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NARRATIVE REVIEW

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# COVID-19 vaccine adverse events: Evaluating the pathophysiology with an emphasis on sulfur metabolism and endotheliopathy

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**Abstract**

In this narrative review, we assess the pathophysiology of severe adverse events that presented after vaccination with DNA and mRNA vaccines against COVID-19. The focus is on the perspective of an undersulfated and degraded glycocalyx, considering its impact on immunomodulation, inflammatory responses, coagulation and oxidative stress. The paper explores various factors that lead to glutathione and inorganic sulfate depletion and their subsequent effect on glycocalyx sulfation and other metabolites, including hormones. Components of COVID-19 vaccines, such as DNA and mRNA material, spike protein antigen and lipid nanoparticles, are involved in possible cytotoxic effects. The common thread connecting these adverse events is endotheliopathy or glycocalyx degradation, caused by depleted glutathione and inorganic sulfate levels, shear stress from circulating nanoparticles, aggregation and formation of protein coronas; leading to imbalanced immune responses and chronic release of pro-inflammatory cytokines, ultimately resulting in oxidative stress and systemic inflammatory response syndrome. By understanding the underlying pathophysiology of severe adverse events, better treatment options can be explored.

**KEYWORDS**

albumin, COVID-19 vaccine, endotheliopathy, glutathione, glycocalyx, lipid nanoparticles

## 1 | INTRODUCTION

During the past 3 years, the deployment of DNA and mRNA vaccines has occurred at an unparalleled pace and scope to fortify immunological defences against SARS-CoV-2. Despite the documented efficacy of this vaccination strategy,[1–3] there have been reports of serious adverse events (AEs)[4–12] and unexpected deaths after vaccination.[5,13–17] Several studies communicated the existence of symptoms related to rare, or never-described before syndromes, which developed after COVID-19 vaccination.[18,19] There were too many serious AEs that warrant attention. Systematic reviews by Hulscher et al.[20,21] and a report by Rancourt et al.[22] found a high probability of a causal link between COVID-19 vaccines and death. The critical question therefore arises: What is the underlying

pathophysiology of severe AEs and deaths experienced by many COVID-19 vaccine recipients?

Here, we approach this question from an undersulfated, or degraded, epithelial glycocalyx (EpGL) and endothelial glycocalyx (EnGL) perspective. In a previous review article,[23] we examined how an undersulfated and degraded EpGL and EnGL predispose to COVID-19 and various chronic diseases. This review focuses on the effect of an undersulfated and degraded glycocalyx (GL) on susceptibility to AEs postvaccination, and the impact of the various ingredients of the COVID-19 vaccine on causing epithelial (Ep) or endothelial (En) degradation.

Under healthy conditions, an adequately sulfated Ep- and EnGL possesses immunomodulating, anti-inflammatory, anticoagulant and vasodilatory mechanisms. Endotheliopathy,[24–26] evidence of SARS-CoV-2 infection of pulmonary and extrapulmonary endothelial cells (EnCs),[26–28] reports of viraemia[29,30] and multiorgan injury,[27,31] all confirm the hypothesis that COVID-19 and COVID-19 vaccine AEs are, in part, vascular diseases of the endothelium. Clinical studies have shown that En dysfunction is a major determinant of severe COVID-19[23,32–34] and vaccine-induced AEs.[13,24,35] At the vascular surface, the EnGL is essential in regulating barrier function, immunomodulation, nitric oxide (NO) production and vasorelaxation, mechanotransduction, and resistance to oxidative stress, coagulation and inflammation.

The GL undergoes constant constitutive remodelling, and a balance of synthesis and degradation of the GL components maintains homeostasis. However, various factors, such as infection, inflammation, toxins and heavy metals, malnutrition, ischemia/reperfusion and hyperglycaemia, can lead to degradation of the GL and release, or shed, bioactive GL fragments, exacerbating disease.[23] Furthermore, GL degradation fragments can act as damage-associated molecular patterns, amplifying pro-inflammatory responses and En injury, resulting in barrier dysfunction. The GL is an extremely delicate layer, where changing or removing one specific component can result in the loss of function of the total.[23]

The degree of sulfation and the position of the sulfate groups on the glycosaminoglycan (GAG) chains determine the biological function of the GL.[23] In chronic inflammation, high levels of 3-O-sulfotransferase 3B (3OST-3B) and unsulfated N-unsubstituted glucosamine units (GlcNH2) lead to reduced heparan sulfate (HS) sulfation. This facilitates the binding of SARS-CoV-2 to receptors of angiotensin-converting enzyme 2 (ACE2).[23] The availability of inorganic sulfate ($SO_4^{2-}$) is crucial for GAG sulfation. Various dietary and environmental factors decrease the availability of inorganic sulfate, causing protein catabolism, such as human serum albumin (HSA), glutathione (GSH) and Secreted Protein Acidic and Rich in Cysteine (SPARC), to provide sulfur amino acids (SAAs) for inorganic sulfate synthesis (Figure 1).

The most popular vaccines commercially used against SARS-CoV-2 were the nonreplicating mRNA vaccines from Moderna (mRNA 1273) and Pfizer-BioNTech – BNT162b2 (Comirnaty™), the prophylactic DNA vaccine, INO-4800, as well as the replicating DNA viral vector vaccine, ChAdOx1 nCoV-19 (Oxford/AstraZeneca or Vaxzevria®), for the expression of spike protein (Sp) by target cells in vivo. This review emphasizes the Pfizer-BioNTech (referred to as 'Pfizer' in the rest of this review) and Moderna vaccines, as they are the most widely used and comprehensive published data are available. However, most of the concerns raised also apply to the other COVID-19 vaccines, and DNA and mRNA vaccines in general.

Additional degradation complications of the GL can be expected because of the introduction of nanoparticles (NPs) through nanomedicine and vaccines. NPs form an integral part of the delivery system of mRNA vaccines. Various liposomal NPs have been approved for inclusion in nanodrugs,[36–39] while lipid nanoparticles (LNPs) received FDA approval[40] for inclusion in COVID-19 mRNA vaccines.[41,42] The patents on the Pfizer[43] and Moderna[44] mRNA vaccines can confirm this.

We hypothesize that after COVID-19 vaccination, the combination of the genetic-vaccine-generated (GVG) Sp antigen, the genetic material and LNPs, will ultimately contribute to GL degradation; mainly through the generation of chronic, skewed or excessive inflammatory responses, and oxidative stress. Therefore, AEs experienced postvaccination results from compromised barrier functions, circulating pro-inflammatory cytokines, reactive oxygen species (ROS), GL fragments, harmful NPs, and soluble GVG Sp and its fragments, all of which cause various cytotoxic effects. These effects will be exacerbated against a milieu of existing inflammation, chronic infection, genetic variability, malnutrition and toxicity, explaining the variation in severity of AEs experienced. Many studies underscore the importance of adequately sulfated GAGs and other metabolites as endogenous regulators of cancer, thrombosis, myocarditis, neurodegeneration and other disease conditions. Therefore, GL injury is a pathological manifestation capable of exacerbating disease, with circulating GL fragments causing GL injury in multiple organs.[23,32]

This narrative review article aims to highlight the potential effect of COVID-19 DNA and mRNA vaccines on sulfation, the GL and innate immunity; and how these factors could explain the various AEs experienced by many COVID-19 vaccine recipients. Therefore, the main focus of this review article is on understanding the etiological factors and pathophysiology of the AEs experienced by many recipients of the various COVID-19 vaccines.

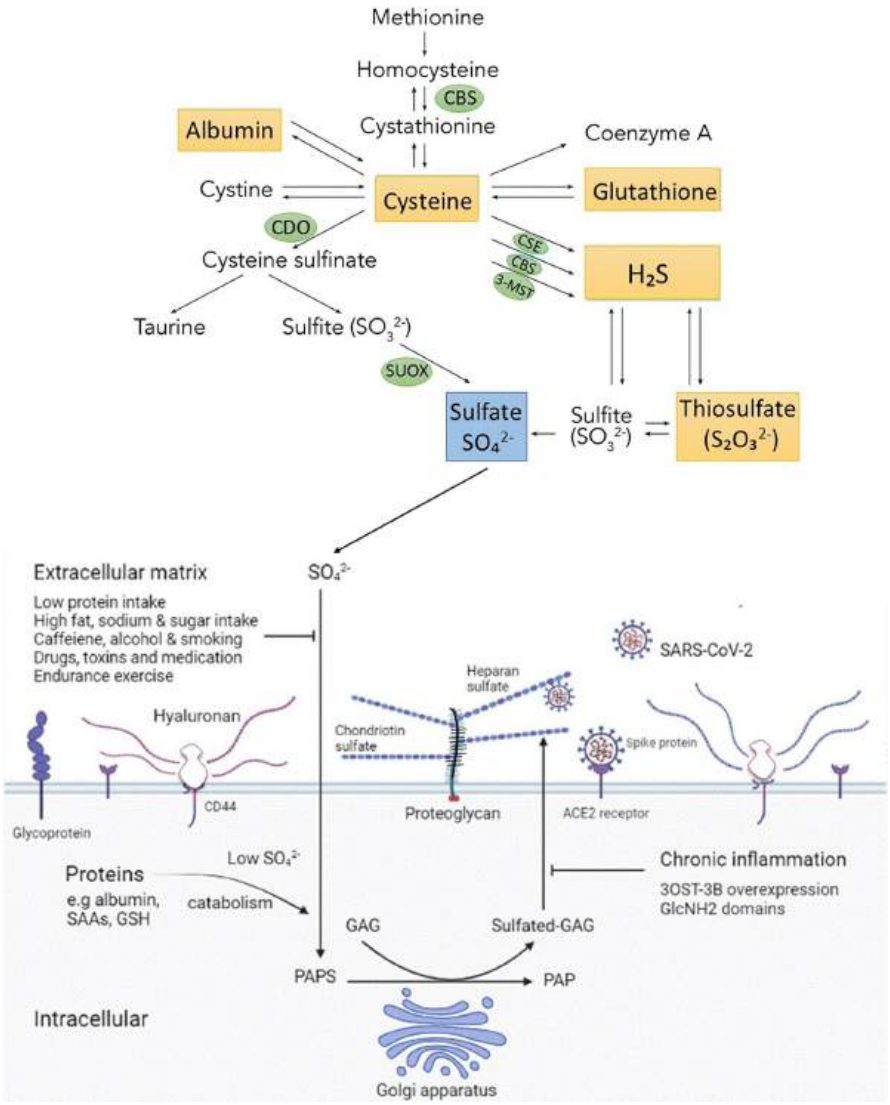


**FIGURE 1** Sulfur metabolism and sulfation diagram. The essential sulfur amino acid (SAA) methionine converts to cysteine, which is a precursor to human serum albumin (HSA), cystine, coenzyme A, glutathione (GSH), hydrogen sulfide ($H_2S$), taurine and inorganic sulfate ($SO_4^{2-}$). $H_2S$ can be converted to thiosulfate ($S_2O_3^{2-}$) and sulfite ($SO_3^{2-}$), which is oxidized to sulfate. The enzyme cystathionine-β-synthase (CBS) converts homocysteine to cystathionine, while cysteine dioxygenase (CDO) is responsible for the conversion of cysteine to cysteine sulfinate. Sulfite oxidase (SUOX) oxidizes sulfite to sulfate.[23] The availability of inorganic sulfate ($SO_4^{2-}$) is the rate-limiting factor for sulfation of the glycosaminoglycan (GAG) heparan sulfate (HS). During chronic inflammation with overexpression of 3-O-sulfotransferase 3B (3OST-3B) and the presence of unsulfated *N*-unsubstituted glucosamine units (GlcNH2), decreased HS sulfation will facilitate the binding of SARS-CoV-2 to receptors of the angiotensin-converting enzyme 2 (ACE2). With limited availability of inorganic sulfate, proteins such as HSA, GSH and Secreted Protein Acidic and Rich in Cysteine (SPARC) would be catabolized to provide SAAs for inorganic sulfate synthesis. Adapted by CC BY 4.0.[23]

## 2 | DNA AND MRNA VACCINES—SAFETY CONCERNS

mRNA technology is not new. Before the SARS-CoV-2 pandemic, Moderna had already conducted a clinical trial with an LNP-formulated mRNA vaccine against the influenza virus.[45,46] The Pfizer and Moderna COVID-19 mRNA vaccines are synthetic nucleoside-modified mRNA vaccines formulated in LNPs, which encode either the trimerized receptor-binding domain (RBD) of the Sp in S1 (BNT162b1) or the prefusion stabilized full-length Sp of SARS-CoV-2 (Moderna and BNT162b2). LNPs ensure stability and facilitate passage of nonreplicating and non-self-amplified RNA through the cell membrane to direct transient expression of the SARS-CoV-2 Sp antigen.[4,47–49] With increased improvements and stability of mRNA vaccines, protein expression can be achieved for days after direct in vivo administration.[50,51] There exist subtle differences between the Moderna and Pfizer vaccines, in relation to the RNA and LNP carriers, and the Moderna

vaccine used a higher amount of RNA per dose (100 μg) compared to the Pfizer vaccine (30 μg).[47]

Although preclinical and clinical trials were conducted, several concerns have been raised in the scientific community about the safety and long-term side effects of COVID-19 vaccines.[4,5,52–54] The two main aspects covered in this review about the COVID-19 vaccines that are likely linked to toxic effects are the delivery vehicle of the genes and the GVG Sp antigen—see more regarding the impact of the Sp in Section 3.1. The main ingredients used in the delivery hydrogel that are possibly linked to cytotoxic effects are LNPs[55–57] and polyethylene glycol (PEG).[47,58,59]

RNA and DNA viruses are known for their cytotoxicity and potent immunogenicity, leading to the popularity of nonviral vectors such as cationic lipids and polymers as gene carriers. However, until recently, cytotoxicity remained a significant challenge for nonviral vector use in gene therapy.[39] The cytotoxicity of lipid-based nanomaterials will depend on the dose, lipid properties and cell types studied.[39,60] The in vivo application of LNPs has been reported to induce liver and lung injuries in animals,[61,62] attributed to lipid-material cytotoxicity and induction of oxidative stress and pro-inflammatory cytokines.[63,64] However, it is crucial to note that in vitro toxicity does not always correlate well with in vivo cytotoxicity,[63,65] and additional human clinical data are necessary to form a comprehensive understanding of the safety profile of LNPs.

Recent advances in LNP technology have led to significant improvements in its stability, along with efforts to mitigate potential adverse effects. To reduce the cytotoxicity of cationic lipid-based NPs and improve targeted delivery, ionizable and PEGylated LNPs became popular. Ionizable cationic LNPs are pH-sensitive; the lipid-rich ionizable core structure is triggered by a pH change that induces endosomal DNA or RNA release due to the lower pH of the endosome (Figure 2).

Therefore, ionizable cationic lipids are deprotonated under neutral conditions and positively charged under low pH conditions, thus below the acid dissociation constant (pKa) of the lipid.[66–69] The LNPs used in the Pfizer and Moderna COVID-19 vaccines contain an ionizable lipid, a structural phospholipid, cholesterol and a PEG-lipid in molar ratio.[70] The ionizable lipid used in the Pfizer vaccine is ALC-0315, while SM-102 was used in the Moderna vaccine.[71] They differed in stability due to steric factors: ALC-0315 has four very short tails compared to SM-102, which is more stable with only three tails, while one is long enough to stabilize the lipid in the lipid leaflet.[66] Small changes in LNP chemistry or formulation, the use of modified nucleosides, the 5′-cytosinephospho-guanine-3′ (CpG) content and the length differences between conventional mRNA, plus the molar ratios and excipients used, will affect targetability, transfection and cytotoxicity.[67,72] The cytotoxicity, structural and biological properties of LNPs are not attributed to a single lipid component alone, but to the combination of lipids.[68]

## 2.1 | Adsorption and distribution

The absorption, distribution and excretion of NPs will be affected by various factors, such as the administration routes, physicochemical properties, particle agglomeration and surface coatings. After entering the body, LNPs can be systemically distributed through the lymph system, blood circulation and cross biological barriers, resulting in varying degrees of retention in different organs. Due to their very small nanosize, LNPs can pass through normal physiological barriers, such as the blood–air barrier, blood–testis barrier, blood–brain barrier (BBB) and the blood-placental barrier,[4,72–75] thus reaching various organs where they can induce acute or chronic injuries in tissues.[76]

LNP's main route of entry into cells is endocytosis or phagocytosis, depending on its size and lipid properties. Once the LNPs are internalized, they are transported through endosomes and lysosomes, where the LNP cargo is digested or exocytosed. Therefore, endosomal escape is a crucial step for effective gene delivery through LNPs.[71]

## 2.2 | Membrane and cellular damage

Cationic lipids are known to be excellent surfactants with the potential to cause solubilization, poration and lysis of the lipid bilayer cell membrane.[63,71] Ionizable LNPs also have endosome lytic properties to ensure the endosomal escape of the cargo.[69]

One of the hallmarks of ionizable LNPs is their active approach to endosomal escape. The ionizable lipid component becomes charged upon endosomal acidification, promoting electrostatic interactions between the LNP's ionizable cationic lipids and the anionic lipids in the endosomal membrane, thus destabilizing the endosomal membrane to promote endosomal escape of nucleic acids.[68,69,71] However, interactions of LNPs with cell membranes, in general, can cause systemic cytotoxicity with related toxic side effects in vivo if the amphiphilic properties of the lipids and the surface property of the LNP are not accurately controlled in the physiological pH (7.4) and endosomal pH range.[71] Cytotoxicity will also depend on the charge ratio between the cationic lipid species and the nucleic acids, where higher charge ratios are generally more toxic to various cell types.[39]

Endosomal signalling is tightly regulated by mechanisms that are not yet fully understood and differ from

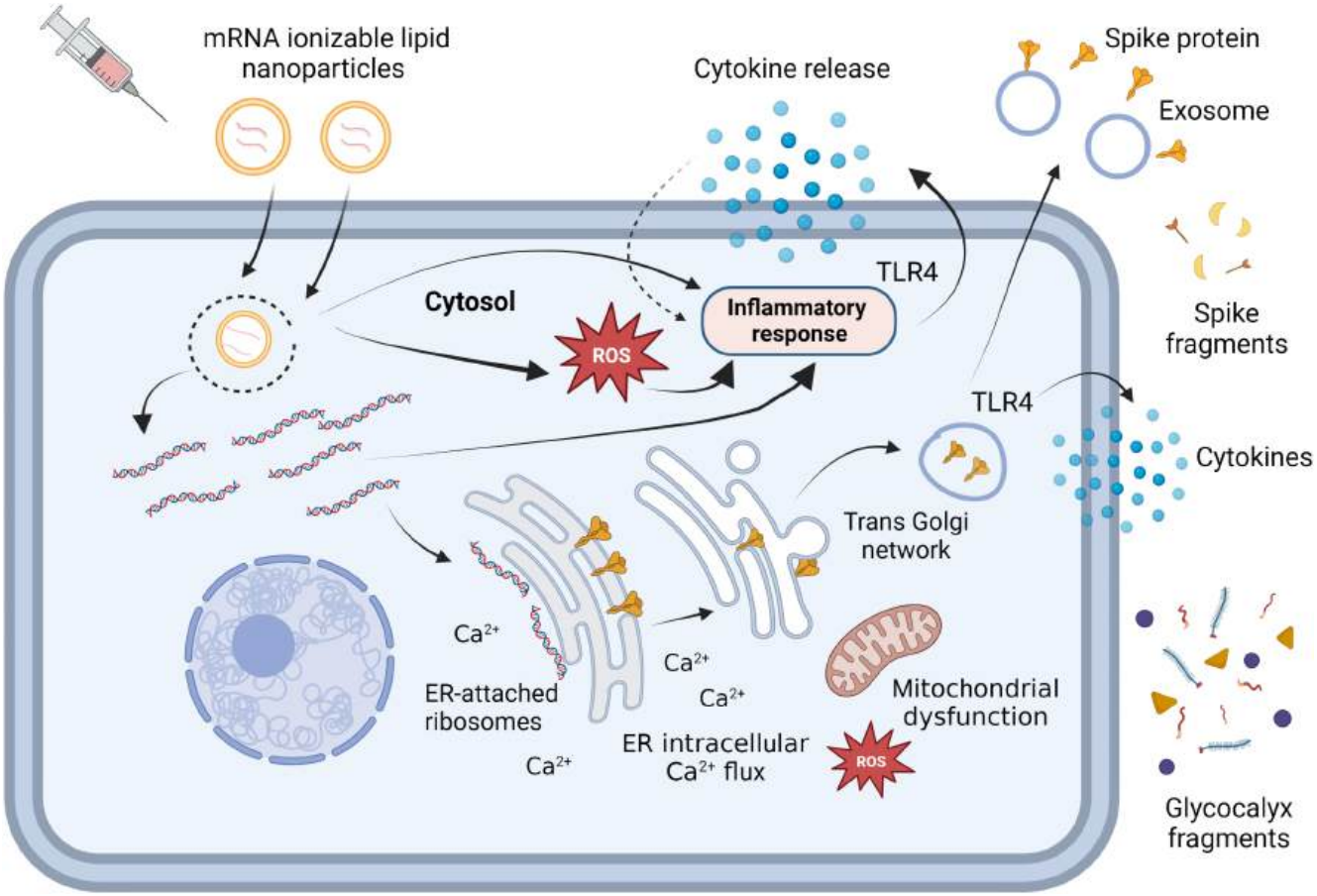


**FIGURE 2** Schematic illustration of the endosomal release of mRNA from ionizable lipid nanoparticles (LNPs); the consequent inflammatory responses and oxidative stress, with cellular release of spike protein (Sp) fragments, cytokines and glycocalyx fragments into the circulation.

those originating from receptors on the plasma membrane.[77] Signals from the diverse families of endosomal receptors control essential growth, differentiation, survival, inflammation and immunity processes. Since defects in these mechanisms can cause disease, the potential adverse effects of ionizable LNPs on endosomal membrane destabilization must be considered. However, the mechanism of endocytosis will specify the outcome of endosomal signalling. Endosomes are important sites where receptor signalling can be initiated, sustained and terminated, such as TLR signalling, which are major mediators of innate immunity.[77]

## 2.3 | Inflammation and cell death

LNPs can cause cell death through autophagy, necrosis triggered by inflammatory responses and ROS, or apoptosis through damage to the plasma membrane.[78] It has been shown that the so-called 'mix and match' strategies of combining two or more existing vaccine platforms, such as the adenoviral and mRNA-LNP vaccines, resulted in exceptionally potent immune responses,[79,80] with a consequent higher tendency for AEs.[81] When inflammation is activated through ROS or primed by antigens, macrophages trigger a natural innate immune response (Figure 2). Immunotoxicity may arise following vaccination if the immune response becomes excessive, chronic, or leads to a skewed T-cell response favouring Th1. The innate immune response triggered by an antigen is primarily transient, and an adequately sulfated Ep- and EnGL can successfully modulate the response. However, in individuals with compromised GL barriers and preexisting chronic inflammation, AEs can be expected after vaccination due to a 'systemic inflammatory response syndrome'.

In addition to stabilizing mRNA and facilitating intracellular delivery, LNPs can exert an adjuvant effect on mRNA vaccines.[68] Although LNPs are more effective and demonstrate less immunogenicity and cytotoxicity than liposomes,[82] they trigger pro-inflammatory responses. Evidence shows that ionizable cationic lipids within LNPs can induce pro-inflammatory cytokines by activating TLRs within endosomes.[83–85] The autophagy pathway is related to phagocytosis by TLR signalling in

macrophages.[86] Endosomes serve as a site for coordinated activation of signalling pathways stimulated by TLR4.[77] LNPs were shown to cause TLR4 activation that induces pro-inflammatory cytokines, such as IL-6,[68] and type I IFN[61,77] (Figure 2). Ota et al. and Li et al.[64,87] pointed out that LNP-induced cytotoxicity is caused by the indirect effect of pro-inflammatory cytokines, such as TNFα and IFNγ, which can result in apoptosis of EnCs in a dose-dependent manner in vitro and in vivo. Terentes-Printzios et al.[88] demonstrated a significant increase in high-sensitive C-reactive protein (hs-CRP) levels 48 h after Pfizer vaccination, compared to the control. This increase in inflammatory markers was most prominent after the second dose and was associated with a moderate transient degradation of En function. More long-term studies are needed to determine the effect of mRNA vaccination on inflammatory markers, oxidative stress and En integrity.

Barmada et al. observed elevations in circulating interleukins (IL-1ß, IL-1RA and IL-15), chemokines (CCL4, CXCL1 and CXCL10) and matrix metalloproteinase (MMP1, MMP8, MMP9 and TIMP1) in a cohort of patients who developed myocarditis and/or pericarditis after vaccination with SARS-CoV-2 LNP-mRNA. Since IL-1ß was elevated and the LNP component of LNP-mRNA vaccines alone was found to be highly inflammatory, with responses centring around IL-6 and IL-1ß, upstream activation of the NLRP3 inflammasome and associated cytokines may play a role in the pathogenesis of COVID-19 vaccine-induced myocarditis.[89] He et al.[90] demonstrated in a murine model that an adenovector-based vaccine elicited a different immune response from a LNP-mRNA vaccine, when sequentially inoculated. They speculated that this may be related to the different natural immune responses activated by the delivery system.

Kedmi et al. observed in vitro that cationic LNPs interact directly with immune cells and lead to immune activation, favouring a Th1 (IL-2, IFNγ, TNFα) and Th17 (IL-17 and IL-6) cytokine response. IL-17 has been shown to play a crucial role in the induction of autoimmune diseases. They confirmed that cationic LNP-siRNA complexes induce immune responses by up-regulating Th1 cytokines and IFN-responsive genes.[61] It was shown that mRNA-LNP immunogenicity would depend on the structure and pKa of the ionizable lipid.[68] Dokka et al. reported that highly charged multivalent cationic liposomes caused a marked inflammatory response, determined by neutrophil influx and oxidative burst of lung cells, where the effect was charge-related.[63]

In a mouse nucleoside-modified mRNA vaccine study, it was confirmed that LNPs are highly inflammatory, independent of the delivery route, evidenced by excessive neutrophil infiltration, activation of various inflammatory pathways and production of various inflammatory cytokines and chemokines.[55] It should be noted that inflammatory responses can be exacerbated on a background of preexisting inflammatory conditions, where this effect was proven to be specific to the LNP platform, acting independently of the mRNA cargo.[55–57] Sedic et al. confirmed in animal studies that the observed pro-inflammatory response and mild liver toxicity were primarily driven by the LNP vehicle, with repeated administration of hEPO-mRNA in LNPs. They also found that repeated dosing increases complement activation (C3a, C5b-9). They concluded that given the similarities observed in LNP-related toxicities between rats and monkeys, it is likely that similar effects will translate to the clinic.[62]

In vitro, it has been shown that cationic LNPs, whether or not they are complexed with nucleic acids, are highly toxic to macrophages.[60] Macrophages can phagocytose a large amount of LNPs, and this phagocytic activity of macrophages is responsible for the high degree of toxicity. Noncationic LNPs are also toxic to phagocytic cells, but to a lesser extent than cationic LNPs. Although the addition of PEG 2000 might seem to abolish toxicity to some degree, the presence of pH-sensitive lipids in ionizable LNPs can enhance cationic LNP toxicity by destabilizing the endosomal membrane, releasing cationic lipids into the cytoplasm.[60] Yavuz et al.[70] demonstrated that, independently of the type of ionizable lipid used to formulate LNPs, intramuscularly immunized mice induced Th1-biased polarization. This aligns with the mRNA COVID-19 vaccines that caused Th1-cellular-biased innate immune responses, with the secretion of IL-6, IL-18, TNFα and IFNγ.[91–93] Therefore, LNPs can induce macrophage M1 polarization through the foreign body response. It is known that continuous M1 polarization can release excessive pro-inflammatory cytokines, such as IL-1, NO and TNFα, as well as ROS, to induce a severe or chronic inflammatory response.[94] In M1-activated macrophages, 3OST-3B is up-regulated, with less HS and a lower degree of sulfation, compared to reparative M2 macrophages.[23] Therefore, M1 macrophages with less HS and lower sulfation, exacerbated by LNPs and ROS, will be more vulnerable to viral and SARS-CoV-2 infection and internalization of the GVG Sp.

## 2.4 | Oxidative stress

Generated ROS leads to oxidative stress capable of activating innate immune responses. However, as noted above, the release of pro-inflammatory cytokines can be induced by LNPs independently of ROS production (Figure 2). LNPs also stimulate ROS generation,[61] which induces cytotoxicity and affects intracellular signalling pathways.[63,78] ROS acts as a second messenger in many intracellular

signalling cascades. It can lead to cellular macromolecular damage, such as DNA fragmentation, membrane lipid breakdown, protein denaturation and mitochondrial dysfunction, significantly affecting cell metabolism and signalling,[95–97] resulting in deleterious effects on cell viability, proliferation and cell death.[98–100] The general belief is that the excessive ROS levels produced by NPs are the main reason for their cytotoxicity.[101,102]

Oxidative stress and the associated inflammation resulting from increased ROS production and/or decreased antioxidant defence contribute to cytotoxicity. GSH, the most abundant antioxidant that plays a crucial role in antioxidant defence against oxidative damage of ROS, regulates various metabolic pathways essential for whole-body homeostasis. GSH is responsible for maintaining mitochondrial function, antiviral defence, regulation of cellular proliferation, apoptosis, DNA synthesis, microtubular-related processes and immune responses. Variations in GSH levels are a hallmark of many pathological disorders, including cancer, metabolic abnormalities and cardiovascular disease.[103,104] The SAA, cysteine (Cys), is a precursor to GSH and inorganic sulfate, hydrogen sulfide ($H_2S$), HSA and taurine (Figure 1).[104] It is important to understand the homeostatic interaction between these sulfur compounds and the effect of inorganic sulfate on cellular health, GL intetrity and innate immune defences against infectious disease. Inorganic sulfate levels are directly correlated with GSH levels and are the rate-limiting factor for sulfation. This topic has extensively been reviewed elsewhere.[23,104]

## 2.5 | Functionalization of nanoparticles

To improve serum stability, prevent aggregation and extend the circulation time of genetic material, PEG polymer chains are attached to LNPs (PEGylation).[105] However, PEG's immunogenicity and shortened biocirculation have been demonstrated by repeated doses.[106] Administration of repeated doses of immune-stimulatory nucleic acids encapsulated with PEG-LNP, as in the case of mRNA vaccines, can generate a robust and long-lived antibody (Ab) response against PEG.[38] Therefore, there is an increased risk of acute hypersensitivity or anaphylaxis upon subsequent administration of mRNA vaccines.[47,58]

Although some of the cytotoxic effects of LNPs are reduced through functionalization with polymers, such as PEG,[68,73,107,108] PEG-lipids can dissociate in biological environments, thus increasing the toxicity of the LNPs.[68,71,109,110] Even if LNPs have been functionalized with PEG to decrease toxicity and increase circulation time,[68] PEG can cause severe allergy-like symptoms.[68,111] Furthermore, these functionalized surface modifications and cloaking techniques can allow NPs to avoid recognition and clearance through detoxification pathways,[58,112] extending circulation time,[72,113] or they can accumulate in the system.[114] Although this might be favourable from a drug development perspective, the systemic consequences in vivo from the hampered detoxification of NPs are not considered.

Seen that both mRNA and PEG will be degraded over time, the long-term cytotoxicity of COVID-19 vaccines and AE experienced months after vaccination[81] could probably be best ascribed to the presence of LNPs and the GVG Sp antigen, as well as the generated ROS,[102] protein coronas, pro-inflammatory immune responses and complement activation.[68] The fact that the failure of the COVID-19 vaccines to determine durable immunity longer than 3–4 months, if at all, for vectorial vaccines and 6 months for mRNA vaccines, required a third boosting dose, without any consideration of the possible build-up of the vaccine toxicological substances in the system. However, it has been shown that, after repeated administration, PEGylated LNPs can undergo accelerated blood clearance and complement activation-related pseudoallergy, triggered by the immune system in reaction to PEG. Therefore, accelerated blood clearance is mediated by Abs raised against PEGylated LNPs after the first injection.[68]

## 2.6 | Protein coronas

NPs have a high capacity to adsorb small molecules from physiological fluids that are partially hydrophobic, and with low solubility, by interacting with the NP surface through electrostatic, hydrophobic and van der Waals forces.[107,108] It is well established that NPs, due to their high free surface charge, can aggregate and interact with proteins in biological fluids, forming protein coronas, which will have a considerable impact on many physiological processes,[107,110,115,116] as well as biodistribution and clearance of the NPs.[68,108] The formed protein coronas will result in protein aggregation, clustering and fibrillation, affecting innate immune responses, macrophage recognition, circulation, biodistribution, cellular uptake, clearance and therefore systemic toxicity of the NPs.[107,108,110,117–119]

In addition to protein interactions with NPs, interactions exist between neighbouring proteins on the protein corona, creating a dynamic system as proteins continuously adsorb and desorb from a protein corona. Furthermore, NP-induced conformational changes in proteins can cause proteins to expose hidden binding sites that may trigger immune responses.[108]

Aliakbarinodehi et al.[119] demonstrated that the adsorption of serum proteins to the surface of LNPs is pH

dependent. According to the Henderson–Hasselbalch equation, if ionizable LNPs are used with an apparent pKa of 6.4, about 10% of the ionizable LNPs are positively charged in blood (at pH 7.4).[68] The fact that LNPs form strong bonds with platelets, haemoglobin, antithrombin (AT) III, HSA and other plasma proteins essential for major physiological functions[68,107,110] means that these bound proteins cannot perform their functions, which will affect many physiological pathways and the availability of these and other molecules (Figure 3). HSA is a precursor to Cys and consequently affects the availability of GSH and inorganic sulfate.[104] Thus, if HSA is consumed by protein corona formation, it will negatively impact the integrity of the GL.

In addition, it has been shown that the secondary structure of HSA changes after adsorption onto NPs, and the presence of HSA and apolipoproteins in protein coronas provides LNPs with a stealth effect, promoting a prolonged circulation time.[108] In addition to HSA and platelets, various proteins and other cellular materials, such as immunoglobulins, fibrinogen, plasma fibronectin and vitronectin, can form protein coronas on NPs.[68,107,108,110,119] These molecules present in the corona bestow NPs with new properties, transforming their interactions at the bio–nano interface and biodistribution while interfering with both the designed properties of NP and innate biomolecular functions.[108,110,117] For example, NPs that bind to apolipoprotein E are often trafficked to the liver, producing liver toxicity.[108] Bashiri et al.[108] critically reviewed the complexity and effects of protein coronas; however, more in vivo studies are required to understand the complex and dynamic nature of the LNP–protein complex and its interactions with biomolecules.

Many different properties, such as the type of NP, chemical concentration, surface functionalization and molecular composition, shape, curvature, size and surface charge of the NPs, will all play a role in the composition and evolution of biomaterials that adsorb on their surface, explaining in part the variation in biocompatibility and effects seemingly exerted by NPs.[68,107,108,110] Environmental conditions, such as pH, ionic strength, shear flow and temperature, protein concentration, size, and glycosylation, will impact protein corona formation.[108] Although PEGylation may show antifouling capacity, it cannot fully prevent protein binding and immunogenicity.[68,110] Apolipoproteins have been shown to be enriched in liposome corona–NP complexes, regardless of PEGylation. There has also been evidence of PEG accumulation in protein corona complexes and uncontrolled oxidative degradation of PEG into toxic products.[108]

As in the case of Onpattro,[68] the FDA-approved liver-targeting LNP, NPs can be exploited for targeted delivery and protein binding. Onpattro was designed to exploit the binding of apolipoprotein E to LNPs in circulation to deliver siRNA to hepatocytes via the LDL R receptor. Before administering Onpattro, a predosing immunosuppressive cocktail is necessary, consisting of acetaminophen, a glucocorticoid and an H1/H2 blocker, to offset potential infusion-related reactions,[68] which confirms the immunogenicity of LNPs.

The fact that NPs can induce conformational changes in adsorbed proteins, remove them out of circulation and affect their functionality, can cause unwanted effects, such as generating a pro-inflammatory immune response, altering enzyme activity and causing aggregation of blood components (Figure 3). The design and synthesis of LNPs with enhanced potency and reduced cytotoxicity is a major focus of current LNP research. However, the considerations of intellectual property surrounding them present an additional barrier to clinical translation.

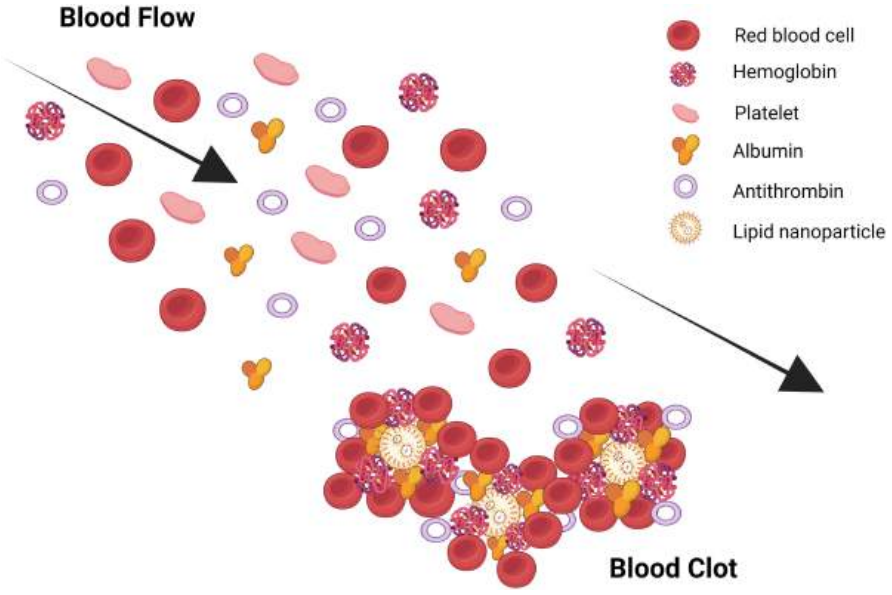


**FIGURE 3** Schematic illustration of protein corona formation and the blood clot inducing potential of lipid nanoparticles (LNPs).

## 2.7 | DNA and synthetic mRNA

Although DNA has a chance of potential genome integration,[67,120,121] it is well established that DNA and mRNA can be immunotoxic[61,64,79] (Figure 2). The main intrinsic limitations of mRNA vaccines are the instability of mRNA molecules and activation of innate immune responses.[122] Even though LNPs have been actively recruited to overcome such limitations, they also have drawbacks, such as a short half-life in the body and a low loading efficiency.[121,123] To overcome these disadvantages, graphene oxide and graphene quantum dots are often used to transfect genes.[121] However, du Preez and Halma warned that when NPs are used as the delivery vehicle for genes, the side effects on normal healthy cells, other than the target cells, must be considered.[65] Furthermore, Pfizer and Moderna use N1-methylpseudouridine-modified mRNA to minimize inherent mRNA immunogenicity; however, internalization of foreign mRNA into the cytosol is detected by intracellular RNA sensors, such as endosomal TLR and cytoplasmic nucleic acid sensors. Binding of mRNA to these host defence receptors will activate innate immune pathways,[61] leading to the expression of hundreds of genes.[67] In fact, Abramczyk et al.[124] observed increased cell signalling when cells were incubated with the Pfizer mRNA vaccine. In addition, innate immune responses have been shown to inhibit in vivo gene transfer and expression.[64] Moreover, these activated innate immune responses may further degrade the GL.

Antisense RNA can interact directly or indirectly with DNA methyltransferase, interfering with DNA methylation and gene transcription. Therefore, synthetic mRNA can eventually lead to epigenetic and/or genomic modifications in dividing and nondividing cells. It can lead to modifications of the chromatin structure, chromosomal integration of retrotranscribed synthetic mRNA, genotoxicity and oncogenesis following mRNA vaccine uptake.[53] Synthetic mRNA has been shown to activate the expression of endogenous transposable elements, undergo reverse transcription and enter the cell nucleus.[125] Mulroney et al.[126] raised concerns about their observations of ribosomal frameshifting that results in mistranslation of COVID-19 mRNA-based vaccines in humans, which can cause potential off-target effects.

Free plasmid DNA also induces the production of proinflammatory cytokines, where the immune response is significantly enhanced when lipid-DNA complexes are used. Cytokine production was observed to be mainly due to unmethylated CpG sequences in plasmid DNA.[64] Therefore, plasmid DNA can serve as a potent immunogen when delivered to immune cells in an 'intact' form, while cationic lipid-DNA complexes largely induce toxicity. McKernan et al.[125] found high levels of DNA contamination that exceed the European Medicines Agency (EMA) 330 ng/mg requirement and the FDAs 10 ng/dose requirements, when they examined the nucleic acid composition of expired vials of the Moderna and Pfizer mRNA vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is unknown. However, there is a risk of genome integration, since double-stranded DNA contamination of the sequence encoding the GVG Sp will not require LINE-1 for reverse transcription. Furthermore, an SV40 nuclear localization signal in Pfizer's vaccine vector[127] will also increase the risk of integration, while SV40 is a cancer promotor. Furthermore, plasmid DNA contamination from E.coli preps is often co-contaminated with lipopolysaccharide, leading to anaphylaxis after injection.[125]

The safety profile of nucleoside-modified synthetic mRNA is far from completely understood, and there are no studies available on what happens when mRNA-LNP formulations are stored for long periods.[67] Before the rollout of the COVID-19 vaccines, only limited in vivo research studies or trials were conducted to evaluate the biodistribution, cell uptake, translation rates, endosomal escape, functional half-life and inactivation kinetics of synthetic mRNA and DNA vaccines.[128] Neither were the rates and duration of vaccine-induced antigen expression evaluated in different cell types, nor potential interactions with the host genome.[53]

In the next section, the possible effects of the genetic material and LNPs, as well as the Sp antigen, are correlated with AEs experienced by the COVID-19 vaccine recipients.

# 3 | ADVERSE EVENTS (AES)

Since billions of people have been vaccinated with one of the COVID-19 vaccines in a short time frame, it is easier to identify AEs linked to COVID-19 vaccination. Rare events of anaphylactic shock have been reported, above the average normal incidence in the population, after COVID-19 vaccination,[5,47,129] in addition to various serious AEs. Although correlation does not necessarily mean causation, active monitoring and awareness of reported postvaccination AEs are essential. It is important to note that when AEs were analysed on the EudraVigilance database[130] for the Pfizer, Moderna, AstraZeneca and Johnson & Johnson vaccines, the percentage frequencies of specific AEs and fatal outcomes for all four COVID-19 vaccines were close to each other. Therefore, the AE profiles of these four vaccines are very similar, if not identical. Although cardiovascular AEs are among the most dangerous, nervous system and musculoskeletal disorders were

the most common organ system-related AEs, followed by gastrointestinal, infectious and skin disorders.[130]

The capacity of the COVID-19 vaccines to protect against SARS-CoV-2 infection and spread is still a matter of debate.[131–133] While various studies demonstrated the efficacy of COVID-19 vaccines against SARS-CoV-2 infection, with less severe symptoms, a reduction in COVID-19–related hospitalization and deaths,[134–137] the effect of the vaccines on all-cause mortality rates was not effectively determined. In the initial phase III randomized control adult trials of the Pfizer and Moderna vaccines, 37 people died in the vaccine group, compared to 33 in the placebo arm. This indicates that neither of the vaccines decreased or increased the absolute risk of death by more than 0.08%.[138] Although the study by Ioannou et al. confirmed the very high effectiveness of mRNA vaccines against COVID-19–related death, it did not look at all-cause mortality rates. Furthermore, they found that the effectiveness of the COVID-19 vaccine against SARS-CoV-2 infection was substantially lower than previously reported,[134] while several studies indicated that the immunity conferred by the COVID-19 vaccines wanes over time.[139,140] Adhikari et al.[141] found an increase in in-hospital mortality rates when they compared vaccinated versus nonvaccinated patients with severe COVID-19. Alquraan et al.[142] reported how mutations in the SARS-CoV-2 Sp domain result in a reduction in vaccine efficiency. Moreover, they established that although higher transmission rates were observed with the Omnicron variant, compared to previous variants, disease severity declined. Compared to Delta variants, Ward et al.[143] reported a 66% reduction in death risk with Omicron BA.1. Therefore, a decrease in hospitalizations could more likely be ascribed to the lower virulence of mutated variants than to COVID-19 vaccination.

Most published research determining the effectiveness of the COVID-19 vaccines was short-term observational studies with known limitations. Fung & Doshi[144] and Høeg et al.[145] pointed out the bias in the initial observational studies, which overstated the effectiveness of the COVID-19 vaccines. To accurately determine the effectiveness of COVID-19 vaccines, all-cause mortality and the risk of severe AEs, as the most objective and important outcomes, should be determined in long-term quality randomized controlled trials. There is no point in being saved from COVID-19 through COVID-19 vaccination, but dying of a heart attack or crippled lifelong with a neurological disease due to an AE from the vaccines.

Fraiman et al. evaluated the risk of severe AEs in the mRNA COVID-19 vaccine group, relative to placebo, in both the Pfizer and Moderna adult phase III trials and found 10.1 (Pfizer) and 15.1 (Moderna) additional AEs for every 10,000 individuals vaccinated.[5] Furthermore, they identified a 36% higher risk of severe AEs in vaccinated participants in the Pfizer trial. The excess risk of serious AEs exceeded the reduction in COVID-19 hospitalizations in both the Pfizer and Moderna trials. It is clear from these results that COVID-19 vaccines are associated with more harm than initially estimated at the time of emergency authorization.[5]

To draw a parallel between serious AEs[146] and deaths[15,16,20,147] with COVID-19 vaccines, the etiological factors possibly underlying the various AEs must be explored and better understood. In addition to the effects of the GVG Sp, genetic material, immune responses and general health status of the vaccinee, it is necessary to consider the possible consequences of the LNPs and ROS generation,[102] as well as the impact of the vaccines on the integrity of the GL, when evaluating the pathophysiology of COVID-19 vaccine-induced severe AEs. This review paper focuses on understanding the pathophysiology of documented severe AEs experienced by many vaccine recipients, as summarized in Table 1.

## 3.1 | The role of the spike protein in adverse events

Some aspects explicitly of the GVG Sp, such as stability, charge potential, its immunostimulating properties and possible systemic toxicity, are highlighted below. The fact that older people (>65) are generally more susceptible to COVID-19,[23,148] while a younger generation (aged 18–64)[149] seems to be more at risk for COVID-19 vaccine AEs,[4,150] indicates that other factors are also at play, other than the neutralizing antibody (nAb) response to the Sp.

Although the ACE2 receptor is the primary entry receptor, various other receptors, ligands, proteases and cofactors interact with SARS-CoV-2 Sp to facilitate entry into the cell, such as heparan sulfate proteoglycans (HSPGs), integrins, TLRs, neuropilin 1 (NRP1), CD147, CD26, aminopeptidase N, glutamyl aminopeptidase, C-type lectins, DC-SIGN and L-SIGN.[30,33,151–154] It is important to note that the precise mechanisms of viral entry and the role of various receptors are still under investigation, and the available knowledge is evolving. The SARS-CoV-2 Sp ectodomain was shown to interact with cell surface HS through RBD in the S1 subunit, favouring the RBD open conformation to facilitate receptor binding. Therefore, HS acts as a co-receptor priming the Sp for receptor interaction.[23]

CD147, in particular, is widely expressed in human tissues, with higher levels seen primarily in the cardiovascular system. It participates in many physiological and pathological processes due to its numerous interacting partners.[152] CD147, a versatile transmembrane glycoprotein, promotes the activation of MMP, myofibroblast differentiation, fibrosis and oxidative stress.[155] It is important

**TABLE 1** Summary of the potential effect of the COVID-19 DNA and mRNA vaccine ingredients on the pathophysiology of the serious adverse events experienced postvaccination.

| Spike protein (Sp) | Adverse events/site of action | Mode of action |
|---|---|---|
| CD147 binding | Cardiovascular system, immune cells, neurological disorders, kidney disease, pulmonary hypertension, digestive tract, rheumatoid arthritis, conjunctivitis, skin health and tumour progression | Activation of MMPs with consequent endothelial glycocalyx degradation<br>Myofibroblast differentiation and fibrosis<br>Oxidative stress, chronic inflammation mediation and procoagulant<br>Facilitate viral infection and internalization of the Sp into various cells<br>Metabolism modulation and vascularization induction<br>Pleiotropic molecular effects in spermatogenesis, fertilization, neuronal networks and retinal development<br>Platelet activation and aggregation |
| Heparan sulfate proteoglycan binding | Breakthrough infections, vascular leakage, clot formation, neurological function, cancer | Heparan sulfate prime the Sp for receptor interaction<br>Barrier dysfunction and Sp dissemination<br>Glycocalyx degradation with shed glycosaminoglycan fragments and soluble Sp resulting in pathology<br>Stimulate inflammation by affecting the complement pathway<br>Affect the binding and retention of many growth factors, morphogens, cytokines and chemokines<br>Regulate cell behaviour and cancer progression |
| ACE2 interaction | Breakthrough infections, hypertension, diabetes and cardiovascular disease. Also affecting the small intestine, neurological system, kidneys and lungs | Stimulate MAP4K3/GLK and other signalling molecules, plus induce RAAS activation<br>Sp act as endocrine disruptor and may affect fertility and spermatogenesis<br>Inflammatory responses, increased oxidative stress, vasoconstriction, barrier dysfunction, lung injury, fibrosis, platelet aggregation, hypertrophy of cardiomyocytes and smooth muscle cells and/or thrombosis |
| Sp binds to various host factors and binding receptors | Systemically | Mediate signalling pathways resulting in pathology<br>Potent stimulator of pro-inflammatory responses<br>Result in NETosis |
| **Lipid nanoparticles (LNPs)** | **Adverse events/site of action** | **Mode of action** |
| Cytotoxicity | Systemically distributed through the lymph system, blood circulation and cross biological barriers<br>Diabetes, neuroinflammation, cancer promotion and progression, cardiovascular, lung and liver disease | Induction of oxidative stress, with excessive ROS and pro-inflammatory cytokine release<br>Complement activation and immunogenicity—toxic to macrophages<br>Solubilization, poration, and lysis of the lipid bilayer cell membrane, causing cell death<br>Endosome lytic properties with endosomal membrane destabilization, affecting endosomal signalling<br>Epithelial and endothelial cell injury and consequent glycocalyx degradation<br>NETosis |
| PEGylation | Acute hypersensitivity, allergy or anaphylaxis | PEGylation inhibits clearance through detoxification, extending circulation time, resulting in accumulation of LNPs and complement activation-related pseudoallergy<br>Immunogenicity<br>Oxidative degradation of polyethylene glycol into toxic products |

(Continues)

**TABLE 1** (Continued)

| Lipid nanoparticles (LNPs) | Adverse events/site of action | Mode of action |
|---|---|---|
| Protein coronas | Systemically | Small molecules and proteins interact with LNP surface, impacting many physiological processes, biodistribution and clearance of the LNPs<br>Resulting in protein aggregation, clustering and fibrillation, affecting innate immune responses, macrophage recognition, circulation, biodistribution, cellular uptake, clearance and thus systemic toxicity of the LNPs<br>Albumin is consumed by protein corona formation, negatively impacting the integrity of the glycocalyx, fetal development and create a procoagulatory phenotype<br>Protein corona consumed platelets results in thrombocytopenia<br>Generating pro-inflammatory immune responses, altering enzyme activity and causing aggregation of blood components |
| **DNA & mRNA** | **Adverse events/site of action** | **Mode of action** |
| DNA | Systemically | Genome integration<br>Immunotoxic—stimulate release of pro-inflammatory cytokines |
| mRNA | Genotoxicity and oncogenesis<br>Neurodegeneration and heart disease | Instability of mRNA molecules<br>Activate innate immune responses, resulting in gene expression<br>Interfering with DNA methylation and gene transcription, resulting in epigenetic and/or genomic modifications<br>Possible procoagulant and miRNA dysregulation<br>Potential frameshifting with off-target effects |

to note that shedding or degradation of the EnGL results primarily from MMP activity.[156] CD147 is also highly expressed in immune cells, EnCs in the brain, tissues of the gastrointestinal tract, platelets, conjunctival tissues, kidney glomerular cells and podocytes, and cardiac pericytes. Its expression level is up-regulated during pathological conditions, including several disorders of the central nervous system.[157] During inflammation and oxidative stress, secreted cyclophilins A (CypA) and B (CypB) interact with CD147 and can facilitate viral infection or cellular internalization of GVG Sp. For HIV-1 and SARS-CoV to infect host cells, the viral nucleocapsid protein was found to first bind to CypA, which then in turn recognizes the CD147 receptor expressed on the surface of host cells.[152]

In addition, CD147 was shown to be involved in SARS-CoV-2 infection of immune cells.[151,158] In the lungs, small intestine, kidney and heart, ACE2 is expressed, whereas it is not found in innate and adaptive immune cells. However, CD147 can modulate the abundance of ACE2.[152] Various researchers demonstrated that CD147 is critical to promoting SARS-CoV-2 infection through interaction with the Sp RBD.[152,159,160] CD147 exerts its influence on tissues through various mechanisms, including: (1) Metabolism modulation—achieved by binding to monocarboxylate transporters and the amino acid transporter CD98; (2) permeability regulation—where it controls the levels and activity of MMPs; (3) vascularization induction—leading to the synthesis and release of vascular endothelial growth factor (VEGF), as well as the expression of the VEGF receptor; and (4) inflammation mediation—involving leukocyte recruitment and infiltration of leukocytes by interacting with chemokines and adhesion molecules, such as integrins, selectins and CD44.[152]

Therefore, it is possible that the GVG Sp binds to CD147 receptors in cardiovascular tissue, in addition to other inflammatory ligands, resulting in chronic inflammation of the cardiomyocytes and degradation of the EnGL through activation of MMPs. In response to inflammatory stimuli, up-regulation of CD147 mediates leukocyte infiltration by binding to E-selectin. Furthermore, the GVG Sp can potentially act as a signalling molecule, affecting glycosylation, resulting in overexpression of CD147 and consequent cardiac maladaptive hypertrophy and remodelling, as well as increased oxidative stress and ferroptosis.[151] Several studies demonstrated that when SARS-CoV-2 Sp, administered to rodents as a soluble molecule or presented with a carrier, resulted in microvascular damage and induced inflammation[160] and haemagglutination.[161] CD147 has been shown to be involved in various cardiovascular diseases, including atherosclerosis and myocardial infarction, as

well as kidney disease during both acute ischemic and chronic fibrotic injuries; and plays a role in pulmonary hypertension, neurological disorders, digestive tract vascular damage and conjunctivitis.[30,152,157]

Furthermore, CD147 plays pleiotropic molecular roles in various physiological conditions, such as spermatogenesis, fertilization, neural networks and retinal development. It can result in pathological conditions, such as tumour progression, inflammatory response, plasmodium invasion and rheumatoid arthritis, in addition to facilitating viral infection.[151,155] Endocytosis is a vital entry mode for viral infection, and Zhou et al.[151] demonstrated that SARS-CoV-2 enters host cells through CD147-mediated endocytosis. The assumption can be made that COVID-19 vaccines might cause an up-regulation of CD147 due to stimulation of immune responses with consequent inflammation, although circulating GVG Sp can cause pathological conditions by further up-regulating CD147 expression and binding to the receptor, possibly entering host cells through endocytosis. Human platelets express CD147, and it has been observed that SARS-CoV-2 Sp causes platelet activation, aggregation, granule release and expression of soluble P-selectin, as well as platelet extracellular vesicles.[161,162]

The involvement of GVG Sp with the various host factors and binding receptors can mediate many signalling pathways that contribute to pathology. It has been shown that SARS-CoV-2 Sp engagement with soluble or cell membrane-attached ACE2 resulted in depletion of ACE2 from the cell surface, leading to an imbalance in the renin–angiotensin system with consequent inflammatory responses, increased oxidative stress, vasoconstriction, barrier dysfunction, lung injury, hypertrophy of cardiomyocytes and smooth muscle cells and/or thrombosis due to unopposed ACE2 and angiotensin-2-mediated effects.[4,33,130,154] Furthermore, Versteeg et al.[163] demonstrated that expression of SARS-CoV Sp can induce endoplasmic reticulum stress, consequently triggering innate immune responses. It has been documented that SARS-CoV-2 Sp is associated with increased degradation of IκB, resulting in NF-κB signalling pathway activation.[164] The GVG Sp is therefore potentially a potent stimulator of pro-inflammatory processes. More research studies are needed to establish how isolated circulating GVG Sp could affect the various receptors and consequent immune and inflammatory responses and signalling. It is important to note that the soluble GVG Sp can remain engaged with cellular receptors and other cofactors for much longer than the whole coronavirus, with consequent prolonged stimulation of intracellular signalling.[160]

Chuang et al.[165] proposed that the Sp-ACE2 interaction can induce vaccine AEs by stimulating MAP4K3 (also known as GLK) or other signalling molecules. MAP4K3 may be involved in the pathogenesis of hypertension, diabetes and cardiovascular disease.[165] It has been suggested that the shed Sp particles and the GVG Sp can promote pathology via interactions with the Ep- and EnGL. Various studies demonstrated that isolated full-length Sp and S1 subunit from SARS-CoV-2 could mediate barrier dysfunction and vascular leak in vivo in an ACE2-independent manner.[33,166,167] Shed viral particles, or Sp, can act as 'viral toxins', mediating barrier dysfunction and promoting Sp dissemination and AEs.[166,168] In a mouse model, it has been observed that administration of Sp into their lungs resulted in a systemic leak in the spleen and small intestine.[33] It seems probable that the binding of Sp to GAGs will cause GL degradation and that both shed GAG fragments and soluble Sp could lead to severe manifestations of disease (Figure 2).[166,168] Even though Biering et al. and Robles et al.[33,154] believe that the levels of GVG Sp circulating in patients following COVID-19 vaccination are too low (pg/mL) to trigger vascular leak and AEs, more research in this area is required.[166] With booster vaccinations recommended every 60–90 days, higher circulating levels of GVG Sp can be expected. GVG Sp was no longer detected in circulation after the second vaccination dose, presumably because Abs generated by the first vaccination quickly and effectively removed the small amounts of Sp reaching circulation.[154] Nonetheless, it would be a combination of circulating GL fragments, pro-inflammatory cytokines, ROS and soluble GVG Sp that all contribute to various AEs (Figure 2), apart from the vaccine adjuvants and LNPs.

Trougakos et al.[4] suggested that after vaccination, a cell can present the GVG Sp and its subunits, or peptide fragments, to the immune system to stimulate responses or be destroyed via cytotoxic T lymphocytes (T cells). They believe that the subsequent debris produced and the direct secretion of the transfected cells, including shedding of the Sp antigen, can release large amounts of the Sp and/or its subunits and peptide fragments, into circulation. When LNPs of the COVID-19 mRNA vaccine are injected into the deltoid muscle, it can affect muscle tissue itself, the lymphatic system[6] and the spleen. However, Sp and its subunits and fragments can also collect in the liver and other tissues, from which it can enter the circulation and distribute throughout the body. The GVG Sp was observed in the plasma of Pfizer and Moderna vaccine recipients, on Day 1 after the first vaccine injection, confirming the distribution of the Sp antigen throughout the body.[4]

Therefore, an extensive range of interactions could be expected between the soluble free-floating GVG Sp and its subunits and peptide fragments, the pro-inflammatory cytokines, and the various binding receptors and other cofactors in the circulatory system and multiple organs. The fact that numerous AEs occur far from the injection

site shows that the GVG Sp, or its production sites are systemically distributed. It is highly probable that some LNPs only release their genetic payload once it enters the systemic circulation. It was proposed that if the vaccine produces a high amount of Sp at a speed that exceeds the capacity to produce nAbs, the GVG Sp can spread to various tissues throughout the body, including the brain, with the potential of causing inflammation, mitochondrial damage and coagulopathies.[169] It was demonstrated that in adenovirus-vectored vaccines, the GVG Sp has the native-like mimicry of SARS-CoV-2 Sp's receptor-binding functionality and perfusion structure. It is plausible that molecular mimicry may occur through additional interactions with other proteins in circulation or even the presentation to the immune system of Sp antigenic epitopes mimicking human proteins.[4] Sp antigen is believed to trigger an autoimmune response to the cell itself. At the same time, it was demonstrated in vitro that some Abs against SARS-CoV Sp have the potential to mediate FcγRII-dependent entry into B lymphocytes, thus causing antibody-dependent enhancement.[170] Then, there is the concern of possible development of anti-idiotype Ab against vaccination-induced Abs as a means of downregulation; anti-idiotype Abs—in addition to binding to the protective SARS-CoV-2 nAbs—can also mirror the Sp itself and attach to the various binding receptors, possibly triggering a wide array of AEs.[4]

The electrostatic forces of the Ep- and EnGL will also play a major role in the binding capacity of soluble GVG Sp to the various receptors. Du Preez et al.[23] indicated previously that an undersulfated GL, with overexpression of 3OST-3B, will facilitate SARS-CoV-2 Sp binding to the various receptors and negatively impact innate immune responses. The Wuhan-strain SARS-CoV-2 Sp is hydrophilic with a grand average hydropathicity (GRAVY) score of −0.079.[171] One could expect that the whole virus would have a higher negative net GRAVY score,[172,173] which will be easier repelled by the negative charge of an intact, highly sulfated GL. However, soluble GVG Sp and its fragments would have a lower charge potential. Therefore, they should be able to penetrate the GL easier and have a higher binding affinity to the various receptors and other cofactors, stimulating immune responses and signalling pathways, with consequent degradation of the barrier functions of the GL.[33,168] It should be noted that both positive values exist in the Sp domain, while other Sp domains showed negative GRAVY scores.[171] Also, with more Sp mutations, higher negative GRAVY score values could be expected,[174] with consequent increase in receptor binding and/or Ab resistance[153] when the GL is compromised. The GVG Sp-induced degradation of the Ep- and EnGL will result in the release of various enzymes, GL fragments, chemoattractants, pro-inflammatory cytokines, ROS and exposure of adhesion molecules, leading to vascular leakage, dissemination of the GVG Sp, clot formation, inflammation and leukocyte infiltration.[4,32,154,161,168]

These pathological conditions of systemic inflammation will trigger the release of histones, which can exert further cytotoxic activity on the EnGL.[175] There is a reasonable probability that GVG Sp and LNPs induce neutrophil extracellular traps (NETs) after vaccination via a ROS-dependent mechanism,[102,176] apart from GVG Sp binding to innate immune receptors.[30,177] SARS-CoV-2, as well as sera from patients with COVID-19, was found to directly trigger NET formation.[175,176] Neutrophils from these patients seemed activated and primed, making them more sensitive to NET stimuli. This pro-NETotic phenotype can probably continue for a while after vaccination. NETosis has been associated with the formation of thrombosis in patients with vaccine-induced immune thrombotic thrombocytopenia (VITT).[13] Given that HS plays an important role in modulating the cytotoxic effects of histones within NETs and coagulation,[36] one can assume that undersulfation of GAGs would contribute to the hyperinflammatory response and abnormal coagulopathy seen in vaccines[178] with serious AEs, which needs to be further investigated.

The GVG Sp appears to be highly toxic on its own.[35] Shed GVG Sp has been detected in multiple organs. There is evidence that the GVG Sp can penetrate ovaries, testes, brain, spinal cord, nervous system, heart, lungs, intestines, kidneys and cross the placenta in pregnant women.[35,128,168,179] Several studies observed the GVG Sp at a considerable distance from the injection site for up to 6 months after the injection.[26,35,179–181] This long persistence of the GVG Sp raises the possibility of sustained inflammation and organ damage. There is a high probability that the circulating hydrophilic GVG Sp affects gene expression and can act as an endocrine disruptor. Fernandes et al.[182] demonstrated through in vivo toxicological studies in zebrafish that isolated recombinant SARS-CoV-2 Sp caused adverse effects on the liver, kidney, nervous and reproduction system. Ota et al.[87] indicated through three case reports that mRNA COVID-19 vaccines could trigger activation of glomerulonephritis through vascular En damage, which persisted for several weeks. Vornicu et al.[183] observed severe En swelling, loss of fenestrations and widening of subendothelial space in the kidneys after COVID-19 mRNA vaccination. Therefore, the isolated Sp and its fragments can interact with HS and various receptors and ligands, mediating and inhibiting various cell signalling and inflammatory pathways,[23,182] with pathological consequences.

In summary, the GVG Sp can cause AEs in multiple ways, including (1) signalling and inflammatory cascades; (2) by triggering vascular leak and En barrier dysfunction; (3) molecular mimicry with human proteins or as a ligand

for various receptors and co-receptors; and (4) by producing NETosis.

## 3.2 | Breakthrough infections and lung diseases

Despite high vaccine coverage, various cases were reported of vaccinated people who became infected with SARS-CoV-2, with breakthrough infections appearing within the first 2 weeks to several months after injection.[131,184,185]

Irrgang et al. found that anti-spike IgG4 Abs and IgG4-switched memory B lymphocytes increased after several months postvaccination with the second Pfizer dose, where the response was boosted after a third vaccination.[184] It has been described that IL-4, together with IL-10, can switch to IgG4. When these Th2-associated cytokines are seen to switch to IgG4, it may negatively impact the anti-inflammatory M2 resolving macrophage phenotype, thereby skewing the immune response. Furthermore, IgG4 has a lower potential to mediate FcγR-dependent secondary effector functions to clear viral infections, indicating a less effective Ab response. Current COVID-19 vaccination regimens do not confer sterilizing protection, as evidenced by the many breakthrough infections caused by the Omicron variant.[184] An IgG4 response can be pathogenic or protective, where IgG4 Abs can lead to cancer and serious illness in several autoimmune disorders.[186] More research is needed to determine the significance of IgG4 Abs in vaccine-induced immunity and viral clearance.

In a previous review, du Preez et al.[23] provided a comprehensive overview of the effect that an undersulfated and degraded lung EpGL would have on susceptibility to SARS-CoV-2 infection. In addition to the GVG Sp, the LNPs, genetic material, pro-inflammatory cytokines and ROS may cause Ep and En degradation,[25,33] as well as result in depletion of GSH and, consequently, inorganic sulfate, thereby decreasing sulfation ability.[104] Upon inflammation and with an undersulfated EpGL, 3OST-3B would be overexpressed, facilitating the binding of SARS-CoV-2 Sp to the ACE2 receptors[23] (Figure 1). Apart from compromising the EpGL, the first-line of defence, undersulfation also negatively affects innate immune responses. The bactericidal activity of neutrophils and their recruitment are influenced by the sulfation pattern of HS. Inactivation of heparan sulfate 2-O-sulfotransferase (HS2ST) in neutrophils substantially reduced their bactericidal activity, increasing susceptibility to systemic infection.[36] Furthermore, GSH depletion has been associated with impaired immune function, especially affecting T cells and macrophages.[164] This is probably associated with the high incidence of secondary infections and viral reactivation in vaccinated individuals. Several cases of herpes zoster[187] and persistent varicella-zoster viral infection[188] have been reported following COVID-19 mRNA vaccination.

Lung macrophages, which reside in the interstitium and alveoli, are recruited by inflammatory stimuli. Various stimuli, such as TNFα and IL-1, were found to affect HS configuration and sulfation in M1 pro-inflammatory macrophages. This resulted in up-regulation of 3OST-3B, with a consequent increase in 3-O-sulfation and hypoxia, which in turn significantly reduces the expression of biosynthetic enzymes and the total HS content,[189] thus increasing susceptibility to infection and inflammation. Multiple vaccine platforms and viral infection were shown to induce SARS-CoV–specific immune responses that enhanced lung inflammation, following homologous challenge in mice and African green monkeys.[190–192] Liu et al. previously identified epitopes in the Sp that elicited both nAbs and Abs that enhanced SARS-CoV infection.[193]

Chronic inflammation induced by the vaccine platform and excessive ROS generation may favour the activation of M1 macrophages, producing uncontrolled pro-inflammatory mediators and persistent injury.[194] Indeed, Liu et al.[193] showed that despite markedly reduced SARS-CoV titres, anti–S-IgG caused lung injury during the early stages of infection by abrogating an M2 wound-healing macrophage response and TGF-β production, while promoting pro-inflammatory cytokine IL-8 and accumulation of MCP1 production and inflammatory M1 macrophages. Li et al.[195] also demonstrated that Sp-activated platelets skewed monocytes towards the M1 macrophage phenotype, with increased TNFα levels, bacterial phagocytic activity and reduced healing capacity. Furthermore, chronic inflammation alone can alter EnGL responsiveness through changes in HS composition and degree of sulfation.[196] Overexpression of 3OST-3B in inflamed cells of the compromised lung was indicated to enhance Sp fusion with cells, even without binding receptors, thus increasing the chances of infection.[23]

It was observed that the expression of 3OST-3B is up-regulated in many cell types exposed to inflammatory stimuli, such as monocytes and macrophages, fibroblasts and EnCs. Chronic inflammation is therefore a predisposing factor to viral infection, and more so, the pro-inflammatory state induced by the entry of viral or GVG Sp into the cell, will potentially further up-regulate the expression of 3OST-3B, creating a vicious cycle that increases infectivity and aggravates the cytokine storm.[23] In vivo, SARS-CoV-2 Sp has been shown to activate macrophages, plus it contributed to the induction of acute lung inflammation in mouse studies. While in transgenic

mice overexpressing human ACE2, intratracheal instillation of the S1 subunit induced severe acute lung injury similar to COVID-19, and inflammation.[4] Biancatelli et al.[197] demonstrated that the S1 subunit decreased cultured human pulmonary microvascular transendothelial resistance (TER), as well as barrier function.

Reactivation of other viruses after COVID-19 vaccination, such as varicella-zoster virus,[188] can further be explained by the fact that the GVG Sp promotes a pro-inflammatory activation profile on the most potent antigen-presenting cells (APCs), such as the dendritic cells. Consequent overexpression of 3OST-3B and binding receptors, such as CD147 and CD4, will facilitate infection of T helper lymphocytes.[4] Therefore, infected immune cells, such as CD4+ T cells, can also be a source of transmission of SARS-CoV-2 throughout the body and result in lymphopenia. A degraded GL allows immune cells to spread through the circulatory system, and undersulfated immune cells will facilitate better binding and spread of the virus and GVG Sp.[198] The failure of innate immune mechanisms, such as natural killer cells that typically control viral infections, is indicated by unregulated viral replication. Furthermore, viruses, bacteria, microbial fragments and pro-inflammatory cytokines, such as IL-1β and TNFα, induce the expression of defensins in various cells.[199] The immunomodulatory action of defensins can negatively impact the function of the Ep barrier, thus contributing to viral dissemination and infectivity through increased uptake of viruses via stromal fibroblasts and recruitment of susceptible target cells.[198,200]

Furthermore, the GVG Sp can activate the Raf/MEK/ERK signal transduction pathway in cells of the vaccinee. The ERK1/2 signalling pathway plays a crucial role in viral replication and triggers the induction of cyclooxygenase-2, an inflammatory prostaglandin synthetase. Increased inflammation will up-regulate 3OST-3B and expression of the CD147 receptor, resulting in increased susceptibility to SARS-CoV-2 infection.[23,160] Chuang et al. demonstrated through in vitro and in vivo studies that the SARS-CoV-2 Sp induces serine/threonine kinase MAP4K3 overexpression in epithelial cells (EpCs), which can facilitate infection of otherwise less susceptible cells. MAP4K3 overexpression in EpCs is positively correlated with COVID-19 severity.[165] Innate immune responses may also result in excessive caspase-1 activation, resulting in various pathological conditions, such as increased viral infection and cardiovascular disease.[201]

### 3.3 | Circulatory problems: Thrombosis, stroke & vasculitis

Damage to the GL substantially increases intravascular adhesion of leukocytes and platelets,[189,202] while rolling leukocytes, lymphocytes and platelets along the vessel wall are supported by an adequate sulfated GL. However, the binding of chemokines and cytokines to the EnGL may represent sequestration, inactivation or local concentration for presentation to rolling cells.[203] The transcytosis process depends on HS expression. The nature of these interactions has been challenging to unravel, as HS is present in both EnCs and immune cells, such as leukocytes and macrophages.[189,204] HS's role in regulating the signalling of various inflammatory mediators through their cell surface receptors is dual functional: they either promote the ligand interaction with its receptor or inhibit receptor–ligand interactions. For example, the EnGL keep neutrophils away from En adhesion molecules.

Moreover, the barrier function of the EnGL can be overcome through mechanical compression by neutrophils and LNPs; and with a protein corona traversing capillaries that are smaller than their own diameter[205]; or shedding of the EnGL through the action of ROS and released enzymes.[23] Modifying HS sulfation patterns and EnGL shedding is important in the regulation of leukocyte/neutrophil rolling in the arterial system. Furthermore, experiments have shown that EnGL shedding and reduced HS sulfation can diminish neutrophil arrest.[196] The systemic knockout of Ndst-1 is lethal in mice, but En inactivation resulted in an impaired response in various inflammatory models. Ndst-1-deficient EnCs showed reduced HS sulfation, reduced IL-8 chemokine presentation on the EnGL and impaired neutrophil arrest.[196] Another study showed that altering the 2-O-sulfation of uronic acids in HS can enhance inflammation and neutrophil recruitment. The HS2ST enzyme was inactivated in EnCs, resulting in decreased 2-O-sulfation, with increased N-sulfation and 6-O-sulfation of HS. In the mutant, the rearrangement of sulfated sequences resulted in a gain-of-function phenotype, characterized by increased inflammation in the animals. Furthermore, IL-8 exhibited improved binding to the EnGL, leading to the observation of increased neutrophil arrest.[196] Therefore, the response to inflammatory stimuli, shear stress or tissue damage can alter the configuration and degree of HS sulfation, thus regulating immune cell infiltration. The relevance of the variation of the HS structure in different tissues extends to chemokine binding. For example, aortic and venous EnCs exhibit different degrees of sulfation in their HS, resulting in the formation of chemokine binding sites exclusively in postcapillary venules and small veins where leukocyte migration occurs, while capillaries and arteries lack such sites.[189]

During COVID-19, it was observed that an HSA-nutrient deficit could result in degradation of the EnGL, which can promote infection across barriers, such as the gut and BBB.[148] Since Cys is a rate-limiting precursor to HSA and inorganic sulfate,[104] hypoalbuminemia may correlate with an undersulfated GL (Figure 1). An

undersulfated EnGL will result in a hyperinflammatory response, vascular permeability and the shedding of GL components, leading to a procoagulant and antifibrinolytic state, with eventual multiple organ failure.[23] Given that GVG Sp and LNPs can induce inflammation and ROS,[102] one could expect a global depletion of GSH in vaccinated individuals.[206]

Furthermore, HSA is the main component in the regulation of interstitial pressure and is the primary transporter of endogenous and exogenous ligands. It is a large transport protein that binds to nutrient ligands in the intestine and liver and then transports ligands to the small capillaries and interstitial spaces. HSA has a high binding affinity for the GVG Sp and LNPs,[107,110,207] hypoalbuminemia could consequently be expected in COVID-19 vaccine recipients.[208,209] A mere 20% drop in normal HSA levels can cause physiological damage in healthy individuals.[148] In addition to HSA depletion due to increased oxidative stress, oxidative damage can also impair the binding properties of HSA.

Moreover, the potential binding of the GVG Sp, cytokines, Abs and LNPs to HSA will decrease available nutrient-HSA binding. HSA carries important ions such as $Ca^{2+}$ and haem, so hypoalbuminemia would exhibit a procoagulatory phenotype and can result in venous thromboembolism.[210,211] Furthermore, reabsorption of HSA in the kidney is usually through clathrin-mediated endocytosis, which thus requires binding of HSA to clathrin. Therefore, any ligand that competes with clathrin for the binding of HSA will change this equilibrium and allow HSA to pass into the urine, further exacerbating hypoalbuminemia.[148] Moreover, the biomolecular corona formed in LNPs can activate coagulation cascades[110] (Figure 3). As is expected in the case of long-COVID, the immune-inflammatory processes and decreased antioxidant defences in vaccinated individuals are accompanied by elevated neutrophils, with reduced HSA, GSH and inorganic sulfate.[212] This aspect needs further research.

Nonetheless, these oxidative stress pathways will negatively impact the vascular endothelium and result in long-term nonresolving inflammatory processes.[167] Various researchers found that the Sp alone can mediate En dysfunction and vascular leak.[33,35,167] Likewise, it was observed that in vitro treatment of vascular EnCs with plasma of COVID-19 patients induced endotheliopathy,[213,214] and it is plausible that nonstructural proteins of SARS-CoV-2 might also mediate these effects, apart from activated platelets, enzymes and cytokines.[215] Therefore, increased serum levels of syndecan (sdc)1, HS and hyaluronan in the acute phase of vaccine-induced AEs,[13,29] due to dysregulation of the immune response and consequent EnGL degradation can be expected.[32] Seeing that vaccine recipients with only brief and mild symptoms showed elevated levels of sdc1, compared to a 2-fold increase in sdc1 level in VITT patients, it is clear that some initial endotheliopathy has developed, which then increased significantly in the VITT group.[13] As in the case of severe COVID-19 disease, therefore, increased levels of soluble En activation markers, such as sdc1, endocan, selectin and angiopoietin-2, can be expected in vaccine recipients with severe AEs.[34,215] The effect of DNA and mRNA vaccines on the endothelium warrants further research. Angiopoietin-*2* can induce the EnGL to secrete heparanase (HPSE), contributing to EnGL shedding and leading to increased vascular leakage and leukocyte diapedesis.[216,217] Angiopoietin-*2* levels will be positively associated with increased CRP and D-dimers, the latter possibly reflecting the link between vaccine-induced coagulopathy and En dysfunction. There may also be a positive correlation in kidney microthrombosis between elevated serum creatinine levels and increased D-dimer levels.[218]

Endotheliopathy will probably also play an important role in VITT and thrombosis, while the Sp binds to the same binding domain than AT in the GL.[13,23,168,219,220] AT may also be bound to protein coronas,[108] further inhibiting its functionality as an anticoagulant. Several reports indicated that S1 could directly induce coagulation by competitive binding to soluble and cellular HS.[4] The interaction between Sp and HSPGs on the cell membrane can also disrupt factor H protein, which serves as a negative regulator of the complement alternative pathway, triggering an inflammatory response mediated by the downstream C3-convertase protein.[219] Therefore, the full-length GVG Sp could trigger the complement pathway causing En dysfunction and leading to thrombotic events,[168] while its interaction with multiple membrane components could induce thrombocytopenia. Platelets bound in LNP–protein corona (Figure 3) will also be a factor in the development of thrombocytopenia. In the classic heparin-induced thrombocytopenia (HIT) model, platelet factor 4 (PF4) interacts with EnC HSPGs, thus displacing AT. When PF4 binds to HSPGs, it induces a conformational change in PF4, exposing a new antigen. This, in turn, leads to the generation of IgG auto-Abs against this complex. The complex formed, consisting of heparin (HP), PF4 and auto-Abs, subsequently crosslinks numerous FcγRIIa receptors in platelets and monocytes, triggering intracellular signalling, thromboxane biosynthesis, as well as platelet activation and aggregation.[215] The activated platelets degranulate, releasing more PF4 molecules and procoagulant microparticles in the plasma, leading to increased generation of thrombin. In addition, anti-PF4 Ab activation of monocytes and EnCs leads to accelerated thrombin generation through expression of tissue factor. This can result in hypercoagulation and potentially life-threatening thrombosis.[219,221] Cesari et al. demonstrated

that platelet activation in their VITT patients was not HP dependent. In some patients with VITT, they found antibodies that cross-react with HP, strongly suggesting contraindication to HP treatment.[222] It was also shown that the Sp binds the blood coagulation factor, fibrinogen, while Sp can enhance fibrin-mediated microglia activation and induce fibrinogen-dependent lung pathology in mice.[4] More studies are needed to evaluate the effect of the GVG Sp on coagulation.

Leung et al. demonstrated that VITT was induced by anti-PF4 Abs and mediated by platelet and neutrophil activation and NETosis.[223] NETs are highly prothrombotic in vivo, aggregating with platelets and the activated endothelium to form microthrombi, occluding the vasculature, and further perpetuating inflammation.[175] Therefore, there are important interrelations between platelet aggregation, increased neutrophil recruitment, NET formation and thrombin activation within the microvasculature. While activated neutrophils and platelets have been shown to induce NETosis, NET components can further regulate platelet and neutrophil function. NETosis promotes venous and arterial thrombosis by showing an important procoagulant and prothrombotic activity. They can activate platelets and other immune cells, damage EnCs and activate blood coagulation pathways.[223] NETs further enhance coagulation by providing a scaffold for platelets, red blood cells, von Willebrand factor (vWf) and tissue factor. This creates a positive feedback loop, resulting in a hypercoagulable state and thrombosis.[221] NETosis is therefore present in patients with active VITT, where NET levels positively correlate with the severity of AEs.[13] It has been demonstrated that VITT IgG triggered a significant increase in DNA release to form NETs to control IgG. Confocal microscopy imaging of thrombi from healthy donors' whole blood, following treatment with VITT IgG, confirmed that platelets, fulminant neutrophil activation and extracellular DNA formed the thrombi. Data from this study suggested that VITT IgG is primarily responsible for thrombosis and NETosis in vivo.[223] Platelet–neutrophil interactions have been proposed to be at the centre of the VITT pathology, with platelet–neutrophil aggregation, NETosis, plus platelet EV activation and generation. EV tissue factor and increased D-Dimer levels would correlate with severity, thrombosis and mortality in VITT.[162,223] Thrombosis and thrombocytopenia in VITT are two distinct processes, where VITT Abs induce thrombocytopenia by binding to platelet FcγRIIa.[223] Hetland et al.[13] found a robust negative correlation between the severity of AEs and platelet counts in COVID-19 vaccines. In addition, there are negative correlations between inflammatory marker levels and platelet counts.

Neutrophils and NETs are present in thrombi in various conditions, such as stroke, acute myocardial infarction and deep vein thrombosis. It was speculated that the adenovirus in DNA vaccines and/or the GVG Sp could have triggered pronounced inflammatory processes in VITT patients, including NETosis.[223,224] It was also demonstrated that purified recombinant SARS-CoV-2 Sp S1 subunits can elicit unconventional CD147-dependent platelet activation, increasing the risk of thrombosis in various organs.[157,160,161,224] The GVG Sp can play a role in VITT in the same way,[219] while binding of S1 to platelet ACE2 receptors triggers its aggregation.[4] The GVG Sp has the potential to activate platelets by binding to TLRs, favouring the occurrence of thrombosis-related cardiovascular events. Activated platelets interact with circulating monocytes, stimulating the release of pro-inflammatory cytokines. Li et al.[225] demonstrated that such crosstalk indeed occurred, where they found that the Sp, both soluble or as part of a virus envelope, induced platelet activation through engagement with CD42b, in addition to the other receptors, such as PF4, CD147 and CD26. It was further demonstrated that the Sp competitively antagonizes vWf binding to CD42b and interferes with platelet adherence to vWF. Platelets actively participate in both haemostasis and immune regulation. Sp-activated platelets induced monocyte differentiation to a pro-inflammatory phenotype.[195]

Activated platelets degranulate and express various membrane receptors, enabling them to bind through P selectin to circulating leukocytes. This induces monocyte differentiation towards a pro-inflammatory phenotype, featuring a higher expression of CD86, HLA-DR and IL-1β.[195] VITT and acute deep vein thrombosis, resulting from inflammatory responses, platelet activation and aggregation, was reported after injections of both DNA and mRNA COVID-19 vaccines.[162,168,211,221,226] It was found that both the AstraZeneca and Pfizer vaccines could elicit anti-PF4 Ab production, even in recipients without clinical manifestation of thrombosis.[4] To abolish VITT IgG-induced thrombosis, one would need to inhibit platelet and neutrophil activation by blocking FcγRIIa, or NETosis and aggregates. It seems probable that an undersulfated GL in VITT will impede in vivo modulation of these pathological events.[23] Moreover, extracellular RNA has been established to promote the activation of coagulation proteases. At the same time, different forms of eukaryotic and prokaryotic RNA serve as promoters of pathological blood coagulation and thrombus formation.[227] More research is needed to establish the possible role of injected mRNA in promoting thrombosis.

## 3.4 | Myocarditis

Myocarditis in adolescents[8,146,150,228,229] and elderly,[230] as well as pericarditis or myopericarditis,[8,89,231,232] has

been clinically diagnosed in many recipients of the various COVID-19 vaccines. Yu et al.[233] observed impaired LV and RV myocardial deformation and persistent late gadolinium enhancement (LGE) in a subset of patients vaccinated against COVID-19 up to 1-year follow-up. The growing evidence indicates a worse prognosis with altered myocardial deformation and LGE in patients with myocarditis. Mörz and Barmada et al.[35,89] demonstrated an up-regulation in inflammatory cytokines and the corresponding lymphocytes with tissue damage capacity after COVID-19 mRNA vaccination. This suggests a cytokine-dependent pathology that may also be accompanied by myeloid cell–associated cardiac fibrosis. They noted that these findings were different from previously reported forms of vaccine-associated myocarditis, where the pathologies were largely eosinophilic.[89]

CD147 has been shown to be involved in the development and progression of various cardiovascular diseases, such as atherosclerosis, ischemic cardiomyopathy and heart failure.[155,234] Since CD147 is expressed in both cardiomyocytes and EnCs, the binding of GVG Sp to these receptors[160,225] may correlate with haemodynamic instability and cardiovascular abnormalities observed in COVID-19 vaccinees. Zhong et al.[155] demonstrated that in response to pressure overload, overexpression of cardiac CD147 promoted cardiac maladaptive hypertrophy and remodelling, along with increased oxidative stress and ferroptosis. Apart from overexpressed CD147 resulting in oxidative stress, it is known that LNPs can also generate excessive ROS.[61] It has been well established that ROS overproduction, resulting in oxidative stress, is a crucial trigger during the pathogenesis of cardiac hypertrophy and the transition to heart failure. In cardiovascular disease, excessive ROS has been shown to cause protein denaturation, lipid peroxidation, DNA damage and eventual cell death.[235] ROS can directly impair the heart's contractile function by oxidizing proteins central to excitation-contraction coupling. Lipid peroxidation will result in membrane destabilization and ferroptotic cell death, a pathological process associated with ROS-induced heart tissue injury.[155,236]

Sp involvement is irrefutable, because acute pericarditis and myocarditis, or myopericarditis, were also observed in predominantly men and young people with COVID-19.[89,236] In fact, adolescents who developed myocarditis after mRNA vaccination had markedly higher levels of full-length free Sp in their plasma, compared to asymptomatic vaccinated control subjects without detectable free Sp.[12] In a postmortem study, Mörz observed Sp-induced En damage in the heart and brain, with adhering plasma coagulates/fibrin clots present on the En surface. The patient received one Astra-Zeneca and two Pfizer mRNA vaccinations, with no evidence of previous SARS-CoV-2 infection.[35] Avolio et al.[160] found that the shed Sp was more abundant than the whole SARS-CoV-2 particles in COVID-19 patients' serum. It was observed that shed Sp disrupted human cardiac pericyte function and triggered increased production of pro-apoptotic factors in pericytes, resulting in the death of EnCs. The GVG Sp can, therefore, act as a ligand to induce noninfective cellular stress. In support of this, administration of the Sp promoted dysfunction of human EnCs through increased expression of vWf and CD147.[4,160] Expressed CD147 promotes MMP activation and will consequently result in considerable EnGL degradation, further exacerbating inflammatory responses, ROS generation and vascular NO abnormalities, resulting in a procoagulant and pro-inflammatory phenotype of the endothelium.[12,23,237] Dursun et al. reported that COVID-19 vaccine-induced acute pericarditis exhibited pleuritic chest pain, pericardial effusion, increased white blood cell count and increased CRP levels, while serum troponin levels were around normal.[236] However, unlike classical stages of acute pericarditis ECG, vaccinated patients showed no ECG changes. Elevated D-dimer and serum troponin levels were also observed in vaccine-induced acute myocarditis and myopericarditis. Their ECG findings were correlated with those who have stage 1 acute pericarditis.[236] These conditions were typically observed within 3 days after mainly the second dose of an mRNA vaccine, and mainly in younger and male adults. The prevalence of young adults has been defined in various studies.[236,238–240] Oxidative stress was the underlying cause of these conditions, with low NO levels indicating the inflammatory and procoagulant state in mRNA vaccine-induced heart inflammation.[236]

It is well established that the pathogenesis of viral myocarditis is caused by direct virus-mediated injury and/or a toxin, and indirect damage through secondary immune and autoimmune responses, as well as influenced by the oxidative state of the host.[241,242] It is very plausible that the GVG Sp and LNPs would stimulate the release of pro-inflammatory cytokines, but that an undersulfated and degraded GL will not be able to modulate the inflammatory response,[160] which, together with oxidative stress, will contribute to the pathogenesis of cardiomyopathy.[243] Salah and Mehta speculated that high expression of ACE2 in the heart will facilitate the interaction between HS and the Sp, resulting in HS consumption that will reduce AT activation and anti-inflammatory activity. This may result in endothelialitis, subsequent En injury and intracardiac thrombus formation.[220,244] However, the fact that the GVG Sp would likely bind to the same binding domain than AT will probably have a greater effect on inflammation and coagulation, in addition to the role of the other Sp-binding receptors, and the increase in oxidative stress. Avolio et al.[160] demonstrated that the CD147 receptor, not ACE2,

directs Sp signalling in cardiac pericytes. The expression of CD147 was shown to be increased in a variety of cardiovascular diseases, which could serve as compensation for any age or disease-related reductions in ACE2 during viral infections.[234]

Nonetheless, Lehmann et al.[130] pointed out that binding of the GVG Sp to ACE2 receptors can induce RAAS activation, particularly an increase in angiotensin-2, resulting in angiotensin-2/NA-dependent acute vasoconstriction, and the progression of inflammatory, fibrotic and thrombotic processes. A histopathological examination of two teenage boys who died suddenly after their second Pfizer vaccination revealed stress cardiomyopathy caused by catecholamine-induced myocardial injury, which differed from typical findings of myocarditis.[130,150] It is plausible that the catecholamines were not deactivated by sulfation.

The coxsackievirus-adenovirus receptor, an adhesion molecule located predominantly in the heart, is required for viral entry into different cell types. The young adult heart has relatively high levels of coxsackievirus-adenovirus receptors, which may partially explain the increased susceptibility of young adults to myocarditis.[241] However, seeing that COVID-19 vaccine-induced myocarditis predominantly affects young males,[8,12,241,245] other factors would probably play a role. Animal studies indicated that sex differences in TLR signalling play an essential role in differential susceptibility to viral-induced myocarditis.[241] Sex differences in innate immune responses have been observed, where an M1 activating immune response was favoured in male mice, compared to an M2 resolving response in female mice.[194]

Various studies found an increase in oxidative stress in patients with dilated cardiomyopathic heart failure, with a decrease in circulating GSH concentrations.[103] The fact that myocarditis accounts for 5%–12% of sudden deaths of young athletes[194] indicates that oxidative stress is probably the main etiological factor underlying myocarditis (see Section 3.5). Research has revealed that males are more susceptible to oxidative stress and possess a lower antioxidant capacity compared to females. Some researchers attribute these sex differences to oestrogen, which is known to facilitate the activation of antioxidant systems and regulate the expression and activity of various antioxidant enzymes.[103] However, it is important to note that high testosterone levels, alcohol, smoking, recreational drugs and certain medications will deplete GSH, altering the redox status.[23,246] During puberty, there is an approximate 20-fold increase in endogenous testosterone in males, while there is only a modest increase in females. Testosterone has been shown to decrease cystathione β-synthase (CBS) activity (Figure 1), decrease GSH concentrations and increase susceptibility to oxidative stress.[247] In steroidogenic cells, one can expect ROS production to be particularly high, since steroid hydroxylations by cytochrome P450 enzymes produce ROS,[248] in addition to the mitochondrial electron transport chain.

Furthermore, after biosynthesis, hydrophobic steroids undergo sulfation to facilitate their circulatory transit. Mueller et al. gave a good account of steroid sulfation and desulfation.[248] Most dihydroepiandrosterone (DHEA) is stored as DHEA sulfate, the inactive form, but with decreased levels of inorganic sulfate, DHEA is preferentially converted to testosterone instead of DHEA sulfate.[247] Moreover, higher testosterone levels will not only deplete GSH. It may also reduce available inorganic sulfate levels, exacerbating inorganic sulfate deficiency and altering EnGL sulfation (Figure 1) and steroid hormone sulfation. Therefore, high testosterone levels can stimulate pro-inflammatory cytokines in the vasculature and generate ROS in vascular smooth muscle cells, consequently decreasing NO bioavailability, resulting in increased blood pressure and renal dysfunction, activation of vasoconstrictor signalling pathways and increased vasoconstriction.[246]

Men have been shown to have lower plasma levels of reduced GSH than women, making them more susceptible to oxidative stress and inflammation.[249] Both epidemiological and immunological evidence indicate that steroids can influence the pathogenesis of various chronic inflammatory diseases. However, research on the cardiovascular actions of testosterone is still controversial, showing its effects as protective to deleterious. Although numerous studies have shown an increased cardiovascular risk and mortality with testosterone deficiency, testosterone therapy has been verified to attenuate cardiovascular risk factors and cardiovascular outcomes.[246] Moreover, high oxidative stress has been associated with adverse testosterone effects, while low oxidative stress is associated with cardioprotective testosterone effects.[103]

It seems that testosterone's positive or negative effects on the heart may depend on the testosterone levels and whether or not testosterone is acting through a nuclear receptor. However, the role of testosterone in the regulation of oxidative stress in cardiomyocytes is far from clear, where testosterone can act as an antioxidant or pro-oxidant.[103] Indeed, a positive correlation has been observed between GSH and testosterone levels. Depletion of the intracellular GSH pool, both in young and old cells, has been found to significantly decrease testosterone production.[250] It is also important to consider the regulation of sulfatases (SULFs) by inflammatory mediators, since sex steroids play a role in immune function and inflammatory processes, where SULF activity is often dysregulated and associated with inflammation. For example, in the vascular smooth muscle cells from patients with atherosclerosis, SULF expression was found to be higher in females with mild atherosclerotic changes, compared to

severe disease and in male aortas. However, the counterpart of SULF, oestrogen sulfotransferase (SULT-1E1), was lower in women with severe disease, indicating the importance of the SULT/SULF ratio in the local regulation of steroid formation in states of inflammatory diseases.[248]

Rienks et al. revealed that the extracellular matrix (ECM) protein, SPARC, regulates inflammation, vascular permeability and consequently mortality in a murine coxsackievirus B3-induced myocarditis model by maintaining the EnGL's integrity (Figure 1).[243] They found that a lack of SPARC resulted in a loss of GL integrity and consequent barrier function. These alterations in GL integrity resulted in increased cardiac inflammation and mortality during viral myocarditis. They also noted a relationship between SPARC and HS, seeing that both could restore EnGL integrity.[243] SPARC probably serves as a Cys donor for oxidation to both GSH and inorganic sulfate (Figure 1). The degree of GAG sulfation in the GL regulates inflammation, vascular permeability, coagulation and mechanotransduction. Depleted levels of GSH, through increased inflammation and oxidative stress, would also mean impaired availability of inorganic sulfate, resulting in an undersulfated and degraded GL. In animal studies, taurine deficiency, with Cys as a precursor to taurine (Figure 1), was also shown to result in cardiomyopathy.[251]

GVG Sp and LNPs can activate platelets and generate ROS, in addition to the pro-inflammatory response, resulting in degradation of the EnGL and coronary artery lesions.[235] In this model, the overexpression of pro-inflammatory cytokines, such as TNFα and IFNγ, may induce cellular hypertrophy and myocardial damage and possibly causes ventricular remodelling. Since TNFα is a potent inducer of NO and ROS, this inflammatory process can promote cardiac injury, myocardial fibrosis and electrical remodelling, through a hyperoxidative state.[252,253] As the EnGL degrades, the protective barrier of the EnCs is compromised, leading to increased interstitial oedema, capillary leakage and a higher risk of multiple organ failure. Furthermore, loss of ability to sense shear stress in the endothelium results in NO release, leading to systemic vasodilation.[244]

In addition to the function of MMPs in ECM remodelling, a critical process involved in the progression of myocarditis to dilated cardiomyopathy, they are also important modulators of the antiviral immune response. A novel role for MMP-12 in innate immunity is mediating the secretion of IFNα, by transcriptional regulation of NF-κB inhibition.[241] Pro-inflammatory cytokines can also lead to aberrant mitochondrial metabolism of cardiomyocytes, further causing heart dysfunction.[201] EnGL degradation and prolonged and increased activity of MMPs and HPSE would be predominant in systemic vascular leakage.[254] Circulating HS can act as DAMP ligands, binding to TLR4 and increasing the release of pro-inflammatory cytokines. Furthermore, serum HS fragments can induce mitochondrial dysfunction in cardiomyocytes in a TLR4-dependent manner.[255] Degradation of the GL will also result in shedding of endogenous protective enzymes, such as extracellular SOD, which will increase oxidative stress in the endothelium.[256,257]

It is also probable that overexpression of IFN in the heart can lead to autoimmune cardiomyopathy. TREX1-deficient mice were shown to develop lethal lymphocytic inflammatory myocarditis, with progressive dilated cardiomyopathy and circulatory failure, in addition to pathological changes in lymphoid organs, consistent with autoimmune cardiomyopathy.[258] Fung et al.[241] gave a good overview of the immunopathogenesis of viral myocarditis. A persistent and excessive immune response after vaccination can have harmful consequences, contributing to the progression of myocarditis and dilated cardiomyopathy.[241] Yonker et al.[12] observed significantly elevated levels of IL-8, IL-6, TNFα, IL-10, IFNγ and IL-1, with lower IL-4 levels, in adolescents and young adults presenting with myocarditis after COVID-19 mRNA vaccination, compared to healthy vaccinated control subjects. The same Th1-polarized immune response was observed with DNA vaccine (AstraZeneca) immune assays,[128] and in a Pfizer COVID-19 vaccination trial.[92,259] This cytokine and chemokine environment represents a pro-inflammatory M1 macrophage phenotype, with Th1 IFNγ potentiating the macrophage microbicidal activity, thus promoting antigen presentation. When the levels of Th2-associated cytokines IL-4 are low, the anti-inflammatory macrophage phenotype M2 is not activated to stimulate myocardial healing, or to blunt the inflammatory response.[194] IFNγ–producing CD4+ T cells producing IFN were shown to predominate in autoimmune myocarditis in animal and human subjects, where IFNγ–overexpressing mice developed spontaneous cardiac inflammation.[260] Barmada et al. observed an elevation in sCD163, which is indicative of cardiac macrophage activation after mRNA vaccination. Released damage-associated signals and elevated pro-inflammatory cytokine IL-1ß, as seen in their patient cohort, can induce recruitment of monocytes and macrophages. This may exacerbate inflammation in myocarditis and/or result in cardiac tissue fibrosis, as was observed in patients with prominent T cell and macrophage infiltration of cardiac tissue.[89] It is known that IL-1 may enhance atherogenesis and exacerbate left ventricular dysfunction by contributing to En damage.[261]

Furthermore, Yonker et al. discovered that total leukocytes, especially neutrophils, were significantly elevated in the postvaccine myocarditis cohort. On the contrary, platelet counts were found to decrease in vaccinated control subjects.[12] These profiles probably suggest innate

inflammatory activation in individuals who developed postvaccine myocarditis. At the same time, adaptive immunity and T-cell responses in the postvaccine myocarditis cohort were found to be indistinguishable from those of asymptomatic age-matched vaccinated control subjects. However, the circulating free GVG Sp antigen evaded Ab recognition in postvaccine myocarditis.[12]

In addition, dysregulation of specific miRNAs has been demonstrated to contribute to developing viral myocarditis, without altering viral replication dynamics.[241] It seems probable that mRNA vaccines can up-regulate miRNAs, which can induce myocarditis.[194] Furthermore, increasing evidence shows that aberrant accumulation of protein aggregates is important in the development of human heart diseases.[241] LNPs are known to aggregate to form protein coronas,[262] cross the BBB and potentially induce strokes and/or cerebral haemorrhages and damage the endothelium of the heart muscle.[263] However, Nahab et al. found that the AstraZeneca DNA vaccine was associated with a higher risk of early postvaccination ischemic stroke, compared to the Pfizer mRNA vaccine.[264] It is clear that other factors are also at play, apart from the presence of LNPs.

Vaccine-induced cardiac injury can, therefore, be related to direct cardiomyocyte damage, coronary plaque destabilization, cytokine inflammatory responses, oxidative stress, EnGL dysfunction and degradation, and intracoronary microthrombi formation.[35,89,220] In fact, in addition to inflammation, increased ROS production has been directly associated with various critical characteristics of the pathophysiology of cardiovascular disease. These include vascular remodelling, En dysfunction, altered vasoconstrictor responses, increased inflammation and modifications of the ECM. All these factors play a significant role in the development and progression of cardiovascular diseases.[246] It is important to note that heart failure is the final mutual outcome of various primary cardiovascular diseases, regardless of the underlying nature of cardiomyopathy. The long-term prognosis of myocarditis in vaccinated individuals remains unclear, and it seems probable that accidental IV administration of the COVID-19 mRNA vaccines would increase the risk of myopericarditis.[225] Longitudinal studies of COVID-19-vaccinated patients with myocarditis and myopericarditis will be necessary to better evaluate long-term risks. Given the low turnover rate of cardiomyocytes (only 1% annually, even at the age of 25) and the fact that fewer than 50% of cardiomyocytes are exchanged during a normal human lifespan, it becomes evident that loss of cardiomyocytes cannot be adequately replenished in a timely manner. As a result, such loss would be detrimental to maintaining cardiac function. The limited regenerative capacity of cardiomyocytes poses challenges for the heart's ability to recover from significant damage or injury.[265] More research is needed to fully understand the complex interplay between oxidative stress, cytokine-driven immune responses and vaccine-induced myocarditis.

## 3.5 | Adverse events in athletes

Myocarditis is ranked as the third leading cause of sudden cardiac death in competitive athletes by the American College of Cardiology.[241,266] More research is required on the reported increased incidence of sudden death among athletes after COVID-19 vaccination.[20,229,267]

Because athletes often use testosterone and other anabolic steroids to improve performance, lower levels of GSH and reduced redox status could be expected. Nonmedical testosterone has been shown to increase arterial blood pressure and induce left ventricular hypertrophy and myocardial infarction, due to coronary vasospasm or thrombosis.[246] Low levels of GSH have also been established to cause increased inflammation, microglial activation, neuroinflammation and expression of NO,[247] indicating an increase in ROS. Interestingly, steroid SULFs are regulated by hypoxic conditions and inflammatory mediators, such as TNFα. Once intracellular, steroid conjugates can be desulfated during intense exercise,[23] increasing levels of active steroid metabolites.[248]

Tostes et al. reported that testosterone also induces mitochondria-associated ROS generation and apoptosis in vascular smooth muscle cells, by activating androgen receptors.[246] Bille et al.[266] indicated a female/male ratio of 1/9 for sudden cardiac death among athletes. Sen et al.[268] studied the association between exercise intensity and related oxidative stress in healthy young men. A 50% decrease in blood GSH levels was observed in moderately trained men during the first 15 min of exercise.[268]

Notably, Cys will favour GSH synthesis during exercise-induced oxidative stress. A higher demand for GSH will have an inhibitory effect on inorganic sulfate synthesis. Besides, stress and hypoxic states favour $H_2S$ as an 'emergency' substrate for ATP production, while Cys also serves as a precursor for mitochondrial production of $H_2S$.[104] Under hypoxic conditions, it can be assumed that there will be an increased demand for $H_2S$ as an electron donor. This increased demand may result in the inhibition of inorganic sulfate and GSH synthesis, with Cys acting as the rate-limiting factor (Figure 1). This will affect the degree of HS sulfation and negatively impact GSH levels. Moreover, mediated by hypoxic conditions, increases in intracellular $H_2S$ and ROS levels can synergistically induce membrane depolarization. This may result in increased levels of cytosolic $Ca^{2+}$ ions, leading to activation of the endoplasmic reticulum stress response involved in initiating apoptosis[104] (Figure 2). Furthermore, prolonged hypoxia states

increase the expression of tissue factors in monocytes and macrophages, as well as pulmonary vascular EnCs, which can lead to increased fibrin accumulation and consequent pulmonary thrombosis.[23]

As discussed in this review, various factors will lead to a lower redox status in recipients of DNA and mRNA vaccines. Coupled with the increased oxidative stress generated during exercise, it is very likely that these events are the perfect storm to result in a heart attack. Increased inflammation and oxidative stress would increase blood viscosity and decrease myocardial perfusion and supply. This would be further exacerbated through dehydration during exercise. In addition, electrolyte imbalances would also affect the renin–angiotensin–aldosterone system, possibly contributing to congestion and worsening of heart failure.[130]

### 3.6 | Immunocompromised patients and autoimmune diseases

Many researchers have looked at the possible link between SARS-CoV-2 and autoimmunity, such as vasculitis. Various autoimmune diseases have been reported after COVID-19 vaccination, such as alopecia areata, Guillain–Barre syndrome (GBS), autoimmune-induced hepatitis, acute autoimmune transverse myelitis, idiopathic thrombocytopenic purpura, arthritis and Ab-associated antineutrophil cytoplasmic vasculitis.[14,269,270] This raises the question of whether COVID-19 vaccines might affect the immune system in the same way as SARS-CoV-2 infection.

Serum from severely ill patients with COVID-19 revealed high autoAb titres, such as antinuclear antibody (ANA), lupus anticoagulant and antineutrophil cytoplasm antibodies (ANCA).[271,272] In plasma isolated from patients with autoimmune conditions, myeloperoxidase (MPO) and NOX2 were associated with the formation of NET. Enhanced NETosis was associated with the onset of acute and chronic inflammation and autoimmune disorders. At the same time, the presence of auto-Abs inhibited NET degradation, increasing the risk of immune-mediated thrombosis.[177] Although patients affected by immune suppression and immune-mediated inflammatory disorders are encouraged to be vaccinated, there exists a paradox, since it is known that COVID-19 vaccines can trigger disease relapse in patients with an established immune-mediated inflammatory disorder. In patients predisposed to develop an autoimmune disease, vaccination can potentially shift the balance towards self-reaction, leading to the initiation or exacerbation of autoimmune responses.[273,274] Ironically, the initial Pfizer and Moderna vaccine trials excluded immunocompromised patients, including those on immunosuppressive medications, and patients with autoimmune conditions. It has been established that autoimmune reactivity in response to viral antigens, after infection or vaccination, can be easily derived in various tissues from cross-reaction with human tissue antigens that share sequence homology with the virus. Therefore, it seems probable that the GVG Sp is a potential epitopic target for biomimicry-induced autoimmunological processes.[275] Therefore, chronic autoimmune disease can result from excessive Ab production in response to the vaccine. Vaccines have been associated with chronic immune-mediated disorders that can develop years after vaccination.[232,276] Moreover, IL-17 cytokines, induced by cationic LNPs, will further exacerbate the autoimmune milieu.[61]

It is also important to note that the CDO-catalysed step, which is responsible for producing the majority of inorganic sulfate in vivo from Cys, is rate-limiting (Figure 1). Pro-inflammatory cytokines, such as IL-1β, TNFα and TGFβ, downregulate CDO at the mRNA level. Therefore, higher plasma levels of Cys could be expected, compared to inorganic sulfate concentrations, after vaccination. A lower degree of HS sulfation is evident in various autoimmune and inflammatory health conditions.[104]

### 3.7 | Immune-mediated hepatitis

Chow et al.[277] documented case reports of individuals who developed autoimmune hepatitis after COVID-19 vaccination. The latency time to onset of the first symptoms ranged from as little as 2 days to 2 months after receiving the first dose of the COVID-19 vaccine. After vaccination, various factors and pathways would cause immune-mediated hepatitis, fibrosis and liver necrosis, such as increased pro-inflammatory cytokines, oxidative stress,[278] circulating LNPs and soluble GAGs in circulation due to EnGL damage. Soluble plasma GAGs are cleared from circulation through clearance receptors present in sinusoidal liver EnCs, including stabilin-2. However, in instances of liver cell injury or infection, such as in the case of hepatitis, there may be an associated increase in circulating GAGs due to impaired clearance mechanisms.[32] Decreased sulfation and degradation of the EnGL would be a substantial contributing factor in mediating hepatitis. SAAs play a crucial role in liver health, where increased ROS or lower levels of GSH, and the other SAAs, will also contribute to hepatitis. The loss of the BPNT1 gene in mice was shown to lead to impaired protein synthesis, which subsequently resulted in impaired liver function and reduced levels of HSA.[248]

LNPs can be cleared in a healthy liver through efficient immune responses, for example, by the convectional APCs in the liver, such as dendritic cells, B lymphocytes

and Kupfer cells. Additionally, sinusoidal EnCs from the liver, liver stellate cells and hepatocytes can act as APCs. However, sustained expression of GVG Sp-related antigens after vaccination, as well as increased levels of GAG fragments and LNPs, can skew the immune response to autoimmune tissue damage, such as through IL-17,[61] as observed in cases of autoimmune hepatitis after COVID-19 vaccination.[4,277,279] It has been shown that PEG-lipids with short lipid tails can desorb from LNPs, allowing the NPs to adsorb apolipoprotein E, which will cause LNPs to endogenously target liver hepatocytes.[68] nAb responses elicited after SARS-CoV vaccination have been shown to induce harmful immune responses that result in liver damage.[280] Fernandes et al.[182] injected SARS-CoV-2 rSp into zebrafish and observed mild lobular infiltration of lymphocytes in the liver, centrilobular sinusoidal dilation, patchy necrosis, moderate microvesicular steatosis and mild inflammatory infiltrates in the hepatic lobule and the portal tract.[182]

## 3.8 | Neurological symptoms

Various factors, such as genetics, oxidative stress, neuroinflammation, mitochondrial damage and abnormal protein folding, underlie neurodegenerative disease. Multiple researchers have linked vaccine adjuvants, such as aluminium salts, to neurological symptoms.[116] Even though neurotoxicity due to LNPs crossing the BBB is possible,[76] one could more likely expect decreased levels of inorganic sulfate as an underlying etiological factor. Sulfate plays an important role in the brain as a vital component of the GL and ECM,[54] and as a deactivator of neurotransmitters, such as serotonin, dopamine, DHEA and pregnenolone. HSPGs play a crucial role in neurite outgrowth and connectivity between neurons and their target cells. They have been shown to be vital in processes such as neurogenesis, axonal guidance and synapse development.[247] The depletion of available inorganic sulfate due to vaccine-induced systemic inflammation and oxidative stress will lead to many neurological conditions. Reduced serotonin sulfation would impair its inactivation, resulting in increased serotonin levels or serotonin syndrome. Increased serotonin levels would not only affect daily brain functioning, it can also cause focal seizures and motor changes, and a loss of oxytocin-containing cells in the hypothalamus, leading to deficits in oxytocin processing with behavioural consequences.

In addition, sulfated steroids, such as DHEA sulfate, have been shown to inhibit GABAa receptors in the nervous system. Decreased sulfate levels might result in anxiety, depression, apathy, insomnia and mood disorders. Impaired N-methyl-D-aspartate (NMDA)-dependent long-term potentiation (LTP) was associated with sodium and potassium current perturbations. Sulfate deficiency might alter memory, since the glycoprotein, N-sdc, enables LTP by transmitting signals from ligands binding to attached HSPGs.[247] Sulfated galactocerebroside, known as sulfatide, is a critical myelin lipid component[54] and is involved in various biological processes, such as cell growth, inflammation and immune modulation, protein trafficking, signal transduction, cell–cell recognition, neuronal plasticity and cell morphogenesis.[281] Mice lacking sulfatides display disorganized paranodes and a deficiency in septate-like junctions, which results in a notable decrease in nerve conduction velocity as a functional outcome. The absence of galactolipid sulfation in the Golgi apparatus appears to disrupt the selective association of NDK/Nm23 proteins with myelin-destined vesicles, leading to random and unregulated associations of NDK/Nm23 with any myelin-destined vesicle. This dysregulation of protein association may contribute to the observed impairment in nerve conduction.[282] Substantial depletion (92 mol%) of sulfatide mass was found in the temporal grey matter of subjects with a clinical dementia rating of 0.5, relative to controls.[281]

Although excessive production and release of pro-inflammatory chemokines and cytokines after vaccination, particularly TNF-α, IL-1 and IL-6, can trigger neuropsychiatric symptoms in itself, they also negatively affect thiamine metabolism.[283] A deficiency in sulfur-containing thiamine has been linked to many neuropsychiatric adverse effects.

As in long-COVID, activated immune-inflammatory and oxidative and nitrosative stress pathways (IO&NS) can underlie the physiological symptoms of COVID-19 vaccine-induced chronic fatigue syndrome, major depression and generalized anxiety disorder,[169,284,285] which are characterized by activated IO&NS pathways. Therefore, the combination of toxicity from neuro-oxidative stress and diminished antioxidant defences can result in increased neurotoxicity, ultimately leading to physiological symptoms.[212] Although Selvakumar et al. did not find a correlation of long-COVID conditions between infected and noninfected individuals, their vaccination status was not taken into account as a possible contributing factor to symptoms.[286] Jangnin et al.[287] observed a high incidence of long-COVID after extensive distribution of vaccines and antiviral therapies.

An overactive immune system is known to contribute to the emergence or aggravation of neurodegenerative diseases, such as Alzheimer's disease, haemorrhagic and ischemic strokes, multiple sclerosis (MS) and GBS, as well as neuropsychiatric symptoms, as cytokines can pass through the BBB and cause acute necrotizing encephalopathy.[4,124,237]

In research studies, S1 has been shown to act as a pathogen-associated molecular pattern that induces

viral infection-independent neuroinflammation through the participation of pattern recognition receptor engagement.[4,166,169] Furthermore, Sp, S1 of SARS-CoV-2 and mRNA-LNPs can cross the BBB.[4,26,95,182] Fernandes et al.[182] showed that rSp generated an inflammatory process in the brain, with a severe influx of mononuclear cells. This profile was correlated with acute necrotizing and transverse myelitis related to SARS-CoV-2 infection, confirming the neurotropism of the Sp.[182]

Furthermore, circulating shed HS fragments can bind to Aβ fibrils and α-Synuclein, thereby competitively inhibiting FGF2-mediated neuroprotection and promote protein aggregation.[288] Moreover, binding of the GVG Sp and LNPs to HSA can affect HSA transport across the BBB, resulting in increased plaque formation and impaired amyloid processing, thus affecting cognition.[148,288] Low HSA, LNPs and increased expression of MPO could also exacerbate capillary leak in the brain,[289] which can lead to thrombosis, severe encephalitis, toxic encephalopathy and rupture.[35] The brain contains the largest number of plasmalogens,[281] which are susceptible to MPO-induced oxidative stress.[290] Plasmalogen deficiency in cerebral grey matter can be directly related to neurodegeneration and loss of synapses, as a decrease in plasmalogen levels can induce membrane instability.[281] Therefore, by enriching neuronal cell membranes with plasmalogens, neuronal function can be improved through modulation of nonlamellar membrane transformations and synaptic plasticity.[291] A weak BBB, through HSA depletion and MPO-induced oxidative stress, may promote secondary infection, leading to meningitis.[95,148,182] Xu et al.[36] illustrated that loss of brain EnC integrity and decreased host defence mediated by NETs would synergistically render HS2ST-deficient mice susceptible to infection. Furthermore, inactivation of HS2ST in neutrophils affects the binding capacity of histones, which are vital antimicrobial molecules and structural components of NETs, revealing a novel function of HS in neutrophil NET biology.[36] There exists a direct relationship between HSA deficiency and decreased sulfation.[104]

Neuronal cells are highly susceptible to exposure to hypochlorous acid (HOCl) induced by MPO, which can release pro-inflammatory cytokines, exacerbating neuroinflammation. HOCl also chlorinates the amine and catechol groups of dopamine, which may selectively kill dopaminergic neurons by inhibiting mitochondrial respiration.[289] It is important to note that endosomes provide a specialized NGF/TrkA platform for sustained signalling, which is required for neuronal survival.[77] The influence of ionized LNPs on these signalling pathways through membrane and endosomal destabilization needs further investigation.

Additionally, various Sp-binding receptors are expressed in the brain, such as ACE2, ephrin (Eph) receptors and ligands, NRP-1, TMPRSS2 and CD147. The GVG Sp, LNPs and activated IO&NS pathways can result in neuroinflammatory processes characterized by neuron demyelination, hyperactivation of microglia and stimulation of astrocytes. Specific pathological changes and symptoms vary depending on the type of cell and the brain region affected. Symptoms related to pituitary gland involvement may include headaches and vision changes, while damage to the cerebellum and cortex can cause neurological symptoms such as ataxia, dizziness and impaired consciousness.[237,292] Numerous cases of acute, temporary, unilateral peripheral facial paralysis or Bell's palsy have been reported as AE.[51,285,293,294]

Various reports of Creutzfeldt–Jakob disease have been reported after COVID-19 vaccination,[295] with symptoms of rapidly progressive dementia, ataxia, pyramidal symptoms and akinetic mutism. It has been hypothesized that neuroinflammatory transcriptional signatures and loss of homeostatic identities in astrocytes could be triggered by systemic inflammatory mediators, which can contribute to neurodegeneration and prion disease pathogenesis.[237]

Abramczyk et al.[124] observed alterations in the reduction–oxidation pathways associated with Cytochrome c in glial cells of astrocytes, astrocytoma and glioblastoma, incubated with the Pfizer mRNA vaccine, similar to that of brain cancer. Glioblastoma multiforme is a common primary human brain cancer characterized by resistance to apoptosis by chemotherapeutic treatment and radiation. The pathogenesis of glioblastoma multiforme is associated with pro-inflammatory cytokines, chemokines and Eph receptors. Elevated levels of pro-inflammatory cytokines, such as IL-1β and TNFα, were found in patients with glioblastoma multiforme.[238] It would be important not to misdiagnose glioblastoma multiforme for acute disseminated encephalomyelitis, which could also be associated with COVID-19 vaccination.[296]

Reports of acute ischemic stroke cases have emerged after COVID-19 vaccination.[14,232,264,297] There is a long-standing association between systemic infections, inflammation and acute ischemic stroke. TLRs can also indirectly damage neurons, resulting in ischemic stroke. EphA2 receptors have also been shown to play an important role in ischemic stroke pathology.[237]

GBS is a commonly reported vaccine-induced AE.[14,285,298–300] GBS is linked to the release of cytokines and chemokines induced by vaccination, resulting in damage to the central and peripheral nervous system.[237] It is the most common acquired inflammatory neuropathy, characterized by demyelination or damage to the myelin sheath and/or axonopathy. It has been speculated that the

GVG Sp can lead to an autoimmune response due to its structural similarities with the ganglioside components of peripheral nerves, thereby damaging them. This hypothesis was confirmed by detecting auto-Abs against gangliosides in the case of tetraparesis.[130]

The fact that these neurodegenerative diseases were also observed in COVID-19 patients[237,269,292] indicates the involvement of the Sp with various receptors, with consequent neuroinflammation. Postvaccination, the integrity of the BBB is probably weakened by LNPs, thereby exacerbating neuroinflammation. Increased immunogenicity can also be expected due to mistranslation of mRNA and protein misfolding, which has been linked to neurodegeneration[301] and heart disease.[302]

The acute neurological presentation of Churg–Strauss syndrome following COVID-19 vaccination has been reported.[269,273,303] Although the cause-and-effect association with COVID-19 vaccines still needs to be proven with certainty, clinicians should suspect autoimmune-related polyneuritis in patients with asthma and rhinosinusitis, who present with sensorimotor symptoms and poor balance after COVID-19 vaccination.[269,303]

There exists an important relationship between HS and Churg–Strauss syndrome. It is known that the EnGL is damaged during inflammatory conditions, with very high levels of extracellular histones and pro-inflammatory cytokines, resulting in the degradation and shedding of HS and other GL fragments. However, En-expressed HS plays a role in allergic airway inflammation, such as asthma and sinusitis, by mediating the interaction of leukocytes with the vascular endothelium, thus regulating the recruitment of inflammatory cells to the airways, as well as sequestering and modulating the activity of pro-inflammatory cytokines.[304] In Churg–Strauss syndrome, the membrane basic protein (MBP) and eosinophil cationic protein (ECP) block HS, thereby inhibiting HS binding to AT, and consequently facilitate coagulation through unhindered factor X activation and thrombin generation. Furthermore, MBP/ECP stimulates platelets to release PF4, which also blocks HS with subsequent coagulation,[305] and an attenuated ability to regulate inflammation. It is clear that after COVID-19 vaccination, these overstimulated immune-mediated pathways would intensify, with less available HS to modulate the response.

## 3.9 | Endocrine disorders, reproductive health and pregnancy

During pregnancy, the blood-placental barrier mediates the exchange of nutrients and metabolic waste products, exerts vital metabolic functions, and secretes hormones and is therefore crucial in maintaining pregnancy. There is a relationship between SAAs, where an HSA deficiency will affect Cys and taurine levels (Figure 1).[104] Interestingly, lower birth weight and length of infants have been linked to maternal taurine deficiency. Since it has been found that increased size of newborns at birth may be protective against the development of coronary heart disease, taurine or HSA deficiency in utero, and in infants, can lead to the progression of coronary heart disease in adulthood. This link between taurine deficiency and cardiac pathologies was demonstrated in taurine transporter (TauT) gene knockout mice.[251] However, a taurine deficiency would also mean lower levels of inorganic sulfate, where undersulfation is also associated with cardiovascular diseases.[23] Infants also depend on taurine for their neurodevelopment.[251] Therefore, diets that lack adequate SAAs and taurine levels, compounded by increased oxidative stress after vaccination, can adversely affect the fetus's health status during pregnancy and lead to an increased risk of pathologies in adulthood.

Due to chronic inflammation and oxidative stress, a reduced supply of inorganic sulfate will also impair serotonin sulfation, affecting its inactivation. Increased levels of serotonin are one of the most consistently replicated biochemical findings in autism. Increased serotonin levels would affect day-to-day brain functioning, as well as the development and outgrowth of serotonergic neurons, resulting in a loss of serotonin terminals.[247] Increased levels of maternal serotonin may result in developmental and behavioural changes in children born from COVID-19-vaccinated mothers. Erdogan et al. concluded from their rat study that the Pfizer mRNA vaccine induced autism-like behaviours in the male offspring of vaccinated dams, while the WNT and BDNF pathways were impacted in both genders.[306]

In addition, spontaneous abortions and stillbirths could be expected when the mother has been vaccinated,[284,307] either recently before, or during pregnancy.[308] The movement of nutrient-rich HSA across the placental barrier is controlled by clathrin-enabled endocytosis. The binding of LNPs and GVG Sp to HSA will reduce the amount of HSA and clathrin binding sites available. This blocks HSA from entering the cell and passing the placental barrier. Therefore, less HSA and nutrients would be available to support healthy growth and development of the fetus and negatively impact the mother.[148] Furthermore, various studies demonstrated the achievability of designing LNPs as a platform for the delivery of mRNA to the placenta.[72,309] The potential effect of LNPs and mRNA on the fetus post-COVID-19 vaccination needs more research.

Although normal Ep and fetal tissues have low expression of CD147, it is significantly up-regulated during inflammatory conditions.[157] Therefore, there

is a possibility that the GVG Sp might directly impact the fetus by binding to CD147. More research is needed to test the immunogenicity, reactogenicity and safety of COVID-19 vaccines during pregnancy,[310] especially since pregnant and lactating women were excluded from the initial COVID-19 vaccine trials.[311] In recipients of the Pfizer vaccine, a wide-ranging immune response was observed, including stimulation of nAb responses, stimulation of CD4+ cells and expansion of effector memory CD8+ T cells in men and nonpregnant women. However, the extent of a comparable immunological response in pregnant women remains uncertain. This uncertainty is concerning, since favourable perinatal outcomes are highly dependent on enhanced helper T-cell type 2 and regulatory T-cell activity, combined with reduced Th1 responses. Alterations in CD4+ T-cell responses during pregnancy have been associated with unfavourable pregnancy outcomes, such as preterm birth and fetal loss. Furthermore, there is some evidence to suggest that infants born to mothers with variant CD4+ T-cell responses may experience long-term adverse consequences.[51]

Various research studies have clarified that HSPGs mediate the retention of many growth factors and morphogens. Carboxyl and sulfate groups of the GL contribute to the negative charge essential for interactions between HS and basic amino acid residues in proteins, such as VEGF-A.[312] In a murine study, it has been demonstrated that dramatically reduced HS sulfation resulted in brain malformations and skeletal defects. This implicates the importance of highly sulfated NS domains for growth factor-binding.[312] Macrophage subsets express differential expression of HS, where reparative M2 macrophages will bind more 2-O-sulfation-dependent FGF-2, augmenting the FGF-2-dependent proliferation of a target cell.[189] This suggests that aberrant regulation of leukocyte HS sulfation during chronic inflammation and oxidative stress, possibly induced by GVG Sp and LNPs, can result in excessive or inhibited growth factor activity, consequently affecting fetal development. Activated macrophages mediate both CD4+ and CD8+ T-cell responses. Therefore, HS and its sulfation configuration are essential for cell growth, tissue homeostasis, immune response and embryonic development.[288]

There is evidence that ACE2 is expressed during all stages of follicle maturation in the ovary and endometrium.[182] This increases the likelihood that the GVG Sp affects female and male fertility, with evidence that the Sp can distribute to the ovaries and testes.[313,314] The dramatic increase in oxidative stress and pro-inflammatory cytokines after COVID-19 vaccination, such as IL-6,[178] can damage spermatozoa. Increased levels of ROS and MPO can damage the sperm membrane[289,290] and induce apoptosis, thus affecting sperm quality, sperm function, and motility. If spermatogenesis is affected, it could eventually result in nonfunctional sperm, thus negatively impacting male reproductive health.[315] Furthermore, Sertoli and Leydig cells are involved in spermatogenesis and express ACE2, TMPRSS2 and CD147.[155] There have also been observations that COVID-19 vaccines caused a decrease in fertility in women.[316] The effect that binding of overexpressed CD147 and GVG Sp might have on spermatogenesis and fertilization would require further research. However, in vitro fertilization studies would not truly reflect the in vivo environment after vaccination. Although the proposed mechanism for infertility is the presumed similarity between GVG Sp and syncytin-1,[317] the up-regulation of Sp-binding receptors, and the effect of steroid sulfation and desulfation, would likely better explain the variation in fertility issues experienced by COVID-19 vaccinated women.

Furthermore, the reduced availability of inorganic sulfate for steroid sulfation could explain the menstrual irregularities and breakthrough bleeding observed in female COVID-19 vaccine recipients.[23,318–320] During the female menstrual cycle, SULF activity peaks in endometrial tissue at the early secretory stage and then declines afterwards. The pro-inflammatory cytokine IL-1β increases during the secretory phase of menstruation, which is known to suppress SULF mRNA and activity in human endometrial stromal cells.[248] This implies that IL-1β may control the endometrium steroid microenvironment by reducing oestrogen's biological action. However, both hypoxic conditions and inflammatory mediators, such as TNFα and IL-6, also influence steroid SULFs. SULF suppression results in decreased maternal-fetal oestrogen during pregnancy due to lack of SULF activity in the placenta. This deficiency of oestrogen would result in delayed progression of parturition. Furthermore, it has been found that SULF activity is increased by up to 12 times in endometrial cancer tissue.[248] Sulfation and desulfation play an essential role in balancing the availability of free steroid hormones near target sites and would affect menstruation regularity. The rate-limiting step for all sulfation reactions is the availability of active sulfate in the form of sulfonucleotide 3-phosphoadenosine 5-phosphosulfate (PAPS), where the responsible PAPS synthases are known to be fragile enzymes.[23,248] Furthermore, cellular efflux of conjugated steroids occurs through the multidrug-resistant protein (MRP) of ABC transporters. Estrone sulfate and DHEA sulfate transport depend on GSH availability, but whether or not the sulfated steroid requires GSH for MRP-mediated efflux remains unresolved.[248]

Since oestrogen is inactivated by sulfation, aberrant sulfation after vaccination would be a leading factor

predisposing to cerebral venous sinus thrombosis (CVST). At the same time, thromboembolic complications associated with the GVG Sp can lead to fetal vascular malperfusion or fetal vascular thrombosis.[321] In vascular development, peptide growth factors of the VEGF and platelet-derived growth factor (PDGF) families have been found to regulate the migration and proliferation of EnCs and support mural cells, such as pericytes (PC) and vascular smooth muscle cells. HS and the overall degree of sulfation play a vital role in PDGF binding and are responsible for PC recruitment and attachment in vascular development.[312]

The fact that insulin could easily interact with LNPs through electrostatic interactions[322,323] may partly explain the high incidence of hyperglycaemic crisis and type 2 diabetes after COVID-19 vaccination, apart from the destructive effect that oxidative stress, endoplasmic reticulum stress and low inorganic sulfate levels would have on beta cells in pancreatic islet.[23,163,288,324–326] It is also necessary to note that HSA transports both insulin and glucose. With more HSA being consumed during inflammation and oxidative stress, and through binding to LNPs, a reduced concentration of insulin delivered to the liver could be expected, with the subsequent elevation of glucose. Moreover, this excess glucose can result in HSA glycosylation, which further reduces the binding sites. A reduction in HSA is associated with type 2 diabetes.[288] Glycated HSA also suppresses glucose-induced insulin secretion by altering glucose metabolism and result in pancreatic beta cell dysfunction through autophagy. Glycated HSA has a very high binding affinity; therefore, with more GVG Sp and LNPs binding to glycated HSA, glucose and insulin metabolism would be further impaired.[58,148]

Type 1 diabetes have also been reported as an AE after vaccination.[326,327] Seen that high levels of sulfated HS protect beta cells in the pancreatic islet from ROS and cell death, this protective antiapoptotic effect would be neutralized during excessive oxidative stress and inflammation postvaccination, when nearby autoreactive T cells secrete HPSE that subsequently degrade HS, leading to the onset of Type 1 diabetes.[23,246]

## 3.10 | Cutaneous adverse effects

Various skin reactions were reported in recipients of the COVID-19 vaccines, such as petechiae, bruising or haemorrhages, haemangiomas, rashes, increased spider veins, redness, blue discoloration and/or peeling of the skin.[130,328,329] These symptoms mainly occurred associated with other complex symptoms and were usually clustered. The onset of symptoms occurred from 12 h to 9 weeks after vaccination, where Moderna elicited the most frequent reactions, followed by AstraZeneca and Pfizer.[130]

A case of Darier's disease flare-up was reported after the first dose of the AstraZeneca vaccine. Darier's disease is a rare autosomal dominantly inherited dermatosis, which is due to a mutation in the ATP2A2 gene on chromosome 12q23-24 that encodes SERCA2, a $Ca^{2+}$ ATPase in the sarcoplasmic/endoplasmic reticulum.[330] Other mechanisms would also be involved, since an antigen-immune-mediated flare was observed after vaccination. Syndecan (sdc) HSPGs regulate cytosolic $Ca^{2+}$ to control cell adhesion, actin cytoskeleton and junction formation. This is achieved by controlling transient receptor potential canonical (TRPC) channels. An sdc–TRPC4 complex in epidermal keratinocytes controls adhesion, adherent junction composition and early differentiation. HSPGs, and the degree of HS sulfation, will determine the binding of growth factors, morphogens, cytokines and chemokines, which synergistically combine with high-affinity receptors to affect intracellular signalling.[331] It is yet again clear that sulfation would affect practically every cellular process. A shift in sulfur metabolism, due to inflammation and oxidative stress, would exacerbate underlying genetic vulnerabilities, such as Darier's disease.

CD147 has been observed to increase with age in the skin.[234] Chronic inflammation after COVID-19 vaccination would increase the expression of CD147. When CD147 induce extracellular MMPs, an excessive breakdown of connective tissue components in the skin could be expected. Even though, most of the skin eruptions following vaccination could be drug-induced, where vaccination might have a synergistic immunologic effect on an adverse drug reaction. Most drugs used to treat inflammatory conditions, such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen and aspirin, inhibit, or are metabolized through sulfation and can thus negatively impact GAG sulfation in the epidermis. The consequent vascular inflammation would alter the regulation of keratinocyte proliferation, with increased epidermal differentiation. Toxic epidermal necrolysis (TEN) is often observed when using acetaminophen, which is associated with inorganic sulfate depletion.[23] However, Sano et al. found the S1 protein in vascular EnCs and eccrine glands in the deep dermis of a woman with confluent maculopapular erythema, which presented after the second dose of the Pfizer vaccine. Therefore, the involvement of Sp in persistent skin inflammation is clear, which remained in this case for at least 100 days after vaccination.[329]

## 3.11 | Ocular adverse events

Li et al.[332] established that after the first and second doses of mRNA vaccines, these vaccinated individuals had a significantly increased risk of retinal vascular occlusion up to 2 years after vaccination. Sp-induced disruption of retinal

capillary barrier function is similar to En damage following intravitreal administration of vascular En growth factor, a prominent vascular hyperpermeability factor in diabetic retinopathy, an inflammatory eye disease.[154] The visual disturbances observed after vaccination[332,333] could be caused by microvascular supply disturbances of the retina and/or optical centre, after exposure to GVG Sp and cytokines. Acute visual disturbances with retinal haemorrhages have been reported in association with AstraZeneca vaccination and autoimmune thrombocytopenia.[130] Given that a diet deficient in taurine is associated with the development of retinopathy,[251] there is a clear link between the availability of SAAs, inorganic sulfate, inflammation, vascular and ocular health.

## 3.12 | Cancer

Carcinogenesis is a complex multifactorial and multistage process that consists of initiation, promotion and progression. Very specific interactions between tumour cells and the microenvironment are required during cancer development, growth, metastasis and invasion. The GL is the critical effector of the tumour cell surface and microenvironment.[288] Therefore, the GL is involved in tumour growth and metastasis, by interacting with growth factors, growth factor receptors and cytokines. Chronic inflammation is underlying many cancers and is a driving force for metastasis of cancer cells. HSPGs play a central role in regulating cell behaviour and cancer progression, where the pattern and degree of GAG sulfation are strongly related to the type of cancer, the tumour regulatory effect and the level of differentiation.[288]

Significant alteration of steroid metabolism in many endocrine-related cancers is a well-established fact. Evidence suggests that sulfation pathways are down-regulated,[334] while SULF activity is up-regulated in numerous tumours.[335] This leads to a preference for desulfation and subsequent conversion of steroids into more active metabolites.[248] Many factors can contribute to dysregulation of sulfation pathways.[23] However, after COVID-19 vaccination, the chronic pro-inflammatory response and oxidative stress would probably be the main factors influencing sulfation. Furthermore, various factors, in addition to pro-inflammatory cytokines, would affect the signalling of SULTs and SULFs.[248] There has been an increase in breast and lung cancer cases reported to VAERS, while pancreatic, ovarian and bladder cancers were directly linked to COVID-19 vaccines.[54,336] One could also expect more cancers related to sarcoma, leukaemia and lymphoma after COVID-19 vaccination.[294,337–345] The fact that the c-Mpl–JAK2 pathway is activated after COVID-19 vaccination,[346] may be another underlying factor in the pathogenesis of leukaemia,[347] myelofibrosis[348] and myeloproliferative neoplasms[349] observed in COVID-19 vaccine recipients.

The fact that the ECM protein SPARC regulates tumour development, progression and angiogenesis[229] confirms the vital role of SAAs in cancer. SPARC would serve as a Cys donor to maintain homeostatic balance in the sulfation pathways (Figure 1). SPARC, which is overexpressed during cancer, exhibits anticancer properties and modulates inflammatory processes.[350]

CD147, a stimulator of MMP1 production in fibroblasts, plays well-characterized roles in tumour metastasis, angiogenesis and chemoresistance. Glycosylation plays a key role in regulating the pro-hypertrophic effects of CD147, such as the involvement of glycosylated CD147 in tumour metastasis.[155] While Sp can initiate gene expression changes, it might also affect glycosylation. Liu et al. showed[159] that CyPA and CD147 have higher levels of expression in pancreatic cancer tissues. Seeing that this proliferation of pancreatic cancer cells can be effectively blocked by a CD147 Ab,[159] confirms that the GVG Sp might be a driving factor in carcinogenesis, by up-regulating the expression of CD147. It has also been shown that the SARS-CoV-2 Sp increases GLK levels in EpCs. GLK overexpression in lung EpCs is correlated with human lung cancer recurrence and poor prognosis, while up-regulated expression of GLK has been observed in human liver cancer. Chuang et al. demonstrated that GLK directly phosphorylates the cytoskeleton regulator IQGAP1, leading to increased cell migration and promoting cancer metastasis.[165]

The formation of NETs has been observed in some cancers.[224] DNA enriched with 8-hydroxy deoxyguanosine in NET was shown to bind to a transmembrane protein such as Cdc25 in tumour cells, thereby facilitating its potential for metastasis. NETs induce the production of pro-inflammatory cytokines, which in turn stimulate more NETosis, leading to a cascading 'feedback' effect. Elevated levels of NETs were found in metastatic lesions. NETosis and increased NET formation were shown to precede cancer metastasis, with the NET-DNA complex acting as a chemotactic factor, attracting metastatic cells to new sites. Enhanced NET formation compromises the adaptive immune system. Additionally, NETs coat tumour cells, providing protection against cytotoxic lymphocytes and/or natural killer cells that typically target tumour cells. Mounting evidence suggests that neutrophils promote tumour growth and metastatic progression through the formation of NETs.[177] The formation of new tumours and the aggravation of existing malignancies can thus be expected postvaccination.

Cationic LNP-RNAs cause TLR4 activation, which can lead to cancer promotion and progression, and increase

angiogenesis. TLR4 activation results in triggering the NF-kB pathway, chemoresistance in ovarian cancer, and increased migration and invasion of both colon and pancreatic cancer cells.[61] Although PEG is relatively stable at physiological pH, it is collapsible under acidic conditions because of the protonation of imidazole groups. Therefore, PEG-coated ionizable LNPs will be destabilized in the acidic tumoural environment,[351] thus amplifying the cytotoxic effects of these NPs.

It has also been postulated that COVID-19 vaccines may interfere with the target cell genome, activating oncogenes and/or deactivating anticarcinogenic gene sequences, thus increasing cancer risks.[352,353] In fact, numerous studies documented how dysregulation of miRNA is associated with cancer development and metastasis.[354] Cancer pathogenesis is related to several biomolecular processes, for example, genomic alterations, transcription of oncogenic factors and inhibition of repressor transcription, such as P53 and hypoxia.[355] The p53 tumour suppressor molecule is responsible for the regulation of various basic cell signalling processes in oncogenesis, where coronavirus infection is known to alter the activity of p53 or its upstream and downstream proteins.[356] Miyashita et al. suggested that miR-92a-2-5p and miR-148a play a role in immune responses to components of the Pfizer vaccine. Of particular interest, miR-92a-2-5p has been identified as a biomarker for small cell lung cancer.[357] However, the direct induction of miRNA dysregulation resulting from mRNA vaccines can potentially have significant consequences for millions of people, including children, by triggering the pathogenesis of tumours or cancer relapses. This warrants careful monitoring and further investigation to ensure vaccine safety and efficacy in the long term.

### 3.13 | Variation in adverse events

Many factors would contribute to the variation in AEs experienced by COVID-19 vaccine recipients. Although most recipients of the COVID-19 vaccines seem to have experienced no adverse effects, more and more reports of severe AEs, and death, due to COVID-19 vaccination are being confirmed.[179]

The sulfation pathways' important role in disease prevention has been highlighted in this review and elsewhere.[23] In this review, the effect of COVID-19 vaccines on inorganic sulfate depletion has been discussed, where the pathological consequences would be aggravated by existing underlying degradation of the GL, sulfate deficiency or dysregulated sulfation pathways. The various epigenetic and genetic factors that influence sulfation should be considered, such as dietary factors/nutritional deficiencies, ageing, male sex, comorbidity, genetic variation, inflammatory insults and oxidative stress, and various environmental factors.[23] Where adolescents are more inclined to drink, smoke and use recreational drugs that will deplete GSH and sulfate, athletes' overall health and redox status will determine their response to vaccination. Impaired redox homeostasis and associated oxidative stress seem critical to explaining increased susceptibility to AE postvaccination.

AEs will also be aggravated by specific medications taken by the vaccine recipient. Drugs used to treat AEs, such as fever, inflammation and pain, can exacerbate the cytotoxicity of vaccines and immune responses, leading to more severe AEs. In an AstraZeneca study, higher rates of solicited reactogenicity were observed in vaccinated participants who received acetaminophen prophylaxis, compared to the no acetaminophen control group.[128] Medications that require sulfation for their metabolism, such as acetaminophen and NSAIDs, can deplete the liver PAPS pool within 2 h.[358] This will negatively impact sulfation of the GL, and other molecules, due to the decreased levels of available inorganic sulfate or PAPS (Figure 1),[23,248] exacerbating AEs.

Various factors will affect NP biomolecular corona formation and, therefore, the in vivo behaviour of NPs. Apart from the NP composition, the biofluid protein concentration, temperature, pH, ionic strength and disease state of the host are all critical parameters that can influence the corona and, therefore, the subsequent nano-bio interactions.[110,119] This will potentially have important implications for LNP clearance, tissue accumulation, efficacy and effect on AEs. Since proprietary ingredients and manufacturing processes are being used in the COVID-19 vaccines, it is difficult to match the outcome with the physicochemical properties of NPs, and other possible ingredients in DNA and mRNA vaccines. As intellectual property or proprietary novel functional excipients, graphene-based NPs could be functionalized with the complex multilayered polymeric lipid delivery structures[65,109,359,360] used in COVID-19 vaccines.[40,361–364]

## 4 | DIAGNOSTIC TESTS

Through anecdotal evidence, a high tendency of *Helicobacter pylori* infection has been noted among COVID-19 vaccinated individuals. Physicians should, therefore, consider screening for this pathogen, especially if gut-related symptoms accompany AEs. Since sulfated gastrin stimulate acid secretion from gastric fistulas and pepsin production,[365] undersulfation and low stomach acid levels could predispose to *Helicobacter pylori* infection.

Markers of NETosis and serum NET levels will be elevated levels of cell-free DNA, MPO or MPO-DNA, citrullinated histone H3 (CitH3) and neutrophil elastase.[175–177,223] MPO forms HOCl, by catalysing the reaction of $H_2O_2$ with chloride ions ($Cl^-$), to facilitate the destruction of pathogens. HOCl rapidly depletes GSH. Glutathione sulfonamide is a sensitive marker for MPO-mediated reactions, while 3-chlorotyrosine and elevated levels of chlorinated aldehydes would indicate HOCl-mediated damage.[289] Teo et al. found that MPO levels and activity in plasma from COVID-19 patients were significantly correlated with soluble EnGL fragments, and by inhibiting MPO activity in vitro, sdc1 shedding was reduced.[366] Cell-free DNA levels should show a strong correlation with other markers of inflammation, such as CRP and D-dimer.[177] Furthermore, higher levels of double-stranded DNA and calprotectin could be expected, together with DNA histone, granular enzymes and sdc1, as found in VITT.[13]

High serum levels of CRP would indicate macrophage activation syndrome, while high serum levels of cytokines, especially IL-6, would be another biomarker of inflammation and robust immune response. Increased activity of pro-oxidative enzymes, such as MPO, points to oxidative stress, while oxidative damage is linked to higher production of malondialdehyde (MDA), protein carbonyls and advanced protein oxidation products. Elevated levels of NO metabolites and IgM directed to nitroso neoepitope adducts indicate increased nitrosative stress. The lower total antioxidant capacity (TAC) of plasma is reflected in reduced levels of glutathione peroxidase (GPx), zinc, GSH and $H_2S$.[212] Increased serum transaminase levels are correlated with low systemic toxicity. AST and ALT levels were elevated in mice treated with PEG-LNP.[38] To identify EnGL damage, elevated plasma levels of sdc1, P-selectin, endocan, chondroitin sulfate, hyaluronan, HS and vWf would be useful markers,[156,244] as in the case of COVID-19.[34,175] It is well established that elevated plasma concentrations of GL degradation fragments are associated with a poor prognosis in severe disease.[156]

Furthermore, increased levels of fibrinogen, fibrin degradation products, D-dimer, vWf and soluble thrombomodulin indicate En injury. At the same time, impaired En function would be related to apoptotic ECs, decreased NO availability and vascular leakage.[32] When sdc4 is expressed by EnCs, smooth muscle cells and cardiac myocytes, there is a positive correlation between increased serum levels of sdc4 and heart failure, making it a possible useful biomarker for predicting cardiovascular events. Sdc4 expression and shedding would increase due to mechanical stress on the walls of the vessel,[367] such as the physical forces that LNPs and protein coronas would apply. Elevated troponin I level or abnormal wall motion on echocardiography may indicate myocardial involvement.[368] Cardiac troponin levels can therefore support a diagnosis of myocarditis.[194]

## 5 | TREATMENT CONSIDERATIONS

Various antioxidants will be beneficial in the treatment of COVID-19 vaccine-induced AEs, by scavenging or chain-breaking ROS and peroxynitrite species, thereby converting them to less reactive products. Enzymatic antioxidants, such as superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase and glutathione transferase, as well as nonenzymatic endogenous molecules, such as N-acetylcysteine (NAC), taurine and GSH, could be used. Thiols and low molecular weight antioxidants, such as methylsulfonylmethane, tocopherols, ascorbate, retinol, urate and reduced GSH, would act as 'the second line of defense' against ROS.[246]

The application of taurine will be an important treatment consideration for cardiovascular-related AEs. The beneficial effects of taurine have been demonstrated in many cardiovascular conditions, such as decreased serum low-density lipoprotein, decreased progression of atherosclerosis, anti-inflammatory effects, regulation of blood pressure and protection against ischemia–reperfusion injury of the myocardium. Therefore, taurine protects against coronary heart disease.[251,369] Furthermore, studies have demonstrated that taurine supplementation can reduce macrophage infiltration, elastin fragmentation and MMP activation, which are linked to MPO overexpression. Taurine acts as a competitive target for HOCl.[289] The antioxidant and scavenging effect of taurine makes it an important treatment option to combat vaccine-induced oxidative stress, restore mitochondrial dysfunction and improve cardiac energy metabolism.

Anaphylaxis events after Pfizer vaccination have responded to epinephrine treatment, although many cases required more than one dose of epinephrine.[51] Imai et al. described a case of vaccine-induced cytopenia in the presence of clozapine, which resolved after discontinuing the drug.[370] Immunological mechanisms of drug-related leukopenia have previously been reported after vaccination.[370] This phenomenon could be expected with drugs such as steroids, NSAIDs and aspirin, which deplete or require sulfation for their metabolism.[23] Furthermore, suppose a vaccine recipient presents with any signs of serotonin syndrome, such as focal seizures or tremors, fasciculation and muscle weakness; they should not be given a selective serotonin reuptake inhibitor (SSRI). Inorganic sulfate is needed to inactivate serotonin through sulfation.

A case of vaccine-induced eosinophilic granulomatosis with Polyangiitis (EGPA) was effectively treated with pulsed intravenous corticosteroids, starting with 250 mg of methylprednisolone for 3 days and transitioning to oral prednisone at a dose of 1 mg/kg/day. The patient experienced gradual recovery from myositis and blood eosinophil counts decreased significantly. Prednisone was reduced and eventually discontinued after 6 months.[273] This case indicates that COVID-19 vaccines might potentially overstimulate immune-inflammatory pathways involved in the pathophysiology of the disease. Adding a sulfur donor, such as NAC, would ensure a better outcome during steroid treatment.[23,104] Patients taking moderate or high doses of corticosteroids have an increased risk of COVID-19 and vaccine-induced complications.[51,371] In acute viral myocarditis, the use of NSAIDs remains controversial and has also shown increased myocardial inflammation and mortality in murine models and human case studies.[241,368] NSAIDs deplete inorganic sulfate, thus exacerbating the underlying condition of inflammation, En damage and thrombosis.[23] Drugs, such as acetaminophen, with limited anti-inflammatory effects, may have inadequate analgesic function, increase blood pressure, deplete a vast amount of inorganic sulfate, impair renal function and affect left ventricular performance.[368,372]

Although IFNs are commonly used to treat viral myocarditis,[241] they are not recommended for vaccine-induced myocarditis, in which case the antigens are responsible for robust inflammatory responses and oxidative stress. Since adequately sulfated HS is important in regulating the threshold for IFN stimulation of macrophages,[23] one could expect that undersulfated macrophage HS, and a degraded GL, will drive the immune response towards a pro-inflammatory state when IFNs are used as treatment.

Inhibition of MMP13 in CVB3 infected mice has been shown to increase myocarditis.[253] Since HS plays a role in modulating MMPs, the application of NAC should be considered. NAC is the acetylated form of Cys. Cys would serve as a decoy, blocking the binding of GVG Sp to the cell surface and soluble HS, and as a potent anti-inflammatory, antioxidant and anticoagulant, plus precursor to inorganic sulfate.[23,104] Furthermore, due to its excellent antioxidant properties, NAC's application significantly reduced ROS.[373] It is important to address mitochondrial-dependent damage that could be triggered via oxidative stress, ischemia and DNA damage. At the same time, mitochondrial dysfunction could induce mitoROS burst, further aggravating mitochondrial disorders, in turn.[373] Since thiol groups are established targets for MPO-derived oxidants, thiol-based therapeutics have broad applications in biomedicine.[289]

NAC is bioavailable and can cross the BBB, where it acts via multiple pathways in the brain. Several animal studies have shown evidence of increased brain GSH after oral administration of NAC. Oral and transdermal GSH supplementation, administered to children on the autism spectrum, has been found to lead to significant increases in plasma reduced GSH, inorganic sulfate, Cys and taurine levels.[247] NAC has been shown to be effective in treating many psychiatric and neurological disorders and can be beneficial in preventing cognitive decline associated with acute physiological insults and dementia-related conditions. NAC has been shown to modulate several neurological pathways, including glutamate dysregulation, oxidative stress and inflammation.[374] NAC also has anti-inflammatory activity, independently of its antioxidant activity.[104,375] NAC has antithrombotic effects and potentiates En NO's vasodilator and antiaggregatory effects. Intravenous NAC has been shown to promote lysis of arterial thrombi that is resistant to conventional methods. It has been suggested that the main molecular target of the antithrombotic activity of NAC is vWF, which crosslinks platelets in arterial thrombi.[375] However, since NAC also serves as a precursor to inorganic sulfate, the various antithrombotic properties of an adequately sulfated GL must also be taken into account.[23] In hospitalized patients, with moderate to severe COVID-19 pneumonia, NAC 600 mg bid orally for 14 days, improved the PO2/FiO2 ratio over time and decreased the white blood cell, CRP, D-dimer and lactose dehydrogenase levels.[375]

Research has shown that patients with coronary heart disease, stroke and myocardial infarction exhibit significantly reduced total GSH levels, making their erythrocytes more susceptible to haemolysis. This suggests that the administration of NAC or GSH could serve as a potential therapeutic strategy to prevent adverse cardiovascular events. Additionally, GSH levels are found to be decreased in asymptomatic cardiac patients with structural abnormalities, even before the onset of full-blown heart failure. Clinical data have demonstrated that intravenous administration of GSH during the acute phase of myocardial infarction, prior to coronary recanalization, can ameliorate reperfusion damage. This indicates that timely exogenous administration of GSH or NAC can potentially improve outcomes after myocardial infarction and slow the progression of cardiac abnormalities leading to heart failure.[103] Niwano et al. demonstrated that NAC suppressed myocarditis and electrical remodelling in a dose-dependent manner in immunized rats,[252] which confirms that hyperoxidative stress plays an important role in promoting electrical and structural remodelling after vaccination. NAC has also effectively treated acute liver failure, spermatogenesis disorders and dermatological diseases.[164,201] The various antiviral, anti-inflammatory, antioxidant and anticoagulatory properties of NAC, and

its role and application in COVID-19, have been discussed elsewhere.[23,104]

Hypercatabolic states, such as cytokine storm and sepsis, are known to result in thiamine deficiency.[376,377] In addition to having direct antioxidant properties, thiamine is also essential for GSH production.[378] Oliveira et al. observed immediate neurological improvement of encephalopathy in COVID-19 patients with high-dose intravenous thiamine administration.[376] Sulaiman et al. found that the use of thiamine as an adjunct therapy in critically ill patients with COVID-19 was associated with a lower incidence of thrombosis.[377] Chen et al. successfully treated a patient with right facial weakness, after the second dose of the Moderna vaccine, with thiamine, riboflavin and prednisolone.[379]

To effectively decrease elevated D-dimer levels, it is necessary to first address the underlying inflammation, as well as NETosis. Low-dose naltrexone offers promise in the treatment of severe inflammatory conditions in patients injected with a COVID-19 vaccine, as it can dampen innate immune responses and TLR signalling, and reduce IL-1, TNFα and IFN levels.[380] Although dexamethasone has previously been shown to reduce neutrophil recruitment and NETosis, both in in vitro and in vivo murine models,[177] Dowey et al. found that dexamethasone did not affect NETosis in neutrophils isolated from hospitalized patients with COVID-19.[176] Since NETs drive inflammation, thrombosis and disease severity, finding effective solutions to address NETosis is important. Ruboxistaurin, a protein kinase C (PKC) inhibitor, could reduce NET formation, thus diminishing airway inflammation and other events, including microvascular thrombosis.[176] Rapid initiation of treatment similar to severe HIT is recommended for patients with suspected or confirmed VITT. However, it is recommended to avoid HP and products in VITT management due to case reports describing thrombosis progression after HP use, similar to HIT and autoimmune HIT complications. Non-HP anticoagulants are currently considered better therapeutic options. Although JAK2 inhibitors, such as ruxolitinib, have been found to reduce aggregation to VITT IgG and PF4, they are not recommended in the treatment of VITT or HIT, due to their partial action and associated thrombocytopenia. Full blockade-inducing agents that target FcγRIIA, such as the monoclonal antibody IV.3, would be a better option to consider.[346]

Aspirin as prophylaxis for VITT in vaccine recipients is not recommended, as a population-based study in Scotland demonstrated around 50% increased frequency of haemorrhagic events up to 1 month after vaccination. Aspirin increases the risk of bleeding and has no clear benefit,[221] plus it increases urinary excretion of inorganic sulfate.[23]

To remove NETs, treatment with deoxyribonuclease-1, or pharmacological inhibitors, has also been suggested, which was shown to inhibit tumour-induced inflammation and metastasis.[177] Interestingly, a proposed mechanism for tumour enhancement involves NETs that stimulate mitochondrial biogenesis and bioenergetics in tumour cells, through the induction and activation of TLRs and peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α), a key regulator of cellular energy metabolism. It has been suggested that this mechanism is how metformin inhibits the adhesion of cancer cells to NETs and consequently prevents metastasis.[177] Bioidentical progesterone will decrease the activity of SULFs, which have been implicated as the main drivers of hormonal cancers and metastasis. Furthermore, sulfur donors, such as NAC, GSH and methylsulfonylmethane, will increase the availability of inorganic sulfate and upregulate SULTs, resulting in the inactivation of oestrogen through sulfation.[23,248,381]

Elevated oxidative stress, as a result of increased expression of MPO, LNP activity and inflammatory mediators, can result in phospholipid oxidation. LNP and HOCl-induced haemolysis, and cell lysis in general, can be addressed by the application of plasmalogens. HOCl-induced cytotoxicity has also been reported in immune and EnCs, lung and bronchial EpCs, chondrocytes, fibroblasts and vascular smooth muscle cells.[289] Cellular plasmalogen lipids are a target for HOCl, which cleaves the plasmalogen vinyl ether linkage, resulting in elevated levels of chlorinated aldehydes.[289,382] Plasmalogens are especially abundant in neuronal, cardiac and immune cells.[291] A deficiency, or destruction of plasmalogen, an antioxidant of the cellular membrane, has been linked to various diseases, such as respiratory disorders, neurodegenerative, cardiovascular, cancer and various inflammatory diseases, and metabolic syndrome. Both oxidative stress and chronic inflammatory conditions will result in plasmalogen deficiency,[290,383–386] resulting in membrane defects.[281] Plasmalogen replacement therapy will be an important treatment option to consider, by administering purified plasmalogens, and/or plasmalogen precursors, to increase plasmalogen levels. Chimyl alcohol and alkylglycerol are important precursors that have been shown to restore plasmalogen levels, while DHA-enriched lipids effectively increased plasmalogen levels in the brain.[290,291,383,387] Precursors are better absorbed than purified plasmalogens.[382,387] Since low-density lipoprotein (LDL) is a major transport protein of plasmalogens, the use of statin drugs might not be advisable to treat vaccine-induced AEs.[386,388] Furthermore, plasmalogen replacement therapy has been shown to be approximately twice as effective as statins in lowering cholesterol levels, where plasmalogen precursors

reduced membrane cholesterol levels through increased membrane cholesterol esterification and transport.[386,389]

The protease inhibitor, doxycycline, significantly reduces GL PG shedding and should be investigated as a treatment option in AE associated with the vascular system. HPSE-2 should also be up-regulated, as it will inactivate HPSE-1 and thus reduce EnGL degradation.[244] Yuan et al. successfully treated EnGL degradation with HS mimetics.[214] Since a degraded EnGL will favour the migration of cytokines, vaccine antigens and ingredients, focusing treatment options on restoring the GL will be necessary. To effectively address the various AEs caused by COVID-19 vaccination, it is clear that a comprehensive and personalized treatment protocol is needed, rather than a single-drug approach.

## 6 | CONCLUSIONS

With substantial evidence indicating that the GVG Sp antigen, genetic material and LNPs used in COVID-19 vaccines can lead to endotheliopathy,[13] a re-evaluation of DNA and mRNA vaccination is warranted. These components in the vaccines trigger the release of pro-inflammatory cytokines and excessive oxidative stress, negatively affecting the integrity of the GL through reduced sulfation and degradation. This impairment of GL function may result in skewed inflammatory responses, compromised immunity, oxidative stress, a procoagulatory state and various disease processes. In particular, COVID-19 vaccines did not prevent infection[390,391] or transmission,[132,392] and were associated with a significant risk of chronic disease, serious AEs or death.[15,20,146,179,393,394]

Nanotechnology, which has been hailed as a major advancement in medical research and nanovaccinology, has been rapidly deployed in the fight against COVID-19.[82,395] However, lipid-based mRNA delivery systems, despite demonstrating high transfection efficiency in vitro, faced toxicity issues and poor pharmacokinetic profiles in vivo, resulting in unwanted inflammatory and immune responses. Inadequate research on the biocompatibility of these NPs prior to clinical application led to severe adverse reactions and fatalities related to COVID-19 vaccination.[15,65] It is most likely that the LNP platforms resulted in acquired cellular immunopathology and severe oxidative stress. Although there is scope for using NPs in precision medicine with intelligent NP design, much more research is needed to gain valuable insights regarding its possible adverse effects in vivo at the molecular level. It is also essential to fully understand the molecular–cellular basis of the rare, and severe, AEs experienced after vaccination with the first generation COVID-19 vaccines, with more research urgently needed in this area. However, it will only be possible to conclude by fully disclosing the precise content of the various COVID-19 vaccines and manufacturing procedures.

The maintenance and restoration of GL integrity is proposed as a primary therapeutic approach against COVID-19 and vaccine-induced AEs. GAGs and proteoglycans, essential components of the GL, are highly complex biomolecules with significant structural and functional heterogeneity. Taking into account the complexity of the innate immune system, GL and inflammatory responses, the current focus on only adaptive immune responses in vaccinology should be revisited.

Synthetically induced chronic inflammation, as seen postvaccination, can have detrimental effects on tissues, organs and normal cells, leading to increased morbidity and mortality. While a natural inflammatory response is beneficial, the pathological consequences of induced chronic inflammation highlight the importance of rather addressing the underlying causes of infectious disease susceptibility, such as promoting balanced wholefood nutrition, effective supplementation, moderate exercise, hygiene, rest and autonomic balance.

Concerns arise from the continued development of various COVID-19 vaccines and plans for DNA and mRNA vaccines for other diseases. Although inactivated vaccines have a relatively good safety profile, they have low immunogenicity; mRNA vaccines demonstrate greater immunogenicity, but relatively higher adverse event rates.[90] The risks associated with gene therapy and vaccination may outweigh the potential benefits, necessitating careful consideration and critical evaluation of their impact on the innate immune system, and the general body system. The focus should be on addressing the root causes of diseases, rather than pursuing profit-driven approaches. Additionally, the compatibility of synthetically engineered NPs with the complexity of the human body's physiology must be thoroughly examined to ensure patient safety and well-being.

### AUTHOR CONTRIBUTIONS

HN du Preez conceived, researched and wrote the manuscript. She also conceptualized and produced the Table and all Figures with BioRender.com. The other authors extensively reviewed and approved the final manuscript.

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The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review article, as no datasets were generated or analysed during the current study. All articles referred to in this review of the literature are listed in the list of references and are in the public domain.

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