1   **A cautionary note on recall vaccination in ex-COVID-19 subjects**

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21

Abstract

22 **Abstract**

23 Currently approved COVID-19 vaccines based on mRNA or adenovirus require a first jab followed

24 by recall immunization. There is no indication as to whether individuals who have recovered from

25 COVID-19 should be vaccinated, and if so, if they should receive one or two vaccine doses.

26 Here, we tested the antibody response developed after the first dose of the mRNA based vaccine

27 encoding the SARS-CoV-2 full-length spike protein (BNT162b2) in 124 healthcare professionals of

28 which 57 had a previous history of COVID-19 (ExCOVID). Post-vaccine antibodies in ExCOVID

29 individuals increase exponentially within 7-15 days after the first dose compared to naïve subjects

30 ($p<0.0001$). We developed a multivariate Linear Regression (LR) model with l2 regularization to

31 predict the IgG response for SARS-COV-2 vaccine. We found that the antibody response of

32 ExCOVID patients depends on the IgG pre-vaccine titer and on the symptoms that they developed

33 during the disorder, with anosmia/dysgeusia and gastrointestinal disorders being the most

34 significantly positively correlated in the LR. Thus, one vaccine dose is sufficient to induce a good

35 antibody response in ExCOVID subjects. This poses caution for ExCOVID subjects to receive a

36 second jab both because they may have a overreaction of the inflammatory response and also in

37 light of the current vaccine shortage.

38

Introduction

39 **Introduction**

40 Currently approved COVID-19 vaccines based on mRNA [1-3] or adenovirus [4] require a first jab

41 followed by recall immunization. The impact of previous exposure to SARS-CoV-2 on immune

42 response elicited the vaccines has not been assessed.

43

Methods

44 **Methods**

45 We tested the antibody response developed after the first dose of the mRNA based vaccine

46 encoding the SARS-CoV-2 full-length spike protein (BNT162b2)[1] in 124 healthcare professionals of

47 which 57 had a previous history of COVID-19 (ExCOVID) (Table 1), as part of an observational

48 study (clinicaltrial.gov NCT04387929) conducted at Istituto Clinico Humanitas in which healthcare

49   professionals were followed for serology and for any occurring COVID-19 related symptoms every

50   three months[5]. We recorded the antibody response to Spike 1/2 with a quantitative test (Liaison

51   SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Italy) which allowed us to evaluate even large amounts

52   of plasma IgG. To predict the IgG response for SARS-COV-2 vaccine, a multivariate Linear

53   Regression (LR) model with l2 regularization (also known as Ridge Regression) was developed.

54   Numerical variables were standardized (z-score algorithm) and the target variable was log

55   transformed due to right asymmetry of the distribution. The subjects without the serological

56   analysis before vaccination were excluded from this analysis (n=11). The final number of subjects

57   analyzed in LR was 113.

58

59   **Results**

60   As shown in Fig.1A-B ExCOVID individuals had a much higher antibody response after the first

61   dose of vaccine than naïve subjects (p<0.0001), regardless of when they developed the COVID-

62   19. They displayed an exponential increase of anti-Spike 1/2 antibody response within 7-15 days

63   after the first dose of vaccine. The pre-vaccine antibody amount of the ExCOVID population was

64   on average 44.+/-37.7 while that after the vaccine was 1055.7+/-1004.2 (p<0.0001) (Table 1), with

65   higher levels in symptomatic ExCOVID (Fig. 1C, p=0.028).

66   We investigated the relationship between the amount of IgG after vaccination with COVID-19, sex,

67   age and symptoms related to disease. The final LR shows a good prediction of the target variable

68   (R^2=0.88, F-statistic = 39.18, p-value<0.001) and the most significant features were history of

69   COVID-19 (1.48, 95% CI 1.07-1.93), the value of IgG before vaccination (0.87, 95% CI 0.59-1.13),

70   the difference between the date of vaccination and the date of serology post-vax (0.87, 95% CI

71   0.65-1.03), and age (-0.13, 95% CI -0.24 - -0.001) as well as COVID-19 related symptoms:

72   gastrointestinal disorders (0.59, 95% CI 0.16-0.97), anosmia/dysgeusia (0.50, 95% CI 0.14-0.87),

73   tachycardia (0.26, 95% CI 0.02-0.60) and sore throat (-0.35, 95% CI -0.53 - -0.11) (Fig. 1D, Suppl.

74   Table1).

75

76   **Discussion**

3

77   The antibody response of ExCOVID patients depends on the IgG pre-vaccine titer and on the

78   symptoms that they developed during the disorder, with anosmia/dysgeusia and gastrointestinal

79   disorders being the most significantly positively correlated in the LR, while sore throat was

80   negatively correlated because 45% non-COVID individuals reported it. Young subjects had a

81   higher antibody response. We previously observed that anosmia/dysgeusia was associated with

82   an increase of antibodies over time, independently of vaccination (Levi et al. submitted). Thus, one

83   vaccine dose is sufficient to induce a good antibody response in ExCOVID subjects and poses

84   caution for a second dose: over stimulation with high amount of antigens could switch-off the

85   immune response due to antigen exhaustion, which occurs in response to several viruses

86   (reviewed in[6]). Alternatively, overactivation of the immune response may drive the development of

87   low-affinity antibodies for SARS-CoV-2 which may foster an antibody dependent enhancement

88   (ADE) reaction when re-exposed to the virus (reviewed in[7]). These results question whether a

89   second shot in ExCOVID subjects is indeed required and suggest to post-pone it while monitoring

90   antibody response longevity. At a time of vaccine scarcity, these findings may have public health

91   implications.

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A

		counts	%	min	max	mean	std	TEST	p
					Serology pre VAX				
COVID-19	NO (Healthy)	67	59.29	3	7.66	3.35	1.02	MW	5E-12
COVID-19	YES (ExCOVID)	46	40.71	3	140	44.58	37.72		
GENDER	F	63	55.75	3	132	18.86	28.46	MW	0.295
GENDER	M	50	44.25	3	140	21.74	34.99		
Age_class	21-30	24	21.24	3	140	29.25	38.82	MW	0.027
Age_class	31-40	37	32.74	3	132	16.56	28.14	MW	0.086
Age_class	41-50	31	27.43	3	81.7	14.76	22.91	MW	0.074
Age_class	51-60	13	11.5	3	123	25.73	42.37	KS	0.146
Age_class	60+	8	7.08	3	78.1	21.12	29.81	KS	0.986

B

		counts	%	min	max	mean	std	TEST	p
					Serology post VAX				
COVID-19	NO (Healthy)	67	54.03	3	104	13.29	19.73	MW	3E-19
COVID-19	YES (ExCOVID)	57	45.97	3	4000	1055.7	1004.2		
GENDER	F	71	57.26	3	3800	466.97	875.9	MW	0.455
GENDER	M	53	42.74	3	4000	526.62	833.75		
Age_class	21-30	30	24.19	3	4000	601.13	901.65	MW	0.015
Age_class	31-40	39	31.45	3	3800	553.52	1046.9	MW	0.115
Age_class	41-50	33	26.61	3	2320	351.12	644.59	MW	0.234
Age_class	51-60	14	11.29	3	2240	437.95	740.98	KS	0.986
Age_class	60+	8	6.45	3	1690	465.76	664.78	KS	0.696
CLASS SYMPTOMS (for ExCOVID)	a/paucisymptomatic	15	26.32	3	1800	507.73	598.39	KS	0.029
CLASS SYMPTOMS (for ExCOVID)	symptomatic	42	73.68	10.7	4000	1251.4	1051.6		
ExCOVID — Fever	0	30	52.63	3	3800	865.65	942.76	MW	0.034
ExCOVID — Fever	1	27	47.37	10.7	4000	1266.9	1045.2		
ExCOVID — Low-grade Fever	0	44	77.19	3	4000	954.8	1060.1	KS	0.016
ExCOVID — Low-grade Fever	1	13	22.81	27	2630	1397.2	717.47		
ExCOVID — Headache	0	31	54.39	3	2520	825.63	734.51	MW	0.038
ExCOVID — Headache	1	26	45.61	27	4000	1330	1211.4		
ExCOVID — Cough	0	40	70.18	3	3800	921.12	959.53	KS	0.176
ExCOVID — Cough	1	17	29.82	8.18	4000	1372.4	1064.5		
ExCOVID — Sore throat	0	31	54.39	3	3330	1040.9	857.41	MW	0.365
ExCOVID — Sore throat	1	26	45.61	8.18	4000	1073.4	1173		
ExCOVID — Muscle pain	0	27	47.37	3	4000	864.43	1100.9	MW	0.013
ExCOVID — Muscle pain	1	30	52.63	10.7	3330	1227.9	892.15		
ExCOVID — Asthenia	0	25	43.86	3	2520	717.64	784.71	MW	0.008
ExCOVID — Asthenia	1	32	56.14	10.7	4000	1319.8	1086.6		
ExCOVID — Anosmia/dysgeusia	0	29	50.88	3	4000	824.28	1044.4	MW	0.009
ExCOVID — Anosmia/dysgeusia	1	28	49.12	27	3800	1295.4	918.26		
ExCOVID — Gastrointestinal disorders	0	33	57.89	8.18	2520	902.12	753.36	MW	0.212
ExCOVID — Gastrointestinal disorders	1	24	42.11	3	4000	1266.9	1259		
ExCOVID — Conjunctivitis	0	49	85.96	3	4000	1018.2	984.56	KS	0.896
ExCOVID — Conjunctivitis	1	8	14.04	27	3330	1285.8	1161.6		

Group	Symptom		counts	%	min	max	mean	std	TEST	p
	Dyspnea	0	41	71.93	3	4000	952.52	1017.5	KS	0.179
		1	16	28.07	27	3330	1320.1	948.76		
	Chest pain	0	42	73.68	3	4000	989.3	988.56	KS	0.819
		1	15	26.32	27	3330	1241.6	1058.9		
	Tachycardia	0	47	82.46	3	3800	982.11	870.62	KS	0.395
		1	10	17.54	27	4000	1401.6	1496.3		
	Pneumonia	0	50	87.72	3	4000	1013.9	1036.5	KS	0.218
		1	7	12.28	27	2240	1354.3	720.25		
Healthy	Fever	0	63	94.03	3	104	13.45	20.13	KS	0.995
		1	4	5.97	3	30.7	10.74	13.4		
	Low-grade Fever	0	62	92.54	3	104	14.04	20.33	KS	0.559
		1	5	7.46	3	6.26	3.9	1.42		
	Headache	0	42	62.69	3	104	12.68	20.73	MW	0.438
		1	25	37.31	3	60.5	14.3	18.28		
	Cough	0	51	76.12	3	104	12.52	20.12	KS	0.126
		1	16	23.88	3	63.8	15.72	18.85		
	Sore throath	0	37	55.22	3	60.5	11.02	15.69	MW	0.256
		1	30	44.78	3	104	16.08	23.78		
	Muscle pain	0	58	86.57	3	104	12.54	19.49	KS	0.724
		1	9	13.43	3	55.9	18.11	21.79		
	Asthenia	0	56	83.58	3	104	12.23	19.47	KS	0.419
		1	11	16.42	3	49.7	18.68	21.11		
	Anosmia/dysgeusia	0	66	98.51	3	104	13.4	19.86	KS	0.687
		1	1	1.49	6.13	6.13	6.13			
	Gastrointestinal disorders	0	58	86.57	3	104	13.3	20.1	KS	0.656
		1	9	13.43	3	55.9	13.2	18.24		
	Conjunctivitis	0	60	89.55	3	63.8	11.5	16.12	KS	0.419
		1	7	10.45	3	104	28.62	37.68		
	Dyspnea	0	64	95.52	3	104	13.75	20.07	KS	0.399
		1	3	4.48	3	4.5	3.5	0.87		
	Chest pain	0	63	94.03	3	104	13.92	20.19	KS	0.205
		1	4	5.97	3	4.4	3.35	0.7		
	Tachycardia	0	62	92.54	3	104	14.07	20.32	KS	0.195
		1	5	7.46	3	4.57	3.61	0.84		
	Pneumonia	0	67	100	3	104	13.29	19.73	na	na
		1	0	0	0	0	0	0		

C

		counts	%	min	max	mean	std	TEST	p
Days between VAX and serology post VAX	Healthy	67	54.03	4.8	16.9	8.9	2.91	MW	3E-08
	ExCOVID	57	45.97	4.89	20.4	13.81	4.91		

114

115 **Table 1.** A.B. Demographic distribution of anti-Spike 1/2 IgG plasma levels. C. Days between
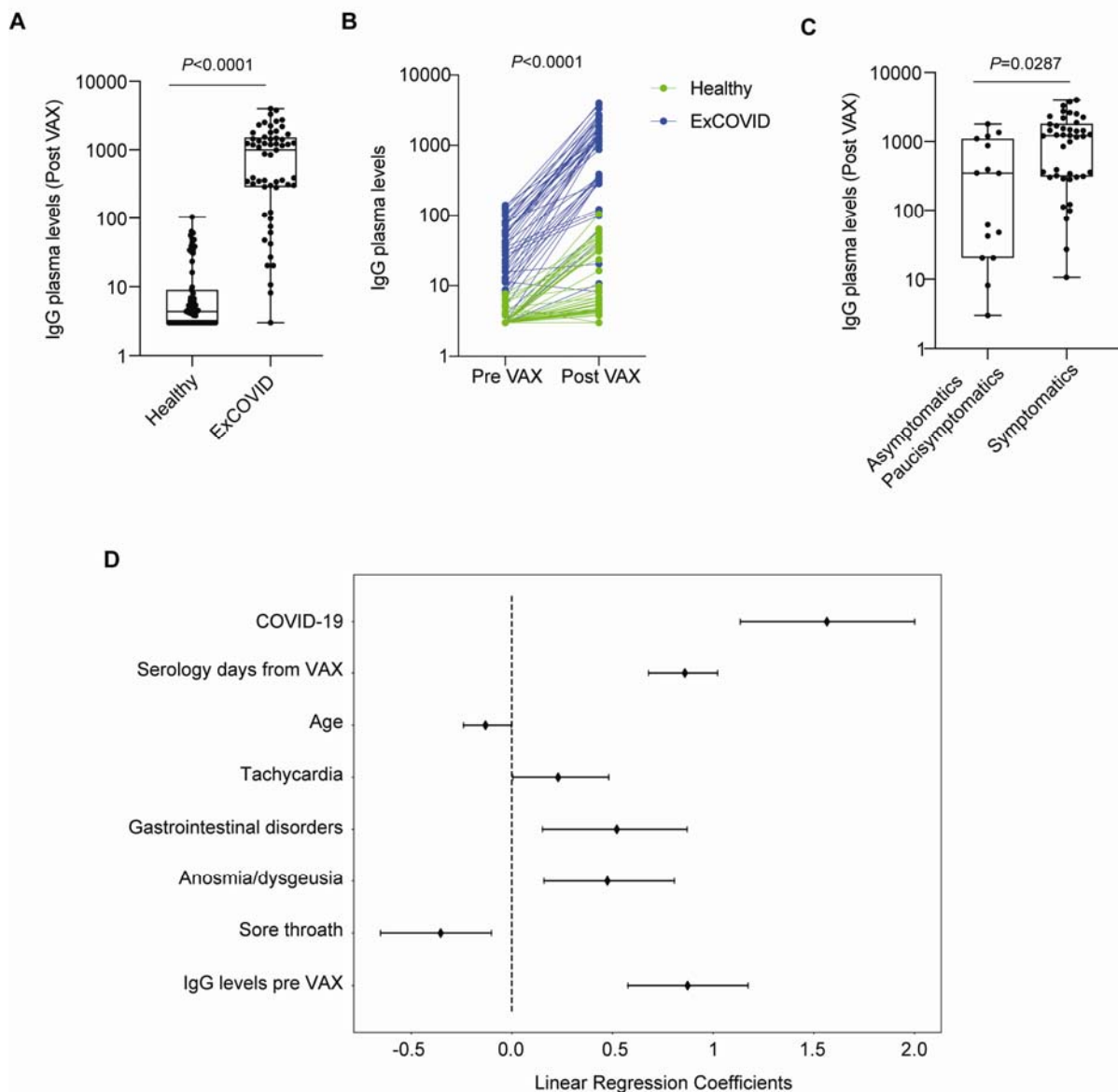
116 vaccination and serology post vaccination. *P* values determined using two-tailed unpaired Mann–

117 Whitney test (MW) or two-tailed unpaired Kolmogorov-Smirnov test (KS). NA: not applicable.

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121 **Figure 1: ExCOVID subjects increase exponentially anti-Spike 1/2 IgG levels after the first**

122 **dose of vaccine.** A, anti-Spike 1/2 IgG plasma levels in healthy (n=67) and ExCOVID individuals

123 (n=57) measured after the first dose of vaccine. B, anti-Spike 1/2 IgG plasma levels in healthy

124 (n=67) and ExCOVID individuals (n=46) measured before and after the first dose of vaccine. C,

125 anti-Spike 1/2 IgG plasma levels in asymptomatic / paucisymptomatic (n=15) and in symptomatic

126 (n=42) ExCOVID individuals measured after the first dose of vaccine. D, Multivariate linear

127 regression coefficients for the most significant variables ($p<0.05$). Dot points represent the mean

128 values and the lines the 95% CI.

8

129   The box plots (A, C) show the interquartile range, the horizontal lines show the median values and

130   the whiskers indicate the minimum-to-maximum range. *P* values were determined using two-tailed

131   unpaired Mann–Whitney test (A) or two-tailed Wilcoxon matched-pairs signed rank test (B) or two-

132   tailed unpaired Kolmogorov-Smirnov test (C).

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