Review

The Persistence of COVID-19 Vaccine Artifacts in Bodily Fluids and Tissues: A Systematic Review

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Review

The Persistence of COVID-19 Vaccine Artifacts in Bodily Fluids and Tissues: A Systematic Review

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Abstract

Several studies have reported the initially unexpected presence of COVID-19 vaccine artifacts (modified mRNA, spike protein) in human tissues and bodily fluids. Questions remain regarding the true persistence of both spike protein and mRNA, and the mechanism of this phenomenon, which defies initial expectations. While n1-methyl-pseudouridine was chosen for its ability to resist immune degradation, it was still expected to decay at a time course somewhat similar to that characterized for RNA or pseudouridinylated RNA. Recent studies have indicated possible persistence exceeding one year in duration, motivating explanation. This review examines the methodologies for which spike protein, mRNA, or other vaccine contents (e.g., lipid nanoparticles) have been detected at long durations since the last injection, the tissues or bodily fluids in which they have been identified, and the accompanying sensitivities of the assays. This review also discusses potential explanations for the persistent vaccine artifacts.

Keywords: COVID-19 vaccines; mRNA vaccines; BNT162b2; mRNA1273; spike protein

Introduction

Bahl et al. evaluated mRNA concentration in mice following injection, though the formulation used in that study lacked nucleoside modifications [6]. Other studies have provided limited information regarding the production of the target antigen or the persistence of vaccine genetic material (Table 1). Studies since the vaccination campaign have revealed persistence of injection-related artifacts in individuals for months or even years past their last dose [10,11].

To date, the persistence of both the vaccine antigen and the modified mRNA defies explanation [10,12]. While mechanisms have been offered , including prolonged stability of n1-methyl-pseudouridinylated RNA [12], or expression of plasmid DNA contamination discovered in vaccine vials [13,14], the phenomenon is not well understood.

Prior to the COVID-19 mRNA vaccine trials in 2020, there were only five years of data on the behaviour of modified n1-methyl-pseudouridinylated mRNA [15]. While it was known that its decay curve was significantly slower than that of unmodified mRNA, the observed results only sufficiently explained differences in lifespan within an order of magnitude [15].

Throughout the development of vaccines during 2020 and their wide rollout in 2021, there was significant public interest in how the novel mRNA platform compared to traditional vaccines [16]. One proposed advantage of RNA vaccines was the transience of the genetic material, as RNA is less stable than DNA [17] and, in principle, avoids the risk of genomic integration [18].

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Table 1. Non-COVID mRNA viral vaccines test duration ATI: Analytical treatment interruption; bDNA: Branched DNA assay; CI: Confidence interval; CFSE: Carboxyfluorescein succinimidyl ester; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; ELISA: Enzyme-linked immunosorbent assay; ELISPOT: Enzyme-linked immunospot assay; Gag: Group-specific antigen (HIV structural protein); GMT: Geometric mean titer; HA: Hemagglutinin (Influenza virus surface protein); HAI: Hemagglutination inhibition assay; HTI: HIVACAT T-cell immunogen (conserved HIV-1 epitopes); ICS: Intracellular cytokine staining; ID: Intradermal; IM: Intramuscular; IU: International unit; LNP: Lipid nanoparticle; MN: Microneutralization assay; Mod: mRNA modification; Nef: Negative factor (HIV regulatory protein); NHP: Nonhuman primate; PCR: Polymerase chain reaction; pVL: Plasma viral load; RABV-G: Rabies virus glycoprotein; Rev: Regulator of virion protein (HIV regulatory protein); RFFIT: Rapid fluorescent focus inhibition test; RT-PCR: Reverse transcription polymerase chain reaction; SFC: Spot-forming cells; TCID50: 50% tissue culture infectious dose; VL: Viral load; Vpr: Viral protein R (HIV regulatory protein); ↑: Increase (used to indicate change from baseline); ↓: Decrease (used to indicate change from baseline).The initial trials varied in their duration of subject monitoring, ranging from a few days to a full 12 months post-vaccination. In atrial of an HIV RNA vaccine, the immune response was monitored up to week 24 [9]. Lorna et al. also measured the expression of proviral HIV-RNA, but that cannot be attributed to the vaccine, instead the proviral reservoir is the likely source [9]. A phase I trial of influenza vaccines tracked antibody in two vaccines for 183 and 205 days, respectively [3]. In this case, immune titers were measured, but not the antigen itself. Another HIV RNA vaccine trial monitored immune parameters for 12 weeks post-vaccination [4].

Messenger RNA vaccines were first deployed on a large scale during the COVID-19 pandemic. Any novel technology can expect to experience technical challenges during its first iterations; and COVID-19 pandemic marked the first deployment of mRNA vaccines, aside from clinical trials involving 385 participants [1]. The first mRNA vaccine trial began in 2013	**Substance Found**	**Population**	**Intervention**	**Outcome measures**	**Max Duration Vaccine-Test**	**Reference**

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(published in 2017), testing a rabies vaccine [2]; therefore, at the time of approval for the COVID-19 vaccines in 2020, mRNA vaccines had approximately seven years of clinical observation [1]. Initial trials did not characterize biodistribution of vaccinal components in human subjects, or test for the persistence of antigens or vaccinal mRNA over time. Instead, studies evaluated immune response at durations up to one year (Table 1).						
Analytical Technique						
RFFIT, IgG and IgM ELISA, ICS	RABV-G-specific IgG/IgM; T cells (IFN-γ, TNF-α, IL-2, CD107a)	**Human:** 101 adults (18–40 y), rabies-naïve, BMI: 18–32 kg/m² **Groups:** needle-syringe (n=42; 18 ID, 24 IM),	**Vaccine:** CV7201 (mRNA-RABV-G) **Packaging:** lyophilized, protamine-complexed mRNA **Mod:** protamine-stabilized **Antigen:** RABV-G	**Neutralizing titers (RFFIT ≥0.5 IU/mL):** ID (needle-free): GMT 0.83 IU/mL (95% CI: 0.51–1.34); IM: GMT 0.44 IU/mL (95% CI: 0.10–1.90); Responders: 71% ID, 46% IM	1 year	[2]

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		needle-free (n=59; 46 ID, 13 IM)	**Route:** ID/IM via needle-syringe or needle-free injector **Doses:** 80–640 μg, 3 doses **Schedule:** days 0, 7, 28 or 0, 28, 56 **Booster:** 80 μg at 1 y (n=14)	**Cytokines:** ↑ at day 42; polyfunctionality ↑ ($p<0.0001$)		
HAI, MN, ELISPOT	Antibody responses (HAI, MN titers); RNA not directly measured	**Human:** 201 adults (18–64 y) **Groups:** 25–400 μg IM (n=23–30), 25–50 μg ID (n=16–23), placebo (n=35)	**Vaccine:** H10N8 mRNA or H7N9 mRNA **Packaging:** LNP **Mod:** not specified **Antigen:** H10N8 or H7N9 HA **Route:** IM or ID **Doses:** IM: 10–400 μg; ID: 25–50 μg, 2 doses **Schedule:** days 1 and 21 **Booster:** 25 or 50 μg H7N9 at 6 months (n=53)	**HAI titers:** H10N8 100 μg IM: 100% ≥1:40, GMT 68.8; H7N9 25 μg IM: 96.3% ≥1:40, GMT 87.0 **MN:** H10N8: 87.0% ≥1:20, GMT 38.8; H7N9: 100% ≥1:20, GMT ≥1280 (post-booster, 6 months)	1 y	[3]
ELISPOT, ICS, pVL PCR, ultrasensitive viral PCR	HIV-1 RNA (cell-associated RNA); no RNA measured in vivo	**Human:** 70 HIV-1 adults (≥18 y), Stable cART ≥3 y, Nadir $CD4^+$ ≥350 cells/μL, current $CD4^+$ ≥450 cells/μL, HIV RNA <50 copies/mL **Groups:** iHIVARNA-01 (n=40), TriMix (n=15), placebo (n=15)	**Vaccine:** iHIVARNA-01 (HTI-TriMix mRNA) **Packaging:** naked mRNA (no LNP) **Mod:** not specified **Antigen:** HTI **Adjuvant:** TriMix (CD40L, caTLR4, CD70) **Route:** intranodal inguinal **Doses:** not specified, 3 doses **Schedule:** weeks 0, 2, 4	**HTI-specific T cell (ELISPOT):** weeks 0, 6, 18, ↑ **$CD4^+$/$CD8^+$ T cell (ELISPOT, ICS):** weeks 0, 6, 30, ↑ **$CD8^+$ suppression (in vitro):** weeks 6, 18, ↑ **VL rebound, % with <50 copies/mL:** weeks 18, 30, partial control **Viral reservoir:** proviral DNA, RNA: : weeks 0, 6, 18, no significant change **Viral escape, host mRNA expression:** weeks 0, 6, 18, no significant change	30 weeks	[4]
RFFIT, ELISA	RABV-G-specific IgG	**Human:** 55 adults (18–40 y), rabies-naive, BMI: 18–32 kg/m² **Groups:** 1 μg (n=16), 2 μg (n=16), 5 μg (n=10), Rabipur control (n=11)	**Vaccine:** CV7202 (mRNA-RABV-G) **Packaging:** LNP **Mod:** unmodified mRNA **Antigen:** RABV-G **Route:** IM, needle-syringe **Doses:** 1–5 μg, 1–2 doses	**Neutralizing titers (RFFIT ≥0.5 IU/mL, days 1–57):** 1 μg: 63–100%, GMT 4.8 IU/mL; 2 μg: 83–100%, GMT 4.2 IU/mL; 5 μg (1 dose): 22%; Rabipur: 100%, GMT 13.5 IU/mL **IgG (day 43):**	57 days (interim); long-term follow-up to 2 y	[5]

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			Schedule: days 1, 29 **Control:** Rabipur (≥2.5 IU/mL; days 1, 8, 29)	1 μg: 34,186 U/mL; 2 μg: 20,707 U/mL; Rabipur: 33,373 U/mL		
HAI, MN, ELISA (IgG1/IgG2a), ELISPOT, $TCID_{50}$, bDNA, Western blot	Vaccine mRNA; HA protein (Western blot)	**Mouse:** BALB/c, F, 5–8 weeks (n not specified) **Ferret:** M, 13–15 weeks (n not specified) **NHP:** Cynomolgus, 2–4 y (n=1/group) **Human:** 31 adults (23 vaccine, 8 placebo)	**Vaccine:** H10N8 or H7N9 mRNA **Packaging:** LNP (50:10:38.5:1.5 ionizable lipid:DSPC:cholesterol:PEG-lipid ratio), 80–100 nm, >90% encapsulation **Mod:** pseudouridine, 5-methylcytidine, cap-1 **Antigen:** HA **Route**: IM **Doses**: Mouse: not specified, single dose; Ferret: 100 μg H10N8 mRNA or 50 μg H7N9 mRNA, 2 doses; NHPs: 6mg H10N8 mRNA, single dose; Human: not specified, 2 doses **Schedule**: days 1 and 21 (2 doses)	**NHP tissue mRNA conc. (ng/mL):** Muscle: 5,680; Lymph nodes: 2,120; Spleen: 86.9; Liver: 47.2; Plasma: 5.47; Heart: 0.799; Brain: 0.429; Lung: 1.82 **HAI titers (ferret):** ↑ at days 21-49 ($p < 0.0001$) **Viral load ($TCID_{50}$, lung – mouse, ferret):** ↓ post-vaccination	Mouse: 11 days; Ferret: 49 days; Human: 43 days (interim); Titers stable 1 y (mouse)	[6]
Flow cytometry, RT-PCR, ELISA, cytokine multiplex	HIV-1 RNA (Gag, Nef, Rev, Vpr mRNA in DCs)	**Human:** 54 HIV-1 adults (37 vaccine, 17 placebo), median CD4 count 450 cells/mm^3	**Vaccine:** AGS-004 (DCs loaded with HIV Gag, Nef, Rev, Vpr RNA) **Packaging:** autologous DCs **Mod:** none **Antigen:** autologous HIV-1 Gag, Nef, Rev, Vpr **Route:** ID **Dosage:** 1.2×10^7 DCs/mL, 4 doses **Schedule**: every 4 weeks (q4w)	**CTL ($CD28^+/CD45RA^-$):** 69% at week 8, 83% at week 18, 92% at week 26 (vs 25% placebo) **VL (week 11/12 ATI):** 4.39 vs 4.47 $\log_{10}$ HIV RNA (p=0.73) **HIV DNA:** no difference at weeks 8, 18, 28	28 weeks	[7]
ELISPOT, CFSE proliferation, ICS	HIV-1 Gag and Nef mRNA (in DCs)	**Human:** 15 HIV-1 adults (median age: 47 y), median CD4 count 556 cells/mm^3	**Vaccine:** DCs loaded with HIV-1 Gag/Nef mRNA **Packaging:** autologous DCs	**CD4 to Nef:** 2.3-fold ↑ from baseline (95% CI: 1.04–5.04); 6.3-fold ↑ vs placebo (95% CI: 1.6–24.6), p=0.009	48 weeks; Peak effect: week 14	[8]

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	Mod: Kozak sequence, codon-optimized, lysosomal targeting **Antigen:** Gag, Nef (Clade B, 2001) **Route:** ID **Dosage:** 5–15M DCs, 4 doses **Schedule**: weeks 0, 2, 6, 10	**CD4 to Gag:** 2.5-fold ↑ from baseline (95% CI: 1.17–5.21), p=0.054 **CD8 to Nef:** 4.8-fold ↑ vs placebo (95% CI: 0.9–24.7), p=0.062 **ELISPOT:** no ↑

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After the initial clinical trials for the Pfizer and Moderna vaccines [19,20], they were soon granted emergency use authorization (EUA) in the United States [21], as well as the respective authorization in countries across the globe. Approximately 70% of the global populationhas received a COVID-19 vaccine, with about 30% of the doses being mRNA vaccines [22]. Following the mass vaccination campaigns, several articles were published demonstrating the persistence of vaccine-derived mRNA or spike protein in recipients [10,11,23].

This article aims to summarize the current findings on the decay kinetics of nucleoside-modified mRNA and spike protein after injection with a COVID-19 mRNA vaccine. The question of vaccine artifact persistence has ramifications for vaccine safety and for the future use and regulation of the mRNA vaccine platform.

Methods

To identify records for the 16 types of bodily fluids identified in [24], we performed a literature search using the PUBMED search terms:

("[Name of bodily fluid or tissue/organ]" AND "spike protein") OR ("[Name of bodily fluid or tissue/organ]" AND "COVID-19 vaccine mRNA")

The search timeframe was kept general to allow shorter term records to be included, which may inform about the time course of mRNA degradation and spike protein expression.

We specified the following inclusion criteria.

(1) Article must use an analytical technique to show direct or proxy evidence for spike protein or vaccinal mRNA.
(2) Sample must be taken from a vaccinated individual.

We specified the following exclusion criteria.

(1) Spike protein is attributed to SARS-CoV-2 infection by the study authors.

For spike protein persistence in tissue and organs, we conducted a separate literature search focusing on autopsies and biopsies. Prior meta-analyses performed on autopsies of people plausibly deceased due to COVID-19 vaccines are available in the literature and provide a curated summary of the autopsy reports published up to the date of writing (17–19). For this literature search, we analyzed individual cases in the cited literature reviews on autopsy.

For the case of spike protein or RNA found in organs and tissues, we employed the following search terms:

("biopsy" AND "spike protein" AND "vaccine") OR ("biopsy" AND "mRNA" AND "vaccine").

The same inclusion and exclusion criteria described above for bodily fluids were applied to tissue and organ studies.

Results

Our search revealed 11 fitting results for studies testing the persistence of vaccine artifacts in bodily fluids (Table 2).

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Table 2. Vaccine artifacts found in bodily fluids. CD16⁺: Cluster of differentiation 16 positive; ddPCR: Droplet digital polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; EV: Extracellular vesicle; fM: Femtomolar; HR2: Heptad-repeat 2 (domain of spike protein); IHC: Immunohistochemistry; LC–MS: Liquid chromatography–mass spectrometry; LC–SACI–MS: Liquid chromatography–surface-activated chemical ionization–mass spectrometry; LN: Lymph node; LLOD: Lower limit of detection; LLOQ: Lower limit of quantification; LOD: Limit of detection; LoQ: Limit of quantification; PACVS: Post-acute COVID-19 vaccine syndrome; PB: Peripheral blood; qPCR: Quantitative polymerase chain reaction; RNA-seq: RNA sequencing; RT-qPCR: Reverse transcription quantitative PCR; SIMOA: Single molecule array; SM-102: Ionizable lipid in Moderna lipid nanoparticle formulation; SPEAR: Successive Proximity Extension Amplification Reaction; TEM: Transmission electron microscopy; WT: Wild-typeVaccine artifacts were detected in blood components up to 709 days post-vaccination (spike protein) [29], and breast milk for up to 7 days (mRNA) [33] after vaccination (Table 2). However, it should be noted that sequence-confirmed vaccinal spike protein fragments have been observed in blood for the duration of 187 days post-immunization [12]. Vaccine artifacts were also found in lymph nodes up to two months after injection [32], though this is expected due to normal immune processing. No reports were found documenting the vaccine artifacts in other bodily fluids.

Analytical Technique	Substance Found	Fluid/Tissue	Vaccine	Sensitivity	Specificity	Concentration Observed	Duration Since Last Vaccination	Max Duration Observed	Reference
RT-qPCR, IHC	mRNA	Axillary LN (lymph)	BNT162b2; mRNA-1273 (HR2)	LOD: BNT162b2: 6 ssRNA copies/reaction; mRNA-1273: 11 ssRNA copies/reaction	Vaccine-specific sequences; none detected in unvaccinated or uninjected tissues	~1–30 copies/ng total RNA (lymph); heart values not numerically reported	Detected primarily within 30 days	30 days	[25]
SIMOA, Flow cytometry, ELISA	Spike protein (free and total)	Plasma (adolescents/young adults)	BNT162b2; mRNA-1273	SIMOA LOD not stated	Free spike detected only in myocarditis cases; not in vaccinated controls	Free spike (myocarditis): 33.9 ± 22.4 pg/mL	Up to 24 days	24 days	[26]
Flow cytometry, LC-MS	S1 subunit, S2 subunit, Mutant S1 peptides	CD16⁺ monocytes (PB)	mRNA-1273, Janssen, AstraZeneca	>99% sort purity; high-resolution peptide matching	S1 only in symptomatic group ($p < 0.0007$); cytokines matched PASC	S1 CD16⁺ monocytes: 92% of symptomatic (11/12); not in 9/10 controls	Range: 38–245 days; mean: 105 days	245 days	[23]
LC–SACI–MS	Spike protein (vaccine-specific)	Blood (dried spot)	BNT162b2; mRNA-1273	Not reported	100% specificity: not found in unvaccinated controls	Detected in 50% of vaccinated subjects	69–187 days	187 days	[12]

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ddPCR	mRNA (intact and degraded)	Plasma	mRNA-1273 bivalent	LLOQ: 0.001 ng/mL	ddPCR primers specific to vaccine mRNA; no WT S-gene amplification	Peak: 0.529 ng/mL (731 copies/µL); Range (4h): 6.5–112 copies/µL; 37% had 0.001–0.01 ng/mL at day 14–15	0–15 days	15 days	(29)
LC-MS	SM-102 (ionizable lipid)	Plasma	mRNA-1273 bivalent	LLOQ: 0.01 ng/mL	$R^2 = 0.9852$ correlation to SM-102 concentration	Peak median: 3.22 ng/mL; Range (4h): 0.39–8.39 ng/mL; ≥0.12 ng/mL at day 7	0–7 days	7 days	[27]
RT-qPCR, EV isolation	mRNA	Breast milk	BNT162b2; mRNA-1273	Detection limit: 1 pg/mL	Trace mRNA in 3/11 participants	1.3–11.7 pg/mL	1 to 45 hours	45 hours	[28]
SPEAR	Spike protein	Plasma	BNT162b2; mRNA-1273	LLOD: 1.81 fM; LLOQ: 8.24 fM	Detected in 36% of controls; 43% of PACVS patients	Mean: 17 pM vs 10 fM (controls)	26 to 709 days	709 days	[29]
SPEAR	S1 subunit	Plasma	BNT162b2; mRNA-1273	LLOD: 5.64 fM	Detected in 32% of controls; 36% of PACVS patients	Mean: 60 pM vs 3 fM (controls)	26 to 709 days	709 days	[29]
qPCR (codon-optimized), TEM	mRNA	Plasma and blood	BNT162b2	LoQ: 10 copies/reaction (~5.26% CV); Efficiency 1.92–2.14; R^2: 0.991–0.999	No amplification of SARS-CoV-2 RNA or human genome	50–300 copies/µg input RNA (plasma)	1–15 days	15 days	[30]
RNA-seq	mRNA	Plasma	BNT162b2; mRNA-1273	Not stated	Unknown	Detected, not quantified	Up to 28 days	28 days	[31]
RT-qPCR	mRNA	LN germinal centers	BNT162b2	Not reported	BNT162b2-specific sequence targeted	Detected, not quantified	Up to 60 days	60 days	[32]
RT-qPCR	mRNA	Breast milk	BNT162b2	Not quantified; standard curve with ≥6 replicates	BNT162b2-specific sequence targeted	Max: 2 ng/mL in few samples	Up to 6 weeks post 2nd dose	7 days post 2nd dose	[33]

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Analytical Techniques

Several different analytical techniques were used to identify the presence of injection artifacts in bodily fluids, depending on the substance observed [34–38]. Spike protein in biological samples was identified using ELISA [26], SIMOA [11,26], Western blotting [11,30], mass spectrometry [10,12,23,27], immunohistochemistry [11,25], immunofluorescence [39], flow cytometry [26,40], and ELISpot [40,41].

Detection and quantification of vaccine-derived mRNA were achieved using a combination of RT-qPCR [10,25,32,33], droplet digital PCR (ddPCR) [11,27], RT-PCR [28,39], in situ hybridization [11,42,43], and branched DNA (bDNA) assays [11].

Detection of lipid nanoparticles (LNPs) was primarily accomplished through extracellular vesicle (EV) isolation via sequential centrifugation and electron microscopy [28].

Tissue/Organ Spike/mRNA Persistency

For vaccine artifacts found in biopsies or autopsies, our search revealed 6 results (Table 3).

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Table 3. Vaccine artifacts found in autopsies or tissue biopsies. 3'UTR: 3' untranslated region; ALN: axillary lymph node; AU: arbitrary units; bDNA: branched DNA assay; bp: base pairs; dpi: days post-infection; ELISA: enzyme-linked immunosorbent assay; ELISpot: enzyme-linked immunospot assay; FISH: fluorescence in situ hybridization; FNA: fine needle aspiration; H&E: hematoxylin and eosin; HAI: hemagglutination inhibition; IHC: immunohistochemistry; IDMS: isotope dilution mass spectrometry; IgG: immunoglobulin G; ISH: in situ hybridization; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LLOQ: lower limit of quantification; LN: lymph nodes; LOD: limit of detection; LV: left ventricle; qRT-PCR: quantitative reverse-transcription polymerase chain reaction; RNA-seq: RNA sequencing; RNAscope: RNA in situ hybridization; RT-qPCR: reverse-transcription quantitative polymerase chain reaction; RV: right ventricle; SIMOA: single molecule array; ssRNA: single-stranded RNA.

Analytical Technique	Substance Found	Tissue/Organ	Vaccine	Sensitivity	Concentration Observed / Signal	Duration Sampled	Max Duration Observed	Reference
RT-qPCR, IHC	mRNA	Axillary LN (ALN), LV, RV, mediastinal LN, liver, spleen	BNT162b2; mRNA-1273 (HR2 domain)	LOD: BNT162b2: 6 ssRNA copies/reaction; mRNA-1273: 11 ssRNA copies/reaction	~1–30 copies/ng total RNA (ALN); heart tissue values not explicitly reported	1–154 days	ALN: 26 days; LV: 12 days; RV: 19 days	[25]
IHC	Spike protein	Brain (frontal cortex, nucleus ruber, capillary endothelium, microglia, astrocytes); heart (LV, endothelial cells); vasculature (aorta, iliac, basal cerebral arteries)	BNT162b2; ChAdOx1 nCov-19	Not reported (described as “abundant deposits” in affected tissues)	Spike protein observed in brain, heart, and vessels; N protein absent	3 weeks post 3rd dose	3 weeks	[44]
IHC	Spike protein	Lungs	mRNA-1273	Not reported	Spike protein not detected	18 days (autopsy)	Negative at 18 days	[45]
FISH	mRNA	Liver	BNT162b2	Probes targeted spike mRNA sequence; cross-reactivity with viral mRNA possible	Qualitative detection of cytoplasmic mRNA in hepatocytes	12 days	12 days	[43]
IHC, ISH	Spike protein; mRNA	Cerebral arteries	BNT162b2; mRNA-1273	Spike: 43.8% (7/16 cases); mRNA: detected in 3/3 tested cases	Spike in vessel intima/smooth muscle (7/16); vaccine mRNA confirmed in 3 cases via FISH	Months after vaccination	Up to 17 months	[42]

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IHC	Spike protein	Liver parenchyma	mRNA-1273	Not reported	Qualitative detection of spike protein in liver tissue	5 weeks	5 weeks	[46]
RNA-seq	mRNA fragments	Right deltoid and quadriceps muscles	BNT162b2	75 bp vaccine-specific fragment aligned to spike mRNA	Qualitative detection of mRNA fragments	4 weeks	4 weeks	[47]

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Characterizing the Decay Curves

Experimental data exists for the presence of three vaccinal artefacts for various time points: spike protein, mRNA sequences and LNP. While traditional sequencing technologies typically cannot distinguish between modified n1-methyl-pseudouridinylated RNA and unmodified mRNA, recent advances in nanopore sequencing have made this differentiation possible [48].

A recent study [27] characterized the decay kinetics of modified mRNA and the ionizable lipids composing the LNP in 19 individuals. By day 28 post-vaccination, all subjects exhibited vaccinal mRNA concentrations <0.001ng/mL in blood plasma (Figure 1A). The study also tracked levels of SM-102, an ionizable lipid composing part of the LNP (Figure 1B). The same study reported, that in day 14-15 post-immunization 37% of recipients still had detectable mRNA. Notably, fragmented or non-intact mRNA was detectable for longer time periods than the intact mRNA [27] (Figure 1C).

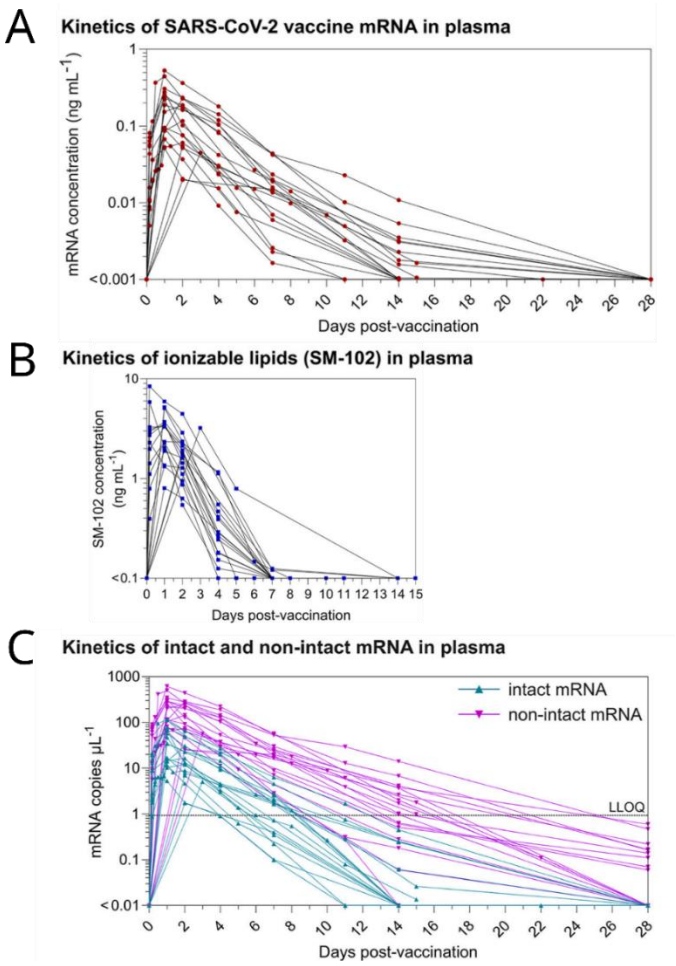

Figure 1. Decay curve of Vaccine mRNA and LNP. (A) Decay curve of SARS-CoV-2 vaccine mRNA in plasma. (B) Kinetics of ionizable lipids in plasma. (C) Kinetics of intact and non-intact vaccinal mRNA in plasma. Reproduced from [27] under a **CC BY 4.0 license** https://creativecommons.org/licenses/by/4.0/.

Li et al. [49] investigated the stability of various SARS-CoV-2 proteins in human lung epithelial cells and found that spike protein is relatively stable, with a half-life exceeding 8 hours. To our knowledge, to date, there are no published studies that measure the decay rate of vaccine-derived spike protein in a quantitative manner over time. However, mutations introduced into the vaccine-encoded spike protein are expected to confer enhanced biochemical stability [49]. It has been shown that vaccine spike protein is more resistant to heat stress and freeze-thaw cycles, persisting in the prefusion state up to 30 minutes, whereas the native viral spike protein aggregates or unfolds more

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rapidly under the same conditions [50]. It can thus be inferred that the have a half-life of the vaccinal spike protein likely exceeds that of the virus-derived spike protein.

Spike as a Potential Biomarker

In individuals with vaccine-associated myocarditis, spike protein has been detected at elevated concentrations for at least three weeks post-vaccination —the longest time point assessed in the study [26]. It appears to be the concentrations of full-length spike protein that differs between myocarditis patients and controls, as measurements for S1 subunit reveal smaller differences between controls and patients. Both Yonker et al. [26] (Figure 2A) and Bhattacharjee et al. [29] (Figure 2B) have identified increased levels of circulating spike in vaccine-associated myocarditis patients and Post-Acute COVID-19 Vaccination Syndrome (PACVS) patients, respectively, when compared to healthy controls. These findings potentially support the use of spike protein levels as a diagnostic biomarker for PACVS and other vaccine-related adverse events. \

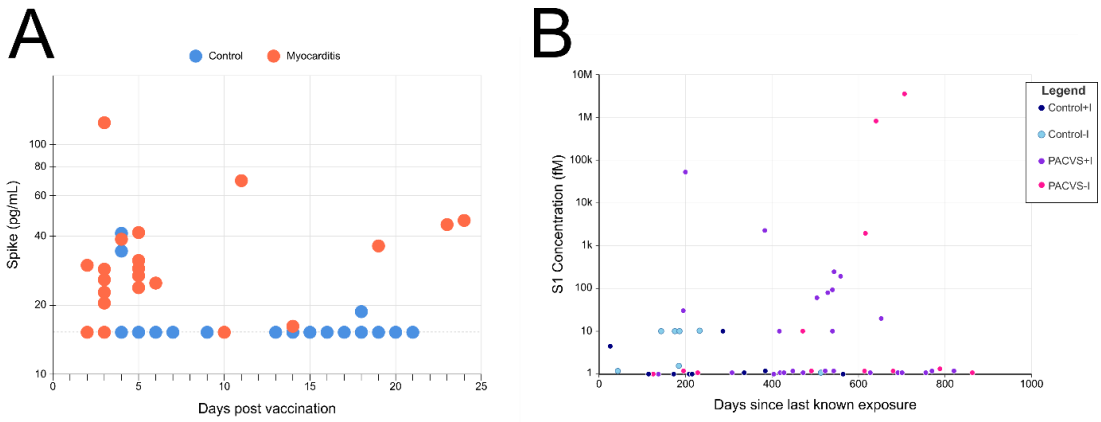

Figure 2. Spike protein concentrations differ between vaccine-injured and healthy vaccine recipients (A) Plasma spike protein concentrations (pg/mL) measured at various days post-vaccination in individuals with vaccine-associated myocarditis (red) and healthy controls (blue), as reported by Yonker et al. [26]. Myocarditis patients exhibited elevated spike levels compared to controls. (B) S1 subunit concentrations (nM) in plasma over time following vaccination, comparing Post-Acute COVID-19 Vaccination Syndrome (PACVS) patients (magenta, purple) and healthy controls (light and dark blue), as reported by Bhattacharjee et al. [29]. + I and – I refer to individuals with a previous Covid-19 infection and individuals without a previous Covid-19 infection, respectively.

While the authors of the above studies generally attribute the presence of the vaccinal artefacts to vaccination, for the case of the spike protein, this can be produced either by infection or injection. The vaccinal spike can be differentiated by testing for vaccine specific sequences [12], which provides unequivocal evidence of the source being the vaccine. A less definitive means of testing for the source of the spike is by testing for the presence of the nucleocapsid (N) protein, which accompanies infection. However, it has been previously shown that the spike protein is one of the more stable SARS-CoV-2 proteins while the nucleocapsid protein can degrade rapidly [51]. Until definitive studies comparing the rate of degradation of spike and nucleocapsid proteins in different tissues are demonstrated, absence of evidence of the nucleocapsid protein cannot be used definitively as evidence of absence of viral infection.

Persistent spike protein and its genetic code may be of concern, as in vitro and in vivo studies support several pathological mechanisms linking it to myocarditis and other adverse events [52–55]. In the case of myocarditis, in vitro studies have shown spike protein inducing damage to cardiac pericytes [56]. In addition to myocarditis [57] and other acute adverse events [58], chronic effects exist as well [59]. One novel condition, termed Post-Acute Vaccination Syndrome (PACVS, also referred to as Post-Vaccination Syndrome; PVS) [60,61], manifests similarly to Post-Acute Covid-19 Syndrome

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(PACS; "long COVID") or Myalgic Encephalomyelitis/ or Chronic fatigue Syndrome (ME/CFS) [59]. PACVS is characterized by exercise intolerance, fatigue and brain fog as major symptoms, though individual presentation differs significantly [62].

In the case of PACS, persisting viral fragments [63], including spike protein [64], may be a driver of the condition. Spike protein may induce PACVS via multiple mechanisms [52]. Should the spike protein be a major driver of disease in PACS and/or PACVS, it is important to investigate mechanisms for its inactivation [65] and removal [52,66], along with addressing any lingering damage [22].

Proposed Persistence Mechanisms

Two foundational assumptions were required to hold for safe and limited biodistribution. Firstly, it was claimed that vaccination contents remained at the site of injection [67]. The findings of systemic biodistribution dispute this claim (Table 2 and 3). Secondly, the claim that the spike protein was short lasting [67], requires additional analysis.

The injection volume of 0.5mL for adults, containing 100ug (Moderna) or 30ug (Pfizer) of n1-methyl-pseudouridinylated RNA, of length 3828nt [68]and 4284nt [69], respectively corresponds to roughly $5x10^{13}$ and $2x10^{13}$ copies of the RNA template respectively. The standard RNA half-life in human blood is 16.4 hours [70], in n1-methyl-pseudouridinylated RNA, this value is 5-6 days [15]. The time to degrade the RNA to <1 copy would be 272 days and 263 days for Pfizer and Moderna, respectively. The slower decay kinetics may partially explain the persistence of spike protein in vaccinees, which has been observed at durations as extreme as 709 days post-vaccination [29], with other studies showing extended durations [23].

To this day, it remains an open question how long n1-methyl-pseudouridinylated RNA lasts in human systems [15], and what factors influence its degradation. The durations observed in Tables 2 and 3 suggest that the vaccinal artifacts resist degradation at durations of months to possibly even years. This finding motivates the question of how the vaccine artifacts are persisting. Brogna et al. hypothesized three possible mechanisms for vaccine artifact persistence from a modified RNA template [12] (Figure 3). The first mechanism is integration of modified mRNA into human cells [10,12]; this has not been observed directly in the scientific literature. However, the recently discovered reverse transcriptase activity of polymerase theta (Polθ) [71,72], and LINE-1 elements [73] theoretically provides a potential mechanism. Recent reports of contamination by bacterial DNA [13,14,74,75] now pose another potential reservoir of spike protein encoding genetic material.

The second mechanism proposed is persistence of the modified mRNA template itself. As mentioned previously, the decay curve of n1-methyl pseudouridinylated RNA is not well described [15], with modest experimental studies beginning in 2015 [15]. Furthermore, very limited biological precedent is available for comparison as the n1-methyl pseudouridine occurs rarely in nature and only at specific sites in tRNA and rRNA [76], i.e. it is present in natural systems at far lower concentrations than would be present in a modified mRNA injection. Until global mass immunization with COVID-19 mRNA vaccines, presence of n1-methyl pseudouridine in humans have never been described.

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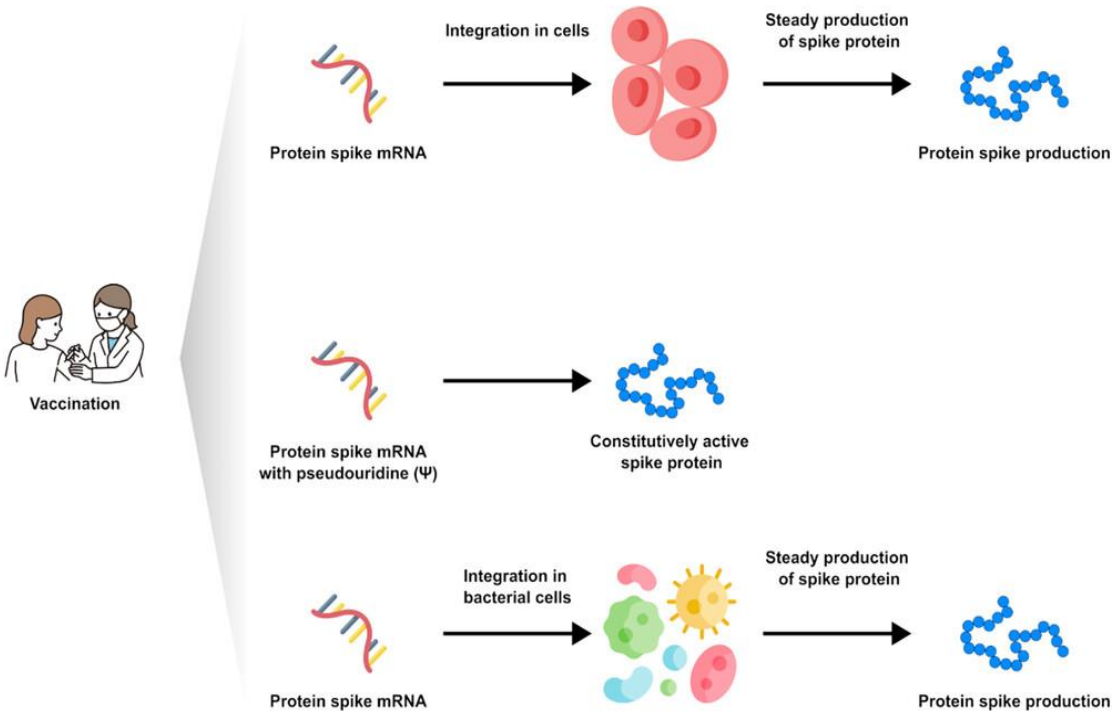

Figure 3. Possible Mechanisms of Spike Protein Persistence in Covid-19 mRNA Vaccination. Reproduced from [12] under an Attribution-NonCommercial-NoDerivatives 4.0 International license.

The third mechanism proposed is integration of the RNA vaccine genetic code into bacterial cells [12]. This may proceed via the endogenous reverse transcription enzymes of the bacteria themselves [77]. An additional possibility is incorporation of bacterial plasmic DNA, present as a contaminant in mRNA vaccines [13], into bacterial cells, serving as a stable reservoir and producing spike protein.

Finally, it is conceivable that spike protein itself could be taken up by certain cells and retained for extended duration depending on co-interaction with other present biological components or the degradation efficiency of a specific cell type. Some evidence exists that spike protein, particularly S1 subunit can persists within non-classical monocytes for extended periods post infection or vaccination [23,29,78].

Regardless of the mechanism of persistence, inadvertent biodistribution and chronic expression of spike protein presents challenges for mRNA technology, which relies on the same delivery mechanism (lipid nanoparticle formulations) and template type (n1-methyl-pseudouridinylated RNA). Unless these issues are fixed, future vaccines using the same technology will have unexpected spillage to other organs and tissues besides the target injection site and lymph nodes. Given that intravenous injection of the vaccine contents can result in myocarditis in a mouse model [79], inadvertent biodistribution presents a safety concern.

Discussion

The use of mRNA technology in humans marks a significant advancement in biomedical science, yet it also introduces a new frontier laden with uncertainty. The persistence and systemic distribution of vaccine components, namely spike protein, modified mRNA, and lipid nanoparticles have defied early expectations that such materials would degrade within days. Instead, studies have found these components in blood, tissues, and other biological fluids months and even years post-injection (Tables 2 and 3). This unexpected longevity raises critical questions about degradation pathways, tissue-specific retention, and immune system interactions with these synthetic biomolecules. The foundational assumptions of rapid decay and localized distribution have not consistently held, suggesting that we still lack a complete understanding of how nucleoside-modified mRNA vaccines

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behave in human systems. These results prompt re-evaluation of the kinetic models of the clearance of these molecules.

A deeper investigation into the mechanisms of action, persistence, and biodistribution of mRNA vaccine components is urgently needed. Further research questions should cover individual variation for degradation of these molecules, which will provide insights into the regulation of the decay pathways. To alleviate these concerns, vaccine delivery systems require improved localization that ensures complete degradation of the genetic material, and verifying absence of off-target effects. Equally important is the continued optimization of translation fidelity [80], and enhancing purification protocols to eliminate residual bacterial DNA contaminants [13] from in vitro–transcribed mRNA to ensure that translation products derive exclusively as intended from the synthetic transcripts. Addressing these gaps will be essential for the safe and effective development of future mRNA-based therapies.

Despite the current challenges, mRNA technology holds immense potential for gene therapy, rapid vaccine development, and treatment of chronic diseases [81,82]. With rigorous research, transparent evaluation, and engineering improvements, this novel platform can be refined into a transformative tool for human health. Moving forward, solving these issues is not only vital for public trust but also for unlocking the full therapeutic potential of mRNA technologies for the benefit of global health [83,84].

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