Robust cellular immune responses following two doses of BNT162b2 mRNA vaccine against SARS-CoV-2 in type 2 diabetic subjects: A longitudinal study in Bangladesh

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Abstract

Background: Diabetes, one of the most common comorbidities of COVID-19, is prioritized for SARS-CoV-2 vaccination. To evaluate the T-cell mediated immune responses, this study assessed and compared IFN-γ and IL-2 secreting PBMCs against the SARS-CoV-2 BNT162b2 mRNA vaccine in individuals with type 2 diabetes mellitus. **Methods:** In this longitudinal study, 19 known cases of type 2 diabetes and 16 healthy controls were included through purposive sampling and were regularly sampled before 1^{st} dose, 4 weeks after 1^{st} dose and 2-4 weeks after 2^{nd}dose of vaccination. Ex-vivo ELISpot assays for IFN-γ and IL-2 were done using isolated PBMCs. HbA1c status was determined by an automated analyzer before 1^{st} dose and SARS-CoV-2 spike-specific IgG antibody were assessed by CLIA 4 weeks after 1^{st}dose. A p -value <0.05 was considered significant. **Results:** All participants were seroconverted after 1^{st} dose and showed a robust rise of IFN-γ and IL-2 secreting PBMCs counts in type 2 diabetic participants irrespective of hypertension, gender and age after two doses of vaccination. However, IFN-γ secreting PBMCs counts were significantly lower in type 2 diabetic group than healthy control group after 1^{st} dose. Substantial negative correlations of HbA1c (%) to IFN-γ secreting PBMCs counts after 2^{nd} dose were also found in this study. **Conclusion:** Although type 2 diabetic participants had a lower response initially, BNT162b2 mRNA vaccine overall induces a robust T-cell mediated immune response.

Background of the study

Since World Health Organization (WHO) called the outbreak a "pandemic" on 11 March, 2020, despite significant movement restrictions, social distancing measures, and stay-at-home orders endorsed in many countries, the COVID-19 pandemic has caused overwhelming morbidity and mortality.[1] To mitigate the escalating encumbrance of COVID-19, vaccine development had taken place at a phenomenal speed and has spawned a slew of vaccines of different platforms and varying degrees of effectiveness and protection; most using the SARS-CoV-2 surface spike as the main antigenic target for the generation of binding and neutralizing antibodies and T cells, as well as an antigen coding sequence based on the Wuhan lineage virus that was first described (Gen Bank accession number M908947).[2]; [3]

SARS-CoV-2 infection has a wide clinical spectrum, ranging from asymptomatic to severe and deadly diseases, especially in case of co-morbidities.[4] Diabetes is undoubtedly one of the most important comorbidities of COVID-19 and the prevalence of SARS-CoV-2 infections range from 5% to 36% among the diabetic patients globally.[5]; [6] The intricate interaction between COVID-19 infection and diabetes get worse significantly with increasing age of patients and longer period of uncontrolled diabetes.[7] Diabetes causes intensification of the severity of COVID-19 disease by compromising innate immunity, triggering an enhanced

pro-inflammatory response, and lowering angiotensin-converting enzyme 2 (ACE2) expressions.[8] Diabetes patients have been reported to have a lower clearance of SARS-CoV-2 from their bloodstream, which along with poor T cell activity, and associated cardiovascular illness lead to the vulnerability of diabetic individuals to COVID-19 infections.[9] Furthermore, dysregulation in immune response is also aided by an adverse hormonal environment in Type 2 diabetes mellitus (T2DM) patients.[10] Hence, T2DM patients are among the priority group to receive vaccination against SARS-CoV-2 worldwide as per recommendation from WHO.[11]

Immunocompromised patients have demonstrated decreased immune response compared to the healthy group. While in prior studies the humoral response (IgG) in healthy control was found to be $> 99\%$ against BNT162b2 (Pfizer-BioNTech), Bergman *et al.* showed that only 72.2% of the patients (solid organ recipients and patients living with the human immunodeficiency virus) had seroconverted 2 weeks after administrating the 2nd dose of the BNT162b2 (Pfizer-BioNTech) vaccine.[12]; [13]; [14] In the case of diabetes, the evidences regarding immune responses are mixed worldwide.[15]; [16]; [17] However, the paucity of data on cell-mediated response to the SARS-CoV-2 vaccine in T2DM patients and data on the immunological vaccine responses related to glycemic control in participants with T2DM in the hitherto available clinical trials of COVID-19 vaccines, calls for more research in this subgroup.[18] Moreover, no study on a comprehensive and integrated analysis of immune parameters with response kinetics to SARS-CoV-2 vaccination in correlation with glycemic control in type 2 diabetic patients has ever been conducted in Bangladesh. In Bangladesh, with more than 13.1 million people living with diabetes, the question regarding the effect of glycemic control over immune response following vaccination against SARS-CoV-2 remains. [19] In these circumstances, this study aimed to assess and compare IFN-γ and IL-2 secreting peripheral blood cells (PBMCs) against the SARS-CoV-2 BNT162b2 (Pfizer-BioNTech) mRNA vaccine in type 2 diabetic individuals attending a tertiary care hospital in Bangladesh.

Materials and Methods

Study design:

This is a longitudinal observational study to evaluate the humoral and T-cell mediated immune response against SARS-CoV-2 mRNA vaccine in individuals at or above 30 years of age with type 2 diabetes mellitus attending a tertiary care hospital of Bangladesh. A total of 19 adults with pre-diagnosed type 2 diabetes mellitus and 16 apparently healthy individuals were included in this study as type 2 diabetic group and healthy control group respectively. Participants were studied before 1^{st} dose (T1), 4 weeks after 1^{st}dose (T2) and 2-4 weeks after 2^{nd} dose (T3) of BNT162b2 mRNA (Pfizer-BioNTech) vaccination. Study participants were selected from November 2021 to April 2022 by purposive sampling method from the Endocrinology outpatient department and COVID-19 Vaccine Centre of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. All laboratory procedures were performed in the Department of Virology, BSMMU. For the purpose of this study, the cut-off value of HbA1c was set at 7% and participants were categorized as follows: type 2 diabetes mellitus patients with HbA1c $>7\%$ as good glycemic control and type 2 diabetes mellitus patients with HbA1c $\leq 7\%$ as inadequate or poor glycemic control.[20] Body Mass Index (BMI) (kg/m^2) was measured and categorized as BMI <25 kg/m^2 and BMI ≥ 25 kg/m^2. Hypertension was defined by either a documented diagnosis of hypertension, the patient taking antihypertensive medications, or the latest (within three months) blood pressure readings (either systolic ≥ 140 or diastolic ≥ 90).

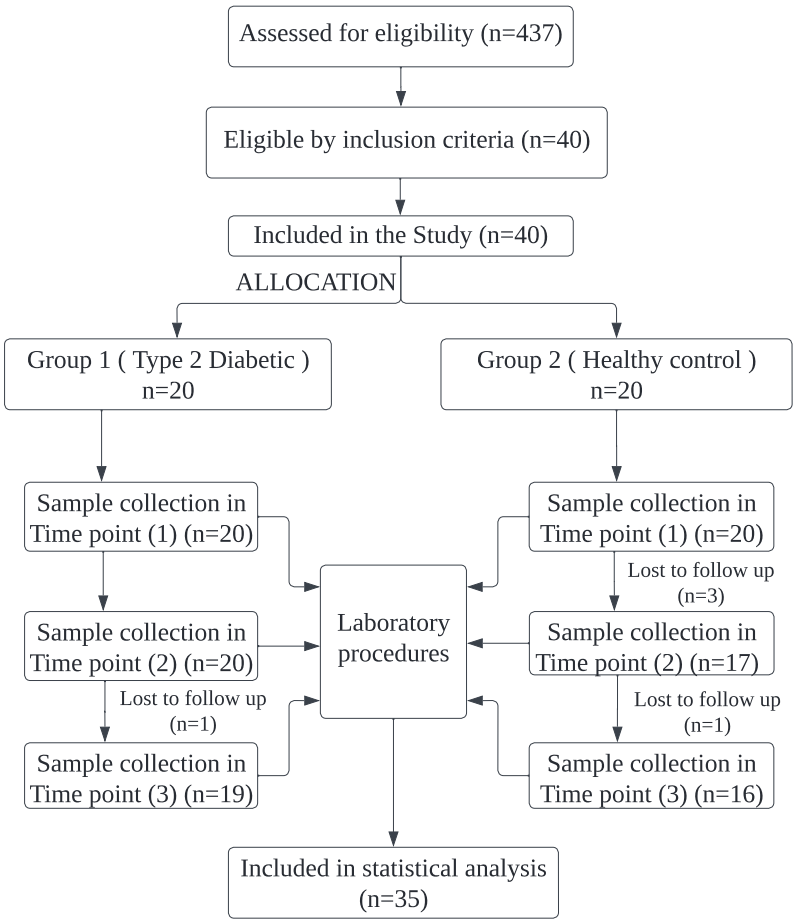

Figure 1: Study flow chart showing the participation of the study subject in different timepoints.

Blood sample collection and processing:

After signing the consent form, with aseptic precautions 4 ml venous blood samples were collected at each time in Plain vacutainer tubes (1ml) and sodium heparin vacutainer tubes(3ml). The blood collected in plain vacutainer tubes were allowed to clot by leaving it undisturbed at room temperature for 30 minutes, followed by centrifugation at 1,000 x g for 10 minutes in a refrigerated centrifuged (cells in pellet), the supernatant was collected and transferred into a clean micro centrifuge tube, aliquoted and stored at –20°C after proper labelling. Sodium heparin containing vacutainer tubes were processed for the collection of PBMCs by density gradient centrifugation and immediately used in ELISpot assay. An extra 1ml Blood was collected in EDTA vacutainer tubes from the participants before 1^{st} dose of vaccination, were labelled properly and sent to Department of Biochemistry, BSMMU for HbA1c determination by an automated analyzer (Sebia capillary).

Detection of IgG antibodies specific to SARS-CoV-2:

Before 1^{st} dose and 4 weeks after 1^{st} dose of BNT162b2 mRNA (Pfizer-BioNTech) vaccination, using processed blood serum, IgG antibody specific to SARS-CoV-2 infection were detected by Automated LIAISON®

XL Analyzer using FDA approved SARS-CoV-2 IgG chemiluminescence enzyme immunoassays kit (LIAISON® SARS-CoV-2 TrimericS IgG assay, REF 311510, Diasorin, Italy) following manufacturer instructions to assess the seroconversion rate. Detection range of assay was 4.80 to 2080 BAU/mL (Binding Antibody Unit/mL) and test results were reported as positive ($\geq$ 33.8 BAU/mL) or negative (<33.8 BAU/mL).

Ex-vivo IFN-γ and IL-2 ELISpot assay

On each time point for each participant, sample collected in the sodium heparin vacutainer tubes was processed for the collection of PBMCs by density gradient centrifugation using Histopaque®-1077 (SigmaAldrich, Germany) by previous method [21] and ex-vivo ELISpot assays for IFN-γ and IL-2 were done using ELISpot assay-kit (AutoImmuneDiagnostika GmbH, Germany) according to manufacturer instructions. The pre-coated multi-test-plate (MTP) was adjusted to room temperature and was taken out of the bag under sterile conditions in Biosafety cabinet class II type A2. A 100µl of RPMI-1640 medium was added in negative control well, a 100µl of mix 1(80µl medium + 20µl Pokeweed mitogen (PWM) (Thermo Fisher Scientific Inc., USA) (final conc. of Mix 1 working solution was 2 µg/mL) was added in positive control[22] and a 100µl of mix 2 (80µl medium + 20µl of SARS-CoV-2 Spike RBD (RBM) Peptide Pool (SinoBiological Inc, China) stock solution (final conc. of Mix 2 working solution was 2 µg/mL)[23] was added in test well. Finally, 100µl of freshly isolated PBMC cell suspension adjusted at about 2x106 cells/ml was added in each well. After 24 hours incubation, plate was removed from the incubator. Wells were emptied and washed for 6 times with 200µl of washing buffer in each well. A 10µl of diluted AP-conjugated secondary antibody was added per well and incubated for 2 h at room temperature in a humid chamber. A 100µl of diluted streptavidin was added per well and incubated for 1 h at room temperature in a humid chamber. Wells were emptied and washed for 6 times with 200µl of washing buffer / well. A 100µL of Substrate was added per well and incubated for 5-20 minutes at room temperature until spots were clearly visible. To stop the reaction, washing was done for 3 times with distilled water. The plate was allowed to dry thoroughly overnight by putting upside down over tissue paper. After drying the plate was evaluated with AID ELISpot Reader 7.0 System and the number of spots in the sample well was calculated at a prefixed count setting which indicates the number of PBMCs secreting IFN-γ or IL-2 accordingly.

Statistical analysis:

After collection of all the required data, these were checked, verified for consistency and tabulated. Collected data processed using Microsoft Excel® 2019. The statistical analysis was conducted and the 95% confidence intervals for each category were calculated at 10% acceptable error level using using IBM® SPSS Statistics 25.0, jamovi 2.2.5 (based on R software), and DATAtab online statistical calculator. Dataset was tested for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and for non-normal data non-parametric test as Mann-Whitney U-Test (two-tailed), Friedman Test etc. was used. A p value of <0.05 were considered significant.

Results

Out of 35 participants, 19 (54.3%) were diagnosed cases of type 2 diabetes mellitus (Group 1) and 16 (45.7%) were non-diabetic healthy control (Group 2). The mean age of type 2 diabetic group and healthy control group was 53.2±9.12 (SD) and 50.4±11.4 (SD) years respectively. Among type 2 diabetic participants (n=19), male and female ratio was 1.11:1, while it was 1:1.29 in case of healthy control group (n=16). Majority of the participants, were from urban areas (n=20, 57.1%) and belonged to middle-income families (n=23, 65.7%). Mean duration of sample collection after 2nd dose was 24.2±3.39 days. Among all participants, 16 (45.7%) were hypertensive and 19 (54.3%) were normotensive. Subdividing based on body mass index (BMI), 12 (34.29%) had a BMI $\geq$25 kg/m^2 and 23 (65.71%) had a BMI <25 kg/m^2 (Table 1).

		Type 2 Diabetic (n=19)		Healthy control (n=16)	
		Male (n=10) (52.63%)	Female (n=9) (47.37%)	Male (n=7) (43.75%)	Female (n=9) (56.25%)
Age (years)(mean±SD)		54.9 ± 6.9	51.33 ± 11.22	53.14 ± 12.56	48.33 ± 10.69
Residence					
	Urban	6 (31.58%)	4 (21.05%)	3 (18.75%)	7 (43.75%)
	Rural	4 (21.05%)	5 (26.32%)	4 (25%)	2 (12.5%)
Socio-economic status					
	High	1 (5.26%)	2 (10.53%)	-	-
	Middle	6 (31.58%)	5 (26.32%)	5 (31.25%)	7 (43.75%)
	Low	3 (15.79%)	2 (10.53%)	2 (12.5%)	2 (12.5%)
Hypertension					
	Hypertensive	5 (26.32%)	6 (31.58%)	1 (6.25%)	4 (25%)
	Normotensive	5 (26.32%)	3 (15.79%)	6 (37.5%)	5 (31.25%)
BMI level					
	<25 kg/m^2	9 (47.37%)	6 (31.58%)	5 (31.25%)	3 (18.75%)
	[?]25 kg/m^2	1 (5.26%)	3 (15.79%)	2 (12.5%)	6 (37.5%)

Table 1: Characteristics of the study participants.

All participants received BNT162b2 (Pfizer-BioNTech) mRNA vaccine, which was well tolerated with no serious AEFI (Adverse events following immunization) was observed. According to the measured HbA1c status, 5 participants (26.32%) with type 2 diabetic had good (<7.0%) glycemic control and 14 (73.68%) had inadequate or poor (≥7.0%) glycemic control. The mean of HbA1c was 6.3±0.45 (SD) in type 2 diabetic participants with good glycemic control and 9.27±1.77 (SD) in type 2 diabetic participants with inadequate or poor glycemic control. Participants of the healthy control group had a mean HbA1c of 5.74±0.294 (SD). All participants of both groups were found seroconverted at 4weeks after 1st dose of vaccination.

Comparisons of IFN-γ and IL-2 secreting PBMCs counts from ex-vivo ELISpot assay in type 2 diabetic participants and healthy control showed significant differences (p <0.001) in the repeated measures of both IFN-γ and IL-2 secreting PBMCs counts in both type 2 diabetic and healthy control groups with time. A significant difference (p =0.021) was also observed in the IFN-γ spot counts between T2DM group and control group at 4 weeks after 1st dose (T2).

Analysis was performed to compare IFN-γ and IL-2 secreting PBMCs against SARS-CoV-2 BNT162b2 (Pfizer-BioNTech) mRNA vaccine in type 2 diabetic participants in context of hypertension, BMI level, gender and age, which showed robust rise of IFN-γ and IL-2 secreting PBMCs counts with time in type 2 diabetic group irrespective of hypertension, BMI level, gender and age. The results of Spearman's correlation test showed significant negative correlation of HbA1c to IFN-γ secreting PBMCs counts (Spearman's rho=-0.47, p =0.043) at T3 in T2DM group. Non-significant correlations of HbA1c to other parameters were shown in control group.

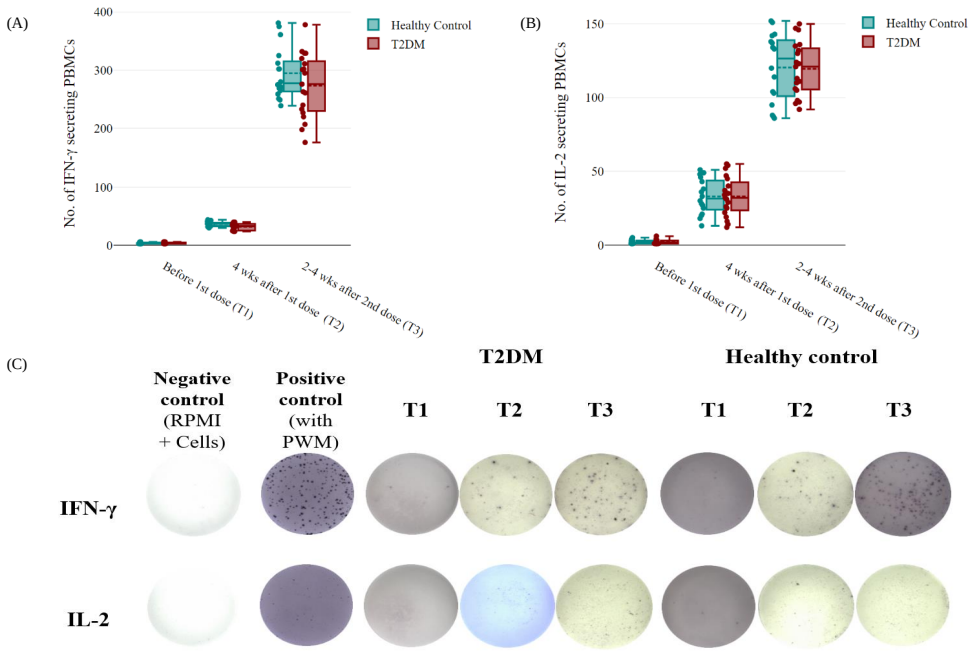

Figure 2: Robust rise of both IFN-γ (A) and IL-2 (B) secreting PBMCs was shown in both healthy control group and T2DM group after ex-vivo ELISpot assays. (C) Representative ELISpot wells of IFN-γ and IL-2 ELISpot assay with positive control (stimulated with PWM) and negative control (RPMI medium and cell suspension).

Discussion

Since the emergency approval, vaccination against SARS-CoV-2 remained the mainstay for preventing COVID-19 notably severe disease, along with social distancing, application of personal protective equipment (e.g., face mask) and maintenance of proper hygiene. The immune response after vaccination, developed by both innate and adaptive immune systems, were found to be slowed and delayed in immunocompromised groups.[18] However, the immunological reactions to COVID-19 vaccinations in type 2 diabetic individuals were little understood up to this point.[24]

In the present study, IFN-γ and IL-2 secreting PBMCs were evaluated in known cases of type 2 diabetes and healthy individuals aged 53.2±9.12 years and 50.4±11.4 years respectively. Through purposive sampling an approximately equivalent distribution of both male and female gender was also taken with a minute female predominance. Most of the participants in this study had a BMI <25 kg/m^2. A major percentage of participants in the type 2 diabetic group had inadequate or poor (HbA1c≥7.0%) glycemic control. Poor glycemic control was an effective indicator of disease severity and mortality in patients with COVID-19.[25] Participants were studied at 3 time points, baseline (T1) being at the day of 1st dose of vaccination, at the day of 2nd dose of vaccination (T2) which was 4 weeks after 1st dose and lastly between the time period of 2-4 weeks after the 2nd dose (T3) with a mean duration of 24.2±3.39 days.

All participants had negative IgG titer at the baseline time point before 1st dose (T1). SARS-CoV-2 specific IgG antibody was assessed in this study to perceive any differences in seroconversion rate between T2DM participants and the healthy control group, and both groups showed a 100% seroconversion rate at 4 weeks

after 1st dose of BNT162b2 (Pfizer-BioNTech) mRNA vaccine. The participants in both type 2 diabetic group and healthy control group had a significant rise in both IFN-γ and IL-2 secreting PBMCs counts from T1 to T3. However, the type 2 diabetic groups showed a lower IFN-γ secreting PBMCs counts after 1st dose (T2) than healthy control group indicating a lower IFN-γ response which was also demonstrated in a study where Van Praet *et al.* showed the association of diabetes with a lower cellular response against BNT162b2 (Pfizer-BioNTech) mRNA vaccine.[26] Prior studies showed that diabetic subjects display a delayed adaptive immune response to pathogen.[27] Dysfunction of both innate immune response (including dysfunction of neutrophils and macrophages) and adaptive immune response (including T cells) are supposed to be accountable for the feeble immune system against invading pathogens in diabetic subjects.[28]

In this study, significant negative correlation of HbA1c (%) to IFN-γ secreting PBMCs count at T3 were observed in type 2 diabetic group which coincides with the CAVEAT study by Marfella *et al* ., in which authors demonstrated significantly reduced immune response, particularly neutralizing antibody, in T2DM patients with HbA1c >7% than normoglycemic subjects and T2DM patients with good glycemic control.[24] Reduced lymphocyte proliferative responses CD4+ to CD8+ lymphocyte ratio, decreased macrophage or monocyte function, and abnormalities with antigen presentation were seen in patients with hyperglycemia and insulin resistance and may resulted in this delayed immune response.[15]; [29] However, Sourij *et al.* demonstrated that HbA1c (%) levels did not have significant impact on antibody levels after COVID-19 vaccination from which the present study differs.[30] In the present study, robust rise of IFN-γ and IL-2 secreting PBMCs counts were observed from T1 to T3, irrespective of hypertension status, BMI level and age of the type 2 diabetic participants. There was also no discernible difference in IFN-γ and IL-2 secreting PBMCs counts from ELISpot assays between the genders in present study which was similar to the findings by Van Praet *et al.*, however, Schwarz *et al.* detected delayed and reduced antibody and T-cell responses in elderly people, to which the present study differs.[26]; [31]

The present study had quite a few limitations, too. Firstly, due to restrictive recruitment along with financial and time constraints, this study was relatively small and was complicated by low statistical power. Larger prospective studies are required to confirm the findings of this study. This study was performed in a single center and only BNT162b2 (Pfizer-BioNTech) mRNA vaccinees were included in this study. A further difficulty was the high individual variability, inherent to human studies. To account for this, very robust statistics, e.g., median value, two-sided Mann-Whitney-U test, etc. were used.

Conclusion

The present study demonstrated that BNT162b2 (Pfizer-BioNTech) mRNA vaccine induces robust T-cell mediated immune response in both type 2 diabetic participants and healthy individuals. However, type 2 diabetic group showed lower immunological responses specially after 1st dose when compared with healthy control. This study also demonstrated that hypertension, gender and age did not affect the cellular immune response in type 2 diabetic participants.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Ethics statement

This study was reviewed and approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (reference: BSMMU/2021/10168, Registration number: 3692). The patients/participants provided their written informed consent to participate in this study.

Author contributions

The manuscript of this study was prepared by SH, revised by TA and reviewed by AN.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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