

**BRIEF REPORT**

# Severe acute respiratory syndrome coronavirus 2 antibody response to a third dose of homologous messenger RNA vaccination in liver transplantation recipients

To the editor,

Liver transplantation (LT) recipients have attenuated antibody response to the two-dose messenger RNA (mRNA) vaccine series against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>[1–3]</sup> Additional vaccine doses, best studied in the kidney transplantation population, may improve response, yet many solid organ transplantation recipients appear to continue to have a poor response despite additional doses.<sup>[4,5]</sup> We aimed to measure antibody levels before and after a third mRNA vaccine dose (D3) in a large cohort of LT recipients to understand vaccine immunogenicity and the demographic, transplantation, and vaccine type associated with sero-response.

## PATIENTS AND METHODS

LT recipients in our longitudinal observational cohort who received three doses of homologous mRNA vaccines and had antibody levels measured both after dose 2 (D2) and after D3 were identified. Participants with multiorgan transplantation ( $n = 37$ ), pre-dose 1 (D1) SARS-CoV-2 infection ( $n = 11$ ), or SARS-CoV-2 breakthrough infection ( $n = 3$  before D3;  $n = 17$  after D3) as antibody interpretation would not be reflective of the overall LT cohort. The pre-D3 level was defined as the last anti-spike antibody level collected between D2 and D3; the level was repeated 1 month after D3. Samples were tested with clinical anti-spike assays: Roche Elecsys Anti-SARS-CoV-2 enzyme immunoassay (EIA) (anti-receptor-binding domain [RBD], positive  $\geq 0.8$  U/ml; ceiling 2500 U/ml) or EUROIMMUN EIA (anti-S1, positive  $\geq 1.1$  AU).

## RESULTS

There were 148 included participants who completed three homologous doses of BNT162b2 (49%) or mRNA-1273 (51%) vaccines between June 5, 2021, and December 21, 2021 (Table 1). Pre-D3 antibody levels were measured at a median (interquartile range [IQR]) of 120 days (92–178) after D2, and post-D3 antibody levels were measured at a median (IQR) of 30 days (26–33) after D3. The median (IQR) time between D2 and D3 was 169 (149–188) days. Overall, 118/148 (80%) were seropositive before D3, increasing to 138/148 (93%) after D3. Specifically, of the 30 participants seronegative before D3, 20 (67%) seroconverted after D3. The median (IQR) time between D2 and the pre-D3 sampling did not significantly differ between those with positive versus negative post-D3 titers (101 [90–164] vs. 121 [92–179] days;  $p = 0.46$ ; Table 1), nor versus those who seroconverted (128 [94–175] days;  $p = 0.91$ ). No participant seroreverted during follow-up. Risk factors significantly associated with seronegativity after D3 were antimetabolite use (90% vs. 41%;  $p = 0.005$ ), and proximity to transplantation (3 vs. 6 years;  $p = 0.014$ ). No patient made substantial decreases to their anti-metabolite dosage before and after D3. Receipt of the BNT162b2 series (80% vs. 47%;  $p = 0.054$ ) was also more common in seronegative participants, but this was not statistically significant. The median anti-RBD level increased from before to after D3 by at least 13.6-fold (from 184 U/ml to  $>2500$  U/ml), and median anti-S1 level increased by 3.6-fold (from 2.50 AU to 8.94 AU) after D3 (Figure 1).

As post hoc analysis, we evaluated the excluded group of participants that had breakthrough infections after D1. Among 20 participants who reported

**Abbreviations:** AU, arbitrary units; D1, first SARS-CoV-2 vaccine dose; D2, second SARS-CoV-2 vaccine dose; D3, third SARS-CoV-2 vaccine dose; EIA, enzyme immunoassay; Ig, immunoglobulin; IQR, interquartile range; LT, liver transplantation; MMF, mycophenolate mofetil; mRNA, messenger RNA; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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**TABLE 1** Baseline characteristics of LT recipients who completed three doses of homologous SARS-CoV-2 vaccines and categorized by positive versus negative levels at 1 month after D3

Recipient characteristic	All participants <i>n</i> = 148	Antibody level after D3		<i>p</i> -value
		Positive, <i>n</i> = 138 (93%)	Negative, <i>n</i> = 10 (7%)	
Age, years	63 (51–69)	63 (51–69)	60 (53–66)	0.93
Female	80 (54)	74 (54)	6 (60)	0.75
White	134 (91)	125 (91)	9 (90)	>0.99
Years between vaccination and transplantation	6 (2–13)	6 (3–14)	3 (1–6)	0.36
Immunosuppression <sup>a</sup>				
Tacrolimus	113 (76)	104 (75)	9 (90)	0.45
Cyclosporine	13 (9)	12 (9)	1 (10)	>0.99
Antimetabolite	66 (45)	57 (41)	9 (90)	<b>0.005</b>
MMF use	43 (29)	37 (27)	6 (60)	0.064
Steroid	26 (18)	23 (17)	3 (30)	0.38
Sirolimus	17 (11)	17 (12)	0	0.61
Everolimus	8 (5)	7 (5)	1 (10)	0.44
Triple immunosuppression <sup>b</sup>	10 (7)	7 (5)	3 (30)	<b>0.021</b>
Vaccine series				
BNT162b2	73 (49)	65 (47)	8 (80)	0.054
mRNA-1273	75 (51)	73 (53)	2 (20)	
Duration, days				
D2 to D3	169 (149–188)	170 (149–189)	164 (147–179)	0.55
D2 to pre-D3 titer	120 (92–178)	121 (92–179)	101 (90–164)	0.46
D3 to post-D3 titer	30 (26–33)	30 (26–33)	32 (23, 35)	0.56
Titers				
Negative pre-D3 <sup>c</sup>	30 (20)	20 (14)	10 (100)	<b>&lt;0.001</b>
Positive pre-D3	118 (80)	118 (86)	0	

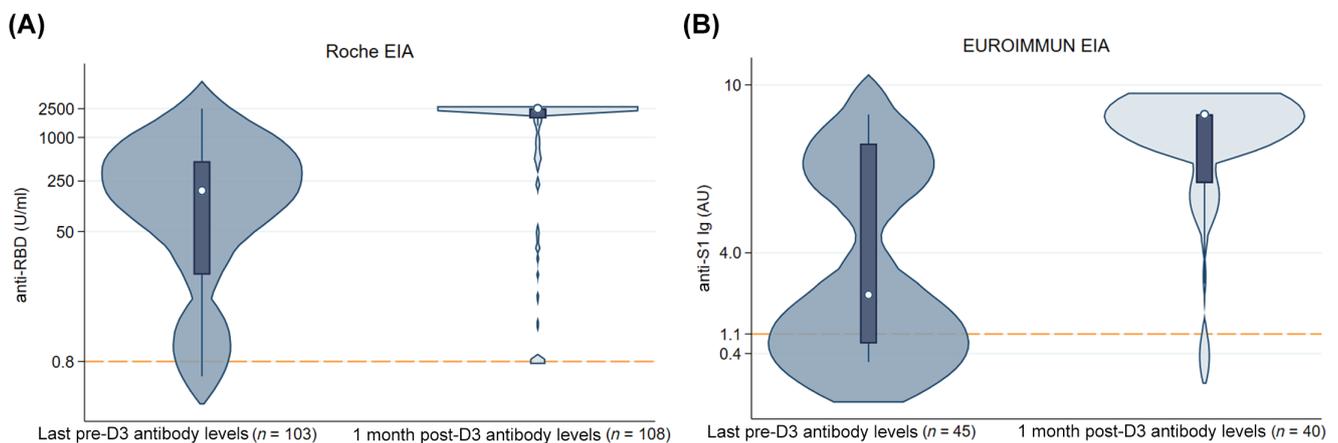
Note: Data are presented as median (IQR) or *n* (%). Fisher exact for small numbers and *p*-values bolded if *p* < 0.05.

Abbreviations: D2, second dose of vaccine; D3, third dose of vaccine; IQR, interquartile range; LT, liver transplantation; MMF, mycophenolate mofetil; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>Listed maintenance immunosuppression is not mutually exclusive and not all dosages are reported.

<sup>b</sup>Triple immunosuppression includes calcineurin inhibitors, steroids, and anti-metabolites.

<sup>c</sup>Of the 30 seronegative recipients before D3, 20 (67%) seroconverted.

**FIGURE 1** Violin plot of pre-D3 versus 1 month post-D3 antibody level, categorized by assay. Reference line delineates positive cut-offs for anti-RBD ( $\geq 0.8$  U/ml) and anti-S1 ( $\geq 1.1$  AU). Anti-RBD assay is measured along a log<sub>10</sub> scale on the y-axis. (A) Roche EIA; (B) EUROIMMUN EIA

breakthrough infection after D1, 17 occurred at a median (IQR) of 125 (116–150) days after D3. Among those 17 participants, 4 (24%) were seronegative after D3. Of the 13 seropositive patients, the median (IQR) post-D3 anti-RBD level was 2500 (1932–2500) U/ml. The median anti-S1 level after D3 was 8.94 AU.

## DISCUSSION

Most LT recipients demonstrated excellent antibody response to mRNA vaccination, with improved seroconversion and titer levels after D3. This response is greater than that reported for other organ recipients, yet, particularly amid the changing variant landscape, this report does not provide direct evidence of seroprotection. Notably, a subgroup of recipients on anti-metabolites and/or closer to transplantation remains seronegative after D3, and thus may warrant additional monitoring and protective interventions given the likely ongoing high risk for SARS-CoV-2 infection. Future directions include durability of vaccine response, including assessing for reversions after additional doses, with a link to clinical outcomes.

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## CONFLICT OF INTEREST

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