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Correspondence

Heterogeneity in protocols for bronchoalveolar lavage & sub-genomic RNA evaluation in non-human primate studies of SARS-CoV-2 vaccine candidates' evaluation

Sir,

I came across the article by Mukhopadhyay *et al*[1] on protective efficacy of various SARS-CoV-2 vaccine candidates in non-human primates (NHPs). The authors have evaluated the pre-clinical NHP studies of various vaccine candidates for SARS-CoV-2 in a comprehensive manner and have also brought out the strengths and limitation of these studies. I would like to highlight a few points that should have been brought out for better analysis as there is significant heterogeneity in the design of the studies considered for review. For conclusively proving the efficacy of vaccine candidates, virus challenge post-immunization is the most important step. Evaluation of genomic RNA (gRNA) and sub-genomic RNA (sgRNA) in the respiratory tract tissues in the throat swab, nasal swab and bronchoalveolar lavage (BAL) fluid aspiration is one of the most important aspects for evaluating the efficacy of the vaccine candidates. sgRNA has been considered as an important marker of active replication of the virus. The detection of sgRNA will be influenced by copy number of actively replicating virus. Of the 19 NHP studies analyzed by the authors, only seven[2-7] have provided the data on sgRNA. Even in the studies that have provided the data for sgRNA, there is gross variation in the values. The probable reason for the major variation in the data provided by various authors could be the volume and dose of the virus used for challenge. The volume of inoculum for challenge in the preclinical studies of SARS-CoV-2 vaccine candidates in the NHP model has varied from 0.5 (BNT162b2[2,8], NVX-CoV2373[2] and RBD[3]), 1.5 (BBV152[5]), 2 (INO-4800[6] and Ad26.CoV2.S[9]), 4 (mRNA-1273[6]) and 6.5 ml (ChAdOx-1nCoV-19[7]). The NHP studies for vaccine candidates carried out for PicoVacc[10], BBIBP-CorV[11] and GX-19[12] have performed the virus challenge, but the volume of virus used has not been mentioned. The NHP studies for vaccine candidates ARCoV[13], MRT5500[14] and LION/repRNA-CoV2S[15] have not carried out virus challenge in the post-immunization period. The route of administration of the virus during the challenge procedure has also not been uniform. In most of the NHP studies, challenge has been done by intranasal and intratracheal instillation[2-6,9]. Only intranasal[3,16] or intratracheal[10,11] routes also have been used. In addition, oral[7,12], intraocular[8,13] and intravenous[13] routes have also been used in the NHP challenge studies. The virus challenge dose has not been uniform across the reported studies apart from quite a few studies that have not performed virus challenge[13-15] altogether or have not mentioned the volume of virus used for challenge[3,6,8,9]. This factor is important in evaluating the sgRNA response of the NHPs to various vaccine candidates and could have been brought out in the article for understanding and proposing an optimum dose/volume of virus used for challenge studies.

Bronchoscopy and collection of the BAL fluid are other major procedures for evaluating the viral load in the lungs by assessing the titres of gRNA and sgRNA. There are no standard guidelines at present for the volume of saline to be used for instillation before aspiration of the BAL fluid. Another factor that is again of significance is the lobes of lung which are used to collect the BAL fluid. As per the published literature of NHP model of SARS-CoV-2, there is no predilection for particular lung lobes that are preferentially involved due the disease[5]. Hence, the detection of sgRNA will depend on the lobes from which the BAL fluid was aspirated. This factor is important because not all the seven lobes of lung of NHPs are easily accessible during bronchoscopy. The authors have not discussed

the details of the bronchoscopy procedure, volume of saline used for instillation before collection of BAL fluid and the lobes of lungs accessed during the procedure in the studies included in this review article. This aspect would have added to strength and would have provided better comparative analysis of the NHP studies.

Notwithstanding the above-mentioned facts, the efforts of the authors are commendable in presenting such an extensive comparative analysis of data in various vaccine candidates against SARS-CoV-2 pre-clinical evaluation in NHP challenge studies.

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Conflicts of Interest**:** None.

Sanjay Kumar
Department of Neurosurgery, Armed Forces Medical College, Command Hospital (Southern Command), Pune 411 040, Maharashtra, India
paraeagles@gmail.com

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