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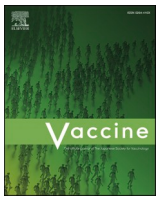

BNT162b2 mRNA COVID-19 vaccine effectiveness in pregnancy: Emulating trial NCT04754594 using observational data from Norwegian health registries

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ABSTRACT

Previous observational studies of mRNA COVID-19 vaccine effectiveness in pregnancy differ from the randomized trial NCT04754594, limiting causal inference. Using nationwide Norwegian health registry data, we emulated trial NCT04754594 to assess agreement of point estimates for BNT162b2 vaccine effectiveness in pregnancy.

We included pregnancies reaching gestational week 24 after January 1, 2021, and delivering before November 30, 2022. Vaccinated individuals received a first dose between weeks 24–34 of gestation, with a second dose assumed within 21 days (intention-to-treat, ITT); the as-treated (AT) analysis required second-dose administration. We sequentially matched vaccinated individuals by gestational week to those who remained unvaccinated until the same gestational week, using propensity scores based on baseline covariates and last menstrual period date. Outcomes were SARS-CoV-2 infection and SARS-CoV-2-related hospitalization until one month post-delivery.

Among 5790 pregnancies (2895 first dose-vaccinated, 2895 unvaccinated), mean maternal ages were 31.1 years (SD 4.3) in the unvaccinated, and 31.4 years (SD 4.6) vaccinated group. Mean BMI was 24.7 (SD 4.6) and 24.9 (SD 5.0), respectively. The ITT analysis showed that vaccination was associated with a 20 % lower risk of infection (IRR: 0.80, 95 % CI: 0.48, 1.13); the AT analysis showed a 39 % lower risk (IRR: 0.61, 95 % CI: 0.27, 0.95), comparable to that of the trial (41 % risk reduction). SARS-CoV-2-related hospitalizations could not be evaluated due to the rarity of the outcome in our population.

This trial emulation demonstrated satisfactory statistical alignment with trial results, supporting the use of observational data to assess vaccine effectiveness in pregnant populations.

Abbreviations: AT, as-treated; ATC, Anatomical Therapeutic Chemical Classification; BMI, Body Mass Index; CI, confidence interval; COVID-19, coronavirus disease of 2019; HR, hazard ratio; ICD-10, International Classification of Diseases, version 10; ICPC-2/ICPC-2B, International Classification of Primary Care; IRR, incidence rate ratio; ITT, intention-to-treat; KUHR, Norwegian Control and Payment of Health Reimbursements Database; LMP, last menstrual period; LMR, Norwegian Prescription registry; MBRN, Medical Birth Registry of Norway; MSIS, Norwegian Surveillance System for Communicable Diseases; NPR, Norwegian Patient Registry; PCR, polymerase chain reaction; RCT, randomized controlled trial; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SD, standard deviation; SMD, standardized mean difference; SSB, Statistics Norway; SYSVAK, Norwegian Immunization Registry; TTE, target trial emulation.

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1. Introduction

Randomized controlled trials (RCTs) constitute the gold standard for establishing causal effects of medications and vaccines. However, ethical constraints often preclude their conduct in pregnant and breastfeeding populations [1]. In such cases, target trial emulation (TTE), mimicking an RCT using observational data, serves as a valuable alternative [2,3]. Nevertheless, drawing causal conclusions from observational studies poses important challenges due to inherent risks of bias in the absence of randomization [4]. Reliable TTE is pivotal when real-world evidence on effectiveness and safety is needed to inform public health interventions and clinical decision-making, particularly in populations underrepresented in randomized controlled trials. Illustrating its potential, the RCT-DUPLICATE initiative emulated 32 drug-effectiveness trials in non-pregnant adults and found that observational estimates closely mirrored the original RCT results, with a Pearson correlation coefficient of 0.82 [5].

The scarcity of RCTs involving pregnant individuals, compared to the general population, has so far hampered similar initiatives [6]. During the COVID-19 pandemic, the need for methodologically sound and timely evidence about the safety and efficacy of the newly introduced vaccines in pregnancy was of paramount importance, given the heightened risks COVID-19 posed to maternal and child health [7,8]. The NCT04754594 RCT evaluating the safety, tolerability, and immunogenicity of the COVID-19 mRNA vaccine BNT162b2 in healthy pregnant individuals commenced on December 16th, 2021, and concluded on July 15th, 2022, with results published in 2024 [9]. The RCT concluded that the vaccine is safe and effective during pregnancy, with an incidence rate ratio (IRR) of 0.59 for asymptomatic SARS-CoV-2 infection within 1 month postpartum among vaccinated pregnant individuals [9]. However, the BNT162b2 vaccine had already received marketing authorization in several countries by December 2020 [10]. In the absence of a conclusive RCT, a large percentage of pregnant individuals were not vaccinated against COVID-19 in early 2021 with the vaccine uptake estimates typically being around 50 % [11–14]. COVID-19 vaccination of pregnant individuals was gradually recommended in mid-late 2021 as more real-world evidence about their safety and efficacy accumulated from observational studies [15–19].

Although prior observational studies varied in study designs, exposure windows and outcome definitions, they constantly showed a reduced risk of maternal COVID-19 disease with mRNA COVID-19 vaccination in pregnancy and no substantial associations with adverse birth outcomes or neonatal mortality. Because these studies differed substantially from the NCT04754594 RCT in terms of design and analysis, they provide only limited insight into how reliably observational data can reproduce causal inferences in pregnant populations. To address this gap, we emulated the NCT04754594 RCT of the COVID-19 mRNA vaccine BNT162b2 in pregnant individuals using observational data from Norwegian health registries, to examine the reliability of causal conclusions on vaccine effectiveness in pregnant individuals drawn from observational research. We aligned our emulation with the RCT design parameters, including eligibility criteria, treatment assignment, time-zero specification, efficacy outcomes, follow-up, and causal contrast, allowing us to assess how closely an observational study can replicate the BNT162b2 vaccine effectiveness in pregnancy demonstrated in the NCT04754594 RCT.

2. Methods

To emulate the target trial, we harnessed observational, individual-level linked data from six nationwide Norwegian health registries. The Medical Birth Registry of Norway (MBRN) is a population-based registry providing comprehensive information on pregnancies including the estimated gestational age at delivery based on ultrasound, which is used to calculate the last menstrual period (LMP) date, and medical conditions of the mother and the child before and during pregnancy as well as during and after delivery. The MBRN is based on mandatory notification of all pregnancies lasting more than 12 weeks and allows direct linkage of child records with those of their mothers [20]. The Norwegian Immunization Registry (SYSVAK) is a national electronic immunization registry based on mandatory records of individual vaccination since birth and supplied details concerning vaccinations and their date of administration [21]. The Norwegian Surveillance System for Communicable Diseases (MSIS) contributed data regarding SARS-CoV-2 PCR-positive tests and the date of the test [22]. The Norwegian Patient Registry (NPR) provided information on specialist outpatient and inpatient clinical diagnoses, and diagnostic codes follow the International Classification of Diseases, version 10 (ICD-10) classification [23]. The Norwegian Control and Payment of Health Reimbursements Database (KUHR) supplemented data on clinical diagnoses during visits to primary care and general practitioners; the diagnosis classification follows the International Classification of Primary Care (ICPC-2/ICPC-2B) [24]. The Norwegian Prescription registry (LMR) was used to obtain information on drug proxies, including detailed records of all prescription drugs dispensed in all community pharmacies in Norway, their Anatomical Therapeutic Chemical (ATC) classification, and dispensing date [25]. Statistics Norway (SSB) is the central agency that provides official statistics in Norway, which provided data about maternal education, profession, and gross income as a measure for socioeconomic status [26]. All these data sources employ a unified identification system of all residents in Norway, facilitating deterministic data linkage. Table 1 details how we operationalized the emulation protocol. Details about clinical diagnostic codes and medication prescription fills used for the definition of the eligibility criteria are shown in **Table S1**. As done in prior research [27], we first describe the elements of the target trial protocol and then we follow it by a detailed description of our emulation that closely resembles the RCT settings.

3. Target trial emulation

3.1. Eligibility criteria

Eligible participants included healthy individuals aged 18 years or older, with a gestational age between 24 and 34 weeks at the time of their first vaccine dose. Eligible unvaccinated participants were those who had reached the same gestational week at which vaccination was administered to their vaccinated counterparts without being vaccinated, nor delivering, nor developing a SARS-CoV-2 infection. We included only individuals who had had an uncomplicated singleton pregnancy up to the start of follow-up. An uncomplicated pregnancy is defined as the absence of conditions such as hyperemesis gravidarum, vaginal bleeding, placenta accreta, abruptio placenta, early preeclampsia, from the day ofLMP up to the gestational week of starting the follow-up. All diagnostic ICPC-2 and ICD-10 codes used to define these conditions can be found in **Table S1**.

Exclusion criteria included a positive SARS-CoV-2 PCR test ever before the week of start of follow-up (including before pregnancy start), psychiatric conditions, bleeding disorders, HIV, chronic hypertension within one year before LMP up to the week of start of follow-up, immunodeficiency, and alcohol or illicit drug abuse from six months before the LMP to the week of start of follow-up. These conditions were identified using ICD-10 and ICPC-2 codes, supplemented by drug proxies to capture additional cases (**Table S1**).

3.2. Treatment strategies

We identified pregnant individuals who were vaccinated with the BNT162b2 vaccine between GW24–34, with at least one (for intention-to-treat; ITT) and two doses with no gap restrictions between the two doses (for as-treated; AT), as recorded in SYSVAK. Pregnant individuals with no records for any COVID-19 vaccine up until the gestational week of enrollment including were considered unvaccinated.

Table 1
Outline of the key protocol elements of the target trial and their respective emulation using real-world observational data.

Protocol component	Target trial definition	Target trial emulation
Eligibility Criteria	Inclusion criteria: 1. Healthy women ≥18 years of age who are between 24 0/7 and 34 0/7 weeks' gestation on the day of planned vaccination, with an uncomplicated, singleton pregnancy. 2. Healthy participants determined by medical history, physical examination, and clinical judgment to be appropriate for inclusion in the study. 3. Documented negative HIV antibody test (Phase 2 only), syphilis test, and HBV surface antigen test during this pregnancy and prior to randomization. Exclusion criteria: 1. Other medical or psychiatric condition including recent (within the past year) or active suicidal ideation/behavior. 2. Previous clinical (based on COVID-19 symptoms/signs alone, if a SARS-CoV-2 NAAT result was not available) or microbiological diagnosis of COVID-19. 3. Participants with known or suspected immunodeficiency. 4. Bleeding diathesis or condition associated with prolonged bleeding that would in the opinion of the investigator contraindicate intramuscular injection. 5. Previous vaccination with any COVID-19 vaccine. 6. Current alcohol abuse or illicit drug use. 7. Participants who receive treatment with immunosuppressive therapy, including cytotoxic agents or systemic corticosteroids, e.g., for cancer or an autoimmune disease, or planned receipt through the postvaccination blood draw.	Same. Full details of the eligibility criteria can be found in **Table S1**, and Fig. 1. Inclusion criteria: 1. Individuals ≥18 years of age receiving at least one dose of the vaccine between gestational weeks 24 and 34, and controls who didn't. Both had an uncomplicated singleton pregnancy. Uncomplicated pregnancies were defined to have no records of hyperemesis gravidarum, Haemorrhage in early pregnancy, Placenta accreta, Abruptio placenta, Early-onset preeclampsia, Chronic hypertension. 2. Participants were deemed healthy if they fit all other eligibility criteria. 3. No record of HIV diagnosis in secondary care. Exclusion criteria: 1. Psychiatric disorders namely Schizophrenia, Bipolar disorder, and suicidality. 2. Previous positive SARS-CoV-2 PCR test, or a clinical diagnosis of COVID-19 infection. 3. Participants with a clinical diagnosis for primary immunodeficiency. 4. Clinical diagnosis of any bleeding condition of the following: Haemophilia, Von Willebrand's, dysfibrinogenemia, hyperheparinemia, hyperthrombinaemia, primary thrombophilia. 5. Previous vaccination with any COVID-19 vaccine. 6. Clinical diagnosis of alcohol abuse or drug use. 7. Immunosuppressants prescription.
Treatment Strategies	Administering 30 μg of BNT162b2 or 30 μg placebo saline in 2 doses, 21 days apart.	No record of vaccination was taken to be equivalent of a placebo. The gap of 21 days apart was assessed in a sensitivity analysis.
Treatment Assignment	The trial is randomized, with triple masking. Participants were therefore assigned to either of the study arms, in a 1:1 ratio, without being aware of the arm they were allocated to.	Participants are allocated to the treatment arm that aligns with their observed exposure. We used propensity score matching based on several baseline covariates measured before week 24 of gestation (**Table S2**).
Outcomes	Incidence of SARS-CoV-2 in pregnant women	Same. Maternal SARS-CoV-2 infection, defined as a positive PCR test and/or COVID-19 diagnosis in primary or secondary care. We assumed that outcome severity was of critical importance, and thereby also assessed severe COVID-19 infection leading to hospitalization, defined as receiving a respiratory diagnosis related to COVID-19 during a hospital visit within 7 days before and up to 14 days after having a positive SARS-CoV-2 PCR test and/or COVID-19 diagnosis in primary or secondary care [29].
Follow-up	Since administering the first dose and up to 1 month post delivery	Same. For the unvaccinated arm, the follow-up started when their matched vaccinated pair was administered the first dose of the vaccine.
Causal Contrast	Per-protocol effect	Both the observational analogue of intention-to-treat and the observational analogue of per-protocol effects.
Statistical Analysis	Incidence rates and their 95 % CIs are reported among vaccinated and unvaccinated pregnant individuals	Same; we used modified Poisson regression models with robust variance to obtain IRRs and their 95 % CIs. Additionally, we accounted for confounding using propensity score matching. Kaplan–Meier estimator was also fit to construct cumulative incidence curves.

3.3. Treatment assignment

To counteract the lack of randomization in our study, we sequentially matched, in each gestational week; in a 1:1 ratio, our vaccinated and unvaccinated cohorts on propensity scores based on multiple baseline confounders, namely maternal age at the gestational week of start of follow-up, parity, education, profession, region of residence, previous influenza vaccination, income, race/immigration status, comorbidities (such as respiratory diseases, thyroid disorders, urinary tract infections, systemic infections, and candidiasis), BMI before pregnancy, marital status, obstetric history, and LMP calendar date. Covariate data were obtained from the MBRN, SSB, KUHR, LMR and NPR (**Table S2**). To ensure a fair comparison between the vaccinated and unvaccinated as gestation progressed, we matched vaccinated individuals at the specific gestational time (weekly) when the first vaccination dose took place with unvaccinated individuals who reached the same gestational time without getting a dose of the vaccine. This means, for example, that a woman vaccinated with the first dose of the BNT162b2 vaccine in gestational week 25 was matched to an unvaccinated woman who reached gestational week 25 +/− 1 week, and their outcomes were later compared. This helped minimize the risk of immortal time bias and control for time-varying confounding [28].

3.4. Follow-up period

Time zero was defined as the date of administering the first vaccine dose in the period of week 24–34 of gestation, with the correspondingly matched unvaccinated who reached same gestational age (weekly) without getting a dose of the vaccine having the same time zero as their vaccinated counterparts. Follow-up started at the treatment assignment and ended at 1 month post-delivery. Matched unvaccinated individuals who got vaccinated at a later gestational week were censored along with their match at the week of switching treatment arms, and then rematched to another unvaccinated individual at that time point. Individuals were censored at developing the outcome. Since our exposure window runs from gestational week 24 to week 34, individuals vaccinated after gestational week 34 were not censored.

3.5. Causal contrast of interest

The causal contrast of interest is both the observational analogues of the intention-to-treat and per-protocol effects.

3.6. Outcomes

The primary outcome was maternal SARS-CoV-2 infection, defined as having a record of at least one positive PCR test for SARS-CoV-2 infection in MSIS and/or having a COVID-19 diagnosis (ICD-10: U07.1, U07.2; ICPC-2: R992) in primary or secondary care (KUHR or NPR) during the follow-up period. Additionally, we examined the more severe outcome of COVID-19 related hospitalization for the same period of follow-up. COVID-19 related hospitalization was ascertained in NPR and defined as having COVID-19 diagnosis or positive PCR test, combined with an inpatient record with respiratory-related diagnosis (**Table S1**) as primary or secondary cause of admission within the period from 7 days before to 14 days after the COVID-19 event, in line with prior research [29]. This approach facilitated the distinction of COVID-19-related hospitalizations from incidental hospitalizations for unrelated conditions or for delivery where the patient tested positive during the screening at admission.

3.7. Statistical analysis

Missing data in baseline confounders ranged from 1.9 % to 11.7 % for each covariate, while 25.9 % had missing data on at least one covariate. Under the assumption that data were missing at random, these were imputed using multiple imputation by chained Eqs. [30]. The imputation model included vaccine exposure, outcome, and auxiliary variables.

In adjusted analyses, we identified all eligible pregnancies and used propensity-score matching to address measured confounding at baseline. In the ITT analysis, individuals receiving at least one dose of the BNT162b2 vaccine in pregnancy were matched to unvaccinated individuals. Matching via the propensity score was performed using the nearest neighbour greedy algorithm on a weekly basis during gestation, in a 1:1 ratio, with a caliper of 0.25 times the standard deviation [31]. In the AT analysis, individuals receiving two BNT162b2 vaccine doses in pregnancy were matched to those unvaccinated in pregnancy. This approach followed a similar design except that those with at least two doses of the vaccine, regardless of the interval between them, were matched to those who remained unvaccinated throughout the pregnancy. After matching, we evaluated covariate balance, which was considered acceptable when the standardized mean difference (SMD) was $\leq$0.1 for all baseline covariates. Unbalanced covariates after matching with an SMD $\geq$ 0.1 were adjusted for in the outcome models. We then fit modified Poisson regression models with robust variance estimators to obtain incidence rate ratios (IRRs) between the vaccinated and unvaccinated. To compare our results to existing literature, we also fit, after matching, the Kaplan–Meier estimator to construct cumulative incidence curves. Fixed-effects meta-analysis was used to pool the effect estimates for all weekly trials before further pooling the effect estimates across 30 imputed data sets based on Rubin's rules [32].

To assess whether our emulation yielded similar results to those of the NCT04754594 RCT, we used three binary metrics proposed by the RCT-DUPLICATE initiative [3]: (1) Full Statistical Significance Agreement: we compared the AT emulation and target trial results to check whether both the estimates and their confidence intervals (CIs) fell on the same side of the null, (2) Estimate Agreement: we evaluated whether the estimates from the emulation were within the 95 % CI of the target trial results, and (3) Standardized Difference Agreement: we calculated the difference between the log-transformed incidence rate ratios (IRRs) from the TTE and the RCT using the formula:

$$|z| < 1.96 \left(z = \frac{\widehat{\theta}_{RCT} - \widehat{\theta}_{TTE}}{\sqrt{\widehat{\sigma}^2_{RCT} + \widehat{\sigma}^2_{TTE}}} \right)$$

where z is the standardized difference, $\widehat{\theta}$ is the effect estimate on the log scale (log (IRR)), the $\widehat{\sigma}^2$ is the associated variance. A standardized difference of less than 1.96 indicated no meaningful difference between the estimates.

3.8. Sensitivity analysis

In our AT assessment of vaccine effectiveness, we did not apply a strict 21-day interval between the first and second doses. This was due to this interval not being strictly applied during the vaccination program in Norway due to logistical challenges (e.g., vaccine availability and distribution). To test the robustness of our results, we conducted a sensitivity analysis for the SARS-CoV-2 infection outcome including only individuals who received their second dose strictly 21 days after the first dose.

4. Results

4.1. Baseline characteristics

Of the 109,130 initial pregnancies occurring in 2021–2022, 30,371 were eligible for inclusion in the trial emulation (Fig. 1). Of these, 2895 were administered at least 1 dose of the BNT162b2 COVID-19 vaccine between week 24–34 of gestation, of which 2236 (2236/2895, 77.2 %) received a second dose before delivery. In the ITT analysis, we matched the 2895 vaccinated individuals with 2895 unvaccinated controls (matched cohort, $n = 5970$) in each of the gestational weeks 24 to 34. Across all weekly trials, 241 individuals in the ITT analysis and 168 in the AT analysis were censored because their matched unvaccinated pair received the vaccine. There was balance in the distribution of most baseline covariates after matching except for the previous influenza vaccination and thus it was included as a covariate in the outcome models (Table 2, **Fig. S1**). The mean age among the unvaccinated and vaccinated individuals at the start of follow-up was 31.1 years (SD = 4.3) and 31.4 years (SD = 4.6). Mean preconception BMI was 24.7 (SD = 4.6) and 24.9 (SD = 5.0) among the matched unvaccinated and vaccinated cohorts, respectively. The baseline characteristics of the individuals included in the AT analysis were also similar (**Table S3, Fig. S2**).

4.2. Incidence of SARS-CoV-2 and SARS-CoV-2 related hospitalizations

In the ITT analysis, the estimated probability of SARS-CoV-2 infection from time zero until 1 month post-delivery was 9.8 % among vaccinated with at least one dose and 13.1 % among matched unvaccinated (Fig. 2). The adjusted IRR for SARS-CoV-2 infection within 1 month post-delivery was 0.80 (95 % CI: 0.48, 1.13) following vaccination during gestational weeks 24–34. The AT analysis yielded a slightly more protective IRR, with SARS-CoV-2 infection having an adjusted IRR of 0.61 (95 % CI: 0.27, 0.95). However, the time interval between the two doses was on average 40 days, almost double the interval used in the target trial. In the NCT04754594 trial, the IRR, after 2 doses 21 days apart, for SARS-CoV-2 infection at 1 month post-delivery was 0.59 (95 % CI: 0.19, 1.88). The probability of SARS-CoV-2-related hospitalization at 1-month post-delivery could not be robustly assessed due to the rare occurrence of this outcome in our matched population (a total of 8 events were identified in our matched population).

In the Kaplan–Meier analyses, we outlined the cumulative incidence of SARS-CoV-2 infection in the matched vaccinated and unvaccinated cohorts in the AT analysis, up to 1 month post-delivery. Fig. 3 shows a clear difference in incidence among the matched unvaccinated and

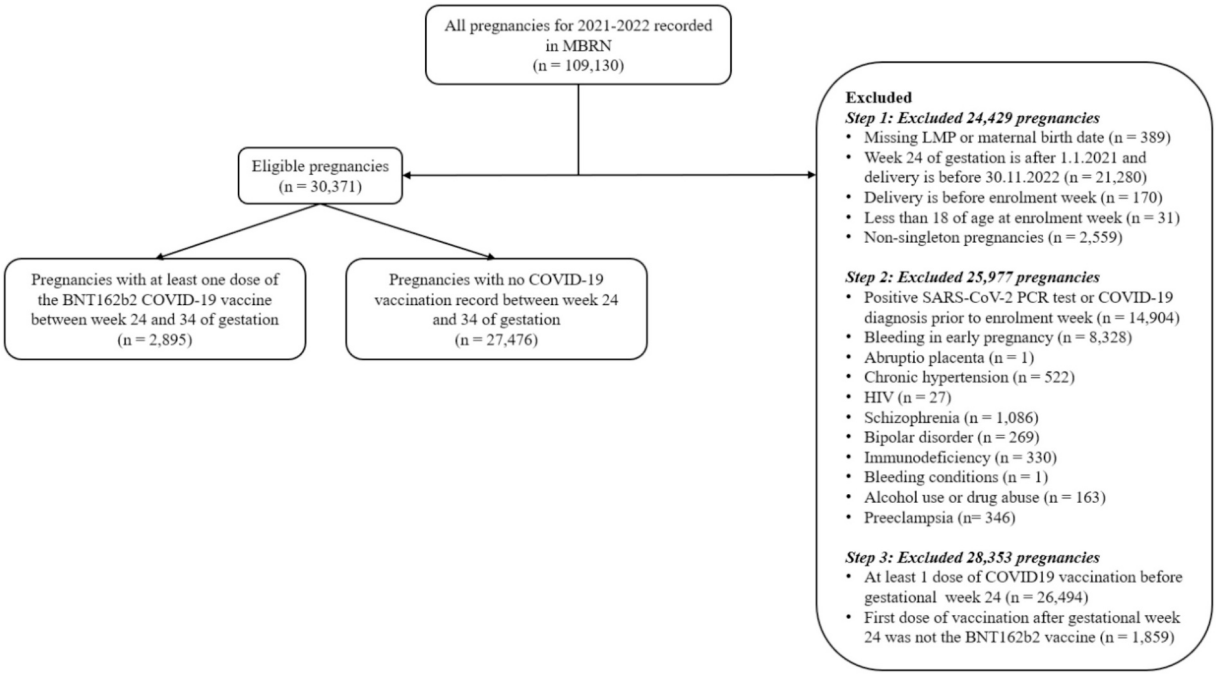

Fig. 1. Flowchart of the eligibility criteria to achieve the final study population. "Delivery is before enrolment week" signifies that delivery happened before the gestational week of being enrolled in the trial: week of vaccination for the vaccinated, and week of being matched for the unvaccinated.

vaccinated populations 14 days after administering the first dose. The cumulative incidence in the vaccinated remains lower than that in the unvaccinated throughout the follow-up period. Follow-up lasted up to 160 days in our matched population.

4.3. Comparison to the target trial

The trial estimate and the AT estimate from our emulation showed partial statistical significance agreement. The IRRs and the lower bounds of the 95 % CIs for both estimates were on the same side of the null, while the upper bounds lied on different sides of the null which could be explained by the small sample size of the RCT for this outcome ($N = 163$). The emulation estimate (IRR: 0.61) also fell within the 95 % CI of the target trial (0.19, 1.88), indicating estimate agreement. Additionally, the standardized difference between the emulation and target trial estimates was 0.04, supporting the null hypothesis of no difference between the two. These findings suggest that the emulation produced results satisfactorily consistent with those of the target trial.

4.4. Sensitivity analysis

In the sensitivity analysis in which we enforced a strict 21-day interval between the two vaccine doses, we matched 235 vaccinated to 235 unvaccinated. We observed a lower reduction in the risk of SARS-CoV-2 infection at 1 month post-delivery (IRR: 0.79, 95 %CI: 0.30, 1.61) compared to the main AT analysis (Fig. 2).

5. Discussion

In this emulation of the NCT04754594 RCT using nationwide Norwegian registry data, we evaluated the agreement of the point estimates for the effectiveness of the SARS-CoV-2 vaccine BNT162b2 in pregnant individuals by comparing TTE estimates to those from the RCT. After closely replicating the inclusion and exclusion criteria of the RCT, and mimicking its design and analysis, we included data from 30,371 eligible pregnancies in 2021–2022 and matched 2895 vaccinated pregnancies to unvaccinated ones, in a 1:1 ratio. Our results show satisfactory alignment between the emulated and trial-based effect estimates, highlighting that high-quality observational data can yield reliable information on vaccine effectiveness in pregnancy, a population often underrepresented in clinical research. To our knowledge, this is the first real-world emulation of a real RCT in pregnant individuals. Our methodological approach and findings are noteworthy for both clinical and regulatory settings. We demonstrated that the use of TTE can provide robust evidence about effectiveness of vaccines taken during pregnancy in a post-marketing setting.

In the AT analysis, which most closely mimics the controlled settings of the RCT requiring administration of two vaccine doses, we found a 39 % reduction of SARS-CoV-2 infection with two doses of the BNT162b2 vaccine during gestation, which closely aligned to the 41 % risk reduction observed in the RCT. We could not prove full statistical significance agreement of the RCT-emulated trial pair estimates, because our CIs were below the null whereas those of the RCT crossed the null. This is likely due to the lower sample size of the target trial (163 pregnant participants only) resulting in imprecise effect estimates. In the ITT analysis, which generally reflects the true treatment effect in the context of RCT designs, we show that at least one dose of the BNT162b2 vaccine during pregnancy reduced the infection risk by 20 % compared to no vaccination. This risk reduction was lower than the one estimated in the target RCT. Cumulative incidence analyses showed an initial decline in infection risk among the vaccinated which lasted until the end of follow-up, similar to that shown by Dagan et al. [15] based on observational data in Israel. Notably, as anticipated, the risk of SARS-CoV-2 infection was comparable between vaccinated and unvaccinated individuals during the first two weeks of follow-up, further reinforcing the biological plausibility and reliability of our results [33].

Although our emulation results satisfactorily fulfill the agreement criteria proposed by the RCT-DUPLICATE initiative, we estimated a smaller effect size for the risk reduction in the AT analysis compared to other studies on vaccine effectiveness in pregnant individuals [15–17]. Dagan et al. reported a hazard ratio (HR) of 0.04 (95 % CI: 0.00, 0.11) for any documented SARS-CoV-2 infection within 7 to 56 days after first dose vaccination, based on Israeli data for the period December 20th, 2020 to June 3rd, 2021 [15]. Another Israeli study in the period from

Table 2
Baseline characteristics of the vaccinated and unvaccinated matched pairs at the start of the target trial emulation ($n = 5790$). Numbers are shown as n (%) unless otherwise indicated.

Maternal covariates	Unvaccinated (n = 2895)	Vaccinated ($n = 2895$)	SMD
Age at week 24 of gestation; mean (SD)	31.1 (4.3)	31.4 (4.6)	0.07
Education: upper secondary or lower	868 (30.0)	850 (29.4)	0.01
Profession			0.02
Military occupations, artisans, cleaners, farmers, fishermen and unspecified	648 (22.4)	626 (21.6)	
Leaders, office jobs, sales and service occupations	841 (29.1)	847 (29.3)	
Academic & university professions	1406 (48.6)	1422 (49.1)	
Region of residency			0.05
Central Norway	206 (7.1)	228 (7.9)	
Eastern Norway	1461 (50.5)	1418 (49.0)	
Northern Norway	216 (7.5)	247 (8.5)	
Southern Norway	160 (5.5)	169 (5.8)	
Western Norway	852 (29.4)	833 (28.8)	
Median annual individual income			0.07
High (≥899,999 NOK)	40 (1.4)	61 (2.1)	
Medium (400,00–899,998 NOK)	1443 (49.8)	1484 (51.3)	
Low (≤399,999 NOK)	1412 (48.8)	1350 (46.6)	
Ethnicity			0.10
Norwegian	2091 (72.2)	2199 (76.0)	
Immigrant	663 (22.9)	541 (18.7)	
Mixed	141 (4.9)	155 (5.4)	
Marital Status			0.04
Married/Cohabiting	2776 (95.9)	2752 (95.1)	
Single/Unmarried	69 (2.4)	89 (3.1)	
Other	61 (1.7)	54 (1.9)	
Parity			0.01
0	1241 (42.9)	1236 (42.7)	
1	1139 (39.3)	1129 (39.0)	
≥ 2	515 (17.8)	530 (18.3)	
Pre-pregnancy BMI; mean (SD)	24.7 (4.6)	24.9 (5.0)	0.03
Smoking status before pregnancy			0.04
No	2770 (95.7)	2774 (95.8)	
Daily	68 (2.3)	68 (2.3)	
Occasionally	55 (1.9)	53 (1.8)	
Unknown	2 (0.1)	0 (0.0)	
Previous miscarriage before 23 weeks of gestation (yes)	683 (23.6)	725 (25.0)	0.03
Previous influenza vaccination, ever before	1306 (45.1)	1487 (51.3)	0.13
Prior candidiasis (yes)[a]	21 (0.7)	34 (1.2)	0.05
Systemic infections (yes)[a]	567 (19.6)	533 (18.4)	0.03
Respiratory diseases (yes)[a]	203 (7.0)	244 (8.4)	0.05
Thyroid disorders (yes)[a]	90 (3.1)	107 (3.7)	0.03
Urinary tract infections (yes)[a]	215 (7.4)	206 (7.1)	0.01

Abbreviations: BMI = Body mass index, NOK = Norwegian Krones, SD = Standard deviation, SMD = Standardized mean difference.
[a] Assessed between 6-months prior to LMP until the gestational week of enrolment.

December 19th, 2020, to February 28th, 2021, reported an aHR of 0.22 (95 % CI: 0.11, 0.43) for symptomatic SARS-CoV-2 infections within 28 to 70 days after first dose vaccination [16]. Several factors can explain these differences. First, we assess SARS-CoV-2 infection up to 1-month post-delivery with an average follow-up time of 160 days after first-dose vaccination, while most other studies only assessed the outcome within shorter follow-up periods. The study by Dagan et al. had the longest follow-up, ending at 77 days after first-dose vaccination. Second, our study cohort included pregnancies during 2021–2022, as done in the target trial, covering periods where multiple SARS-CoV-2 variants were circulating. While the mentioned studies mainly focused on times when the Alpha, Beta, and Delta variants were circulating, our emulation, like the RCT, incorporates times where the Omicron variant was more common. The reduced vaccine efficacy against Omicron likely contributed to the smaller effect size observed in both our emulation and the RCT [34,35]. Additionally, for the AT analysis, the time interval between the two doses was on average 40 days in our data which is almost double the recommended 21 day interval used in the RCT [9]. Yet, our sensitivity analysis showed minimal impact of the interval on the effect estimate. The wider confidence intervals (CIs) in the sensitivity analysis are attributable to a substantially smaller sample size that met the 21-day interval criterion. It also remains unclear how prior studies handled the gap between doses [15,16]. Another difference is that those studies did not only include participants followed up from gestational week 24; instead, any pregnant woman during the study period was eligible. Finally, the influence of growing population immunity to SARS-CoV-2, which could dilute the observed vaccine effectiveness, cannot be excluded [36]. Nevertheless, our estimates align closely with those of the RCT which was the aim of the trial emulation. Furthermore, a recent multinational study using TTE to assess BNT162b2 vaccine effectiveness in pregnancy estimated a HR of 0.74 (95 % CIs: 0.65, 0.84) for SARS-CoV-2 infection during pregnancy following two-dose vaccination, with the Norwegian sub-cohort yielding a HR of 0.72 (95 % CIs: 0.65, 0.81), which is more in line with our results [37].

Our study has several strengths. First, we use a nation-wide medical birth registry that covers all deliveries since gestational week 12, and that has been validated to be a high-quality registry [38–40]. It also provides gestational age at delivery based on ultrasound estimation which is used to calculate LMP. The mandatory registration of COVID-19 vaccination in the SYSVAK minimizes risk of misclassification of vaccine exposure. Similarly, mandatory registration of PCR tests for SARS-CoV-2 infection in MSIS reduces the risk of outcome misclassification. Because in February 2022, COVID-19 testing was no longer mandatory in Norway, we supplemented outcome data in MSIS with clinical diagnosis of confirmed COVID-19 in both primary and secondary care. The comprehensive availability of clinical diagnoses from primary and

Analysis	Matched Vaccinated (n, %)	Matched Unvaccinated (n, %)	Adjusted IRR (95% CI)
Intention-to-treat analysis			
SARS-CoV-2 infection 1 month postpartum	284 (9.81%)	380 (13.13%)	0.80 (0.48, 1.13)
As-treated analysis			
SARS-CoV-2 infection 1 month postpartum	181 (8.09%)	312 (14.00%)	0.61 (0.27, 0.95)
Sensitivity analysis 21-day interval			
SARS-CoV-2 infection 1 month postpartum	21 (8.93%)	27 (11.49%)	0.79 (0.30, 1.61)
Trial NCT04754594			
SARS-CoV-2 infection 1 month postpartum	40 (47.62%)	68 (76.40%)	0.59 (0.19, 1.88)

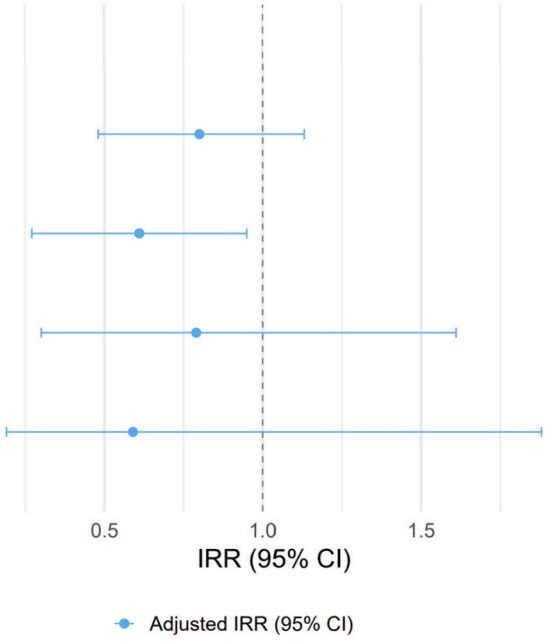

Fig. 2. Adjusted effect estimates for both the ITT and AT analyses. Adjusted analyses included the matched vaccinated and unvaccinated pairs. IRR from the target trial is presented for comparison.

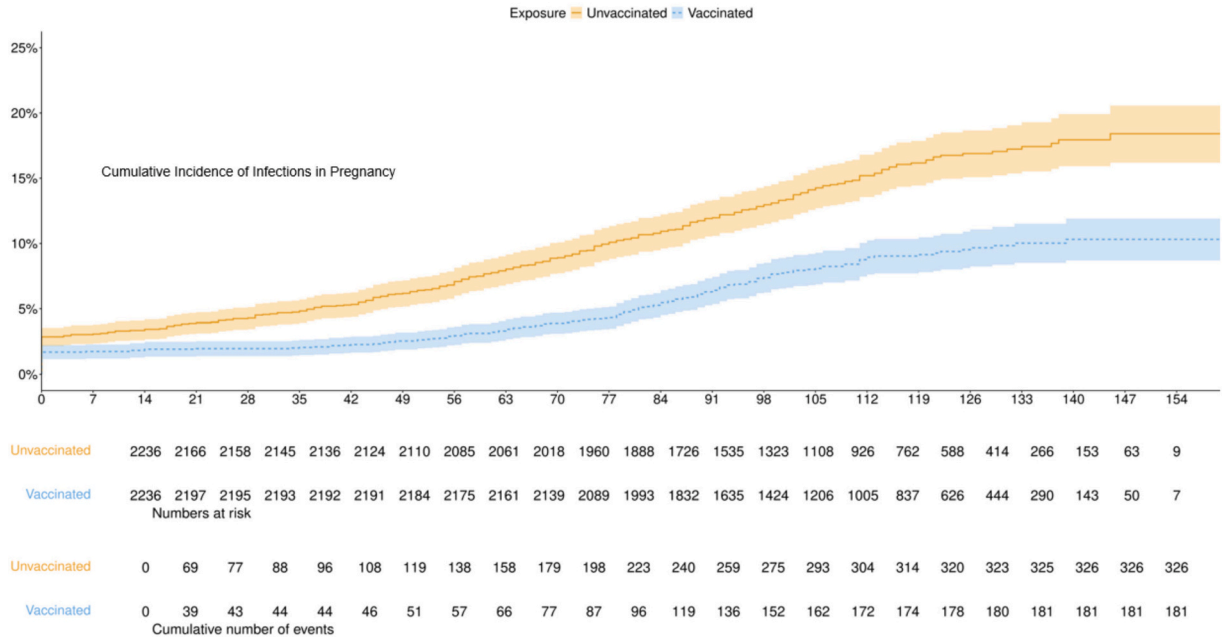

Fig. 3. Cumulative incidence of SARS-CoV-2 infection curves for the matched vaccinated and unvaccinated cohorts up to 1-month post delivery. Below the plot, the population at risk in each cohort is displayed over time, followed by the number of events in both cohorts during the follow-up period.

secondary care, coupled to obstetric records from the MBRN, enabled us to adequately classify maternal inclusion and exclusion criteria, as well as baseline factors used for propensity score matching. Using multiple imputation in our data minimized the risk of bias due to missing data. We applied propensity score matching to control for measured confounding, to closely resemble the conditions of the RCT. Lastly, we conducted both AT and ITT analyses which provided insights about the effectiveness of the vaccine both in controlled settings reflecting an RCT as well as settings better reflecting the real-world effectiveness.

Our study also has several limitations. We could not fully emulate certain eligibility criteria of the target trial, primarily the ones of "healthy" individuals with an "uncomplicated pregnancy", since the RCT did not provide specific diagnoses/illnesses that could be translated into useable diagnostic codes. Nonetheless, we applied a pragmatic and well justified definition, likely reflecting a healthy uncomplicated pregnancy. Moreover, the granularity of the data did not allow for properly making weekly propensity scores since some covariates are only reported on a yearly basis in the data (median annual income, education, profession, marital status and region of residence). However, we updated other covariates weekly where suitable, and the mentioned covariates are likely to be time-fixed for most individuals throughout the study period. Additionally, censoring unvaccinated individuals who get

vaccinated later on along with their matched vaccinated pair might lead to the overall pooled estimate to be more representative of those who get vaccinated later in pregnancy (near week 34). However, it remains the optimal solution for preserving the exchangeability between the vaccinated and unvaccinated pairs. Furthermore, vaccine doses given abroad need manual retrospective registration in SYSVAK, leading to some regarding undocumented vaccinations. However, this risk is considered as minimal, given the strong incentive and widespread adherence to vaccine registration during the study period. This could be more important for assessing effectiveness of other vaccines where a strong incentive for registering vaccines in SYSVAK is less present. Another limitation is that we cannot be sure there is no residual confounding due to the rapidly changing policies and guidelines determining those eligible for the vaccine; a woman eligible in late 2022 might not have been eligible in early 2021. Furthermore, we cannot capture individual-level behavior towards the pandemic. Our data lacks information on adherence to guidelines on mask wearing, social distancing, and remote working conditions, all of which affect the risk of both getting the vaccine and developing the outcome. Finally, previous influenza vaccination was not balanced after matching, and although the outcome models were adjusted for it as a covariate, healthy vaccinee bias might still persist [41].

6. Conclusion

In this study closely emulating the NCT04754594 RCT using nationwide Norwegian registry data, we observed satisfactory agreement in the point estimates for the effectiveness of the vaccine BNT162b2 in pregnant individuals, up to 1 months post-delivery, with those of the target trial. Despite the slight difference in the observed effect size in the AT analysis versus the target RCT, and lack of full statistical agreement mainly due to differences in sample size, our results support that target trial emulation is a robust methodological approach for assessing vaccine effectiveness in pregnancy and informing clinical or regulatory decisions, particularly when randomized trials are unavailable or infeasible.

Authors contribution

AL conceived the study. AL applied for the study data. MZ performed the data analysis, and AL and NTHT contributed to data curation. MZ wrote the initial draft. All authors contributed to study planning, data interpretation and writing the final manuscript. AL obtained funding. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Ethical approvals

The Regional Committee for Medical and Health Ethics of South/East Norway (no. 285687) approved the study. The Norwegian Data Protection Services for research and the University of Oslo approved the Data Protection Impact Assessment – DPIA (no. 341884).

CRediT authorship contribution statement

Mahmoud Zidan: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Nhung T.H. Trinh:** Writing – review & editing, Validation, Supervision, Methodology, Data curation. **Anteneh Desalegn:** Writing – review & editing. **Louisa H. Smith:** Writing – review & editing, Methodology. **Marleen M.H.J. van Gelder:** Writing – review & editing, Methodology. **Hedvig Nordeng:** Writing – review & editing, Methodology. **Angela Lupattelli:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Angela Lupattelli reports financial support was provided by European Union. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127908.

Data availability

The authors do not have permission to share data.

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