in both research and selected clinical-research settings, though their lack of structural specificity is a recognised limitation relative to mass-spectrometry-based quantification (5,6,14,15). Oxysterols and structurally modified apoB-100 are gaining interest as markers more tightly linked to necrotic core biology and plaque vulnerability, but their estimation currently

remains confined largely to specialised or reference laboratories owing to the need for gas chromatography–mass spectrometry or adduct-specific immunoassays (19,20).

III. Inflammatory and Plaque-Specific Enzymatic Markers

The recognition that atherosclerosis is an inflammatory disease at every stage, from endothelial activation to plaque rupture, has driven extensive characterisation of circulating inflammatory mediators and enzymes localised to vulnerable plaque tissue (7,8,9,17). These markers are generally most informative in the intermediate-to-late stages of disease, reflecting active plaque inflammation and instability rather than the initial lipid-retention event.

Table 3. Inflammatory and plaque-specific enzymatic markers: structure/source, mechanism, estimation, and reference ranges.

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
High-sensitivity C-reactive protein (hs-CRP)	Pentameric acute-phase protein synthesized by hepatocytes in response to circulating interleukin-6; non-specific acute-phase reactant	Activates complement, promotes endothelial adhesion molecule expression and monocyte recruitment; may directly opsonise oxidised LDL for macrophage uptake; principally a marker (and possible mediator) of systemic vascular inflammatory burden	High-sensitivity immunoturbidimetric or immunonephelometric assay (detection limit <0.1–0.2 mg/L)	Low risk: <1.0 mg/L; Average risk: 1.0–3.0 mg/L; High risk: >3.0 mg/L (per AHA/CDC risk stratification)
Interleukin-6 (IL-6)	Pleiotropic pro-inflammatory cytokine secreted by macrophages, foam cells, endothelial cells, and adipocytes within and around the atherosclerotic plaque	Principal upstream driver of hepatic CRP synthesis; promotes endothelial dysfunction, smooth muscle cell proliferation, and procoagulant tissue factor expression; central mediator validated as a causal inflammatory target (canakinumab/colchicine trial era)	ELISA or chemiluminescent immunoassay	Typically <7 pg/mL in healthy adults (assay-dependent); levels rise acutely with infection/inflammation, limiting specificity
Tumour necro	Pro-inflammatory	Upregulates endothel	ELISA or multiple	Typically <8.1 pg/mL

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
sis factor-alpha (TNF-α)	cytokine produced by activated macrophages and foam cells within plaque, and by adipose tissue systemically	ial adhesion molecules and MCP-1; promotes insulin resistance and dyslipidaemia; amplifies local plaque inflammation and matrix metalloproteinase expression	x cytokine immunoassay	in healthy adults (assay-dependent)
Lipoprotein-associated phospholipase A2 (Lp-PLA2)	Calcium-independent phospholipase secreted by macrophages, T-lymphocytes, and mast cells; circulates bound predominantly to LDL (>80%)	Hydrolyses oxidised phospholipids within ox-LDL to generate lysophosphatidylcholine and oxidised non-esterified fatty acids, both potent pro-inflammatory	Mass (ELISA) or enzymatic activity assay (colorimetric/fluorometric substrate hydrolysis)	Mass assay: <200 ng/mL (lower risk) to >235 ng/mL (higher risk), per PLAC test categories; activity assay reference ranges are laboratory-specific
	and, to a lesser extent, HDL	mediators; highly expressed in the necrotic core and macrophage-rich shoulder regions of vulnerable, rupture-prone plaque		
Myeloperoxidase (MPO)	Heme-containing enzyme stored in azurophilic granules of neutrophils and monocytes; released during activation/degranulation at sites of vascular inflammation	Catalyses formation of hypochlorous acid and other reactive oxidant species from hydrogen peroxide and chloride, directly oxidising LDL and HDL (impairing HDL's antioxidant/reverse-cholesterol	ELISA (plasma/serum MPO mass) or enzymatic activity assay	Typically <350 pg/mL (mass assay, assay-dependent); levels rise acutely in unstable angina and acute coronary syndromes

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
		rol-transport function); promotes plaque erosion and instability		
Fibrinogen	Soluble plasma glycoprotein synthesised by hepatocytes; acute-phase reactant and the substrate for thrombin-mediated clot formation	Contributes to plasma viscosity and platelet aggregation; deposited within the arterial wall and plaque matrix, where it may be incorporated into the necrotic core; elevated levels associate with both inflammatory burden and prothrombotic risk	Clauss clotting-based functional assay (most common) or immunoturbidimetric antigenic assay	Normal: 200–400 mg/dL
Matrix metalloproteinase-9 (MMP-9)	Zinc-dependent endopeptidase (gelatinase B) secreted by activated macrophages, foam cells, and neutrophils within the plaque shoulder and fibrous cap	Degrades type IV collagen and gelatin within the fibrous cap extracellular matrix, directly thinning and weakening the cap and predisposing to mechanical rupture; a principal late-stage marker of active plaque destabilisation	ELISA (total or pro-/active-MMP-9 specific assay); gelatin zymography (research method)	Typically <600 ng/mL in healthy adults (assay-dependent); markedly elevated in unstable plaque and acute coronary syndromes

Clinical and diagnostic significance

Among inflammatory markers, hs-CRP has accumulated the largest body of outcome data and is incorporated into several validated cardiovascular risk calculators, with values above 3.0 mg/L associated with increased risk independent of LDL-C (17). However, its lack of vascular specificity limits its utility as a stand-alone marker of plaque-specific biology. Lp-PLA2 and myeloperoxidase, by contrast, are enriched within plaque tissue itself and are more specifically associated with plaque vulnerability and rupture risk; meta-analytic data indicate that Lp-PLA2 demonstrates good discriminative performance for unstable versus stable plaque, with pooled diagnostic accuracy metrics supporting its classification as a late, plaque-instability-specific marker (7,8,12,13). MMP-9 similarly functions as a direct biochemical correlate

of active fibrous cap degradation and is most useful in the acute or peri-event setting rather than for early screening (9).

IV. Composite Lipid and Haematological Ratios

Composite ratios derived from routinely available lipid panels and complete blood counts have gained substantial attention owing to their negligible additional cost, since they require no new assay, only arithmetic combination of values already generated during standard testing (10,11). By integrating two complementary biological axes (e.g., lipid burden and HDL functional capacity, or innate immune activation and lymphocyte-mediated immune regulation), these ratios frequently outperform either constituent parameter in isolation.

Table 4. Composite lipid and haematological ratios: derivation, mechanistic rationale, estimation, and reference ranges.

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
TC/HDL-C ratio	Total cholesterol divided by HDL-cholesterol	Integrates total atherogenic lipid burden against the protective, reverse-cholesterol-transport capacity of HDL; a simple proxy for net atherogenic balance	Calculated from standard lipid panel	Desirable: <4.5 (general); <3.5 considered optimal in higher-risk individuals
LDL/HDL ratio	LDL-cholesterol divided by HDL-cholesterol	Similar rationale to TC/HDL ratio but specific to the principal atherogen	Calculated from standard lipid panel	Desirable: <2.5–3.0 (risk-category dependent)
		ic particle (LDL) relative to the protective HDL fraction		
Triglyceride/HDL-C ratio	Fasting triglycerides divided by HDL-cholesterol	Surrogate marker for small dense LDL phenotype and underlying insulin resistance; elevated ratio associates with the atherogenic dyslipidaemic triad (high TG, low HDL, small dense LDL)	Calculated from standard lipid panel	Optimal: <2.0 (mg/dL units); ratios ≥3.5 strongly suggest insulin resistance and small dense LDL predominance
Neutrophil-to-lymphocyte ratio (NLR)	Absolute neutrophil count divided by absolute lymphocyte count, from routin	Reflects the balance between innate inflammatory activation (neutrophilia, driven by cortisol/catecholamine and cytokine	Calculated from automated CBC differential count	Normal: approximately 1.0–3.0 in healthy adults; values >3.0–4.0 associated with increased cardiovascular risk in multiple

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	e complete blood count	signalling) and adaptive immune/regulatory capacity (relative lymphopenia); elevated NLR associates with plaque burden and acute coronary syndrome severity		cohort studies
Platelet-to-lymphocyte ratio (PLR)	Absolute platelet count divided by absolute lymphocyte count, from routine complete blood count	Reflects combined platelet activation/reactivity (pro-thrombotic) and relative lymphopenia (inflammatory stress); elevated in acute coronary syndromes and correlates with thrombus burden	Calculated from automated CBC	Normal: approximately 50–150 in healthy adults; higher values associated with increased thromboinflammatory risk
Monocyte-to-HDL	Absolute mono	Integrates monocyte-driven	Calculated	No single universal cut-off;

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
ratio (MHR)	cyte count divided by HDL-cholesterol concentration	plaque inflammation/foam cell potential against the antioxidant and anti-inflammatory capacity of HDL; a particularly novel composite marker since it captures both pro-atherogenic cellular and anti-atherogenic lipoprotein axes simultaneously	from CBC monocyte count and lipid panel HDL-C	commonly proposed research threshold approximately 0.4–0.5 (units of 10^3 monocytes/μL per mg/dL HDL-C), with higher MHR associated with greater plaque burden and adverse cardiovascular outcomes
Systemic immune-inflammation index (SII)	Platelet count × neutrophil count, divided by lymphocyte count, from routin	Composite index incorporating three leukocyte/platelet lineages simultaneously (thrombotic, innate inflammatory, and adaptive/regulatory	Calculated from automated CBC differential and platelet	No universally standardised reference range; higher quartiles/tertiles consistently associated with greater

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	e CBC	axes); proposed as a more comprehensive single-value inflammatory burden index than NLR or PLR alone	count	atherosclerotic burden and adverse outcomes in published cohorts
Systemic inflammatory response index (SIRI)	Absolute neutrophil count × absolute monocyte count, divided by absolute lymphocyte count, from routine CBC	Integrates both innate inflammatory cell lineages (neutrophils and monocytes, both centrally implicated in plaque inflammation) against the lymphocyte-mediated regulatory axis; recent comprehensive analyses have identified SIRI as a significant independent predictor of early atherosclerosis	Calculated from automated CBC differential count	No universally standardised reference range; elevated SIRI tertiles/quartiles associated with significantly increased odds of atherosclerosis in published cohort data

Comparative and combinatorial diagnostic performance

Recent comprehensive analyses evaluating inflammatory and lipid biomarkers in early atherosclerosis among young adults have demonstrated that patients with subclinical atherosclerosis exhibit significantly elevated SIRI, NLR, and SII alongside elevated total cholesterol and LDL-C and reduced HDL-C, with logistic regression modelling identifying SIRI as one of the strongest independent predictors of disease presence; combined multivariable models in this literature achieved areas under the receiver operating characteristic curve exceeding 0.80, substantially outperforming any single traditional lipid parameter considered in isolation (9,10,11). This pattern — wherein a composite panel combining an inflammatory index with one or two atherogenic lipid markers outperforms either domain alone — is a recurring and clinically important finding across the appraised literature, and forms the principal rationale for combinatorial biomarker strategies discussed further.

V. Emerging Genomic, Proteomic, and Autoimmune Markers

Alongside the established biomarker domains discussed above, a growing body of literature has examined emerging molecular markers — circulating microRNAs, structural and adhesion-molecule proteins, and autoantibodies directed against oxidation-specific epitopes — that may offer additional mechanistic specificity or earlier detection capability, though most remain confined to research and specialised reference laboratories pending large-scale clinical validation and assay standardisation.

Table 5. Emerging genomic, proteomic, and autoimmune markers: structure/source, mechanism, estimation, and reference ranges.

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
Circulating microRNAs (e.g. miR-126, miR-33, miR-21, miR-145)	Small (~22 nucleotide) non-coding RNA molecules released from vascular endothelial cells, smooth muscle cells, and platelets, circulating free or within exosomes/ microparticles	Post-transcriptionally regulate genes governing endothelial repair (miR-126), cholesterol efflux/ ABCA1 expression (miR-33), inflammatory NF-κB signalling (miR-21), and smooth muscle cell phenotype switching (miR-145); altered circulating profiles reflect active vascular cell stress/injury	Quantitative real-time PCR (qRT-PCR) following RNA extraction; microarray or next-generation sequencing for broader profiling (research setting)	No standardised clinical reference range; typically reported as fold-change relative to control or internal reference miRNA (e.g. miR-16, cel-miR-39 spike-in)
Soluble CD40 ligand (sCD40L)	Trimeric transmembrane protein cleaved and released predominantly from activated platelets, with smaller contributions from activated T-lymphocytes and endothelial cells	Engages CD40 receptor on endothelial cells, macrophages, and smooth muscle cells, upregulating adhesion molecules, tissue factor, and matrix metalloproteinase expression; links platelet activation directly to plaque inflammation and thrombogenicity	ELISA (plasma, collected with careful pre-analytical platelet-activation control)	Typically <5 ng/mL in healthy adults (assay- and pre-analytical-handling-dependent)
Osteopontin	Phosphorylated glycoprotein secreted by activated	Promotes macrophage and smooth	ELISA (plasma/serum)	Typically in the range of 15–80

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
	macrophages, smooth muscle cells, and endothelial cells within plaque; also synthesised in bone and several epithelial tissues	muscle cell migration into the plaque, vascular calcification regulation, and extracellular matrix remodelling; elevated expression localises to calcified and inflamed plaque regions		ng/mL in healthy adults (assay-dependent); elevated in advanced calcified atherosclerotic plaque
Oxidised LDL autoantibodies (anti-oxLDL IgG/IgM)	Circulating immunoglobulins (IgG and IgM isotypes) generated by the adaptive immune system against oxidation-specific epitopes on modified apoB-100	Reflect the magnitude of the humoral autoimmune response to oxidative LDL modification; IgM anti-oxLDL antibodies are generally considered atheroprotective (natural antibody, facilitating clearance), whereas IgG isotypes have been variably associated with both pro- and anti-atherogenic effects depending on study population	ELISA using oxidised-LDL- or malondialdehyde-LDL-coated plates	No standardised clinical reference range; reported as optical density ratio or arbitrary units
Cell-free DNA (cfDNA) / neutrophil extracellular trap	Fragmented extracellular DNA released during cell death or active neutrophil extracellul	NETs directly promote thrombus formation, endothelial	ELISA for MPO-DNA complexes or citrullinated histone H3;	No standardised clinical reference range; an activel

Marker	Structure / source	Mechanism of action	Method of estimation	Reference range
(NET) markers	ar trap (NET) formation (NETosis) within inflamed or unstable plaque	injury, and plaque destabilisation; circulating cfDNA and NET-associated proteins (citrullinated histone H3, MPO-DNA complexes) serve as surrogate markers of this process	fluorometric quantification (e.g. PicoGreen) for total cfDNA	y evolving research biomarker domain, predominantly used in acute coronary syndrome and stroke research cohorts

These emerging markers are presently best regarded as complementary research tools rather than first-line clinical diagnostics, given the lack of large-scale outcome validation, standardised reference intervals, and cost-effective assay platforms suitable for routine clinical laboratories. Nonetheless, microRNA panels and NET-associated markers in particular have shown promising correlation with plaque vulnerability and acute thrombotic events in early validation cohorts and represent active areas for future biomarker development.

VI. Temporal Classification: Early Versus Late Predictive Markers

A practically important and recurring theme across the appraised literature is that no single biomarker performs equally well across the entire natural history of atherosclerosis. Markers that are sensitive to the earliest, subclinical stages of endothelial activation and lipid retention are frequently poorly correlated with acute plaque rupture risk, and vice versa. Table 6 summarises the consensus temporal classification of the major biomarkers discussed, synthesised from their underlying mechanistic stage of action as outlined above.

Table 6. Temporal classification of atherosclerosis biomarkers as early, intermediate, or late predictive markers.

Temporal category	Representative biomarkers	Rationale
Early / subclinical predictive markers	ApoB, non-HDL-C, Lp(a), oxidised LDL, MDA, 4-HNE, soluble VCAM-1/ICAM-1, oxidised LDL autoantibodies (IgM), circulating microRNAs (miR-126, miR-33)	Reflect atherogenic particle burden and the earliest oxidative modification and endothelial activation events, often detectable before structural plaque is apparent on imaging
Intermediate predictive markers	hs-CRP, IL-6, TNF-α, TC/HDL and LDL/HDL ratios, triglyceride/HDL ratio, carotid intima-media thickness (imaging correlate)	Reflect established systemic inflammatory burden and dyslipidaemic phenotype associated with progressive, established plaque accumulation
Late / plaque-instability and acute-event markers	Lp-PLA2, myeloperoxidase, MMP-9, fibrinogen, NLR, PLR, SII, SIRI, sCD40L, NET-associated markers (cfDNA, MPO-DNA complexes), oxysterols (7-ketocholesterol,	Reflect active plaque inflammation, necrotic core expansion, fibrous cap degradation, and thrombogenic potential —

Temporal category	Representative biomarkers	Rationale
	7β-hydroxycholesterol)	the immediate biochemical substrate of plaque rupture or erosion and acute clinical events

It is important to note that this classification reflects the predominant or best-supported point of mechanistic action for each marker, and meaningful overlap exists; several markers (notably MHR, ox-LDL, and hs-CRP) have been reported to carry both early discriminative value (differentiating subclinical atherosclerosis from health) and incremental late prognostic value (predicting recurrent events in established disease), and should not be regarded as confined exclusively to a single temporal category.

Correlation with vascular imaging

Carotid intima-media thickness (CIMT) measured by B-mode ultrasonography and coronary artery calcium (CAC) scoring by non-contrast computed tomography remain the principal non-invasive structural correlates against which circulating biomarkers are typically validated. Across the appraised literature, oxidative markers (ox-LDL) and composite inflammatory indices (SIRI, MHR, NLR) have shown moderate-to-strong correlation with CIMT and plaque presence on ultrasonographic screening, while Lp-PLA2 and myeloperoxidase correlate more specifically with plaque echolucency and lipid-rich necrotic core features on advanced imaging (intravascular ultrasound, optical coherence tomography) associated with vulnerable plaque morphology. This imaging correlation provides an important structural validation layer for biomarker-based risk stratification, particularly in research settings where serial imaging is used as the reference standard for biomarker accuracy assessment.

Discussion

Individual versus combinatorial biomarker performance

A consistent finding across the appraised literature is that combinatorial biomarker panels — typically integrating one atherogenic lipid parameter (ApoB, non-HDL-C, or LDL-C), one inflammatory or oxidative marker (hs-CRP, ox-LDL, or Lp-PLA2), and increasingly one composite haematological index (NLR, MHR, or SIRI) — consistently outperform any single biomarker considered in isolation, with reported areas under the receiver operating characteristic curve in the combined-panel range of 0.75 to above 0.85, compared with typically 0.60–0.75 for individual markers.(5-9) This pattern is biologically coherent: atherosclerosis is a multifactorial process spanning particle retention, oxidative modification, and inflammatory amplification, and no single biochemical axis can be expected to capture this entire spectrum. From a clinical and translational standpoint, this argues against the search for a single "ideal" atherosclerosis biomarker and in favour of structured, tiered panels matched to the specific clinical question (population screening versus secondary prevention risk stratification versus acute plaque-instability assessment).11

Old versus new biomarkers: complementary rather than competing

Traditional lipid markers remain indispensable owing to their near-universal availability, low cost, extensive standardisation, and decades of outcome-validated risk algorithms. However, their principal limitation — capturing lipid quantity rather than lipid biological activity — is precisely the gap addressed by oxidative markers and inflammatory markers.10,12,13,14 Rather than positioning newer biomarkers as replacements for traditional lipid testing, the weight of evidence supports a layered approach: lipid markers for population-level risk stratification and treatment target-setting, oxidative and inflammatory markers for refining risk in intermediate-risk individuals or those with risk-factor-discordant presentations (e.g., normal LDL-C with established or premature disease), and plaque-specific late markers (Lp-PLA2, MPO, MMP-9) for acute-setting assessment of plaque instability in patients with known or suspected unstable coronary or cerebrovascular disease.17,19,23

The particular value of composite haematological ratios

Composite ratios derived from the routine complete blood count (NLR, PLR, MHR, SII, SIRI) merit particular discussion given their exceptionally favourable cost-to-information ratio: they require no additional blood draw, no specialised assay, and no incremental laboratory cost beyond a standard CBC and lipid panel already routinely ordered in clinical practice. The consistent finding that these indices, particularly when combined with one or two lipid parameters, achieve diagnostic accuracy approaching that of considerably more expensive specialised assays (Lp-PLA2 mass assay, ox-LDL ELISA) positions them as an especially attractive option for resource-limited healthcare settings and large-scale population

screening, pending further prospective validation in diverse populations and against hard clinical endpoints rather than surrogate imaging outcomes alone.11,15,16,20

Methodological heterogeneity and standardisation challenges

A substantial barrier to the clinical translation of several biomarkers discussed in this review, particularly within the oxidative modification domain and the emerging molecular domain, is the lack of standardised, harmonised assay methodology and universally accepted reference ranges. Ox-LDL, MDA, and 4-HNE in particular are measured using a range of platforms (ELISA with varying monoclonal antibody clones, TBARS colorimetric assay, and LC-MS/MS) that yield results not directly comparable across studies or laboratories, complicating meta-analytic pooling and the establishment of clinically actionable cut-off values. Future research priorities should include inter-laboratory standardisation initiatives, harmonisation of assay calibrators, and large prospective multi-ethnic cohort studies establishing population-specific reference ranges and validated risk thresholds, analogous to the standardisation pathway previously achieved for hs-CRP.21,22

Limitations of the review

This review is subject to several limitations inherent to its narrative-systematic design. The marked heterogeneity in study populations, assay platforms, outcome definitions, and follow-up duration across the appraised literature precluded formal quantitative meta-analysis, and reported diagnostic accuracy figures should therefore be interpreted as indicative ranges drawn from heterogeneous source studies rather than pooled, weighted estimates. Publication bias favouring positive biomarker associations cannot be excluded, particularly for emerging markers discussed with a comparatively limited evidence base. Finally, this review focused predominantly on circulating (blood-based) biomarkers and did not comprehensively address tissue-based, urinary, or genetic/polygenic risk score approaches, which represent important complementary avenues for future synthesis.

Conclusion

Atherosclerosis is a biologically multifactorial disease spanning lipoprotein retention, oxidative modification, chronic vascular and systemic inflammation, and ultimately plaque destabilisation and rupture, and no single circulating biomarker comprehensively captures this entire spectrum. Traditional lipid and lipoprotein markers remain the indispensable foundation of risk assessment and treatment target-setting, but the molecular products of oxidative LDL modification — lipid hydroperoxides, reactive aldehydes (malondialdehyde, 4-hydroxynonenal, acrolein), oxysterols, and structurally modified apoB-100 — together with inflammatory mediators (hs-CRP, IL-6, Lp-PLA2, myeloperoxidase) and inexpensive composite haematological ratios (NLR, MHR, SII, SIRI), provide complementary and often incremental diagnostic and prognostic value, particularly for capturing biologically active disease in individuals whose conventional lipid profile alone would underestimate true risk.

The synthesis of evidence presented in this review supports a tiered, combinatorial biomarker strategy rather than reliance on any single test: early/subclinical risk assessment is best served by atherogenic particle markers (ApoB, non-HDL-C, Lp(a)) supplemented where available by oxidative markers; intermediate risk refinement benefits from systemic inflammatory markers and composite ratios; and assessment of acute plaque instability in established or suspected unstable disease is best informed by plaque-specific late markers such as Lp-PLA2, myeloperoxidase, and MMP-9. This staged approach aligns biomarker selection with the specific underlying pathophysiological question being asked, rather than treating the available biomarker armamentarium as interchangeable.

Future research should prioritise the standardisation of oxidative and emerging molecular marker assays across laboratories, the establishment of population- and ethnicity-specific reference ranges, and large prospective outcome studies validating composite biomarker panels — particularly the low-cost haematological ratios — against hard cardiovascular endpoints rather than surrogate imaging measures alone. Such efforts would meaningfully advance the translation of the oxidative modification hypothesis and inflammatory paradigm of atherosclerosis from mechanistic understanding into routine, cost-effective clinical risk stratification.

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