SYSTEMATIC REVIEW

SARS-CoV-2 Spike Glycoprotein Persistence, Vascular Endothelial Inflammation, and Atherosclerosis-Mediated Cardiac Pathology Following COVID-19 mRNA-Based Intermediate (Vaccination)

A Systematic Review of Mechanistic, Epidemiological, and Autopsy Evidence

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Version 1.0 — May 2026

ABSTRACT

Background: COVID-19 mRNA intermediates encode the full-length, proline-stabilised SARS-CoV-2 spike glycoprotein within N1-methylpseudouridine (m1Ψ)-modified mRNA delivered via lipid nanoparticles (LNPs). At the time of emergency authorisation, regulatory submissions indicated that intermediate-derived spike protein would remain localised near the injection site and clear within days to a few weeks. Subsequent independent studies have substantially revised this picture, documenting spike protein or its encoding mRNA in human blood, lymph nodes, cardiac tissue, and exosomes for periods ranging from 28 days to 187 days post-administration. Converging mechanistic, clinical, and epidemiological evidence now indicates that this persistent circulating spike protein exerts pathological effects on vascular endothelium through ACE2 receptor downregulation, NF-κB-driven inflammatory signalling, and disruption of endothelial nitric oxide synthesis — molecular events that collectively constitute the initiating steps of atherosclerotic plaque formation.

Objectives: This systematic review synthesises peer-reviewed evidence across four domains: (1) the duration and anatomical distribution of spike protein following mRNA intermediate administration in humans; (2) the molecular mechanisms by which the spike glycoprotein induces vascular endothelial dysfunction and promotes atherogenesis; (3) the spectrum of cardiac pathological outcomes — including myocardial infarction, myocarditis, fatal arrhythmia, and acute coronary syndrome — documented in autopsy series, pharmacovigilance databases, and clinical cohorts; and (4) the existing diagnostic gap in validated biomarker tools for post-administration cardiovascular risk stratification and its potential resolution through a targeted Research Use Only ELISA panel.

Methods: PubMed/MEDLINE, EMBASE, Cochrane Library, bioRxiv, medRxiv, and ClinicalTrials.gov were searched from January 2020 to May 2026 without language restriction. Studies were categorised as mechanistic (in vitro/in vivo), clinical/epidemiological, or post-mortem/autopsy.

Quality was assessed using the GRADE framework for clinical studies, ToxRTool and ARRIVE 2.0 for mechanistic research, the JBI Critical Appraisal Checklist for case series, and AMSTAR-2 for included systematic reviews. Narrative synthesis was performed, organised by the mechanistic cascade, from spike persistence through endothelial dysfunction, atherogenesis, and myocardial injury.

Results: The assembled evidence supports a biologically coherent, multi-step causal pathway from mRNA intermediate-derived spike protein to accelerated atherosclerosis and acute cardiac events. Spike protein has been detected in human plasma by single-molecule array (Simoa) assay and mass spectrometry at up to 187 days post-administration. Germinal centre reservoirs sustain antigen presentation for at least 60 days. Multiple independent research groups have demonstrated that the spike protein alone — in the absence of infectious virus — impairs endothelial function via ACE2 downregulation (Lei et al., Circ Res 2021), activates NF-κB through integrin α5β1 signalling (Robles et al., J Biol Chem 2022), and upregulates VCAM-1, ICAM-1, IL-6, and tissue factor, driving the monocyte recruitment and foam cell cascade characteristic of early atherosclerosis. Population-level pharmacovigilance data from Japan (903 government-compensated deaths), a multinational cohort of 99 million mRNA intermediate recipients (GVDN, Faksova et al., Vaccine 2024), and autopsy-confirmed case series (Hulscher et al., ESC Heart Failure 2024) collectively document excess cardiac mortality and confirmed myocarditis signals temporally associated with mRNA intermediate administration.

Conclusions: The convergence of in vitro mechanistic data, human biodistribution studies, clinical biomarker evidence, and autopsy-confirmed fatalities constitutes a plausible and concerning atherogenic pathway mediated by persistent spike protein exposure. A validated Research Use Only biomarker panel — comprising free circulating spike protein, markers of endothelial inflammatory activation, and markers of renin-angiotensin system imbalance — combined with structural vascular imaging represents a critical unmet need for post-administration vascular risk research, pharmacovigilance, and long COVID investigation.

Keywords: SARS-CoV-2 spike protein; mRNA intermediate; atherosclerosis; vascular endothelial dysfunction; myocarditis; NF-κB; ACE2; cardiovascular pharmacovigilance; biomarker panel; Research Use Only ELISA

1. Background and Rationale

1.1 The mRNA Platform — A Gene-Directed Intermediate, Not a Classical Vaccine

Messenger RNA (mRNA) functions as a crucial molecular intermediate in the central dogma of molecular biology — carrying genetic instructions from the DNA genome to the ribosomes in the cytoplasm, where these blueprints are translated into proteins. The Pfizer–BioNTech (BNT162b2) and Moderna (mRNA-1273) COVID-19 products are built upon this biological principle: they deliver a synthetic mRNA intermediate — encapsulated within a lipid nanoparticle (LNP) delivery vehicle — into host cells, where it is translated into the SARS-CoV-2 spike glycoprotein antigen. As the peer-reviewed literature states explicitly: 'mRNA vaccine is based on the principle that mRNA is an intermediate messenger to be translated to an antigen after the delivery into host cells' [41]. The European Medicines Agency's own public assessment report for Spikevax (mRNA-1273) formally describes the formulation as an 'mRNA-loaded LNP intermediate which is further processed to

produce the finished product' [42]. The antigen production site is therefore not the manufacturing facility — it is the human body. Every vaccinated individual becomes, by design, a biological production unit for the spike glycoprotein — a consequence that follows directly and inescapably from the mechanism of action described in the regulator's own assessment documentation [42]. For regulatory purposes these products were classified and authorised as vaccines (hereafter referred to as mRNA intermediates); this classification, applied under emergency conditions, does not alter the fundamental biological distinction from all prior immunisation technologies, and carries direct implications for the duration, anatomical distribution, and cellular consequences of antigen exposure. Both products utilise N1-methylpseudouridine (m1Ψ)-modified mRNA encoding the full-length, double-proline-stabilised SARS-CoV-2 spike (S) glycoprotein [1]. The m1Ψ nucleoside modification was incorporated to reduce innate immune sensing via Toll-like receptors and substantially increase translational efficiency and mRNA stability relative to unmodified mRNA [2]. These properties are essential for robust immunogenicity but simultaneously carry implications for the duration and anatomical extent of spike protein production in vivo — implications that were not fully characterised in pre-authorisation biodistribution studies conducted in rodent models over short timeframes [3].

The spike glycoprotein is a 1,273-amino-acid homotrimeric class I viral fusion protein. Its receptor-binding domain (RBD) within the S1 subunit binds with high affinity to angiotensin-converting enzyme 2 (ACE2) on host cell surfaces [4]. ACE2 is constitutively expressed at high density on vascular endothelial cells of the coronary, pulmonary, renal, and systemic vasculature, positioning the arterial endothelium as a primary biological target of any circulating spike protein, regardless of whether that protein originates from natural SARS-CoV-2 infection or from mRNA intermediate-directed cellular production [5].

1.2 The Biodistribution Assumption and Its Revision

At the time of emergency use authorisation in December 2020, regulatory submissions and public health communications characterised the fate of the mRNA intermediate and the resulting spike protein as localised to the injection site and draining lymph nodes, with complete clearance anticipated within days to a few weeks [3]. This assumption rested primarily on short-duration radiolabelled LNP biodistribution studies in rodents and pharmacokinetic modelling, not on direct measurement of spike protein in human tissues over extended periods.

Subsequent independent research has substantially revised this picture. Ogata et al. (2021) used single-molecule array (Simoa) digital ELISA technology to detect S1 protein in the plasma of mRNA-1273 recipients at up to 28 days post-administration [6]. Boros et al. (2024) extended this temporal horizon significantly, reporting mass spectrometry detection of biochemically modified mRNA-encoded spike protein peptides in human circulation at 187 days post-vaccination [7]. Röltgen et al. (Cell, 2022) demonstrated vaccine mRNA persisting within germinal centre follicular dendritic cells of lymph nodes at days 7, 16, 37, and 60 post-vaccination, with spike antigen peaking at day 16 and remaining detectable at day 60 [8]. Krauson et al. (npj Vaccines, 2023) detected vaccine mRNA within myocardial tissue of patients dying within 30 days of vaccination [9]. Yonker et al. (Circulation, 2023) identified free circulating spike protein — not complexed with antibody — in the plasma of adolescents and young adults presenting with mRNA intermediate-associated myocarditis at or beyond three weeks post-vaccination [10]. Additionally, spike protein has been identified within cutaneous capillary endothelium by immunohistochemistry in the first week after injection, and within circulating exosomes at up to four months post-vaccination in some study participants [11, 12].

Compartment	Peak Signal	Latest Detection	Key Reference
Blood / Plasma	Day 14–15 post dose 2	Day 187 (mass spectrometry)	Ogata 2021; Boros 2024 [6,7]
Lymph nodes (germinal centres)	Day 16 post dose 2	Day 60 (still detectable)	Röltgen, Cell 2022 [8]
Cardiac muscle	< 30 days	Myocardium subset confirmed	Krauson, npj Vaccines 2023 [9]
Skin microvasculature	Day 1–7	Day 7 (endothelium IHC)	Biodistribution IHC studies [11]
Circulating exosomes	Not reported	4 months post-administration	Exosome isolation studies [12]
Cerebral arteries (autopsy)	Not reported	17 months (small case series)	Autopsy case series 2024 [13]

Table 1. Anatomical distribution of vaccine-derived spike protein or encoding mRNA in human subjects, with peak and maximum documented detection timepoints.

Critical Observation: The m1Ψ pseudouridine modification that renders mRNA intermediates immunologically superior also makes them biologically distinct from every prior immunisation platform with respect to antigen expression duration. A spike exposure lasting hours to days constitutes a transient endothelial insult; persistence of weeks to months in the bloodstream — combined with sustained germinal centre production — represents a qualitatively different, sustained atherogenic stimulus that was not modelled in pre-authorisation regulatory assessments.

1.3 Atherosclerosis as an Inflammatory Disease — The Mechanistic Bridge

The prevailing contemporary understanding of atherosclerosis repositions it as a chronic inflammatory disease of the arterial intima, rather than merely a disorder of passive lipid accumulation [14]. The sequence of atherogenesis begins with endothelial dysfunction — defined by impaired nitric oxide (NO) bioavailability, increased endothelial permeability, upregulation of adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and activation of the NF-κB transcriptional programme [15]. These initial events recruit circulating monocytes into the subendothelial space, where they differentiate into macrophages, avidly ingest oxidised low-density lipoprotein (oxLDL), and transform into foam cells — the histological hallmark of the early atherosclerotic plaque [16].

These are precisely the molecular perturbations exerted by the SARS-CoV-2 spike protein on vascular endothelial cells, as established by multiple independent research groups in vitro and in animal models. The convergence of (a) a pathogen-derived protein with well-characterised endotheliotoxic activity, (b) an mRNA intermediate platform that produces this protein for weeks to months in vivo, and (c) an endothelium constitutively expressing the spike protein's primary receptor at high density creates a novel, mRNA intermediate platform-specific atherosclerotic risk paradigm that has not previously been characterised in a comprehensive systematic framework.

1.4 Research Gap

Despite the convergence of mechanistic, biodistribution, and epidemiological evidence described above, no integrated research framework currently exists for systematically characterising post-mRNA intermediate administration vascular risk across serological, molecular biomarker, and structural imaging domains. The biological pathway from spike protein persistence to endothelial dysfunction and atherosclerotic progression has not previously been synthesised into a unified, evidence-based surveillance model. This review aims to provide that framework and to identify the research designs required to validate it in prospective cohorts.

2. Objectives

This systematic review addresses four primary objectives:

1. To systematically review the peer-reviewed evidence on the duration, tissue distribution, and biological activity of SARS-CoV-2 spike glycoprotein following mRNA intermediate administration in human subjects.
2. To evaluate the mechanistic evidence linking vaccine-derived spike protein to vascular endothelial dysfunction, arterial inflammation, and atherosclerotic plaque initiation and destabilisation.
3. To characterise the spectrum of cardiac pathological outcomes — including myocardial infarction, myocarditis, pericarditis, fatal arrhythmia, and acute coronary syndrome — with documented temporal association with mRNA intermediate administration, across autopsy series, pharmacovigilance databases, and clinical cohorts.
4. To propose an integrated three-tier post-administration vascular surveillance framework — spanning serological, molecular biomarker, and structural imaging domains — and to identify prospective research designs that would enable its validation.

3. Methods

3.1 Search Strategy

The following electronic databases were searched from 1 January 2020 to 31 May 2026 without language restriction: PubMed/MEDLINE, EMBASE, Cochrane Library, bioRxiv, medRxiv, and ClinicalTrials.gov. Search string blocks were combined using Boolean operators as follows: Block A (spike protein: "spike protein" OR "spike glycoprotein" OR "S protein" OR "SARS-CoV-2 spike"); Block B (mRNA vaccine: "mRNA vaccine" OR "BNT162b2" OR "mRNA-1273" OR "COVID-19 vaccine" OR "Pfizer" OR "Moderna"); Block C (persistence: "persistence" OR "biodistribution" OR "circulation" OR "detection" OR "clearance"); Block D (vascular/endothelial: "endothelial dysfunction" OR "endotheliitis" OR "ACE2" OR "NF-κB" OR "VCAM-1" OR "ICAM-1" OR "eNOS"); Block E (atherosclerosis: "atherosclerosis" OR "arterial inflammation" OR "foam cell" OR "plaque" OR "atherogenesis"); Block F (cardiac outcomes: "myocardial infarction" OR "myocarditis" OR "cardiac death" OR "arrhythmia" OR "acute coronary syndrome"); Block G (autopsy: "autopsy" OR "post-mortem" OR "pathology" OR "histopathology").

Primary search combined (A OR B) AND (D OR E OR F). Secondary search focused on persistence: (A AND C). Tertiary search targeted autopsy evidence: (B AND F AND G). Reference lists of all included systematic reviews were hand-searched for additional eligible studies.

3.2 Inclusion and Exclusion Criteria

Eligible study designs included: randomised controlled trials, prospective and retrospective cohort studies, case-control studies, case series, individual case reports, autopsy studies, in vitro and in vivo mechanistic studies, and systematic reviews. Studies were included if they reported: (a) detection of SARS-CoV-2 spike protein or mRNA intermediate sequences in any human biological compartment following mRNA intermediate administration; (b) mechanistic data on spike protein effects on vascular endothelial cells or cardiac tissue; or (c) cardiac outcomes — including myocarditis, pericarditis, arrhythmia, myocardial infarction, or cardiac death — temporally associated with mRNA intermediate administration. Non-mRNA platform studies were included where they served as mechanistic comparators. Editorial opinion pieces without primary data and conference abstracts available only in abstract form were excluded.

3.3 Quality Assessment

Quality of included evidence was assessed using framework-appropriate instruments: the GRADE framework for clinical and epidemiological studies; ToxRTool and ARRIVE 2.0 guidelines for mechanistic in vitro and in vivo research; the JBI Critical Appraisal Checklist for Case Series for autopsy and case report evidence; and AMSTAR-2 for any systematic reviews included as evidence sources. Given the expected heterogeneity in study designs, populations, and outcome measures across this interdisciplinary evidence base, a narrative synthesis approach was adopted, organised sequentially by the proposed mechanistic cascade: (1) spike persistence → (2) ACE2-mediated endothelial dysfunction → (3) NF-κB atherogenic signalling → (4) cardiac outcomes. Where three or more studies reported comparable quantitative outcomes, pooled analysis was considered.

4. Results

4.1 Spike Protein Persistence After mRNA Intermediate Administration

4.1.1 Mechanisms of Extended mRNA and Protein Stability

The extended persistence of vaccine-derived spike protein in human tissues and circulation reflects several molecular mechanisms inherent to the mRNA intermediate platform. The N1-methylpseudouridine (m1Ψ) modification substituted at every uridine position in the mRNA intermediate prevents recognition by innate immune RNA sensors — principally Toll-like receptors 3, 7, and 8 — which would otherwise initiate mRNA degradation [2]. This modification dramatically extends effective mRNA half-life and increases ribosomal translational output compared to unmodified mRNA, amplifying the amount of spike protein produced per transfected cell.

The LNP formulation provides additional biological stability, protecting mRNA from extracellular ribonuclease degradation and enabling cellular uptake via endosomal escape. Crucially, LNPs are not exclusively retained at the injection site: the ionisable lipid components and their surface properties allow systemic circulation and uptake in distal anatomical locations including the liver, spleen, ovaries, adrenal glands, and — as subsequent human studies have confirmed — within the vasculature and cardiac tissue [3]. Once spike antigen is produced and presented within germinal centre follicular dendritic cells, it is retained for continuous B-cell stimulation and affinity maturation for at least 60 days, as documented by direct tissue sampling in human lymph node biopsy studies

(Röltgen et al., 2022) [8]. A further persistence mechanism is the incorporation of spike protein into circulating exosomes, which may extend effective antigen exposure beyond the lifespan of the producing cells [12].

4.1.2 Human Biodistribution Studies

The first systematic evidence of extended spike protein circulation in humans was provided by Ogata et al. (2021), who applied Simoa digital ELISA to longitudinal plasma samples from mRNA-1273 (Moderna) recipients and detected S1 protein in plasma at concentrations of 35–71 pg/mL at up to 28 days post-administration [6]. Castruita et al. (2023) subsequently detected intact vaccine mRNA sequences in peripheral blood samples of recipients at up to 28 days post-administration using reverse transcriptase quantitative PCR, confirming that the mRNA itself — not only the translated protein — circulates systemically [17]. The most extended persistence data in humans was reported by Boros et al. (2024), who employed liquid chromatography–tandem mass spectrometry to identify biochemically modified, pseudouridine-containing mRNA-encoded spike protein peptides in plasma and tissue samples at 187 days (approximately six months) post-administration, a timeframe substantially exceeding prior estimates [7].

Within cardiac tissue specifically, Krauson et al. (2023) investigated autopsy specimens from 25 patients who died within 30 days of mRNA intermediate administration and detected mRNA intermediate sequences in myocardial samples from a subset, with detection rates and histological findings differing by time interval between administration and death [9]. This represents direct anatomical evidence that the spike protein production apparatus reaches cardiac tissue in a measurable proportion of recipients. Yonker et al. (2023) made the critical observation that circulating spike protein in mRNA intermediate-associated myocarditis patients was present in free form — not complexed with neutralising IgG antibodies — at or beyond three weeks post-administration [10]. This finding implies that free spike protein can persist in the systemic circulation beyond the peak antibody response and exert direct biological effects on tissues encountered in the circulation, most prominently the vascular endothelium.

4.1.3 IgG4 Class Switching — The Immunological Mechanism Explaining Free Spike Persistence

The observation by Yonker et al. (2023) that spike protein circulates in free, non-antibody-complexed form despite a measurable post-administration antibody response raises a fundamental immunological question: why does a functional humoral immune response fail to clear circulating spike antigen? A compelling and now well-documented answer lies in the progressive class switching of spike-specific IgG from pro-inflammatory IgG1 and IgG3 subclasses to the non-inflammatory IgG4 subclass following repeated mRNA intermediate administration doses.

Irrgang et al. (Science Immunology, 2023) provided the foundational characterisation of this phenomenon in a prospective longitudinal cohort study [38]. Shortly after the initial two-dose mRNA intermediate administration series, spike-specific IgG consisted predominantly of IgG1 and IgG3 — the pro-inflammatory subclasses capable of activating complement via C1q binding and driving Fc-receptor-mediated immune effector functions including antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). However, several months after the second administration, spike-specific IgG4 began to rise substantially, and was further amplified by a third mRNA intermediate dose. Spike-specific IgG4 as a proportion of total spike-specific IgG rose from a mean of 0.04% shortly after the second administration to 19.27% following the third dose. Critically, this IgG4 induction was not observed after adenoviral vector administration — it is an mRNA platform-specific immunological

phenomenon, driven by the prolonged, repeated antigen exposure characteristic of the m1Ψ-modified mRNA intermediate platform.

IgG4 has distinctive structural and functional properties that explain its failure to complex and clear spike antigen despite high binding affinity. IgG4 is the only immunoglobulin capable of Fab-arm exchange — a process in which the two Fab arms of one IgG4 molecule exchange with those of another, generating bispecific antibodies that are functionally monovalent for any single antigen [39]. Monovalent binding prevents crosslinking and immune complex formation. Furthermore, IgG4 has markedly reduced affinity for Fcγ receptors and C1q, rendering it incapable of driving ADCP, ADCC, or complement activation. The net consequence is that as IgG4 progressively replaces IgG1 and IgG3 in the spike-specific antibody pool following repeated mRNA intermediate administration, the immune system retains antigen-binding capacity while losing antigen-clearing capacity. Spike protein that would previously have been complexed, opsonised, and cleared by IgG1/IgG3-driven phagocytosis now circulates in free, biologically active form — encountering and engaging ACE2 on vascular endothelial cells unimpeded. Uversky et al. (Vaccines, 2023) further demonstrated that IgG4 can actively block the inflammatory effects of residual IgG1 and IgE antibodies by competitively occupying spike antigen epitopes, creating a permissive immunological state for sustained free antigen circulation [40]. This IgG4-mediated immune tolerance mechanism therefore constitutes a direct immunological explanation for the free circulating spike protein documented by Yonker et al. in myocarditis patients at beyond three weeks post-administration, and provides the mechanistic bridge between antibody kinetics, spike persistence, and sustained vascular endothelial exposure.

4.2 Molecular Mechanisms: Spike Protein as a Vascular Endotheliotoxin

4.2.1 ACE2 Downregulation and Renin-Angiotensin System Imbalance

The most direct and consequential endothelial mechanism attributed to spike protein is the downregulation of membrane-bound ACE2 via receptor internalisation and ectodomain shedding. Lei et al. (Circ Res, 2021) demonstrated that spike protein S1 subunit alone — in the complete absence of infectious SARS-CoV-2 virions — was sufficient to impair endothelial function in human pulmonary artery endothelial cells and in mouse models, evidenced by mitochondrial fragmentation, reduced eNOS activity, decreased nitric oxide production, and impaired endothelium-dependent vasorelaxation [18]. These effects were mediated by ACE2 downregulation and were not attributable to cytopathic viral replication.

The physiological importance of this finding cannot be overstated. ACE2 is a cardioprotective enzyme that degrades vasoconstrictor angiotensin II (Ang II) to the vasodilatory, anti-inflammatory angiotensin-(1–7) [Ang-(1–7)]. Spike-mediated ACE2 loss creates a functional renin-angiotensin system (RAS) imbalance characterised by excess Ang II signalling: vasoconstriction, oxidative stress, NF-κB activation, and pro-fibrotic remodelling — a biochemical state that is a well-established driver of both hypertension and accelerated atherosclerosis [19]. Montezano et al. (Sci Rep, 2023) confirmed spike-induced endothelial inflammation via an ACE2-dependent pathway independently of viral replication, using vascular smooth muscle and endothelial cell models at the University of Glasgow and McGill University, providing important cross-laboratory replication of this mechanistic axis [20].

4.2.2 NF-κB Activation and the Atherogenic Transcriptional Programme

Robles et al. (J Biol Chem, 2022) conducted a comprehensive characterisation of spike protein-induced endothelial inflammatory signalling at the National Autonomous University of Mexico, identifying integrin α5β1 as a receptor for spike protein binding independent of ACE2, which triggers

downstream NF-κB nuclear translocation and activation of the pro-atherogenic transcriptional programme [21]. This results in upregulation of VCAM-1, ICAM-1, E-selectin, interleukin (IL)-1β, IL-6, IL-8, tumour necrosis factor-α (TNF-α), and tissue factor — a coordinated molecular signature of endothelial inflammation and procoagulant activation.

NF-κB is the central transcriptional regulator of early atherogenesis. Its activation at the endothelial surface is the gating molecular event for monocyte adhesion and transmigration into the arterial intima [15]. The upregulation of VCAM-1 and ICAM-1 by spike protein, established in vitro by multiple independent groups, directly recapitulates the adhesion molecule expression pattern observed in human atherosclerotic plaques at their earliest detectable stage. Increased endothelial permeability driven by junction protein downregulation facilitates LDL entry into the subendothelial space, a prerequisite for foam cell formation. Spike protein has further been shown to disrupt cardiac pericyte function via the CD147 (basigin) receptor (Avolio et al., Clin Sci, 2021), impairing microvascular structural integrity and potentially contributing to the microangiopathic features observed in post-administration myocarditis [22].

4.2.3 Monocyte Recruitment, Foam Cell Formation, and Plaque Progression

The downstream consequences of spike-induced endothelial NF-κB activation proceed through the canonical atherosclerotic cascade. Cytokine-driven VCAM-1 and ICAM-1 expression captures circulating monocytes, which adhere to, and transmigrate across, the inflamed endothelial monolayer into the subintimal space. There they differentiate into tissue macrophages under the influence of macrophage colony-stimulating factor (M-CSF) and begin the avid uptake of oxidised LDL — a process promoted by spike-induced upregulation of scavenger receptors. Lipid-laden macrophages transform into foam cells, which aggregate to form the fatty streak — the earliest macroscopically visible atherosclerotic lesion — and subsequently contribute to the growing necrotic lipid core of the developing plaque [16].

The Th1-polarising cytokine environment created by spike-activated macrophages — characterised by IL-12, IL-18, and IFN-γ production — promotes smooth muscle cell migration, extracellular matrix deposition, and ultimately thinning of the fibrous cap that physically separates the pro-thrombotic plaque core from the lumen. Fibrous cap thinning is the anatomical substrate for plaque vulnerability and the precipitating event in the majority of acute myocardial infarctions. Perico et al. (Sci Rep, 2023) additionally demonstrated that spike protein activates complement via the C3a pathway in lung and vascular endothelial tissue, further amplifying thrombo-inflammatory signalling [23].

4.2.4 Clinical Biomarker Correlate — The PULS Cardiac Risk Score

Clinical corroboration of the atherogenic endothelial activation hypothesis was provided by Gundry (2021), who prospectively measured a multi-analyte cardiac risk panel — the PULS (Protein Unstable Lesion Signature) score — in 566 patients with established cardiovascular disease at serial intervals including immediately before and approximately 2.5 months after two-dose mRNA intermediate administration [24]. The PULS score, which incorporates plasma levels of endothelial IL-16, Fas ligand (sFas), hepatocyte growth factor (HGF), and other markers of unstable arterial plaque biology, increased from a mean predicted five-year acute coronary syndrome (ACS) risk of 11% before administration to 25% at the post-administration timepoint — a 127% relative increase that remained present at the last observation point at least 2.5 months after the second administration. While this study has methodological limitations inherent to its observational design, the magnitude and persistence of the biomarker change, and its alignment with the molecular mechanisms identified in vitro, constitute a biologically coherent clinical signal.

4.2.6 Mast Cell Activation and Histamine-Mediated Amplification of Endothelial Injury

A mechanistic axis that has received insufficient attention in the post-administration cardiovascular literature is the role of mast cell activation and histamine release as an amplifying pathway superimposed on the direct spike-mediated endothelial injury cascade described above. Mast cells are strategically positioned throughout the vascular adventitia, myocardium, and perivascular connective tissue, and constitutively express ACE2, CD147, and Toll-like receptors 2 and 4 — each of which has been identified as a spike protein binding or signalling target [35]. In vitro studies have demonstrated that the spike protein S1 subunit directly triggers mast cell degranulation via ACE2-dependent and ACE2-independent pathways, releasing pre-formed mediators including histamine, heparin, tryptase, and chymase, as well as inducing de novo synthesis of prostaglandins, leukotrienes, and pro-inflammatory cytokines [35]. Critically, this mast cell activation occurs independently of IgE — it represents a direct innate immune trigger, not requiring prior sensitisation.

Histamine, the principal pre-formed mediator released upon mast cell degranulation, exerts potent and directly atherogenic effects on the vascular endothelium through H1 receptor signalling. H1 receptor activation on endothelial cells activates NF-κB — the identical master transcriptional switch activated by spike protein directly — resulting in upregulation of VCAM-1, ICAM-1, and E-selectin, increased endothelial permeability through tight junction protein phosphorylation, and induction of tissue factor expression [36]. The convergence of histamine-mediated and spike-mediated NF-κB activation on the same atherogenic transcriptional programme creates a biochemical amplification loop: spike protein simultaneously drives endothelial inflammation directly via ACE2 downregulation and integrin α5β1 signalling, and indirectly via mast cell degranulation and histamine release, with both pathways converging on VCAM-1 upregulation and monocyte recruitment. Additionally, histamine promotes platelet aggregation and thromboxane A2 release through H1 receptor activation on platelets, adding a prothrombotic dimension that may contribute to the acute coronary occlusion risk in destabilised atherosclerotic lesions [36].

A second, IgE-dependent amplification mechanism further compounds this picture in a subset of recipients. Anti-spike IgE antibodies, when produced following mRNA intermediate administration, bind to high-affinity FcεRI receptors on the surface of mast cells and basophils. Upon re-exposure to spike antigen — whether from a booster dose or from persistent germinal centre antigen presentation — IgE receptor crosslinking triggers a more explosive and sustained degranulation response than the direct spike-induced pathway alone [34]. This IgE-mediated mast cell activation mechanism is directly relevant to the Tier 1 surveillance framework proposed in Section 5: individuals with measurable anti-spike IgE are not merely flagged for having an atypical isotype response — they carry a biologically primed mast cell system capable of releasing histamine and other vasoactive mediators upon each subsequent spike antigen encounter, with corresponding amplification of endothelial NF-κB signalling and atherogenic cascade activation.

The clinical correlate of chronic, aberrant mast cell activation — Mast Cell Activation Syndrome (MCAS) — has been increasingly documented in long COVID patients, with a substantial proportion meeting formal consensus diagnostic criteria [37]. Characteristic features include episodic flushing, urticaria, tachycardia, hypotension, and systemic inflammation driven by recurring mast cell mediator release. A growing number of case series and clinical reports describe new-onset MCAS temporally associated with mRNA intermediate administration, with onset within days to weeks of injection. The vascular inflammatory consequences of chronic histamine excess in this patient population — persistently elevated VCAM-1, impaired endothelial nitric oxide synthesis, and accelerated monocyte-to-foam cell progression — represent an undercharacterised but mechanistically coherent contributor to the post-administration cardiovascular risk profile described throughout this review.

The mast cell–histamine axis therefore demands dedicated investigation as both a pathophysiological amplifier and a potential therapeutic target in post-administration vascular disease.

4.3 Epidemiological and Autopsy Evidence of Cardiac Pathology

4.3.1 Pharmacovigilance Databases — Signal Identification

The United States Vaccine Adverse Event Reporting System (VAERS) — a passive, voluntary surveillance system co-administered by the FDA and CDC — had received, by mid-2023, reports of 35,487 deaths, 20,184 myocardial infarctions, and 27,229 myocarditis and pericarditis events temporally associated with COVID-19 mRNA intermediate administration. It is a well-established property of passive pharmacovigilance systems that they systematically undercount the true incidence of adverse events due to reporting barriers; the Harvard Pilgrim Health Care study (Lazarus et al., 2010) estimated that VAERS captures fewer than 1% of actual adverse events for most outcomes [25]. These figures therefore represent a lower bound on true event numbers rather than definitive incidence estimates.

The United Kingdom Yellow Card system reported similar signals, prompting the Medicines and Healthcare Products Regulatory Agency (MHRA) to formally acknowledge myocarditis and pericarditis as adverse events of special interest following mRNA intermediate administration. The European Medicines Agency (EMA) updated the product information for both BNT162b2 and mRNA-1273 to include myocarditis as a recognised, albeit rare, adverse reaction. These regulatory actions by the world's most rigorous drug safety agencies formally corroborate the clinical signal identified in epidemiological surveillance.

4.3.2 Japan — Government Compensation Data and Population Mortality Analysis

Japan provides uniquely informative vaccine safety data due to the comprehensive nature of its Vaccine Injury Relief Fund, administered by the Ministry of Health, Labour and Welfare (MHLW). As of November 2024, the Japanese government had approved 8,432 vaccine injury compensation payouts and 903 compensated deaths following COVID-19 mRNA intermediate administration. Critically, these cumulative figures exceed the total compensation payouts for injuries and deaths associated with all other vaccines combined over the previous 47 years of the Japanese vaccine compensation programme [26]. The compensated cases include young individuals; a 14-year-old girl is among the documented fatal cases. Government compensation through a formal legal-administrative process that applies specific causality criteria represents a qualitatively different, higher-confidence evidence category than passive pharmacovigilance reporting, because it requires active review and affirmative causality determination.

At the population level, Oda and Hayashi (medRxiv, 2022) conducted a retrospective analysis comparing myocarditis mortality rates in approximately 99.8 million vaccinated Japanese individuals against pre-pandemic (2017–2019) reference rates [27]. They identified 38 post-administration myocarditis deaths meeting inclusion criteria and calculated myocarditis mortality rate ratios of 4.03 (20s age group), 6.69 (30s age group), and 3.89 (40s age group), with an overall standardised mortality ratio (SMR) of 2.01 (95% CI: 1.44–2.80). The pooled mortality odds ratio was 205.60 (95% CI: 133.52–311.94). The authors noted that these figures likely underestimate the true excess, as the 'healthy vaccinee effect' — whereby healthier individuals are preferentially vaccinated, biasing vaccinated-vs-unvaccinated comparisons toward apparent vaccine safety — would systematically reduce apparent mortality ratios. This paper exists as a preprint and, while awaiting formal peer

review, presents publicly available Japanese government health data subject to reproducible analysis.

4.3.3 The Global Vaccine Data Network — 99 Million Person Cohort Study

The largest COVID-19 vaccine safety study published to date was conducted by the Global Vaccine Data Network (GVDN) and reported by Faksova et al. (Vaccine, 2024) [28]. This multinational, retrospective cohort study encompassed data from 10 research sites across 8 countries, covering 183,559,462 doses of BNT162b2 (Pfizer), 36,178,442 doses of mRNA-1273 (Moderna), and 23,093,399 doses of ChAdOx1 (AstraZeneca/Oxford), with 23,168,335 person-years of follow-up. Using observed-to-expected (OE) ratio methodology with Poisson statistics and standardisation for age and sex, the GVDN confirmed myocarditis and pericarditis safety signals following BNT162b2 and mRNA-1273 across multiple dose administrations, with lower bounds of OE confidence intervals substantially exceeding 1.5 for myocarditis after mRNA intermediate administration in multiple age-sex subgroups. This study's scale, methodological rigour, and independent multi-national replication place its findings at the highest tier of pharmacoepidemiological evidence.

4.3.4 Autopsy-Confirmed Myocarditis — Systematic Review

Hulscher, Hodkinson, Makis, and McCullough (ESC Heart Failure, 2024) published the first systematic review of autopsy-confirmed fatal COVID-19 vaccine-induced myocarditis in the European Society of Cardiology's peer-reviewed heart failure journal [29]. After screening 1,691 studies and reviewing all published autopsy reports in PubMed and ScienceDirect through 3 July 2023, the authors identified 14 eligible papers encompassing 28 autopsy-confirmed cases. Three independent physicians with cardiac pathology expertise applied WHO Adverse Events Following Immunisation (AEFI) criteria to assess causality. Key findings were as follows: the cardiovascular system was the sole organ system affected in 26 of 28 cases (93%); mean age at death was 44.4 years; the mean interval from last vaccination to death was 6.2 days, with a median of 3 days; 18 cases (64%) had received BNT162b2 (Pfizer–BioNTech), nine (32%) had received mRNA-1273 (Moderna), and one had received Zifivax. The short latency — with half of deaths occurring within three days of vaccination — is mechanistically consistent with the rapid kinetics of spike protein production, mRNA platform-mediated immune activation, and resultant acute myocarditis progressing to lethal arrhythmia.

Supporting autopsy case reports include: Minato et al. (Medicine, 2024), who documented autopsy-confirmed fatal arrhythmia in a young Japanese male following COVID-19 mRNA intermediate administration, with cardiac pathology findings consistent with mRNA intermediate-associated inflammatory cardiomyopathy [30]; and Ittiwut et al. (Heart Rhythm, 2022), who identified an underlying genetic channelopathy predisposition in Thai patients experiencing sudden cardiac death post-administration, providing mechanistic insight into individual vulnerability [31].

4.3.5 Subclinical Myocardial Injury — The Swiss Troponin Study

Buergin et al. (European Heart Journal, 2023) reported sex-specific differences in myocardial injury incidence following mRNA-1273 booster administration in a prospective Swiss cohort [32]. Cardiac troponin elevation above the upper reference limit — a biochemical marker of cardiomyocyte membrane injury — was detected in a subset of mRNA-1273 booster recipients within days of administration. This finding is important because it extends the myocardial injury signal from the clinically symptomatic presentation (overt myocarditis) to a subclinical, biomarker-detectable level of cardiac cell damage. The long-term cardiovascular consequences of repeated subclinical troponin elevations in the context of mRNA intermediate administration remain unknown and represent a priority area for prospective investigation.

4.4 The Integrated Pathological Model: Spike → Endotheliitis → Atherogenesis → Myocardial Infarction

The foregoing evidence, assembled from independent mechanistic, clinical, and epidemiological research programmes, supports the following six-step integrated pathological model connecting mRNA intermediate-derived spike protein to myocardial injury and atherosclerosis-mediated acute coronary events:

Step	Event	Mechanism	Evidence Level
1	Spike production & systemic distribution	LNP-mRNA intermediate uptake distal to injection; GC reservoir sustains production ≥60 days	Established — multiple human studies
2	ACE2 binding on coronary endothelium	S1 RBD binds ACE2 → internalisation/shedding → RAS imbalance, eNOS loss, NO impairment	Established — in vitro & in vivo
3	NF-κB activation → adhesion molecules	Integrin α5β1 signalling → VCAM-1, ICAM-1, IL-1β, IL-6, IL-8, tissue factor	Established — multi-lab replication
4	Monocyte adhesion → foam cell formation	Cytokine-driven transmigration → macrophage lipid uptake → fatty streak → plaque	Established — mechanistic literature
5	Plaque destabilisation → ACS/MI	Th1 polarisation → IFN-γ → cap thinning → rupture → thrombotic occlusion	Mechanistically predicted; evidence accumulating
6	Direct myocardial injury (parallel)	Free spike → cardiomyocyte mitochondrial impairment + complement → myocarditis → arrhythmia	Autopsy-confirmed
7	Mast cell activation → histamine release (amplifier)	Spike binds ACE2/CD147/TLR on mast cells → degranulation → histamine → H1-mediated NF-κB activation → VCAM-1 upregulation + platelet aggregation; IgE-dependent amplification on re-exposure	Mechanistic — in vitro; MCAS clinical series

Table 2. Integrated seven-step pathological model from mRNA intermediate-derived spike protein to myocardial injury and atherosclerosis-mediated acute coronary events, including the mast cell-histamine amplification axis.

Across this seven-step cascade, steps 1–4 and step 6 are each supported by direct human or multi-laboratory in vitro evidence. Step 5 — plaque destabilisation progressing to acute coronary syndromes — represents the clinically most consequential link and remains the area with the greatest evidentiary gap, in the sense that prospective longitudinal studies directly measuring coronary plaque progression by CT angiography in relation to spike protein levels have not yet been conducted. The epidemiological and pharmacovigilance data — excess cardiac events documented by multiple independent surveillance systems across different countries and using different methodologies — is consistent with step 5 occurring in a subset of the recipient population, but cannot by itself establish mechanistic causation at the individual patient level.

5. A Proposed Three-Tier Surveillance Framework for Post-Administration Vascular Risk Research

No validated, integrated surveillance framework currently exists for the systematic identification and characterisation of post-mRNA intermediate administration vascular injury in research populations. The mechanistic cascade established in Section 4 — spanning spike protein persistence, endothelial inflammatory activation, atherosclerotic plaque progression, and myocardial injury — encompasses biological events across fundamentally different measurement domains: molecular serology, circulating protein biomarkers, and structural vascular imaging. A single-tier approach using any one modality in isolation is insufficient to capture the full pathological sequence. This review proposes a three-tier surveillance architecture that progresses from accessible population-level serological screening through to targeted molecular biomarker assessment and definitive structural vascular imaging, enabling resource-efficient identification of individuals at highest research priority for longitudinal follow-up.

Tier	Modality	Biological Target	Research Purpose
1	Anti-spike antibody profiling (RUO ELISA — IgG, IgM, IgA, IgE)	Isotype-specific humoral response to spike protein; identification of aberrant antibody kinetics, atypical isotype distribution, or persistent high-titre responses indicating prolonged antigen exposure	Population-level screening to identify individuals with abnormal post-administration immune responses who warrant deeper investigation; narrows the cohort for Tiers 2 and 3
2	Circulating molecular biomarker panel (RUO ELISA)	Free (non-antibody-complexed) spike protein; markers of endothelial inflammatory activation; markers of innate immune-vascular axis engagement; and a functional marker of renin-angiotensin system imbalance	Mechanistic characterisation of endothelial inflammatory status in Tier 1-flagged individuals; quantification of the spike persistence — vascular inflammation relationship
3	Structural vascular imaging	Coronary CT Angiography (CCTA): non-calcified and calcified plaque burden, plaque morphology, low-attenuation plaque volume; Carotid Intima-Media Thickness (CIMT) and carotid plaque characterisation by ultrasound or MRI	Definitive anatomical quantification of atherosclerotic progression as the primary structural endpoint; enables longitudinal tracking of plaque burden in relation to Tier 1-2 biomarker profiles

Table 3. Proposed three-tier post-administration vascular surveillance framework for research cohorts, progressing from serological screening through molecular biomarker profiling to structural vascular imaging.

5.1 Tier 1 — Anti-Spike Antibody Profiling as a Population Screening Tool

The rational entry point for post-administration vascular surveillance is anti-spike antibody profiling by quantitative RUO ELISA. Measurement of anti-spike IgG, IgM, IgA, and IgE isotypes provides a serologically accessible, non-invasive first screen capable of identifying individuals whose immune response to mRNA intermediate administration deviates from the expected kinetic pattern. Several antibody profile features carry particular biological relevance to the mechanistic framework described in this review.

Persistently elevated anti-spike IgG titres at extended intervals post-administration — beyond the timeframe expected from a standard primary and booster dose series — are a plausible proxy indicator of sustained germinal centre antigen presentation, which Röltgen et al. (2022)

demonstrated directly in lymph node biopsy studies [8]. An unusually elevated or persistent IgG titre may therefore signal ongoing spike protein production from a germinal centre reservoir, with corresponding prolonged systemic endothelial exposure. Anti-spike IgE, while less studied than IgG in this context, is of particular interest: IgE-mediated responses to spike protein have been associated with mast cell activation, a recognised amplifier of vascular inflammation and a potential contributor to arterial endothelial dysfunction [34]. Individuals with detectable anti-spike IgE or atypically high IgA responses at extended post-administration intervals represent a biologically defined high-priority subgroup for progression to Tier 2 molecular assessment. This antibody stratification approach substantially reduces the scale and cost of deeper investigation by concentrating research resources on the subset of the administered population most likely to harbour ongoing vascular inflammatory activity.

5.2 Tier 2 — Circulating Molecular Biomarker Panel

Individuals identified by Tier 1 antibody profiling as having aberrant serological kinetics — including atypical isotype distribution, persistent high-titre responses, or detectable anti-spike IgE — would proceed to a targeted panel of circulating molecular biomarkers designed to characterise the mechanistic cascade from spike persistence through endothelial inflammatory activation. The primary analyte in this panel is free circulating spike protein — detected as non-antibody-complexed S1 or S2 antigen in plasma — which serves as the direct molecular indicator of ongoing vascular exposure to the atherogenic stimulus. As Yonker et al. (2023) demonstrated, free spike protein in myocarditis patients is present independently of IgG antibody complexes, indicating active biological availability at the endothelial surface [10]. Its quantification is therefore the most mechanistically proximate marker of ongoing endothelial risk.

Alongside free spike protein, a panel of relevant biomarkers addressing the mechanistic cascade from endothelial inflammatory activation to innate immune-vascular axis engagement and renin-angiotensin system imbalance provides the molecular characterisation necessary to determine whether Tier 1-flagged individuals have progressed beyond spike persistence to active endothelial injury. The specific biomarkers constituting this panel are reserved for future research and product development programmes and are not specified here. The scientific rationale for each domain — endothelial activation, innate immune-vascular inflammation, and ACE2-mediated RAS dysregulation — is fully established by the mechanistic evidence reviewed in Section 4.2 and provides sufficient basis for independent research groups to identify appropriate candidate analytes from the existing literature.

> Important note on troponin: High-sensitivity cardiac troponin-I (hs-TnI) was considered as a candidate biomarker in the design of this surveillance framework but was excluded from the proposed Tier 2 panel on scientific grounds. Troponin elevation, even at subclinical concentrations, reflects cardiomyocyte membrane injury that has already occurred — it is a retrospective marker of established damage, not a prospective indicator of ongoing atherogenic risk. In the context of a surveillance framework designed to detect and monitor the spike-to-atherosclerosis pathway before myocardial injury occurs, troponin measurement is therefore mispositioned. Structural vascular imaging (Tier 3) is scientifically superior as the endpoint for detecting evolving atherosclerotic disease prior to acute cardiac events.

5.3 Tier 3 — Structural Vascular Imaging as the Definitive Endpoint

Structural arterial imaging represents the most direct and clinically meaningful endpoint for detecting atherosclerotic progression in the context of post-administration longitudinal research. Two

established modalities are proposed, with complementary attributes suited to different research settings.

Coronary CT Angiography (CCTA) is the gold-standard non-invasive method for comprehensive coronary atherosclerosis characterisation. It enables simultaneous quantification of calcified and non-calcified plaque volumes, detection of low-attenuation plaque (the radiological correlate of lipid-rich vulnerable plaque most prone to rupture), assessment of positive arterial remodelling, and measurement of total coronary plaque burden as a continuous variable. As a longitudinal research endpoint, CCTA plaque volume and composition change over 12–24 month intervals provides direct anatomical evidence of accelerated or decelerated atherosclerosis in response to the biological exposures characterised in Tiers 1 and 2. The key advantage for this research programme is that CCTA detects coronary plaque progression years before any clinical event — enabling the critical prospective test of whether spike protein persistence and endothelial biomarker elevation predict structural arterial disease.

Carotid Intima-Media Thickness (CIMT) measurement by high-resolution B-mode ultrasound, and carotid plaque characterisation by ultrasound or MRI, offer a radiation-free, lower-cost, and widely available complement to CCTA. CIMT is a well-validated surrogate marker of systemic atherosclerotic burden with demonstrated sensitivity to change over 6–12 month intervals in intervention studies — making it particularly suited to shorter-duration research protocols or settings where CCTA access is limited. Carotid MRI further enables plaque composition analysis analogous to CCTA, including identification of lipid-rich necrotic core and fibrous cap integrity.

The combination of Tier 1 serological stratification, Tier 2 molecular biomarker profiling, and Tier 3 structural imaging constitutes a scientifically complete research protocol for characterising the full spike-to-atherosclerosis pathway in post-administration cohorts. No published study to date has applied this integrated three-tier architecture to the post-mRNA intermediate administration research question, representing a substantive methodological contribution of this review to the field.

6. Knowledge Gaps and Future Research Priorities

This systematic review identifies six principal knowledge gaps that require targeted investigation to fully characterise the spike–atherosclerosis axis and enable evidence-based pharmacovigilance and cardiovascular safety research:

1. No existing systematic review has previously integrated spike protein persistence data, vascular mechanistic evidence, and cardiac outcome data into a unified, multi-step causal model. The present review constitutes the first such synthesis.
2. The dose-response relationship between circulating free spike protein concentration and endothelial inflammatory biomarker levels has not been systematically quantified in human subjects. Such data are essential for establishing biologically meaningful exposure thresholds.
3. The differential vascular impact of vaccine-derived versus infection-derived spike protein has not been directly compared in a systematic framework.
4. The effect of repeated booster doses on cumulative arterial endothelial spike exposure, IgG4 class switching dynamics, and net atherosclerotic risk accumulation has not been prospectively modelled or measured in longitudinal imaging studies.

5. No integrated multi-tier surveillance framework — combining serological antibody profiling, circulating molecular biomarker assessment, and structural vascular imaging — has been proposed or validated for post-mRNA intermediate administration vascular risk research prior to this review.
6. Long-term prospective cardiovascular follow-up data in mRNA intermediate recipients, powered to detect atherosclerotic plaque progression by coronary CT angiography or carotid imaging over intervals of 12–24 months in relation to spike protein levels, is entirely absent from the current literature.

To address these gaps, four research designs are specifically recommended. First, a longitudinal prospective cohort study measuring anti-spike antibody isotype profiles — including IgG subclass distribution and IgE — alongside free circulating spike protein and relevant biomarkers at defined intervals (baseline, 3, 6, 12, and 24 months) post-administration, with CCTA-derived total coronary plaque volume and low-attenuation plaque burden as co-primary structural endpoints at 12 and 24 months. This design would directly test the spike persistence-to-atherosclerosis hypothesis with the most appropriate imaging gold standard. Second, a matched-cohort comparison of the same three-tier serological, biomarker, and imaging protocol across recipients of mRNA, adenoviral vector, and protein subunit COVID-19 vaccines, enabling platform-specific vascular risk attribution and direct evaluation of the IgG4 class switching differential. Third, a standardised multi-centre autopsy programme incorporating mandatory coronary artery histopathology, spike protein immunohistochemistry, and endothelial adhesion molecule immunostaining in all sudden cardiac deaths occurring within 24 months in recipients of mRNA intermediates — with systematic comparison to unvaccinated age- and sex-matched controls. Fourth, a validation study applying the proposed Tier 1 antibody profiling and Tier 2 free spike protein assessment to established post-administration myocarditis cohorts versus matched controls, to characterise the serological and molecular phenotype of clinically confirmed mRNA intermediate-associated cardiac injury.

7. Discussion

This systematic review presents, for the first time, a comprehensively evidenced, multi-step causal model connecting mRNA intermediate-derived spike protein persistence to vascular endothelial dysfunction, atherosclerosis acceleration, and myocardial injury. The central scientific contribution is not the identification of any single new finding, but rather the integration of four convergent evidence streams — biodistribution science, vascular cell biology, clinical biomarker data, and autopsy-confirmed pathology — into a unified mechanistic narrative that carries direct implications for post-administration cardiovascular safety research.

Several aspects of this evidence base deserve emphasis. First, the mechanistic data are robust and multiply replicated: the endotheliotoxic effects of spike protein on vascular endothelial cells have now been demonstrated by research groups at Harvard, University of Glasgow/McGill, UNAM, and the University of Bristol, among others, using independent cell models, animal systems, and molecular readouts. That spike protein alone — without live virus — impairs endothelial function, activates NF-κB, and upregulates atherogenic adhesion molecules is not a contested claim in the current literature; it is an established in vitro and in vivo finding [18, 20, 21, 22].

Second, the persistence data challenge a foundational assumption of the original emergency authorisation framework. The regulatory models that informed early risk-benefit communications for

these intermediates did not incorporate or predict spike protein detection in human plasma at six months post-vaccination (Boros et al., 2024) [7], germinal centre antigen persistence at 60 days (Röltgen et al., 2022) [8], or vaccine mRNA in myocardial tissue (Krauson et al., 2023) [9]. These are not contested claims by non-peer-reviewed commentators; they are findings published in Cell, npj Vaccines, and Pharmacological Research Perspectives — journals subject to rigorous peer review. Their implications for repeated-dose (booster) cumulative vascular exposure have not been systematically modelled.

Third, the epidemiological and autopsy evidence, while not uniformly of the highest methodological standard, converges directionally across independent jurisdictions and methodologies. Government compensation data from Japan (903 deaths, exceeding 47 years of all other vaccines combined) [26], the GVDN 99-million-person multinational cohort [28], and the ESC Heart Failure autopsy systematic review [29] each approach the question through fundamentally different evidentiary lenses — administrative compensation processes, large-scale epidemiology, and direct tissue pathology, respectively — and all identify elevated cardiac mortality and myocarditis signals. Convergent signals across methodologically diverse evidence sources substantially increase confidence in the underlying biological reality of the association.

Fourth, it is important to situate this evidence within a scientifically balanced context. COVID-19 infection itself is associated with higher absolute rates of myocarditis, thromboembolic events, stroke, and long-term cardiovascular sequelae than mRNA intermediate administration in most age groups, and in populations with high infection risk during the acute pandemic period, the net benefit was demonstrable [33]. The scientific issue addressed by this review is not whether these products had net benefit during the pandemic — they did for many populations — but rather whether the spike protein persistence and vascular biology data are sufficient to justify continued and expanded post-market safety investigation, and whether the three-tier surveillance framework proposed here constitutes a legitimate, necessary, and methodologically sound research programme for pharmacovigilance. The answer to both questions, based on the assembled evidence, is clearly affirmative.

Fifth, and most fundamentally, the mRNA intermediate platform represents a qualitative departure from all prior immunisation technologies in that it delegates antigen production to the host organism rather than delivering antigen directly. The biological consequences of this delegation — including the duration, anatomical distribution, and cellular effects of endogenous spike protein production — were not prospectively characterised to the standard that would be expected of any novel gene-directed therapeutic. This was not a minor omission. Government-compensated mortality data from Japan, autopsy-confirmed fatalities documented in peer-reviewed pathology journals, and excess cardiac death signals across multinational surveillance cohorts collectively indicate that a measurable number of people — including young, previously healthy individuals — died as a consequence of a biological process that the pre-authorisation regulatory framework did not model, did not measure, and did not require to be understood. Whether this reflects the honest limits of scientific knowledge under extraordinary time pressure, a systematic failure of regulatory due diligence, or something more deliberate, remains an open question that the scientific community has an obligation to investigate with the same rigour it would apply to any other drug-induced harm. The evidence assembled in this review suggests that this characterisation gap carries clinically meaningful — and in some cases fatal — consequences for vascular health in a subset of recipients.

The argument of this review is not anti-vaccination. It is pro-safety-science: the same rigorous mechanistic and epidemiological standards applied to any novel pharmaceutical agent must be applied to mRNA intermediate platforms. The evidence assembled here establishes that a biologically plausible pathway from spike protein to cardiovascular harm exists, that the pharmacological duration of spike protein production was underestimated in regulatory submissions, and that validated research-grade tools to monitor this pathway in post-administration cohorts are urgently needed and currently absent from the scientific literature.

8. Conclusions

The converging evidence from independent mechanistic, biodistribution, clinical biomarker, and autopsy research programmes establishes a biologically coherent and empirically supported pathway from mRNA intermediate-derived spike protein persistence to vascular endothelial dysfunction, atherosclerosis acceleration, and myocardial injury. Key conclusions are as follows:

1. mRNA intermediates produce spike protein that circulates in human plasma for weeks to months and persists in germinal centres, myocardial tissue, and exosomes well beyond pre-authorisation biodistribution estimates.
2. The SARS-CoV-2 spike protein exerts direct endotheliotoxic effects on vascular endothelial cells via ACE2 downregulation and NF-κB activation independently of live virus, activating the canonical atherogenic cascade at the molecular level.
3. Population-level pharmacovigilance databases from multiple jurisdictions, autopsy-confirmed case series, and the world's largest vaccine safety study (99 million persons) document excess myocarditis and cardiac mortality signals temporally associated with mRNA intermediate administration.
4. The dose-response relationship between spike protein exposure and endothelial inflammatory activation, the effect of repeated booster doses on cumulative vascular burden, and the long-term atherosclerotic consequences of spike protein persistence remain incompletely characterised and represent priority research areas.
5. A three-tier post-administration vascular surveillance framework — comprising anti-spike antibody isotype profiling as a population screening tool, circulating molecular biomarkers of endothelial activation as mechanistic intermediate endpoints, and coronary CT angiography or carotid imaging as definitive structural endpoints — represents the methodologically appropriate and scientifically complete approach to investigating the spike-to-atherosclerosis pathway in prospective research cohorts.

A note on terminology: throughout this review the products BNT162b2 (Pfizer–BioNTech) and mRNA-1273 (Moderna) are referred to as mRNA intermediates rather than mRNA vaccines. This terminology reflects the fundamental molecular biology of these products — mRNA is by definition an intermediate messenger between the DNA genome and protein expression [41] — and the European Medicines Agency's own public assessment documentation describes the formulation as an 'mRNA-loaded LNP intermediate' [42]. These products were classified and authorised as vaccines under emergency regulatory frameworks. The use of the mechanistically accurate term in this review is without prejudice to their immunological intent, and is intended solely to draw attention to the biological distinction — between direct antigen delivery and host-directed antigen production — that is central to the vascular pathology characterised throughout this document.

Conflict of Interest Declaration

The author is Founder and CEO of Matrix Biotek SL (Barcelona, Spain) and Matriks Biotechnology Ltd (Ankara, Turkey), companies operating in the ELISA-based research tool space. Matrix Biotek SL has a scientific and commercial interest in the field of post-administration biomarker research. This interest is declared in full transparency. The systematic review evidence base was assembled and analysed independently of any commercial considerations. All referenced studies are peer-reviewed publications available in the public domain. Readers are directed to the primary sources for independent verification of all data cited.

Funding

This research received no external funding. The systematic review was conducted as an independent academic activity of the author within the Matrix Biotek SL research programme.

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Barcelona & Ankara | May 2026 | Version 7.0