Title: Using negative control outcomes and difference-in-differences to estimate treatment effects in an entirely treated cohort: the effect of ivacaftor in cystic fibrosis

Authors: Simon J. Newsome, Rhian M. Daniel, Siobhán B. Carr, Diana Bilton, Ruth H. Keogh

Correspondence Address: Dr Ruth H. Keogh, Department of Medical Statistics, London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT, UK. ruth.keogh@lshtm.ac.uk

Affiliations: Department of Medical Statistics, Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, UK (Ruth H. Keogh, Simon J. Newsome); Novartis, Basel, Switzerland (Simon J. Newsome); Division of Population Medicine, School of Medicine, Cardiff University, UK (Rhian M. Daniel); National Heart and Lung Institute, Imperial College London, UK (Siobhán B. Carr, Diana Bilton); Royal Brompton Hospital, London, UK (Siobhán B. Carr, Diana Bilton).
Funding: This work was supported by the Cystic Fibrosis Trust (Strategic Research Centre grant “CF-EpiNet: Harnessing data to improve lives” to SBC); UK Research and Innovation (Future Leaders Fellowship MR/S017968/1 to RHK); the Medical Research Council (Methodology Fellowship MR/M014827/1 to RHK); and the Wellcome Trust and the Royal Society (Sir Henry Dale Fellowship 107617/Z/15/Z to RMD).

Data Availability Statement: This work used anonymized data from the UK Cystic Fibrosis Registry, which has Research Ethics Approval (REC ref: 07/Q0104/2). The use of the data was approved by the Registry Research Committee. Data are available following application to the Registry Research Committee. https://www.cysticfibrosis.org.uk/the-work-we-do.uk-cf-registry/apply-for-data-from-the-uk-cf-registry.

Thanks: We thank the CF Epi-Net Strategic Research Centre data group for contributions to data preparation and cleaning.

Conference presentation: N/A

Disclaimer: N/A

Conflict of Interest: RHK has received funding from a Vertex Circle of Care Award. DB was principal investigator on a UK CF registry based pharmacovigilance study of Ivacaftor funded by Vertex, SBC reports personal fees and other from Chiesi Pharmaceuticals, non-financial support and other from Vertex, other from Zambon, other from Insmed, outside the submitted work.

Running Head: Estimating treatment effects in a treated cohort

Key words: Causal inference; CFTR modulator; Cystic fibrosis; Difference-in-differences; Ivacaftor; Longitudinal data; Negative control outcomes.
ABSTRACT

When an entire cohort of patients receives a treatment it is difficult to estimate the treatment effect in the treated because there are no directly comparable untreated patients. Attempts can be made to find a suitable control group, (e.g. historical controls), but underlying differences between the treated and untreated can result in bias. We show how negative control outcomes (NCO) combined with difference-in-differences analysis can be used to assess bias in treatment effect estimates and obtain unbiased estimates under certain assumptions. Causal diagrams and potential outcomes are used to explain the methods and assumptions. We apply the methods to UK Cystic Fibrosis (CF) Registry data to investigate the effect of ivacaftor, introduced in 2012 for a subset of the CF population with a particular genotype, on lung function and days receiving intravenous antibiotics (IV days). We consider two NCOs: outcomes measured in the pre-ivacaftor period and outcomes in individuals ineligible for ivacaftor due to their genotype. Ivacaftor was found to improve lung function in year one (~6.5 increase in FEV$_1$%), was associated with reduced lung function decline (~0.5 decrease in annual FEV$_1$% decline, though confidence intervals include 0), and reduced the rate of IV days (~60% over 3 years).

BACKGROUND

Randomised controlled trials are the gold standard for estimating treatment effects but typically infeasible for estimating long-term effects. Observational data provide opportunities to estimate such effects, under strong assumptions. A key assumption is positivity, meaning individuals have a probability less than one of receiving (or not) the treatment, given any covariates controlled in the analysis. (1)
One situation where this assumption is not met is when an entire cohort of patients receives treatment. Then, it is difficult to estimate a treatment effect, because we don’t observe contemporary untreated individuals. It may be possible to identify a comparable group who could not receive treatment, e.g. historical controls prior to its availability. However, resultant analyses make the strong assumption of no differences between the control and treatment group that affect the outcome, except the treatment itself and its consequences. We consider estimation of causal treatment effects in this setting through an investigation of the effect of the disease modifying treatment ‘ivacaftor’ in people with cystic fibrosis (CF).

In the UK approximately 10,500 have CF.(2) The most seriously affected organ is the lung, with long-term deterioration in lung function observed. Ivacaftor has been available in the UK since 2012 and approximately 5% of the UK CF population - with a particular CF-causing gating mutation - are eligible to receive it.

Randomised controlled trials have found ivacaftor improves lung function and reduces incidence of pulmonary exacerbations.(3–5) Ramsey et al. reported that in subjects aged 12 years or older with the specified gating mutation, percent predicted forced expiratory volume in one second (ppFEV$_1$) was 10.5% higher after 48 weeks of ivacaftor treatment versus placebo, and the ivacaftor group were 55% less likely to have a pulmonary exacerbation.(3) Similar results have been reported in younger children.(4,5) People will take ivacaftor for many years and it is hoped it will change the slope of lung function decline (“slope-change effect”), as well as improving lung function during the initial treatment phase (“step-change effect”).

In most countries with high CF prevalence almost all eligible patients now receive ivacaftor, meaning that observational data provide no contemporary controls. Four studies using national
patient registry data compared ivacaftor users either to people not receiving ivacaftor because they do not have a gating mutation (“genotype comparison”), or people eligible for ivacaftor but in the time-period prior to its availability (“time-period comparison”).(6–9) The results were similar to randomised controlled trials findings and also suggest longer-term benefits out to four years. However, even after accounting for baseline differences between treated and untreated, these studies are prone to bias due to people with different CF-causing mutations having different disease trajectories, or general improvements over time in the health of the CF population.(10) Lung function decline of people with different CF-genotypes has previously been found to be similar,(11,12) but even small differences could result in biased findings. The health of people with CF has been improving over time, which could impact time-period comparisons.

We use directed acyclic graphs (DAGs) and single world intervention graphs (SWIGs) (13) to illustrate assumptions made in the choice of control groups for the genotype and time-period comparisons. We describe how negative control outcomes (NCO) can be used as a tool to detect bias in treatment effect estimates in this setting, and used in combination with the difference-in-differences approach to obtain unbiased estimates of the causal treatment effect under weaker assumptions. The methods are applied using UK Cystic Fibrosis Registry data (14) to provide more robust estimates of the effect of ivacaftor on lung function and annual rate of intravenous antibiotic use (IV days).

DATA
We use UK CF Registry data from 2008-2016. The registry has been described elsewhere.\(^{(14)}\)

Briefly, patients have an annual assessment, which captures measures of current health status, events since the last assessment, and treatments used.

Our analyses included 8444 people (Web Figure 1): 467 with a gating mutation, and therefore eligible to receive ivacaftor after its introduction, and 7977 with a non-gating mutation.

We define 2008-2012 as the pre-ivacaftor period and 2013-2016 as the post-ivacaftor period. Outcomes measured in 2012 were made prior to ivacaftor becoming available to eligible patients.

Key data for this analysis include treatment (ivacaftor), genotype (gating mutation or non-gating mutation), calendar period (pre- or post-ivacaftor period), and outcomes (lung function; annual number of IV days). For lung function, the main analyses use percent predicted forced expiratory volume in 1 second (ppFEV\(_1\)). We also present results for two other measures of lung function (percent predicted forced vital capacity, percent predicted forced mid-expiratory flow). Adjusted analyses use several demographic and clinical variables, as measured in 2008 for analyses in the pre-ivacaftor period and 2012 for the post-ivacaftor period (see Analysis section).

We divide individuals into four groups defined by genotype and time-period (Table 1). Ivacaftor is only available in group B, this being the group with an eligible genotype \((G = 1)\) in the post-ivacaftor period \((P = 1)\). The other three groups do not receive ivacaftor, either because it was not yet available (A), or due to an ineligible genotype (D), or both (C). Some individuals appear in both A and B, or C and D.

METHODS

Causal treatment effect
The causal treatment effect (CTE) of interest is the effect of ivacaftor on an outcome $Y$ in those who actually receive the treatment ($X = 1$). Let $Y^{X=0}$ denote the potential value of $Y$ had, contrary to fact, a patient not received ivacaftor, and let $Y^{X=1}$ denote the potential value of $Y$ had a patient received ivacaftor. The CTE is the average treatment effect in the treated and for a continuous outcome (ppFEV$_1$) this is measured using the mean difference:

$$\text{CTE} = E(Y^{X=1} | X = 1) - E(Y^{X=0} | X = 1)$$  \hspace{1cm} (1)

The treated cohort corresponds to individuals with the eligible genotype in the post-ivacaftor period. Hence the CTE can be written

$$\text{CTE} = E(Y^{X=1} | P = 1, G = 1) - E(Y^{X=0} | P = 1, G = 1)$$  \hspace{1cm} (2)

Since all patients with the eligible genotype did receive ivacaftor in the post-ivacaftor period, $Y^{X=1}$ is equal to the observed $Y$, therefore

$$\text{CTE} = E(Y | P = 1, G = 1) - E(Y^{X=0} | P = 1, G = 1)$$  \hspace{1cm} (3)

The first expectation, $E(Y | P = 1, G = 1)$, can be estimated directly as the mean outcome in individuals with $(P = 1, G = 1)$ (group B). Assumptions are needed, however, to estimate the second expectation, since $Y^{X=0}$ is entirely unobserved in group B. In groups A, C, and D, $Y^{X=0} = Y$. Hence, we can estimate $E(Y^{X=0} | P = 0, G = 1) = E(Y | P = 0, G = 1)$ using group A, $E(Y^{X=0} | P = 0, G = 0) = E(Y | P = 0, G = 0)$ using group C, and $E(Y^{X=0} | P = 1, G = 0) = E(Y | P = 1, G = 0)$ using group D. Corresponding methods for the count outcome (IV days), where the CTE is a rate ratio, are described in Web Appendix 1.

*Naïve Treatment Effect*
Previous observational studies have compared people receiving ivacaftor to people in the time-period prior to its availability ("time-period comparison") or to people not eligible due to their genotype ("genotype comparison"), typically with adjustment for covariates. We define such comparisons as naïve treatment effects (NTE). The (unadjusted) time-period NTE, comparing groups A and B, is defined as

$$\text{NTE}_P = \mathbb{E}(Y \mid P = 1, G = 1) - \mathbb{E}(Y \mid P = 0, G = 1)$$

(4)

Under the genotype comparison, which compares groups B and D, the NTE is

$$\text{NTE}_G = \mathbb{E}(Y \mid P = 1, G = 1) - \mathbb{E}(Y \mid P = 1, G = 0).$$

(5)

Adjusted comparisons are considered below. The above NTEs only correspond to the CTE under the assumption that $\mathbb{E}(Y^{X=0} \mid P = 1, G = 1)$ is equal to either $\mathbb{E}(Y \mid P = 0, G = 1)$ or $\mathbb{E}(Y \mid P = 1, G = 0)$. This assumption is strong and considerations of its plausibility can be aided by thinking of the three scenarios depicted by the DAGs and corresponding SWIGs in Figure 1. We use these causal diagrams to outline the conditions under which the NTEs correspond to the CTE. When they do not, we describe how NCOs can be used in combination with a difference-in-differences analysis to estimate the CTE.

**Scenario (a): G and P conditionally independent of Y given X**

In DAG A (Figure 1A), receiving treatment $X$ depends deterministically on $G$ and $P$, but $Y$ is independent of $G$ and $P$ conditional on $X$. From the corresponding SWIG (Figure 1D), we see that $Y^{X=0}$ is independent of $G$ and $P$. Thus, $\mathbb{E}(Y^{X=0} \mid P = 1, G = 1) = \mathbb{E}(Y^{X=0} \mid P = p, G = g)$ for any $g, p$. In particular,

$$\mathbb{E}(Y^{X=0} \mid P = 1, G = 1) = \mathbb{E}(Y^{X=0} \mid P = 0, G = 1) = \mathbb{E}(Y^{X=0} \mid P = 1, G = 0).$$

In groups A
\[(P = 0, G = 1) \text{ and } D \ (P = 1, G = 0), \quad Y^{X=0} = Y, \text{ therefore } \mathbb{E}(Y^{X=0}|P = 1, G = 1) = \\
\mathbb{E}(Y|P = 0, G = 1) = \mathbb{E}(Y|P = 1, G = 0). \] It follows that \(\text{NTE}_P\) and \(\text{NTE}_G\) correspond to the CTE.

**Scenario (b): G and P conditionally independent of Y given X and H**

In DAG B (Figure 1B), measured covariates \(H\) (baseline health status) are included that affect \(Y\) and are also dependent on \(G\) and \(P\). We see from the corresponding SWIG (Figure 1E) that \(Y^{X=0}\) is not independent of \(G\) and \(P\), hence neither \(\text{NTE}_P\) nor \(\text{NTE}_G\) corresponds to the CTE. However, \(Y^{X=0}\) is conditionally independent of \(G\) and \(P\) given \(H\), meaning \(\mathbb{E}(Y^{X=0}|P = 1, G = 1, H) = \mathbb{E}(Y^{X=0}|P = p, G = g, H)\) for any \(p, g\). Using this, and standardizing to the distribution of \(H\) in group B \((P = 1, G = 1)\), the second expectation of the CTE can be written

\[
\mathbb{E}(Y^{X=0} | P = 1, G = 1) \quad \quad (6)
= \sum_h \mathbb{E}(Y^{X=0} | P = 1, G = 1, H = h) \Pr(H = h | P = 1, G = 1) \\
= \sum_h \mathbb{E}(Y^{X=0} | P = p, G = g, H = h) \Pr(H = h | P = 1, G = 1)
\]

In all groups except B, \(Y^{X=0} = Y\), allowing us to write

\[
\mathbb{E}(Y^{X=0} | P = 1, G = 1) \quad \quad (7)
= \sum_h \mathbb{E}(Y | P = 0, G = 1, H = h) \Pr(H = h | P = 1, G = 1) \\
= \sum_h \mathbb{E}(Y | P = 1, G = 0, H = h) \Pr(H = h | P = 1, G = 1)
\]

Therefore, the following adjusted NTEs correspond with the CTE under DAG B (Figure 1B):
These results extend to continuous and multivariable $H$. For these adjusted effects to be estimable requires an assumption that there is overlap in the distribution of $H$ in groups defined by $G$ and $P$. Under the assumption that the effect of $H$ on $Y$ is not modified by $G$ or $P$, the adjusted NTEs can be expressed as conditional differences in expectations (for all $h$):

$$NTE_{P}^{Adj} = E(Y \mid P = 1, G)$$

$$= 1 - \sum_{h} E(Y \mid P = 0, G = 1, H = h) \Pr(H = h \mid P = 1, G = 1)$$

$$NTE_{G}^{Adj} = E(Y \mid P = 1, G)$$

$$= 1 - \sum_{h} E(Y \mid P = 1, G = 0, H = h) \Pr(H = h \mid P = 1, G = 1)$$

Scenario (c): $G$ and $P$ are not conditionally independent of $Y$

In DAG C (Figure 1C) there is dependence between $Y$ and $G, P$ conditional on $X$. In an extended version of DAG C in Web Figure 2 we add covariates $H$. The main issue is encompassed in DAG C (Figure 1C) and we focus on this here. The corresponding SWIG (Figure 1F) shows that $Y^{X=0}$ is not independent of $G$ and $P$. Now, the unadjusted NTEs ((4) and (5)) do not correspond to the CTE. If there remains dependence between $Y$ and $G, P$ after conditioning on $H$ as well as $X$, the adjusted NTEs ((8) and (9)) also do not correspond to the CTE.

NCO are tools for detecting bias due to unmeasured confounding and other sources in observational studies, and are defined as outcomes that are not affected by the treatment but have the same associations with other variables as the true outcome of interest. (15) The difference-in-
differences approach can also be used to estimate treatment effects in the presence of unobserved confounding. Sofer et al. (16) showed the link between NCOs and difference-in-differences, using pre-treatment outcome as the NCO. Here we use these tools to detect bias in the NTEs and estimate the CTE under certain assumptions. While these tools have previously been discussed primarily in the context of addressing unmeasured confounding, we use them instead to address bias due to dependence between $Y^{X=0}$ and $G, P$. By noting that $\text{NTE}_G$ can equivalently be written as

$$\text{NTE}_G = E(Y \mid P = 1, X = 1) - E(Y \mid P = 1, X = 0),$$

it is clear that $G$ is uncontrolled in this difference, and similarly that $P$ is uncontrolled in $\text{NTE}_P$. This can be considered a form of unmeasured (or uncontrolled) confounding as there are backdoor paths from $X$ to $Y$ through $G$ and $P$ that cannot be blocked using a standard analysis due to lack of positivity. The uncontrolled confounders $G$ or $P$ take the roles of an unmeasured confounder ($U$ in Sofer et al. (16)).

We consider two NCOs that detect the bias in $\text{NTE}_G$ and $\text{NTE}_P$ due to uncontrolled confounding.

We begin by considering the outcome observed in period $P = 0$ (groups A and C) as the NCO. Any difference between $E(Y \mid P = 0, G = 1)$ (equivalently $E(Y \mid P = 0, X = 1)$) and $E(Y \mid P = 0, G = 0)$ (equivalently $E(Y \mid P = 0, X = 0)$) cannot be due to treatment, because the outcome measure preceded the treatment. We define the “genotype negative control effect (NCE)"

$$\text{NCE}_G = E(Y \mid P = 0, G = 1) - E(Y \mid P = 0, G = 0)$$

A non-zero $\text{NCE}_G$ would indicate that the estimate of $\text{NTE}_G$ is not only due to treatment, but also to dependence between $Y^{X=0}$ and $G$. As in the description of Lipsitch et al. (15), this NCE uses the treatment $X$ and assesses its association with the NCO.
Under certain assumptions, the CTE can be written in terms of the NTE and NCE. The CTE can be written as the difference-in-differences:

\[
CTE = E(Y^{X=1}|P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 1) \\
= \{E(Y^{X=1}|P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 0)\} \\
- \{E(Y^{X=0} |P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 0)\}
\]

The first difference can be written \(E(Y^{X=1}|P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 0)\) = \(E(Y|P = 1, G = 1) - E(Y|P = 1, G = 0)\), which is \(\text{NTE}_G\). The second difference identifies bias in \(\text{NTE}_G\). \(E(Y^{X=0}|P = 1, G = 1)\) cannot be estimated from the data due to lack of positivity.

However, we show that the second difference can be estimated under certain assumptions.

Consider the model for \(Y^{X=0}\)

\[
E(Y^{X=0}|P = p, G = g) = \alpha + \beta_P p + \beta_G g + \gamma_{PG} P g.
\]

Under this model the second difference in the CTE in (13) is \(E(Y^{X=0}|P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 0)\) = \(\beta_G\). It follows that under the assumption that \(\gamma_{PG} = 0\), i.e. if treatment were set to 0, then there is no product term \(P \times G\) in the model for \(Y^{X=0}\), we have

\[
E(Y^{X=0}|P = 1, G = 1) - E(Y^{X=0} |P = 1, G = 0) \\
= E(Y^{X=0}|P = 0, G = 1) - E(Y^{X=0} |P = 0, G = 0),
\]

Under this assumption the second difference in (13) is \(\text{NCE}_G\) and the CTE can be written as \(\text{NTE}_G - \text{NCE}_G\). We call this the negative control corrected treatment effect (NCCTE):

\[
\text{NCCTE}_G = \text{NTE}_G - \text{NCE}_G
\]

An alternative NCO is the outcome observed in genotype group \(G = 0\). This differs from using the outcome in period 0 as an NCO, as it does not involve an outcome that can be observed on all
individuals. Instead of using the outcome measured in one time period on individuals in both genotype groups, it makes use of outcomes measured in two time periods on individuals with \( G = 0 \). As the treatment is not given in either time-period in the \( G = 0 \) group, we do not assess the association between the treatment and the NCO in this case. However, we show that this NCO can be used to obtain an estimator of the CTE under the same assumptions used above. We define the “time-period NCE” as the contrast in the expected outcome in groups C and D:

\[
NCE_P = E(Y|P = 1, G = 0) - E(Y|P = 0, G = 0)
\]

(37)

A non-zero \( NCE_P \) indicates that the estimate of \( NTE_P \) is not only due to treatment, but also to dependence between \( Y^{X=0} \) and \( P \). The CTE can be expressed in terms of another difference-in-differences:

\[
CTE = \{E(Y^{X=1}|P = 1, G = 1) - E(Y^{X=0}|P = 0, G = 1)\} - \{E(Y^{X=0}|P = 1, G = 1) - E(Y^{X=0}|P = 0, G = 1)\}.
\]

(18)

Assuming \( \gamma_{PG} = 0 \) in (14), the CTE can be written as \( NTE_P - NCE_P \), and we define

\[
NCCTE_P = NTE_P - NCE_P
\]

(19)

We have shown how a difference-in-differences approach to estimating the CTE corresponds to using a NCO to detect bias in the NTE when there is a positivity assumption violation. The NCCTEs correspond to the CTE under weaker assumptions than the NTEs. In our situation there are two possible NCOs, corresponding to different difference-in-differences formulae for the CTE. Figure 2 shows a reformulation of DAG C (Figure 1C), such that the outcome is shown separately by time-period or genotype group. Figure 2A corresponds to the DAG of Sofer et al. (16), with \( G \) playing the role of the unmeasured (or uncontrolled) confounder \( U \). This illustrates that the outcome in period 0 is not affected by treatment but that the two outcomes share the
same association with $G$. Our second NCO is illustrated in Figures 2B and 2C, where the outcome in group $G = 0$ (Figure 2B) is not affected by treatment, but the outcomes in the two genotype groups share the same association with $P$, which plays the role of the unmeasured confounder in this case. The model for $Y^{X=0}$ in equation (14), with $γ_{PG} = 0$, is similar to Soler et al.’s (16) model for pre- and post-exposure outcome (their equation 3).

We discussed NCCTEs in the context of DAG C (Figure 1C). In practice, it is not known which scenario we are in. NCOs are a way of investigating the validity of the NTE as an estimate of the CTE and, combined with the difference-in-differences analysis, of correcting for bias in the NTE. In scenarios (a) and (b), the NCE is null.

An extended scenario of interest is when the effects of $G$ and $P$ on $Y$ are partially mediated through measured covariates $H$ (Web Figure 2). The arguments using NCOs and difference-in-differences can be extended to incorporate adjustment for $H$ (Web Appendix 2). Adjusting for $H$ could result in more efficient estimates of the CTE.

ANALYSIS

Our aim was to estimate the causal effect of ivacaftor on those eligible to receive it in the post-ivacaftor period using longitudinal UK CF Registry data. We estimate NTEs, NCEs, and NCCTEs using the time-period and genotype comparisons. We focus here on the continuous outcome $ppFEV_1$. For the count outcome, IV days, the treatment effect is quantified by rate ratios after one, two and three years of treatment (Web Appendix 1).

The outcome is measured at up to 4 visits ($j = 0,1,2,3$) per individual in a given period ($P = 0,1$). Let $Y_{ij}$ denote the outcome measured for individual $i$ at visit $j$ in a given period. For most individuals $j = 0$ corresponds to 2009 for $P = 0$ and 2013 for $P = 1$. $X_i$ denotes the treatment
Observed treatment status is $X_i = 1$ for group B, $X_i = 0$ for groups A, C, and D. We estimate a treatment effect with two components, a step-change effect and a slope-change effect. The analysis model is

$$E(Y_{ij} | X_i) = \beta_0 + \beta_{ST} X_i + \beta_{SL} X_{ij} + \beta_j j,$$

where $\beta_{ST} = E(Y_{i0}|X_i = 1) - E(Y_{i0}|X_i = 0)$ represents the step-change effect and $\beta_{SL} = \{E(Y_{i(j+1)}|X_i = 1) - E(Y_{ij}|X_i = 1)\} - \{E(Y_{i(j+1)}|X_i = 0) - E(Y_{ij}|X_i = 0)\}$ represents the slope-change effect. Each NTE and NCE comprises a step-change effect and a slope-change effect. NTE$_P$ is estimated by fitting the model in groups A and B ($X$ corresponds to $P$), and NTE$_G$ in groups D and B ($X$ corresponds to $G$). To estimate the NCEs the treatment status in one of the groups is switched for the analysis: NCE$_P$ uses groups C and D, setting $X_i = 1$ for group D (so $X$ corresponds to $P$), and NCE$_G$ uses groups C and A, setting $X_{ij} = 1$ for group A (so $X$ corresponds to $G$). Each model was fitted using generalized estimating equations assuming an independence working correlation matrix. Models were refitted with adjustment for variables $H_i$ (Table 2) measured in the year prior to visit 0 in each period, giving adjusted NTEs, NCEs and NCCTEs (see Web Appendix 2).

Non-parametric bootstrapping with 1000 resamples was used to obtain 95% confidence intervals (CI) and p-values.

RESULTS

Table 2 summarises baseline characteristics by group. Comparing groups B and D, a higher proportion of people in the ivacaftor group had an infection (88.2% vs 80.6%) and white ethnicity (98.2% vs 95.5%). Comparing groups A and B, the mean age was higher in group B.
(22.4 vs 20.4), which also had a slightly higher proportion of infections (88.2% vs 81.9%), CF-related diabetes (22.7% vs 15.8%) and mucolytic treatment use (66.5% vs 51.0%). Characteristics were otherwise similar across groups. 7,933/14,594 (54.4%) people (many counted twice) had the maximum of 4 visits post-baseline, with only 1,667 (11.4%) having just one visit.

Results for ppFEV₁ are in Figure 3 (Web Table 1). We focus on the adjusted analysis. The unadjusted results are qualitatively similar, with wider CI. First consider the step-change effect.

In the time-period comparison, the NTE (NTE³Con) estimates a 7.27% absolute increase in ppFEV₁ (95% CI 5.87,8.57) in the ivacaftor group. The corresponding NCE (NCE³Con – see Web Appendix 2) estimates a 0.77% increase (0.44,1.08), indicating a small improvement in mean absolute ppFEV₁ in the post-ivacaftor period in the G = 0 group. The resulting NCCTE³Con estimates a 6.50% increase (5.06,7.85) in ppFEV₁. In the genotype comparison, NTE⁵Con was estimated to be 6.22% (5.17,7.24), NCE⁵Con to be -0.37% (-1.36,0.65), resulting in an NCCTE⁵Con estimate of a 6.59% increase in ppFEV₁ (5.22,7.90). The NCE indicates that in the pre-ivacaftor period, mean ppFEV₁ was slightly lower in those with the ivacaftor eligible genotype compared with the ineligible.

Slope-change effect estimates suggest a small improvement in lung function decline due to ivacaftor, but 95% CIs for NCCTE estimates include 0. In the time-period comparison the NTE³Con estimates a 0.68% absolute improvement in the annual rate of ppFEV₁ decline (0.11,1.32). The corresponding estimated NCE³Con was 0.20% (0.04,0.37), giving a NCCTE³Con estimate of 0.49% (-0.15,1.13). In the genotype comparison, NTE⁵Con was estimated to be 0.67%
(0.27,1.10), $\text{NCE}_G^{\text{Con}}$ to be 0.19% (-0.36,0.70), giving an $\text{NCCTE}_G^{\text{Con}}$ estimate of a 0.49% improvement (-0.14,1.13).

Results for IV days are in Figure 4 (Web Table 2). We focus on the adjusted estimates. In the time-period comparison, according to $\text{NTE}_p^{\text{Con}}$ the rate of IV days was estimated to decrease by 58% (95% CI 46, 71), 72% (59, 79), and 69% (57, 81) after 1, 2, and 3 years of treatment. Corresponding estimates of $\text{NCE}_p^{\text{Con}}$ were 23% (17,26), 25% (20,30) and 26% (21,33), indicating reductions in IV days in the post-ivacaftor period in the $G = 0$ group. The resulting $\text{NCCTE}_p^{\text{Con}}$ estimates of the percentage reduction in the rate of IV days are 45% (31,64), 63% (45,72), and 58% (42,74) after 1, 2, and 3 years of treatment. Results from the genotype comparison were similar.

Results for two other measures of lung function (percent predicted forced vital capacity, percent predicted forced mid-expiratory flow) (Web Tables 3-4) were similar to those for $\text{ppFEV}_1$. We considered sensitivity analyses restricting the $G = 0$ to patients heterozygous or homozygous for f508del (Web Tables 5-10), or patients homozygous for f508del (Web Tables 11-16), corresponding to 90% and 54% of the original $G = 0$ group. The results showed no substantial differences.

**DISCUSSION**

We have shown how NCOs can be used to assess whether a control group is suitable for estimating the treatment effect in a group where everyone receives treatment, and how they can be used in combination with the difference-in-differences approach to provide a more robust treatment effect estimate. Previous descriptions of NCOs have focused on unmeasured confounding bias (15-19). Potential bias in our situation occurs due to an inability to block all
paths from $X$ to $Y$ using a standard analysis, which could be considered a form of unmeasured confounding. A key assumption of our methods is that no genotype-by-period product term in the model for the counterfactual outcome under no treatment (equation (14)). This is a strong assumption that is not verifiable using the data, though it is weaker than the assumptions made when using NTEs. NCCTEs also provide unbiased treatment effect estimates in further scenarios, e.g. allowing for unmeasured variables $U$ affecting $H$ and $Y$ (Web Figure 3). It is of interest to investigate how the assumption made in our difference-in-differences analyses using NCOs could be relaxed. One approach could be through the use of synthetic control methods, which make use of pre- and post- intervention observations in the group receiving the intervention, and observations in multiple time periods for groups that have not received the intervention. (20, 21)

Our NTE estimates for the effect of ivacaftor are similar to results from previous studies, which estimated that ivacaftor results in a step-change absolute improvement in ppFEV$_1$ of 3.2% to 8.2%, and a decrease in the rate of annual ppFEV$_1$ decline of approximately 0.8%. (6,9) However, these are only unbiased estimates of the treatment effect if the assumptions that $G$ and $P$ are conditionally independent of $Y$ given $X$ (or $X$ and $H$) are valid.

The time-period comparison NCE showed a 0.77% absolute increase in ppFEV$_1$, indicating a small non-clinically significant improvement in population average lung function since ivacaftor was introduced. This means that the NTE slightly overestimates the ivacaftor effect. In the genotype comparison, the NCE was negative, indicating slightly lower ppFEV$_1$ in the eligible versus ineligible genotype group in the pre-ivacaftor period, but also small. This resulted in NCCTE estimates of 6.50% and 6.59% step-change improvement in ppFEV$_1$, which are similar
to the NTE estimates (6.22% to 7.27%), but with wider CIs, correctly reflecting uncertainty in the comparability of the groups.

When considering the ivacaftor effect on the slope-change of lung function, the NCE suggested that some of the NTE estimate was not due to ivacaftor but to general improvements in lung function decline over time. The NCCTE suggests a beneficial effect of ivacaftor, with an estimated absolute improvement in annual rate of decline of 0.49%, though with 95% CIs including 0.

Findings for the ivacaftor effect on rate of IV days were similar to those from previous studies, indicating a treatment benefit up to 3 years. The NCE results estimated that in the absence of treatment, the rate of IV days was slightly lower in the $G = 1$ versus $G = 0$ group and in the later time-period. NCCTE estimates were therefore slightly attenuated compared with the NTEs. Our results support evidence of a long-term clinical benefit of ivacaftor.

REFERENCES

1. Hernán MA, Robins JM. Causal Inference: What If. Boca Raton, Florida. Chapman & Hall/CRC. 2020.

2. Cystic Fibrosis Trust. UK Cystic Fibrosis Registry Annual Data Report 2019. https://www.cysticfibrosis.org.uk/the-work-we-do/uk-cf-registry/reporting-and-resources. Accessed 20 September 2021.

3. Ramsey B, Davies J, McElvaney G, Tullis E, Bell S, Drevinke P, et al. A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation. N Engl J Med.
4. Davies JC, Wainwright CE, Canny GJ, Chilvers MA, Howenstine MS, Munck A, et al. Efficacy and safety of ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. Am J Respir Crit Care Med. 2013;187(11):1219–25.

5. Flume P, Wainwright C, Tullis E, Rodriguez S, Davies J, Wagener J. 58 Pulmonary exacerbations in CF patients with the G551D-CFTR mutation treated with ivacaftor. J Cyst Fibros. 2013;12 (SUPPL1): S63.

6. Sawicki GS, McKone EF, Pasta DJ, Millar SJ, Wagener JS, Johnson CA, et al. Sustained benefit from ivacaftor demonstrated by combining clinical trial and cystic fibrosis patient registry data. Am J Respir Crit Care Med. 2015;192(7):836–42.

7. Bessonova L, Volkova N, Higgins M, Bengtsson L, Tian S, Simard C, et al. Data from the US and UK cystic fibrosis registries support disease modification by CFTR modulation with ivacaftor. Thorax. 2018;73(8):731–40.

8. Hubert D, Dehillotte C, Munck A, David V, Baek J, Mely L, et al. Retrospective observational study of French patients with cystic fibrosis and a Gly551Asp-CFTR mutation after 1 and 2 years of treatment with ivacaftor in a real-world setting. J Cyst Fibros. 2018;17(1):89–95.

9. Volkova N, Moy K, Evans J, Campbell D, Tian S, Simard C, et al. Disease progression in patients with cystic fibrosis treated with ivacaftor: Data from national US and UK registries. J Cyst Fibros. 2020; 19 (1): 68-79.

10. Stevens D, Marshall B. A decade of healthcare improvement in cystic fibrosis: Lessons
for other chronic diseases. BMJ Qual Saf. 2014;23(SUPPL1):2–3.

11. McKone E, Emerson S, Edwards K, Aitken M. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. Lancet. 2003;361(9370):1671–6.

12. Sawicki G, McKone E, Millar S, Konstan M, Lubarsky B, Wagener J. Patients with Cystic Fibrosis and a G551D or Homozygous F508del Mutation: Similar Lung Function Decline. Am J Respir Crit Care Med. 2017;195(12):1673–6.

13. Richardson TS, Robins JM. Single world intervention graphs (SWIGs): a unification of the counterfactual and graphical approaches to causality. Center for Statistics and the Social Sciences, University of Washington. Technical Report 128, 2013. https://csss.uw.edu/research/working-papers/single-world-intervention-graphs-swigs-unification-counterfactual-and. Accessed 20 September 2021.

14. Taylor-Robinson D, Archangelidi O, Carr S, Cosgriff R, Gunn E, Keogh R, et al. Data Resource Profile: The UK Cystic Fibrosis Registry. Int J Epidemiol. 2018;47(1):9-10e.

15. Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative Controls: A Tool for Detecting Confounding and Bias in Observational Studies. Epidemiology. 2010;21(3):383–8.

16. Sofer T, Richardson D, Colicino E, Schwartz J, Tchetgen Tchetgen E. On negative outcome control of unobserved confounding as a generalization of difference-in-differences. Stat Sci. 2016;31(3):348–61.

17. Smith GD. Negative Control Exposures in Epidemiologic Studies. Epidemiology. 2012;23(2):351–2.

18. Weisskopf MG, Tchetgen Tchetgen EJ, Raz R. On the use of imperfect negative control
exposures in epidemiologic studies. Epidemiology. 2016;27(3):365–7.

19. Shi X, Miao W, Tchetgen Tchetgen EJ. Multiply robust causal inference with double negative control adjustment for categorical unmeasured confounding. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2020; 82(2): 521-540.

20. Abadie A, Diamond A, Hainmueller J. Synthetic control methods for comparative case studies: Estimating the effect of California’s tobacco control program. Journal of the American Statistical Association. 2010; 105 (490): 493-505.

21. O’Neill S, Kreif N, Grieve R, Sutton M, Sekhon JS. Estimating causal effects: considering three alternatives to difference-in-differences estimation. Health Serv Outcomes Res Methodol 2016;16:1-21.
Table 1. Number of people and total number of longitudinal observations in the UK CF Registry divided into four groups based on genotype (gating (\(G = 1\), or other (\(G = 0\))) and time-period (pre-ivacaftor (2008-2012) \(P = 0\), or post-ivacaftor (2013-2016) \(P = 1\)). Many individuals contribute to both groups A and B or both groups C and D.

| Genotype          | No. of people | No. of longitudinal observations | No. of people | No. of longitudinal observations |
|-------------------|---------------|----------------------------------|---------------|----------------------------------|
|                   |               | Group A \((P = 0, G = 1)\)        |               | Group B \((P = 1, G = 1)\)        |
| Gating \((G = 1)\) | 437           | 1326                             | 397           | 1368                             |
| Other \((G = 0)\)  | 6382          | 19 067                           | 7378          | 24 381                           |
Table 2. Summary of groups at baseline, defined as 2008 for the pre-ivacaftor period and 2012 for the post-ivacaftor period.

| Variable                                      | Group                                                                 |
|-----------------------------------------------|-----------------------------------------------------------------------|
|                                               | A          | B          | C          | D          |
| Ivacaftor Use                                 |            |            |            |            |
| Total Number of Post-baseline Visitsa         | 0          | 3.0 (1.1)  | 3.4 (1.1)  | 3.0 (1.1)  |
| Baseline Age (Years)a                         | 20.4 (10.8) | 22.4 (11.2)| 20.9 (11.6)| 21.9 (12.6)|
| Female                                        | 205        | 186        | 297        | 346        |
| White Ethnicity                               | 428        | 390        | 615        | 704        |
| Baseline ppFEV1a                               | 71.0 (23.2)| 69.7 (23.2)| 71.6 (23.3)| 72.0 (23.4)|
| Baseline Percent Predicted Forced Vital Capacitya,b | 84.8 (19.4)| 84.1 (18.9)| 84.0 (19.5)| 84.4 (19.6)|
| Baseline Percent Predicted Forced Mid-Expiratory Flowa,b | 56.3 (31.3)| 55.9 (32.4)| 60.9 (32.8)| 58.4 (31.0)|
| Baseline IV Daysa                             | 18.4 (28.1)| 20.2 (30.5)| 17.6 (27.7)| 18.6 (28.3)|
| Baseline Infectionc                           | 358        | 350        | 484        | 594        |
| Baseline CF-Related Diabetes                  | 69         | 90         | 119        | 175        |
| Baseline Smoker                               | 9          | 2.1        | 2.3        | 2.4        |
| Baseline Mucolytic Treatmentd                 | 223        | 264        | 299        | 488        |

<sup>a</sup> Values are expressed as mean (standard deviation).
<sup>b</sup> Percent predicted forced vital capacity based on 14 556 individuals, percent predicted forced mid-expiratory flow based on 5711 individuals, out of a total of 14 594 individuals across the four groups.
<sup>c</sup> Baseline infection includes *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, methicillin resistant *Staphylococcus aureus* (MRSA), influenza, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex.
<sup>d</sup> Baseline mucolytic treatment includes acetylcysteine, dornase alfa, hypertonic saline and mannitol.
Figure 1. A), B), and C): Directed acyclic graphs (DAGs) showing three possible causal pathways between ivacaftor ($X$), Genotype ($G$), time-period ($P$), measured covariates of health at baseline ($H$), and outcome ($Y$). D), E), and F): corresponding single world intervention graphs (SWIGs) for the intervention world in which $X$ is set to 0.

Figure 2. Reformulation of the DAG in Figure 1C showing the negative control outcomes (NCO). A) Using the outcome in period 0 as the NCO. B) and C) are the DAGs in groups $G = 0$ and $G = 1$ respectively when using the outcome in genotype group $G = 0$ as the NCO. In A), $Y_{P=0}$ and $Y_{P=1}$ denote the pre- and post-treatment outcomes, and treatment $X$ occurs after $Y_{P=0}$. In A), $G$ is an uncontrolled confounder, and is equivalent to the unmeasured confounder $U$ in the work of Sofer et al (16). In C) $P$ is the uncontrolled confounder.

Figure 3. Estimated naïve treatment effect (NTE), negative control effect (NCE) and negative-control-corrected treatment effect (NCCTE) of ivacaftor on ppFEV$_1$, using the time-period comparison (Unadjusted: NTE$_P$, NCE$_P$, NCCTE$_P$. Adjusted: NTE$_P$\textsuperscript{Con}, NCE$_P$\textsuperscript{Con}, NCCTE$_P$\textsuperscript{Con}) and the genotype comparison (Unadjusted: NTE$_G$, NCE$_G$, NCCTE$_G$. Adjusted: NTE$_G$\textsuperscript{Con}, NCE$_G$\textsuperscript{Con}, NCCTE$_G$\textsuperscript{Con}). A) Absolute step-change in ppFEV$_1$ ($\beta_{ST}$). B) Absolute change in the annual ppFEV$_1$ slope ($\beta_{SL}$). The adjusted analysis adjusts for baseline variables: sex, age, ethnicity, smoking status, CF-related diabetes, ppFEV$_1$, IV days (including an indicator of a non-zero count, and a linear term for the non-zero counts), mucolytic treatment use and bacterial infection.

Figure 4. Estimated naïve treatment effect (NTE), negative control effect (NCE) and negative-control-corrected treatment effect (NCCTE) of ivacaftor on rate of IV days, using the time-period comparison (Unadjusted: NTE$_P$, NCE$_P$, NCCTE$_P$. Adjusted: NTE$_P$\textsuperscript{Con}, NCE$_P$\textsuperscript{Con}, NCCTE$_P$\textsuperscript{Con}) and the genotype comparison (Unadjusted: NTE$_G$, NCE$_G$, NCCTE$_G$. Adjusted: NTE$_G$\textsuperscript{Con}, NCE$_G$\textsuperscript{Con}, NCCTE$_G$\textsuperscript{Con}). A) In year 1 ($\exp(\gamma_{X1})$). B) In year 2 ($\exp(\gamma_{X2})$). C) In year 3 ($\exp(\gamma_{X3})$). The adjusted analysis adjusts for baseline variables: sex, age, ethnicity, smoking status, CF-related diabetes, ppFEV$_1$, IV days (including an indicator of a non-zero count, and a linear term for the non-zero counts), mucolytic treatment use and bacterial infection.
A) $G \rightarrow X \rightarrow Y$

B) $G \rightarrow X \rightarrow Y$

C) $G \rightarrow X \rightarrow Y$

D) $G \rightarrow X|x=0 \rightarrow Y^{x=0}$

E) $G \rightarrow X|x=0 \rightarrow Y^{x=0}$

F) $G \rightarrow X|x=0 \rightarrow Y^{x=0}$
A) Comparison

| Time period | Unadjusted | Adjusted |
|-------------|------------|----------|
| NTE         | 5.83 (3.91, 7.64) | 7.27 (5.87, 8.57) |
| NCE         | 0.60 (0.12, 1.10)  | 0.77 (0.44, 1.08)  |
| NCCTE       | 5.23 (3.21, 7.09)  | 6.50 (5.06, 7.85)  |

| Genotype    | Unadjusted | Adjusted |
|-------------|------------|----------|
| NTE         | 4.12 (1.40, 6.47) | 6.22 (5.17, 7.24) |
| NCE         | −1.11 (−3.58, 1.27)| −0.37 (−1.36, 0.65) |
| NCCTE       | 5.23 (3.21, 7.09)  | 6.59 (5.22, 7.90)  |

Absolute Change in ppFEV1
B) **Comparison**

**Time period**
- **Unadjusted**
  - NTE
  - NCE
  - NCCTE
- **Adjusted**
  - NTE
  - NCE
  - NCCTE

**Genotype**
- **Unadjusted**
  - NTE
  - NCE
  - NCCTE
- **Adjusted**
  - NTE
  - NCE
  - NCCTE

**Absolute Change in Annual ppFEV1 Slope**

| Comparison | Absolute Change (95% CI) |
|------------|--------------------------|
| Time period |                          |
| Unadjusted  | 0.41 (−0.67,1.39)        |
|             | 0.08 (−0.20,0.35)        |
|             | 0.33 (−0.77,1.31)        |
| Adjusted    | 0.68 (0.11,1.32)         |
|             | 0.20 (0.04,0.37)         |
|             | 0.49 (−0.15,1.13)        |
| Genotype    |                          |
| Unadjusted  | 0.76 (0.10,1.39)         |
|             | 0.42 (−0.37,1.26)        |
|             | 0.33 (−0.77,1.31)        |
| Adjusted    | 0.67 (0.27,1.10)         |
|             | 0.19 (−0.36,0.70)        |
|             | 0.49 (−0.14,1.13)        |
| Comparison | Unadjusted | | | | Adjusted | | | | Genotype | Unadjusted | | | | Adjusted | | | |
|------------|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time period | | | | | | | | | | | | | | | | |
| Unadjusted | NTE | 0.50 (0.40, 0.61) | | NCE | 0.93 (0.89, 0.97) | | NCCTE | 0.54 (0.42, 0.66) | | | | | | | | | |
| Adjusted | NTE | 0.42 (0.29, 0.54) | | NCE | 0.77 (0.74, 0.83) | | NCCTE | 0.55 (0.36, 0.69) | | | | | | | | | |
| Genotype | Unadjusted | | | | | | | | | | | | | | | |
| NTE | 0.51 (0.40, 0.63) | | | NCE | 0.96 (0.80, 1.15) | | | NCCTE | 0.54 (0.42, 0.66) | | | | | | | | |
| Adjusted | NTE | 0.43 (0.33, 0.54) | | | | | | | | | | | | | | | |
| NCE | 0.80 (0.68, 0.95) | | | | | | | | | | | | | | | | |
| NCCTE | 0.53 (0.39, 0.70) | | | | | | | | | | | | | | | | |

**Rate Ratio of IV Days**
| Comparison | Rate Ratio (95% CI) |
|------------|--------------------|
| **Time period** |                |
| Unadjusted  |                  |
| NTE        | 0.47 (0.36,0.60)  |
| NCE        | 0.89 (0.85,0.93)  |
| NCCTE      | 0.52 (0.41,0.67)  |
| Adjusted    |                  |
| NTE        | 0.28 (0.21,0.41)  |
| NCE        | 0.75 (0.70,0.80)  |
| NCCTE      | 0.37 (0.28,0.55)  |
| **Genotype** |                 |
| Unadjusted  |                  |
| NTE        | 0.50 (0.39,0.62)  |
| NCE        | 0.95 (0.81,1.11)  |
| NCCTE      | 0.52 (0.41,0.67)  |
| Adjusted    |                  |
| NTE        | 0.38 (0.28,0.53)  |
| NCE        | 0.88 (0.74,1.04)  |
| NCCTE      | 0.43 (0.31,0.61)  |
## Time period

|                | Unadjusted |               | Adjusted |               |
|----------------|------------|---------------|----------|---------------|
|                | NTE        | NCE           | NCCTE    | NTE           | NCE           | NCCTE       |
| **Rate Ratio** | **(95% CI)** |               | **(95% CI)** |               |               |             |
| Unadjusted     | 0.42 (0.31, 0.55) | 0.86 (0.81, 0.91) | 0.49 (0.36, 0.65) |               |               |             |
| Adjusted       | 0.31 (0.19, 0.43) | 0.74 (0.67, 0.79) | 0.42 (0.26, 0.58) |               |               |             |

## Genotype

|                | Unadjusted |               | Adjusted |               |
|----------------|------------|---------------|----------|---------------|
|                | NTE        | NCE           | NCCTE    | NTE           | NCE           | NCCTE       |
| **Rate Ratio** | **(95% CI)** |               | **(95% CI)** |               |               |             |
| Unadjusted     | 0.44 (0.33, 0.54) | 0.89 (0.72, 1.08) | 0.49 (0.36, 0.65) |               |               |             |
| Adjusted       | 0.37 (0.27, 0.47) | 0.89 (0.72, 1.09) | 0.41 (0.29, 0.59) |               |               |             |