Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Post-SARS-CoV-2 vaccination specific antibody decrease — Thresholds for determining seroprevalence and seroneutralization differ

Dear editor,

We would like to respond to the Letter to the Editor of Bene et al. discussing our articles on the evaluation of the serological response 3 months after BNT162b2 vaccination[1-3]. The authors pointed out that we used a "*catchy label*" when referring to the observe decline of antibody titers. The authors added that our analysis can be "*in this touchy context, (be) interpreted as bad news*" and (they) "*would like to re-interpret these data in a more positive way by emphasizing the high antibody titers detected in this study*". The authors then discussed on selected studies supporting their assumptions, even though we previously commented on the limitations of some of these studies to appreciate the antibody response due to inadequate sample dilution[3].

In our opinion, we did not convey "*bad news*" and as implied by the authors of this letter, we did not use deliberately "*catchy*" words. We factually interpreted and discussed the analytical results we obtained. In addition, our manuscript already stipulated that "*it is important to notice that all participants still had a robust antibody response at 3 months*". We also added that "*moreover, the vaccination with BNT162b2 elicited much higher antibody titers at 3 months compared to the titers collected in serum from convalescent patients using the same assay*"[2]. An interim analysis of 75 out of the 231 subjects included in the CRO-Vax HCP study[4] reports a decline of 51% and 20% between day 90 and day 180 for the seronegative and seropositive groups, respectively.

We also noticed that the authors stipulate that the upper positive threshold of the test (without using sample dilution) is already 300-fold higher than the positivity threshold (i.e. 0.8 IU/mL) and seems to use this argument to support their optimistic view on the observed declined in antibody titers. We question on the relevance of such interpretation since the threshold of 0.8 IU/mL has not been determined against the neutralizing capacity but against positive RT-PCR to detect subjects who have been in contact with the virus. According to the manufacturer, a higher threshold is needed to correlate the Roche RBD total antibody assay (Roche Diagnostics, Machelen, Belgium) with the neutralizing capacity[5].

We take the opportunity of this response to report results showing that even high antibody titers as detected by the Roche RBD total antibody assay may not be neutralizing. Neutralizing antibodies are a subset of the antibodies produced against SARS-CoV-2, but they are considered linked to protective immunity due to their ability to block the viruses from entering the host cells[6]. Among our cohort of 75 COVID-19 patients representing in total 114 samples, we determine the neutralizing capacity using a pseudovirus neutralization test (pVNT) and a surrogate virus neutralization test (sVNT), two methods we described previously[7]. Results are presented in Table 1 and Fig. 1. One hundred and four samples were positive with the Roche RBD total assay but among them, 16 (15%) were negative with the pVNT though some showing high Roche RBD total antibody titers (range from 1.13 to 2219 AU/mL). These results are consistent with those obtained with the sVNT. Therefore, such results can provide inaccurate information on the level of protection against SARS-CoV-2 since some patients generate antibodies which are not neutralizing. However, among those who produce neutralizing antibodies, there is a correlation between the level of antibodies against RBD and their neutralizing capacity (Fig. 1), indicating that the waning effect we ob-

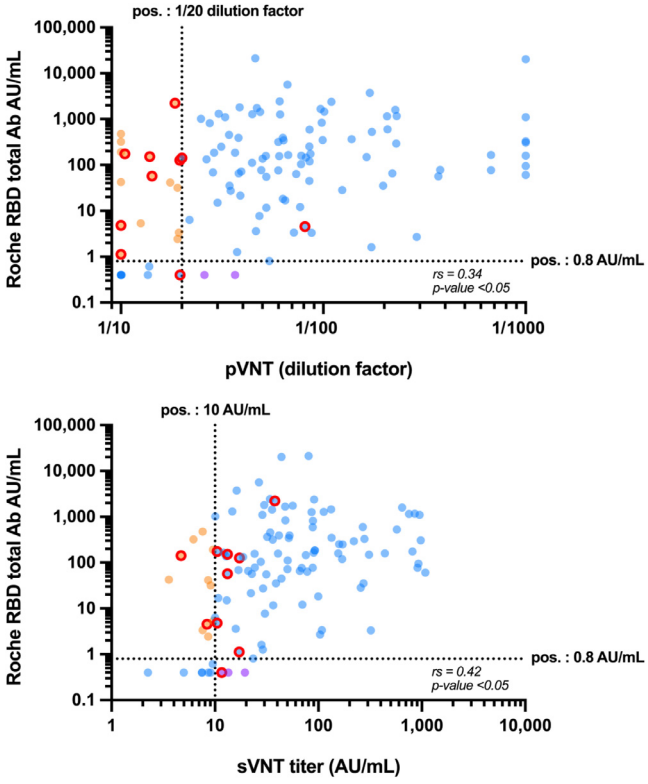

Fig. 1. Comparison of the results obtained with the Roche RBD total antibody assay and those obtained with the pseudovirus neutralization and surrogate virus neutralization tests. Blue dots represent samples for which the Roche assay and the corresponding neutralizing test agreed. Orange dots represent samples positive with the Roche assay and negative with the corresponding neutralizing test. Purple dots represent samples negative with the Roche assay and positive with the corresponding neutralizing assay. Dots which are surrounded by a red ring represent samples for which sVNT and pVNT results are divergent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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Table 1
Summary table of positive and negative results with the Roche RBD total Ab assay against the pVNT and the sVNT assays. Agreement between sVNT and pVNT is 91.23% (95% CI: 84.46—95.71%, 8 false positive and 2 false negative for sVNT vs pVNT, this mainly concerns samples close to the positivity threshold of the pVNT and sVNT assays).

Roche RBD total Ab	**pVNT**		**Total**	**sVNT**		**Total**
	Negative(*F* ≥ 1:20)	**Positive(*F* <1:20)**		**Negative(<10 AU/mL)**	**Positive(≧ 10 AU/mL)**	
Negative(< 0.8 AU/mL)	*8*	*2*	**10**	*7*	*3*	**10**
Positive(≥ 0.8 AU/mL)	*16*	*88*	**104**	*11*	*93*	**104**
Total	**24**	**90**	**114**	**18**	**96**	**114**
Roche RBD total Ab vs pVNT		**Roche RBD total Ab vs sVNT**				
Specificity	**Sensitivity**	**Specificity**	**Sensitivity**			
33.33%(95%CI: 15.63—55.32%)	97.78%(95%CI: 92.20—99.73%)	38,89%(95%CI: 17.30—64.25%)	96,88%(95%CI: 91.14—99.35%)			

served may finally reduce the protection against SARS-CoV-2. Our assumptions are in line with the results of Bergwerk et al. who reported that among fully vaccinated health care workers, the occurrence of breakthrough infection with SARS-CoV-2 was correlated with neutralizing antibody titers during the peri–infection period[8]. The authors of this study found that low titers of neutralizing antibodies and S-specific IgG antibodies may serve as markers of breakthrough infection, an observation which has also been made by Khoury et al.[9]. A study from Rus et al. proposed a Roche RBD total assay titer of 133 BAU/mL to reach the neutralizing threshold[10]. The identification of the correlation (or the lack of) between immunity and protection from SARS-CoV-2 is critical to predicting how the expected antibody decay will affect clinical outcomes, if and when a booster dose will be needed, and whether vaccinated persons are protected. All these observations lead at least to the following conclusions: 1) positivity threshold reported in the instruction of use of the Elecsys anti-SARS-CoV-2 spike is not a threshold for protection and our group and others demonstrated that higher antibody titers are needed to correlate with seroneutralization although this is also subject to the diversity of antibody response among individuals; 2) the neutralizing antibody titer is linked to the occurrence of breakthrough infection and therefore, decline or waning effect should be evaluated and anticipated in order to provide a rationale for the administration of a boost dose of vaccine especially in patients with expected low response to the vaccine like the elderly.

Finally, we would like to stress out that scientific observations should be interpreted as they are and should not be moderated under the pretext of potentially conveying « bad » news for the public. Vaccination against COVID-19 has proven and still prove efficacy but the decline in antibody titers should question the scientific community on the long-term protection against SARS-CoV-2 infection.

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Declaration of Competing Interest

The authors declare no conflict of interest in relation to the present study.

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