

1 SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination

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19 **Summary**

20 Samaniego Castruita JA, Schneider UV, Mollerup S, Leineweber TD, Weis N, Bukh J, Pedersen MS, Westh H. SARS-  
21 CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination

22 In Denmark, vaccination against Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) has been with the  
23 Pfizer-BioNTech (BTN162b2) or the Moderna (mRNA-1273) mRNA vaccines. Patients with chronic hepatitis C virus  
24 (HCV) infection followed in our clinic received mRNA vaccinations according to the Danish roll-out vaccination plan.  
25 To monitor HCV infection, RNA was extracted from patient plasma and RNA sequencing was performed on the  
26 Illumina platform. In 10 of 108 HCV patient samples, full-length or traces of SARS-CoV-2 spike mRNA vaccine  
27 sequences were found in blood up to 28 days after COVID-19 vaccination. Detection of mRNA vaccine sequences in  
28 blood after vaccination adds important knowledge regarding this technology and should lead to further research into  
29 the design of lipid-nanoparticles and the half-life of these and mRNA vaccines in humans.

30 Keywords: SARS-CoV-2, vaccine, blood, mRNA

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34 **Introduction**

35 With the emergency approval by the FDA of two mRNA vaccines in December 2020, and subsequently very large-  
36 scale vaccine production and mass immunization programs, a breakthrough in protective measures against the  
37 global pandemic with Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was achieved. Both the  
38 Pfizer-BioNTech (BTN162b2) and the Moderna (mRNA-1273) vaccines code for production of the full-length SARS-  
39 CoV-2 spike protein. To ensure stability, these vaccines are composed of codon-optimized modified spike mRNA,  
40 have two stabilizing proline substitutions, and the mRNA is encapsulated in lipid nanoparticles (LNPs).<sup>1,2</sup> The  
41 modified nucleotide sequences allow perfect identification of the vaccine sequences as being different from any  
42 coronavirus sequence. Upon intramuscular injection, the vaccine mRNA is taken up by muscle and immune cells, and  
43 transported to the regional lymph nodes and concentrated in the spleen.<sup>3</sup> The vaccines consist of nonreplicating  
44 mRNA and are expected to naturally decompose both within the cytosol after translation and at the injection site.  
45 The half-life of mRNA translation is estimated to be short, from hours to a day and translation is described to span  
46 up to 10 days.<sup>4-6</sup> The Infectious Diseases Society of America (IDSA) informs that the vaccine mRNA is degraded  
47 quickly by normal intracellular processes and states that there is no evidence for long-term detection of mRNA  
48 vaccines in vaccinated individuals by RNA-seq.<sup>1,7</sup>

49 In Denmark, the predominant vaccines against Corona Virus Disease 2019 (COVID-19) have been the two mRNA-  
50 based vaccines from Pfizer-BioNTech and Moderna. As of 28 July 2022, 80.2% of the population has received two  
51 doses and 81.6% at least one dose.<sup>8</sup> At Copenhagen University Hospital, Amager-Hvidovre, Denmark, patients with  
52 chronic hepatitis C virus (HCV) infection are routinely followed at Department of Infectious Diseases, while  
53 measurement of HCV viral load and genotyping by whole RNA-Seq genome sequencing of their HCV RNA, directly  
54 from plasma samples, are performed at Department of Clinical Microbiology.<sup>9</sup> In this paper, we describe the  
55 unexpected finding of SARS-CoV-2 vaccine mRNA sequences in plasma from 10 HCV patient samples up to 28 days  
56 after COVID-19 vaccination. These patients had recently received SARS-CoV-2 mRNA vaccinations according to the  
57 Danish roll-out vaccination plan.

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## 60 **Methods**

61 We analyzed 5 consecutive sequencing runs, from May 2021 to the end of June 2021, with 108 HCV patients, 5  
62 negative controls and 5 HCV positive controls consisting of HCV grown in cell culture.<sup>10</sup> Samples were from patients  
63 that were HCV positive and came for HCV treatment evaluation with no relation to their vaccination characteristics.  
64 The direct RNA sequencing (RNA-seq) procedure has been described previously.<sup>11</sup> Briefly, RNA was extracted with  
65 the ZR viral RNA kit (Zymo Research) and depleted for human rRNA with the NEBNext rRNA depletion kit (New  
66 England BioLabs). Stranded RNA-seq libraries were prepared with the NEBNext Ultra II directional RNA library prep  
67 kit (New England BioLabs). Sequencing was performed with 2x150-bp paired-end reads on a NextSeq instrument  
68 (Illumina).

69 All software was used with default parameters unless specified. Raw reads were trimmed for low-quality bases with  
70 fastp v 0.20.1 with minimum phred quality of 20 and minimum length of 50.<sup>12</sup> Human depletion was done by  
71 mapping the trimmed reads to the human genome hg38 (GenBank accession no. GCA\_000001405.27) with Bowtie2  
72 v 2.3.4.1 adding the parameters -k 1 -X 2000.<sup>13</sup> Paired reads unmapped to human genome sequences were *de novo*  
73 assembled with VICUNA v 1.3 using minimum contig links of 2 and minimum identify of 90.<sup>14</sup> As part of our pipeline,  
74 contigs above 1,000 bp are aligned against NCBI's nt database using BLASTn v2.8.1+ with a minimum value threshold  
75 of 1e-22.<sup>15</sup> Contigs that were found to align to coronavirus sequences were subsequently aligned against two  
76 assemblies of SARS-CoV2-spike-encoding mRNA vaccines, BNT-162b2 and mRNA-1273 using BLASTn for  
77 confirmation.<sup>16</sup> Paired reads were also mapped against the two vaccine assemblies using BWA-mem v 0.7.16 and  
78 SAMtools v 1.2 inside the NASP pipeline v 1.1.2.<sup>17-20</sup> Stats of number of reads mapping and coverage were calculated  
79 only for the SARS-CoV-2 spike gene coding region avoiding the 5' and 3' UTR as these regions share high percentage  
80 of similarity with human gene regions. Coverage was calculated with BEDTools v 2.30.0 and plotted in R v 3.6.1 with  
81 ggplot2.<sup>21-23</sup> SNP calls were generated with HaplotypeCaller from GATK v 4.2.0.0 and filtered by heterozygous  
82 genotype, mapping quality of <30 and symmetric odds ratio of >3.<sup>24</sup> Consensus sequences were made in GATK and  
83 bases covered with less than 10 reads were masked with BEDTools.

84 The best (highest coverage) spike sequence for the Pfizer-BioNTech and similarly the two best spike sequences for  
85 the Moderna vaccine have been uploaded to NCBI (GenBank accession numbers OK120840-OK120842). Gaps in the

86 sequences are represented by Ns. Approval by an institutional review board was not required, as this study was  
87 performed as an operational activity related to patient management.

## 88 **Results**

89 *De novo* assembly of human-depleted RNA-seq reads from two patient samples produced contigs of >1,000 nt with  
90 closest homology to bat coronavirus and 67% homology to the SARS-CoV-2 reference genome (NC\_045512.2).  
91 Translation of the contig nucleotide sequences to amino acid sequences revealed 100% identity to parts of the spike  
92 protein of SARS-CoV-2. From the literature we found the sequences of the two commercial SARS-CoV-2 mRNA  
93 vaccines which were used as references to map reads from all the samples.<sup>16,25</sup> This led to the identification of an  
94 additional eight samples with reads that matched the mRNA vaccine sequences. Both mRNA vaccine sequences have  
95 been modified and are only ~70% identical to the spike reference genome on a nucleotide level, making them  
96 distinct from circulating infectious SARS-CoV-2 sequences. Thus, of the 108 patient samples, 10 samples (9.3%) had  
97 partial or up to full sequences of the vaccine mRNA sequence (Figure 1), identified from one to 28 days post-  
98 vaccination. There was ~100% identity between the detected mRNA nucleotide sequences found in plasma and the  
99 specific mRNA vaccine given. The 10 samples had a median of 5.5 million raw read pairs available (see  
00 Supplementary Table 1). Breadth and depth of coverage of the vaccine mRNA sequences ranged from completeness  
01 and >20,000, respectively, to short fragments with a depth of coverage of 100 (Figure 1). None of the negative or the  
02 HCV positive controls had SARS-CoV-2 matching reads.

## 03 **Discussion**

04 We surprisingly found fragments of COVID-19 vaccine mRNA up to 28 days post-vaccination in blood from chronic  
05 HCV patients vaccinated with mRNA vaccines from both Pfizer-BioNTech and Moderna.

06 Analysis of mRNA vaccine function has focused on the immune response and on protection of vaccinated individuals  
07 from SARS-CoV-2 induced severe COVID-19.<sup>26,27</sup> The LNPs have been reported to be rapidly cleared by immune cells  
08 and mRNA is degraded by exonucleases in tissue and blood.<sup>28-30</sup> A recent study did not detect the vaccine mRNA by  
09 quantitative PCR in human milk after 4-48 hours post dose 1 or 2 with BNT162b2 or mRNA-1273.<sup>31</sup>

10 RNA-seq is widely used in biological and medical research. We routinely use direct total RNA-seq to obtain full length  
11 HCV RNA sequences and from these infer their genotype. Our pipeline includes taxonomic analysis of contigs of

12 more than 1,000 nt which led to the discovery of coronavirus sequences that when first blasted had closest identity  
13 to a bat coronavirus.

14 We expect that vaccine mRNA detected in plasma is contained within LNPs and that the LNPs in plasma have been  
15 slowly released from the injection site either directly to the blood or through the lymph system. Without the LNPs  
16 protecting the mRNA, the mRNA would rapidly degrade. This allows prolonged spike protein production giving an  
17 advantage for a continuous immune response in some persons. Current studies on half-life of mRNA vaccines could  
18 have underestimated the half-life of the LNPs, primarily using results from half-life of studies of mRNA in the cytosol  
19 of human cells.

20 In samples where we observed only fragments of vaccine mRNA, this could indicate that the concentration of LNPs in  
21 plasma is low. This is in accordance with our findings that HCV viral load should be higher than 10,000 IU/ml to  
22 obtain a full-length HCV RNA genome (size ~9,600 nt) as genome coverage correlates with viral load. The number of  
23 SARS-CoV-2 vaccine mRNA molecules is therefore likely lower than ~4,000 IU/ml, explaining the partial read  
24 coverage randomly spread over the coronavirus vaccine sequence.

25 To our knowledge, our study is the first to detect Pfizer-BioNTech and Moderna COVID-19 mRNA vaccine sequences  
26 in blood after vaccination, and therefore provides new knowledge regarding the timeframe in which the mRNA can  
27 be detected. This study examined a cohort of HCV positive patients with presumed functional immune systems, as  
28 most of the patients can be cured, with negative HCV RNA 12 weeks after end of treatment with direct acting  
29 antivirals. A future prospective study to establish the half-life of mRNA vaccines in vaccine recipients could be  
30 performed using mRNA vaccine specific PCRs. These findings are interesting and should lead to further research into  
31 the design of LNPs and the half-life of LNPs and mRNA vaccines, but it should be emphasized that our data does not  
32 in any way change the conclusion that both mRNA vaccines are safe and effective.

### 33 **Author contributions**

34 JASC, HW wrote the initial draft with substantial input from SM, MSP, UVS, TDL, NW, JB. JASC, HW designed the  
35 study. JASC, UVS, SM, TDL, NW, JB, MSP, HW performed data collection and/or analyses. All authors read and  
36 approved the final manuscript.

### 37 **Conflict of interest**

38 Authors have no conflicts of interest to declare.

39 **Data availability statement**

40 The raw datasets presented in this article are not readily available because they contain identifiable human genome  
41 sequences. Requests to access the datasets should be directed to HW (henrik.torkil.westh@regionh.dk). Spike  
42 sequences have been uploaded to NCBI (GenBank accession numbers OK120840-OK120842).

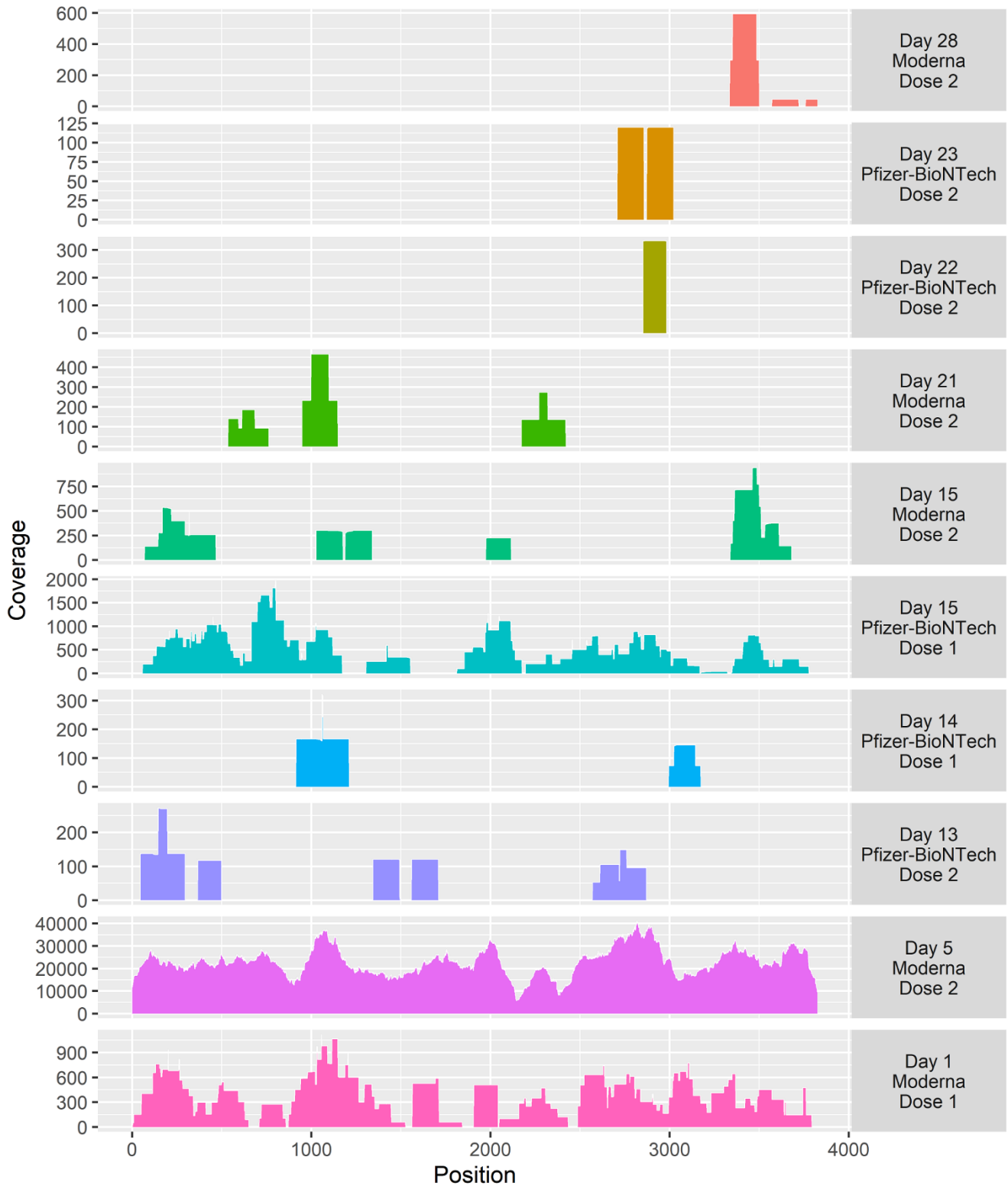
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15 Figure 1. Mapping of trimmed and filtered reads to the coding regions of the spike protein of the Moderna mRNA vaccine or  
 16 Pfizer BioNTech vaccine. Each colored plot is a single sample. The right-hand column shows the day post-vaccination, the vaccine  
 17 given and found in blood and if sample was drawn after 1. vaccination or revaccination. The two bottom samples are from the  
 18 same patient after first and second vaccination with the Moderna vaccine.

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Sample	Vaccine	Raw pair reads	Trimmed pair reads	Nonhuman pair reads	Nonhuman pair reads / Trimmed pair reads	Mapped reads	Mapped reads	Average coverage	Bp covered with 1X	Genome covered with 1X
Day 28 Moderna Dose 2	moderna	5545030	5374136	1016858	18.92%	686	0.03%	26.0993	390	10.19%
Day 23 Pfizer-BioNTech Dose 2	pfizer	6312127	5951246	2430654	40.84%	240	0.00%	9.44967	302	7.90%
Day 22 Pfizer-BioNTech Dose 2	pfizer	5426946	4990105	1048564	21.01%	334	0.02%	11.5218	133	3.48%
Day 21 Moderna Dose 2	moderna	1079678	981613	573806	58.46%	974	0.08%	37.2079	678	17.71%
Day 15 Moderna Dose 2	moderna	20935257	19715636	953070	4.83%	2726	0.14%	106.323	1188	31.03%
Day 15 Pfizer-BioNTech Dose 1	pfizer	3127521	2921033	181365	6.21%	12164	3.35%	466.023	3300	86.27%
Day 14 Pfizer-BioNTech Dose 1	pfizer	10003668	9312132	2388342	25.65%	480	0.01%	18.8894	477	12.47%
Day 13 Pfizer-BioNTech Dose 2	pfizer	725726	640949	261748	40.84%	838	0.16%	32.3318	988	25.83%
Day 5 Moderna Dose 2	moderna	10927169	10506627	531142	5.06%	607615	57.18%	23028.5	3828	100%
Day 1 Moderna Dose 1	moderna	3323201	3197689	278490	8.71%	10064	1.81%	370.845	3566	93.16%

20

21 Supplementary Table 1. Sequencing and mapping report of the ten patient samples. Mapping and coverage statistics are calculated for only the SARS-CoV-2 spike gene coding region of the  
22 vaccine.

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