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## SciScore Report

Below you will find your SciScore report containing three tables. Your score is calculated based on adherence to scientific rigor criteria (Table 1) and identification of key biological resources (Table 2). Table 3 contains statistical tests and oligonucleotides but is not scored. If SciScore makes any mistakes, please [contact us](#) to help us learn and improve.

**Table 1: Rigor Adherence Table**

<u>Ethics</u>
IRB: The CORSIP study received approvals from the University of British Columbia (H20-03620) and the University of Toronto Health Research Ethics Boards (40435).
Consent: The paramedics provided written consent upon enrolment through an internet-based portal.
<u>Inclusion and Exclusion Criteria</u>
Participants who had received any non-mRNA vaccines were excluded.
<u>Attrition</u>
not detected.
<u>Sex as a biological variable</u>
not detected.
<u>Subject Demographics</u>
Age: not detected.
Weight: not detected.
<u>Randomization</u>
not detected.
<u>Blinding</u>
not detected.
<u>Power Analysis</u>
not detected.
<u>Replication</u>
not required.

**Table 2: Key Resources Table**

Your Sentences	REAGENT or RESOURCE	SOURCE	IDENTIFIER
<u>Antibodies</u>			
SARS-CoV-2 infection was defined as: (1) a participant-reported positive SARS-CoV-2 nucleic acid amplification test (NAAT) or rapid antigen test (RAT) result; or (2) the presence of anti-nucleocapsid antibodies in the blood sample (Roche, IND, USA).	anti-nucleocapsid		
Serological testing We tested all blood samples with: (1) the V-PLEX SARS-CoV-2 Panel 28 ACE2 Kit (Meso Scale Discovery, MD, USA) to measure percent inhibition of angiotensin-converting enzyme 2 (ACE-2) binding to spike protein of Omicron (B.1.617.2) BA.4/BA.5 sub-lineages; and, (2) the Elecsys Anti-SARS-CoV-2 Nucleocapsid assay [Roche IND, USA] to identify the presence of anti-nucleocapsid protein antibodies, indicative of preceding SARS-CoV-2 infection.	ACE2		
	Anti-SARS-CoV-2		
	anti-nucleocapsid protein		
<u>Software and Algorithms</u>			
We used GraphPad Prism version 9.5.0 (GraphPad Software, San Diego, CA) and SAS version 9.4 for data analyses.	GraphPad Prism		Suggestion: (GraphPad Prism, RRID:SCR_002798)( <a href="#">link</a> )
	GraphPad		Suggestion: (GraphPad Prism, RRID:SCR_002798)( <a href="#">link</a> )

## Other Entities Detected

Your Sentences	Recognized Entity
Statistical Tests	
We diagramed ACE-2 inhibition to BA.4/5 for the paired comparisons using scatter plots and used the Wilcoxon matched-pairs signed rank test to test the differences between the median ACE-2 inhibitions to BA.4/5 of the groups.	Wilcoxon matched-pairs signed rank test
In addition, we used the Bonferroni correction test to adjust p-values obtained from the Wilcoxon matched-pairs signed rank test in order to account for multiple comparisons of outcomes.	Bonferroni

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## Materials Design Analysis Reporting (MDAR) Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: [doi:10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). The MDAR checklist is a tool for authors, editors and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.

## Materials

<b>Antibodies</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
For commercial reagents, provide supplier name, catalogue number and RRID, if available	Yes, 4 antibodies detected, 0 RRID provided : anti-nucleocapsid : ACE2 : Anti-SARS-CoV-2 : anti-nucleocapsid protein Please add identifiers for all resources where possible	
<b>Cell Materials</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
<b>Cell lines:</b> Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID	No cell lines detected Please add identifiers for all resources where possible	
<b>Primary cultures:</b> Provide species, strain, sex of origin, genetic modification status.	Not currently checked by SciScore	
<b>Experimental Animals</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
<b>Laboratory animals:</b> Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID	No organisms detected Please add identifiers for all resources where possible	
<b>Animal observed in or captured from the field:</b> Provide species, sex and age where possible	Not currently checked by SciScore	
<b>Model organisms:</b> Provide Accession number in repository (where relevant) OR RRID	See laboratory animals section for information.	
<b>Plants and microbes</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
<b>Plants:</b> provide species and strain, unique accession number if available, and source (including location for collected wild specimens)	Not currently checked by SciScore	
<b>Microbes:</b> provide species and strain, unique accession number if available, and source	Not currently checked by SciScore	
<b>Human research participants</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
Identify authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	The CORSIP study received approvals from the University of British Columbia (H20-03620) and the University of Toronto Health Research Ethics Boards (40435).	
Provide statement confirming informed consent obtained from study participants.	The paramedics provided written consent upon enrolment through an internet-based portal.	
Report on age and sex for all study participants.	<b>Age:</b> not detected. <b>Sex:</b> not detected.	

## Design

<b>Study protocol</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
For clinical trials, provide the trial registration number OR cite DOI in manuscript.	Not detected.	
<b>Laboratory protocol</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
Provide DOI or other citation details if detailed step-by-step protocols are available.	Not detected.	
<b>Experimental study design (statistics details)</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
State whether and how the following have been done, or if they were not carried out		
Sample size determination	not detected.	
Randomization	not detected.	
Blinding	not detected.	
inclusion/exclusion criteria	Participants who had received any non-mRNA vaccines were excluded.	
<b>Sample definition and in-laboratory replication</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
State number of times the experiment was replicated in laboratory	Not detected.	
Define whether data describe technical or biological replicates	Not detected.	
<b>Ethics</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	The CORSIP study received approvals from the University of British Columbia (H20-03620) and the University of Toronto Health Research Ethics Boards (40435).	
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Not detected.	
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	Not detected.	
<b>Dual Use Research of Concern (DURC)</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
If study is subject to dual use research of concern, state the authority granting approval and reference number for the regulatory approval	Not currently checked by SciScore	

## Analysis

<b>Attrition</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
State if sample or data point from the analysis is excluded, and whether the criteria for exclusion were determined and specified in advance.	not detected.	

<b>Statistics</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
Describe statistical tests used and justify choice of tests.	We diagramed ACE-2 inhibition to BA.4/5 for the paired comparisons using scatter plots and used the Wilcoxon matched-pairs signed rank test to test the differences between the median ACE-2 inhibitions to BA.4/5 of the groups.	

<b>Data availability</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
State whether newly created datasets are available, including protocols for access or restriction on access.	Not detected.	
If data are publicly available, provide accession number in repository or DOI or URL.	Not detected.	
If publicly available data are reused, provide accession number in repository or DOI or URL, where possible.	Not detected.	

<b>Code availability</b>	<b>Yes (indicate where provided: page no/section/legend)</b>	<b>n/a</b>
For all newly generated code and software essential for replicating the main findings of the study:		
State whether the code or software is available.	Not detected.	
If code is publicly available, provide accession number in repository, or DOI or URL.	Not detected.	



## Analysis

Adherence to community standards	Yes (indicate where provided: page no/section/legend)	n/a
MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.		
State if relevant guidelines (eg., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (eg., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.	Not currently checked by SciScore	

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*By Michael Asamoah-Boaheng*

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**Access Microbiology**

**Immunogenicity of bivalent versus monovalent mRNA booster vaccination among adult paramedics in Canada who had received three prior mRNA wild-type doses**

--Manuscript Draft--

CONFIDENTIAL

1           **Immunogenicity of bivalent versus monovalent mRNA booster vaccination among adult**  
2           **paramedics in Canada who had received three prior mRNA wild-type doses**

3

4

5           <sup>45</sup> **Michael Asamoah-Boaheng<sup>1,2,4,5\*</sup>, David M. Goldfarb<sup>3,4</sup>, Iryna Kayda<sup>4</sup>, Justin Yap<sup>5,6</sup>, Tracy**  
6           **Kirkham<sup>7,8</sup>, Mohammad Ehsanul Karim<sup>2,9</sup>, Paul Demers<sup>7,8</sup>, Jeffrey M. Copp<sup>10</sup>, Brian Grunau**  
7           **1,2,4,5**

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33

## Abstract

34 **Introduction:** Comparative immunogenicity from different mRNA booster vaccines (directed at  
35 wild-type [WT], BA.1, or BA.4/5 antigens) remains unclear.

36 **Methods:** We included blood samples from adult paramedics who received three mRNA WT-  
37 directed vaccines plus a fourth dose of: (1) WT monovalent; (2) Moderna BA.1-WT bivalent; or  
38 (3) Pfizer BA.4/5-WT bivalent vaccine. The primary outcome was angiotensin-converting  
39 enzyme 2 (ACE-2) inhibition to BA.4/5 antigen. We used optimal pair matching (using age, sex-  
40 at-birth, preceding SARS-CoV-2 infection, and fourth vaccine-to-blood collection interval) to  
41 create balanced groups to individually compare each vaccine type to each other vaccine  
42 (overall, within subgroups defined by SARS-CoV-2 infection, and after combining BA.1 and  
43 BA.4/5 cases). We compared outcomes with Wilcoxon matched pairs signed rank test.

44 **Results:** Overall, 158 paramedics (mean age 45 years) were included. ACE-2 inhibition was  
45 higher for BA.1 compared to WT ( $p=0.002$ ); however, no difference was detected between  
46 BA.4/5 vs. WT, or BA.1 vs BA.4/5. Among cases with preceding SARS-CoV-2, there were no  
47 between-group differences. Among cases without preceding SARS-CoV-2, the only difference  
48 was BA.1 > WT ( $p=0.003$ ). BA.1 and BA.4/5 cases combined had higher ACE2 inhibition than WT  
49 ( $p = 0.003$ ).

50 **Conclusion:** Omicron-directed vaccines appear to improve Omicron-specific immunogenicity;  
51 however, this appears limited to SARS-CoV-2-naïve individuals.

52 **Keywords:** mRNA COVID-19 vaccines; Bivalent mRNA vaccines; SARS-CoV-2 Omicron infection;  
53 BA.4/5 Omicron subvariant; immunogenicity.

54 Word count (abstract): **200**

55 Word count (main): **1933**

56

## 57 Introduction

58 <sup>7</sup> The emergence of SARS-CoV-2 variants, including the Omicron sublineages, has led to questions  
59 regarding optimal ongoing vaccination strategies. Recent studies have documented reduced  
60 vaccine effectiveness of two-dose mRNA vaccination (including original Wuhan Hu 1 platform  
61 <sup>3</sup> mRNA-1273 and BNT162b2) against Omicron BA.1 infection, compared with earlier variants;<sup>1</sup>  
62 immunogenicity appears to improve after a third dose but subsequently wanes quickly  
63 thereafter<sup>1-6</sup>. While vaccination with three to four of the original mRNA vaccines remains highly  
64 effective against hospitalization for all variants, effectiveness <sup>43</sup> 14-30 days post fourth dose  
65 against COVID-19 is only moderate for <sup>24</sup> the BA.2, BA.2.12.1, and BA.4 strains, but low against  
66 BA.5, with benefit appearing to disappear after 90 days.<sup>3</sup>

67 To align vaccines with the current SARS-CoV-2 variants, vaccines directed at Omicron strains  
68 were developed<sup>7,8</sup>. The approved bivalent mRNA booster formulations contain both the Wuhan  
69 <sup>6</sup> Hu-1 (wild-type) SARS-CoV-2 and Omicron (B.1.1.529) variant spike sequences. However, few  
70 <sup>32</sup> studies have compared the effectiveness of bivalent booster doses against the wild-type mRNA  
71 monovalent vaccines. Further, the comparative immunogenicity conveyed from different mRNA  
72 booster vaccines (specifically wild-type vaccine vs. Moderna BA.1 vs. Pfizer BA4/5) among  
73 previously vaccinated <sup>33</sup> individuals with or without preceding SARS-CoV-2 infection remains  
74 unclear.

75 For these reasons, we sought to compare the immunogenicity between those who received  
76 wild-type (WT), BA.1, or BA.4/5 booster vaccines among adult paramedics in Canada who had  
77 previously received three wild-type mRNA vaccines.

78

## 79 **Methods**

### 80 **Study design and data source**

81 We used blood sample test results and questionnaire data from adult paramedics participating  
82 in the COVID-19 Occupational Risk, Seroprevalence, and Immunity among Paramedics in  
83 Canada (CORSIP) study. CORSIP is an observational cohort study that recruited adult  
84 paramedics to investigate COVID-19 occupational risk and SARS-CoV-2 seroprevalence among  
85 paramedics in Canada. Recruitment of participants started on January 21, 2021 and ended on  
86 February 11, 2023. The CORSIP study received approvals from the University of British Columbia  
87 (H20-03620) and the University of Toronto Health Research Ethics Boards (40435). The  
88 paramedics provided written consent upon enrolment through an internet-based portal. The  
89 study included paramedics from five provinces in western and central Canada, including Alberta  
90 (AB), British Columbia (BC), Manitoba (MB), Ontario (ON), and Saskatchewan (SK). Participants  
91 provided questionnaire responses regarding demographic characteristics (age, sex at birth,  
92 race/ethnicity), SARS-CoV-2 infections and symptoms, vaccination history (including dates and

93 vaccination types), and medical history through an online portal, in addition to providing  
94 longitudinal blood samples every six months for serological testing <sup>9</sup>.

95

## 96 **Study population**

97 CORSIP participants were included in these analyses if they had received four mRNA vaccine  
98 doses prior to providing a blood sample, with the first three doses being a wild-type (WT) based  
99 mRNA vaccine, and the fourth dose being either: (1) a Moderna BA.1 bivalent vaccine, directed  
100 at BA.1 and WT strains (hereafter referred to as “BA.1 vaccine”); (2) a Pfizer <sup>27</sup> BA.4/5 bivalent  
101 vaccine, directed at BA.4/5 and WT strains (hereafter referred to as “BA.4/5 vaccine”); or, (3) a  
102 WT mRNA monovalent vaccine, directed only at the WT strain (hereafter referred to as “WT  
103 vaccine”). Participants who had received any non-mRNA vaccines were excluded. The study  
104 cohort included both those who had, and had not, previously been infected with <sup>6</sup> SARS-CoV-2.  
105 SARS-CoV-2 infection was defined as: (1) a participant-reported positive SARS-CoV-2 nucleic  
106 acid amplification test (NAAT) or rapid antigen test (RAT) result; or (2) the <sup>5</sup> presence of anti-  
107 nucleocapsid antibodies in the blood sample (Roche, IND, USA).

108 <sup>Given</sup> that we aimed to investigate the impact of the fourth vaccine (after three WT-based  
109 monovalent mRNA vaccines), we categorized cases based on <sup>10</sup> the type of fourth vaccine: BA.1  
110 vaccine, BA.4/5 vaccine, or WT vaccine. We chose samples (as described below) from eligible  
111 participants to create matched groups for three separate comparisons to compare each vaccine  
112 type to each other vaccine type.



113 **Serological testing**

114 We tested all blood samples with: (1) the V-PLEX SARS-CoV-2 Panel 28 ACE2 Kit (Meso Scale  
115 Discovery, MD, USA) to measure percent inhibition of angiotensin-converting enzyme 2 (ACE-2)  
116 binding to spike protein of Omicron (B.1.617.2) BA.4/BA.5 sub-lineages; and, (2) the Elecsys  
117 Anti-SARS-CoV-2 Nucleocapsid assay [Roche IND, USA] to identify the presence of anti-  
118 nucleocapsid protein antibodies, indicative of preceding SARS-CoV-2 infection. This Elecsys  
119 assay has previously been shown to have high sensitivity for detecting preceding SARS-CoV-2  
120 infections<sup>10</sup>.

121 **Outcome variable**

122 The primary outcome was ACE-2 percent (%) inhibition to the BA.4/5 Omicron antigen.

123 **Statistical analysis**

124 We described continuous variables with mean (and standard deviation [SD]) or median (with  
125 interquartile range [IQR]) and represented categorical variables with counts (with percentages).  
126 We used GraphPad Prism version 9.5.0 (GraphPad Software, San Diego, CA) and SAS version 9.4  
127 for data analyses.

128 We performed a 1:1 matching to select the multiple pairs from the three vaccine types using  
129 the optimal pair matching method<sup>11,12</sup>. For the first main comparison, comparing BA.1 vaccine  
130 vs. WT vaccine, we used the optimal pair matching method to create two balanced groups for  
131 comparison, matching based on participant age, sex at birth, preceding SARS-CoV-2 infection,

132 and the number of days from fourth dose to blood collection. We repeated this process for the  
133 second main comparison of BA.1 vaccine vs. BA.4/5 vaccine, and again for the third main  
134 comparison of BA.4/5 vaccine vs. WT vaccine.

135 In addition, we performed several sensitivity analyses. First, we repeated the comparisons but  
136 only included samples from <sup>37</sup> participants who had evidence of preceding SARS-CoV-2 infections.  
137 Second, we repeated the comparisons including only samples from participants who did not  
138 have preceding SARS-CoV-2 infections. Third, we combined all the <sup>41</sup> BA.4/5 and BA.1 vaccine  
139 cases, and compared them to the WT vaccine cases (overall and within subgroups dichotomized  
140 by preceding SARS-CoV-2 infection).

141 We diagrammed ACE-2 inhibition to BA.4/5 for the paired comparisons using scatter plots and  
142 used the <sup>23</sup> Wilcoxon matched-pairs signed rank test to test the differences between the median  
143 ACE-2 inhibitions to BA.4/5 of the groups. Further, we used the Related-Samples Hodges  
144 Lehman test to estimate the confidence interval of the differences in medians. In addition, we  
145 used the Bonferroni correction test to adjust <sup>6</sup> p-values obtained from the Wilcoxon matched-  
146 pairs signed rank test in order to account for multiple comparisons of outcomes.

147

## 148 Results

149 Overall, <sup>5</sup> the study included a total of 158 paramedics, with a mean participant age of 45 years.

150 Characteristics of participants included in the three different comparisons are shown in Table 1

151 and in supplementary Tables 1 and 2. Comparison group characteristics, and the interval  
152 between the fourth vaccine to blood collection, were similar.

153 Figure 1A shows the results of the main comparisons, including all samples (from both  
154 individuals with and without <sup>8</sup> preceding SARS-CoV-2 infections). We observed a significantly  
155 higher <sup>8</sup> percent inhibition to BA.4/5 among participants <sup>9</sup> who received a fourth BA.1 vaccine,  
156 <sup>15</sup> compared to those who received a fourth WT vaccine (median difference [Mdn]: 9.0, 95 %  
157 <sup>15</sup> Confidence Interval (CI): 3.8, 14.4,  $p^* = 0.006$ ; Figure 1A [left]). We did not detect a difference  
158 in the second main comparison, between the BA.4/5 vaccine vs. WT vaccine (Mdn: 7.1, 95% CI:  
159 <sup>40</sup> -0.52, 13.7,  $p^* = 0.186$ ; Figure 1A [middle]), nor in the third main comparison, between the BA.1  
160 vaccine vs. BA.4/5 vaccine (Mdn=0.56, 95% CI: -3.0, 3.9,  $p^* = 0.999$ ; Figure 1A [right]).

161 Figure 1B shows the results of the three comparisons when including only samples from  
162 individuals with preceding SARS-CoV-2 infections. We did not observe a difference in percent  
163 inhibitions for any comparison.

164 Figure 1C shows the results of the three comparisons when including only samples from  
165 individuals without <sup>8</sup> preceding SARS-CoV-2 infections. We observed a significantly higher  
166 <sup>38</sup> percent inhibition for the BA.1 vaccine, when <sup>29</sup> compared to the WT vaccine, however, we did  
167 <sup>38</sup> not detect a difference in the other two comparisons.

168 In the fourth analysis, samples from individuals <sup>10</sup> that received either BA.1 or BA.4/5 vaccines had  
169 <sup>10</sup> higher levels of ACE-2 percent inhibition to BA.4/5, compared to those who received the WT  
170 vaccine (Mdn = 7.8, 95% CI: 1.5, 16,  $p^* = 0.009$ ; Supplementary Figure 1A). This was consistent

171 when comparing samples from <sup>2</sup> individuals who had not previously had a SARS-CoV-2 infection  
172 (Mdn = 17.7, 95% CI: 7.9, 26; Supplementary Figure 1C), however, a difference was not seen  
173 when comparing samples from <sup>2</sup> individuals who had previously had SARS-CoV-2 infections (Mdn  
174 = 2.8, 95% CI: -1.2, 8.1; Supplementary Figure 1B).

175

## 176 Discussion

177 The results of this work provide insights into the humoral immune response to the Omicron  
178 BA.4/5 subvariant among individuals vaccinated with different types of mRNA COVID-19  
179 boosters. A unique aspect of this study is that it occurred in the Canadian context, where both  
180 the <sup>22</sup> Moderna BA.1 and Pfizer BA.4/5 vaccines were introduced, allowing for a comparison of the  
181 humoral response elicited by both of these vaccines against a more recent variant. Among  
182 individuals who had already received three prior wild-type monovalent mRNA vaccines, we  
183 were able to compare those who received an additional wild-type vaccine, <sup>18</sup> a BA.1 bivalent  
184 vaccine, or a BA.4/5 bivalent vaccine for their fourth dose, and examined resultant  
185 immunogenicity against a BA.4/5 antigen. Overall, bivalent fourth vaccines demonstrated  
186 superior immunogenicity <sup>31</sup> compared to the fourth wild-type vaccine among SARS-CoV-2-naïve  
187 individuals. Interestingly, our results did not suggest that <sup>25</sup> the BA.4/5-directed vaccine was  
188 superior to those targeting BA.1. This suggests that both bivalent boosters may be equally  
189 effective in boosting humoral immunity against the Omicron BA.4/5 subvariant, which is in

190 keeping with recent vaccine effectiveness data from Denmark comparing these two bivalent  
191 booster vaccines<sup>13</sup>.

192 We found that neither bivalent vaccine demonstrated an advantage over WT vaccine boosting  
193 <sup>14</sup> in those with evidence of prior SARS-CoV-2 infection; however, the majority of those in our  
194 cohort had evidence of infection during the Omicron era, which may help explain this finding.

195 Although we could not detect a difference between the BA.4/5 vaccine and the WT vaccine, this  
196 may have been due to a smaller sample size compared to the BA.1 vs. WT comparison. This  
197 could also be attributable to the fact that the timing of the fourth monovalent dose to the  
198 blood draw was shorter than that of the fourth dose of BA.4/5 booster to the blood draw,  
199 thereby reducing the expected differences in immunogenicity between the two boosters.

200 Further, the higher dose of the Moderna <sup>10</sup> BA.1 booster (50 mcg) compared to the Pfizer BA.4/5  
201 booster (30 mcg) could also help explain these findings. <sup>34</sup> These results are also consistent with  
202 <sup>17</sup> previous evidence demonstrating that the higher-dosed mRNA-1273 vaccine resulted in  
203 superior serological markers at six months compared to those who received the BNT162b2  
204 vaccine<sup>14</sup>.

205 Overall, our results suggest that the Omicron-specific bivalent booster may be more effective in  
206 boosting humoral immunity against the Omicron BA.4/5 subvariant, than using all four WT  
207 monovalent vaccines. However, the effect appears to be limited to those <sup>20</sup> who have never been  
208 infected with SARS-CoV-2. These findings have important implications for ongoing COVID-19  
209 vaccination strategies, especially in jurisdictions where the Omicron variant is prevalent.  
210 Further research is needed to confirm these results in other occupational groups and to

211 investigate the <sup>39</sup> long-term effectiveness of different COVID-19 booster vaccines in inducing and  
212 maintaining humoral immunity.

### 213 **Limitations**

214 Our study might have been subjected to some limitations. This study may not be generalizable  
215 to the entire population across all age groups since we included <sup>9</sup> a relatively homogenous group  
216 of middle-aged adult paramedics whose immunity may differ from other groups. Other  
217 limitations include lack of randomization, use of self-reported data, and small sample size,  
218 which might have influenced the findings of some comparisons between bivalent and WT  
219 boosters.

220

### 221 **Conclusion**

222 Our study suggests that the Omicron-specific bivalent boosters may induce <sup>28</sup> a higher humoral  
223 immune response against the Omicron BA.4/5 subvariant compared to the mRNA monovalent  
224 boosters. However, this finding appears limited to those without preceding SARS-CoV-2  
225 infections.

226 <sup>1</sup> **Conflict of Interest:** None declared.

227

## 228 Ethical statement

229 The study was approved by the University of British Columbia (Reference number: H20-  
230 03620), and University of Toronto (Reference number: 40435) research ethics boards.  
231 Participants provided electronic consent upon enrolment.

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238 Grunau is supported by the Michael Smith Foundation for Health Research.

## 239 Author contribution

240 <sup>12</sup> *Conceptualization*: M.A-B, D.M.G, I.K, J.Y, T.K, M.E.K, P.D, J.M.C, B.G. <sup>1</sup> *Data curation*: M.A-B, B.G,  
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243 <sup>12</sup> <sup>1</sup> M.E.K, P.D, J.M.C, B.G. *Methodology*: M.A-B, B.G, D.M. G. <sup>1</sup> *Project administration*: B.G, D.G, T.K,  
244 <sup>16</sup> PD. *Resources*: B.G, D.G, T.K, PD. *Software*: M.A-B, D.M.G, I.K, J.Y, T.K, M.E.K, P.D, J.M.C, B.G.  
245 <sup>12</sup> <sup>1</sup> *Validation*: M.A-B, D.M.G, I.K, J.Y, T.K, M.E.K, P.D, J.M.C, B.G. *Visualization*: M.A-B, B.G, D.M. G.  
246 *Writing – original draft*: M.A-B, B.G, D.G. *Writing – review and editing*: all authors.

247 **1**  
**Data summary statement**

248 The CORSIP data used for this study can be publicly assessed through the website of Canada  
249 COVID-19 Immunity Task Force (CITF) website via the link: <https://portal.citf.mcgill.ca/>.

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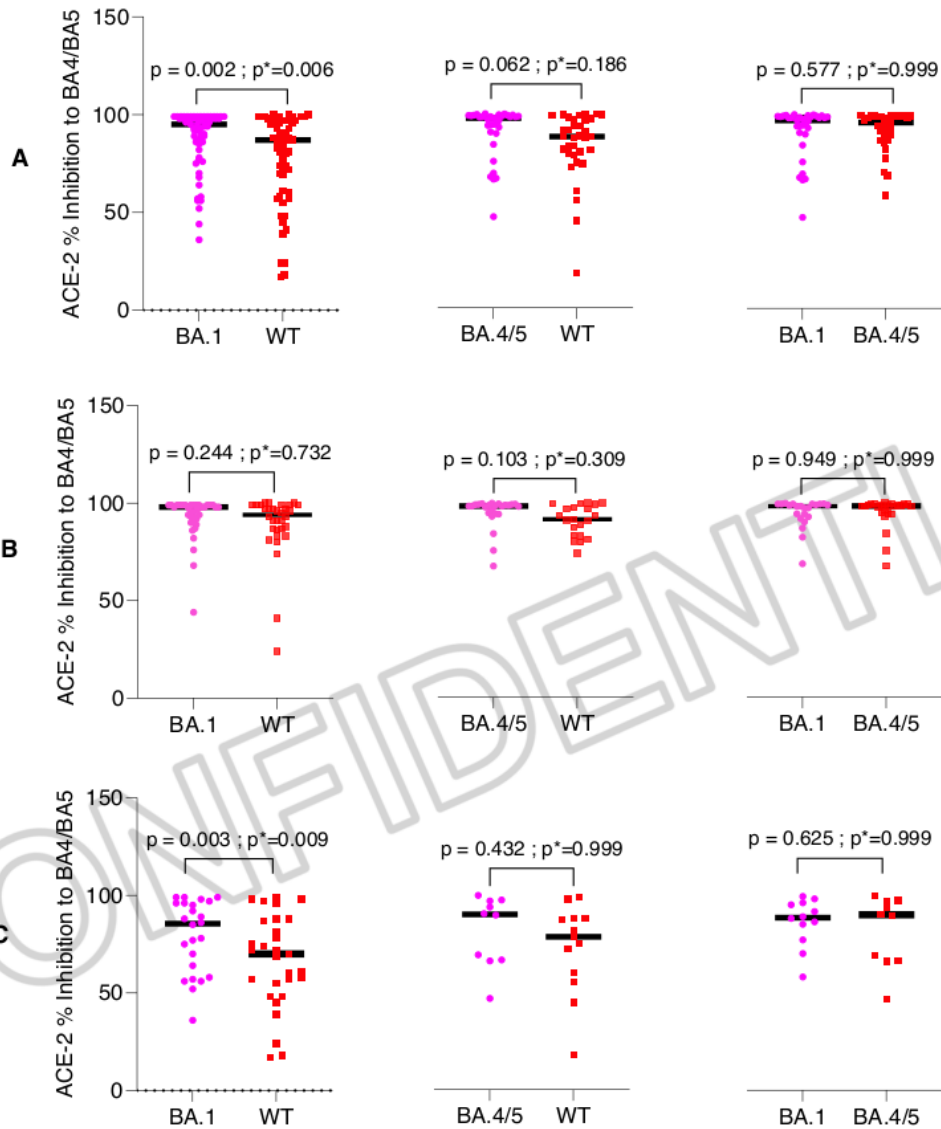
**Table 1:** Participant characteristics stratified by matched pairs of vaccine doses (including those with or without preceding COVID 19).

Variables	Comparison 1*		Comparison 2*		Comparison 3*	
	Moderna BA.1	WT	Moderna BA.1	Pfizer BA.4/5	Pfizer BA.4/5	WT
	n = 61	n = 61	n = 34	n = 34	n = 34	n = 34
<b>Matched variables</b>						
Age in years, Mean (SD)	45 (11)	45 (10)	45 (11)	43 (10)	43 (10)	44 (11)
Sex (at birth)						
Female	22 (36)	23 (38)	13 (38)	15 (44)	15 (44)	16 (47)
Male	39 (64)	38 (62)	21 (62)	19 (56)	19 (56)	18 (53)
COVID-19 diagnosis, total [n (%)]	37 (61)	32 (53)	22 (65)	24 (71)	24 (71)	21 (62)
Pre-Omicron infections**	2 (5)	2 (6)	2 (9)	2 (8)	2 (8)	2 (10)
Omicron Infections**	35 (95)	30 (94)	20 (91)	22 (92)	22 (92)	19 (90)
V4-to-BC (days), Median (IQR)	81 (48, 119)	72 (41, 123)	60 (43,79)	54 (42, 75)	54 (42, 75)	47 (37, 85)
<b>Other variables</b>						
<i>Race/Ethnicity, n (%)</i>						
Racialized	6 (10)	5 (8)	4 (12)	5 (15)	5 (15)	32 (94)
White	55 (90)	56 (92)	30 (88)	29 (85)	29 (85)	2 (6)
Tobacco use, n (%)	3 (5)	4 (7)	2 (6)	1 (3)	1 (3)	3 (9)
Influenza vaccination, n (%)	54 (89)	54 (89)	30 (88)	30 (88)	30 (88)	31 (91)
<i>Medical history, n (%)</i>						
Hypertension	5 (8)	6 (10)	4 (12)	5 (15)	5 (15)	5 (15)
Diabetes	2 (3)	5 (8)	2 (6)	-	1 (2.9)	-
Asthma	10 (16)	5 (8)	4 (12)	1 (3)	1 (2.9)	2 (6)
Chronic Lung Disease	-	-	-	1 (3)	1 (2.9)	1 (3)
Cancer	2 (3)	1 (2)	1 (3)	4 (12)	4 (12)	1 (3)

WT: Wild-type; SD: Standard deviation, IQR: Interquartile range; V4-to-BC: Days from vaccine 4 to the blood collection date;

\*No significant differences between vaccine groups, within comparison, were observed for characteristics.

\*\* Pre-Omicron infection was defined as SARS-CoV-2 infections that occur before November 26, 2021; and Omicron infections were defined as infections that occurred after December 26, 2021.



**Figure 1:** Comparing immunogenicity of bivalent vaccine doses with monovalent vaccine doses among all participants (A); among participants with previous SARS-CoV-2 infections (B); and among participants with no previous SARS-CoV-2 Infection (C).

The solid line denotes the median ACE-2 inhibitions. Outcomes were compared with the Wilcoxon matched pairs signed rank test, with the original p value (p) and Bonferroni corrected p-value (p\*) as shown.

**Supplementary Table 1:** Participants characteristics among individuals without preceding COVID-19 diagnosis

Variables	Comparison 1*		Comparison 2*		Comparison 3*	
	Moderna BA. 1 n = 24	WT n = 29	Moderna BA.1 n = 12	Pfizer BA.4/5 n = 10	Pfizer BA 4/5 n = 10	WT n = 13
<b>Matched variables</b>						
Age, Mean (SD)	47 (11)	44 (9)	48 (10)	40 (8)	40 (8)	43 (10)
Sex (at birth)						
Female	9 (38)	9 (31)	5 (42)	4 (40)	4 (40)	6 (46)
Male	15 (62)	20 (69)	7 (58)	6 (60)	6 (60)	7 (54)
V4-to-BC (days)	77 (53, 118)	70 (41, 88)	67 (45, 78)	56 (51, 90)	56 (51, 90)	42 (36, 80)
<b>Other variables</b>						
<i>Race/Ethnicity, n (%)</i>						
Racialized	3 (12)	1 (3)	3 (25)	3 (30)	3 (30)	1 (8)
White	21 (88)	28 (97)	9 (75)	7 (70)	7 (70)	12 (92)
Tobacco use, n (%)	3 (13)	2 (7)	2 (17)	0 (0)	0 (0)	1 (8)
Influenza vaccination, n (%)	21 (88)	24 (83)	11 (92)	7 (70)	7 (70)	11 (85)
<i>Medical history, n (%)</i>						
Hypertension	1 (4.2)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Diabetes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Asthma	6 (25)	4 (14)	2 (17)	0 (0)	0 (0)	1 (8)
Chronic Lung Disease	-	-	0 (0)	0 (0)	0 (0)	0 (0)
Cancer	1 (4)	1 (3)	0 (0)	2 (20)	2 (20)	1 (8)

**WT:** Wild type; **SD:** Standard deviation, **IQR:** Interquartile range; **V4-to-BC:** Days from vaccine 4 to the last blood collection date.

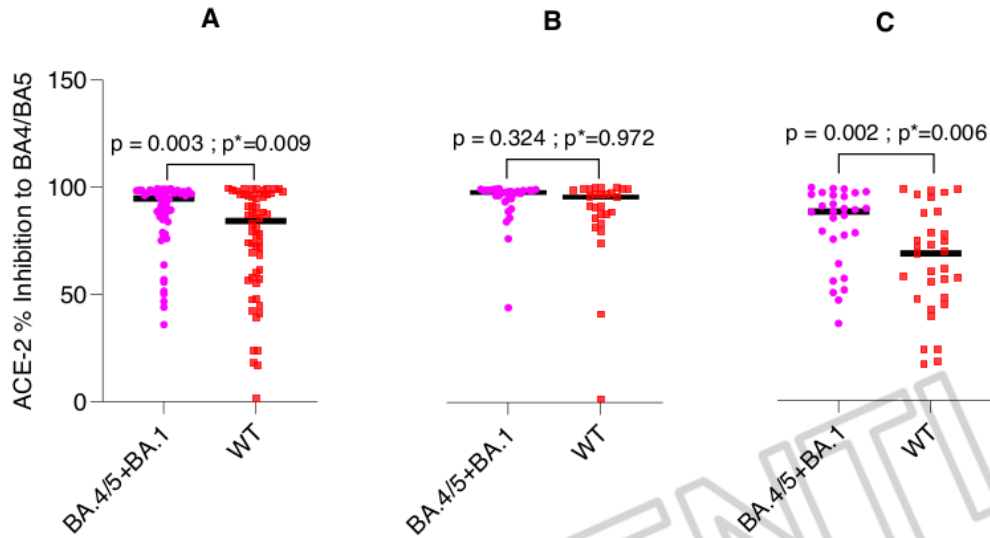
\*No significant differences between vaccine groups, within comparison, was observed for characteristics.

**Supplementary Table 2:** Participants characteristics among individuals with preceding COVID-19 diagnosis

Variables	Comparison 1*		Comparison 2*		Comparison 3*	
	Moderna BA. 1	WT	Moderna BA.1	Pfizer BA.4/5	Pfizer BA 4/5	WT
	n = 37	n = 32	n = 22	n = 24	n = 24	n = 21
<b>Matched variables</b>						
Age, Mean (SD)	44 (10)	45 (11)	43 (11)	44 (11)	44 (11)	44 (12)
Sex (at birth)						
Female	13 (35)	14 (44)	8 (36)	11 (46)	11 (46)	10 (48)
Male	24 (65)	18 (56)	14 (64)	13 (54)	13 (54)	11 (52)
V4-to-BC (days)	83 (47, 120)	93 (41, 129)	53 (42, 82)	47 (41, 74)	43 (36, 51)	42 (36, 54)
<b>Other variables</b>						
<b>Race/Ethnicity, n (%)</b>						
Racialized	3 (8)	4 (12)	1 (4)	2 (8)	2 (8)	1 (5)
White	34 (92)	28 (88)	21 (96)	22 (92)	22 (92)	20 (95)
Tobacco use, n (%)	0 (0)	2 (6)	0 (0)	1 (4)	1 (4)	2 (10)
Influenza vaccination, n (%)	4 (11)	30 (94)	19 (86)	23 (96)	23 (96)	20 (95)
<b>Medical history, n (%)</b>						
Hypertension	4 (11)	5 (16)	4.0 (18)	5.0 (21)	5 (21)	4 (19)
Diabetes	2 (5)	0 (0.0)	2.0 (9)	0 (0)	0 (0)	0 (0)
Asthma	4 (11)	1 (3)	2 (9)	1 (4)	1 (4)	1 (5)
Chronic Lung Disease	0 (0)	0 (0)	0 (0)	1 (4)	1 (4)	1 (5)
Cancer	1 (3)	0 (0)	1 (5)	2 (8)	2 (8)	0 (0)

**WT:** Wild type; **SD:** Standard deviation, **IQR:** Interquartile range; **V4-to-BC:** Days from vaccine 4 to the last blood collection date

\*No significant differences between vaccine groups, within comparison, was observed for characteristics.



**Supplementary Figure 1:** Comparing immunogenicity of the combined bivalent booster with the fourth dose monovalent vaccine booster.

- A:** The plot compares the fourth dose of combined bivalent booster (including BA.1 or BA.4/5) versus the fourth dose of monovalent booster among paramedics with or without preceding SARS-CoV-2 infections.
- B:** The plot compares the fourth dose of combined bivalent booster (including BA.1 or BA.4/5) versus the fourth dose of monovalent booster among paramedics with preceding SARS-CoV-2 infections.
- C:** The plot compares the fourth dose of combined bivalent boosters (including BA.1 or BA.4/5) versus the fourth dose of monovalent booster among paramedics without preceding SARS-CoV-2 infections.

The solid line denotes the median ACE-2 inhibitions. Outcomes were compared with the Wilcoxon matched pairs signed rank test, with the original p value (p) and Bonferroni corrected p-value (p\*) as shown.

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