INTERVENTI

COVID-19 vaccination and all-cause and non-COVID-19 mortality. A revaluation of a study carried out in an Italian Province

Vaccinazione COVID-19 e mortalità per tutte le cause e non COVID-19. Rivalutazione di uno studio condotto in una provincia italiana

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Abstract

The COVID-19 vaccination prevents COVID-19 specific mortality. Well planned population-based studies, however, are necessary to evaluate the overall effectiveness of vaccination programmes. A study carried out in the province of Pescara is used to illustrate the potential biases that may affect such studies. The Pescara study analysed total and non-COVID-19 mortality and the occurrence of Potentially Vaccine-Related Serious Adverse Events (PVR-SAEs) in vaccinated and unvaccinated people, from January 2021, when vaccines became available, to July 2022. The study reported a lower probability of both total and non-COVID-19 death in vaccinated people. However, the authors did not include in the denominator of the unvaccinated cohort the population experience of the vaccinated cohort before vaccination (*immortal time bias*). Correcting the denominator of the unvaccinated cohort, the crude death rate of vaccinated and unvaccinated persons becomes the same. For the same reason, the unvaccinated non-COVID-19 mortality was overestimated, as was the mortality of people receiving only one or two vaccine doses. *Confounding by indication* and the *healthy vaccinee bias* will also be discussed, as well as the bias deriving by not considering the evolution of risk over time.

Keywords: COVID-19 vaccination status, all-cause mortality, non-COVID-19 mortality, COVID-19 vaccinations' adverse events

Keypoints

- **COVID-19 vaccination prevents specific mortality; well-planned population studies are needed to evaluate the overall effectiveness of vaccination programmes.**
- **A study carried out in the province of Pescara allows to illustrate the systematic errors that can influence these studies.**
- **First of all, it is discussed the lack of consideration of the immortal time bias, which overestimates the mortality of the unvaccinated, such as of those who have received only one/two vaccine doses. It is also discussed the confounding by indication bias, the healthy-vaccinees bias and the bias of not considering the evolution of risk over time.**

Riassunto

La vaccinazione COVID-19 previene la mortalità specifica per COVID-19. Sono tuttavia necessari studi di popolazione ben pianificati per valutare l'efficacia complessiva dei programmi di vaccinazione. Uno studio con questo scopo, condotto nella provincia di Pescara, consente di illustrare potenziali errori sistematici che possono influenzare tali studi. Lo studio di Pescara ha analizzato la mortalità totale e non-COVID-19, e il verificarsi di eventi avversi gravi potenzialmente correlati al vaccino (PVR-SAE), nelle persone vaccinate e non vaccinate, da gennaio 2021, quando i vaccini sono diventati disponibili, a luglio 2022. Lo studio ha riportato una probabilità inferiore di morte sia totale che non-COVID-19 nelle persone vaccinate.
Tuttavia, gli autori non hanno incluso nel denominatore della coorte non vaccinata l'esperienza della popolazione della coorte vaccinata prima della vaccinazione (*immortal time bias*). Correggendo il denominatore della coorte non vaccinata, il tasso grezzo di mortalità delle persone vaccinate e non vaccinate diventa lo stesso. Per lo stesso motivo, la mortalità non-COVID-19 dei non vaccinati è stata sovrastimata, così come la mortalità delle persone che hanno ricevuto solo una o due dosi di vaccino. Si discutono, inoltre, il confondimento da indicazione, il bias del vaccinato sano, e il bias derivante dal fatto di non considerare l'evoluzione del rischio nel tempo.

Parole chiave: stato di vaccinazione COVID-19, mortalità per tutte le cause, mortalità non da COVID-19, eventi avversi alle vaccinazioni COVID-19

While the Randomized Controlled Trial (RCT) is widely accepted as the design that provides the most definitive results, the generalizability of its findings to the real-world setting can be challenging. Indeed, RCTs often use strict selection criteria and treatment adherence that may differ with the real-world setting. Well-conducted observational studies will provide further results that can inform clinical decision-making, health policies, and future research, but can be methodologically complex.
The COVID-19 vaccines have been developed during 2020 and evaluated in randomized trials that demonstrated their efficacy in preventing COVID-19 infection and hospitalization for serious diseases.

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However, important questions, such as the overall effectiveness of vaccination programmes in the general population, the effectiveness against new virus variants, the effectiveness of booster vaccination, and long-term side effects could not be answered by randomized trials and must therefore be addressed by observational studies. However, analyses of observational data can be biased by confounding and inadequate design that does not consider the evolution of the pandemic over time and the rapid uptake of vaccination.

To illustrate these complexities and the biases that can be corrected a recently published population-based study is used to analyse total and non-COVID-19 mortality and the occurrence of Potentially Vaccine-Related Serious Adverse Events (PVR-SAEs) in vaccinated and unvaccinated people. The study was conducted by Flacco et al.[1] in the whole population of the province of Pescara, Italy, from January 2021, when vaccines became available, until July 2022.

This is the type of study that needs to be done to monitor the long-term effects of vaccination campaigns, but the analysis is flawed, because of the so-called immortal time bias[2,3] and because the evolution of the epidemic over time was not taken into account.[4,5] Immortal time is a period of follow-up (person-time) during which, by design, the outcome of interest (death or another outcome) cannot occur. Vaccinated people, for instance, cannot die between the start of observation and the date of vaccination (immortal time). The immortal bias may occur if this time period is included in the denominator of vaccinated people or it is not included in the denominator of unvaccinated persons.

Unfortunately, the authors of the Pescara province study did not consider that the pre-vaccination population experience of the vaccinated cohort belongs to the unvaccinated cohort. It can be considered their unadjusted analysis of mortality in unvaccinated individuals versus those who received ≥1 vaccine dose (Table 2 of the Flacco et al. paper).

Over the entire study period 259,821 persons were vaccinated with at least one dose, while 56,494 persons were not vaccinated (never vaccinated). Flacco et al. Table 1 shows that the average follow-up time for those never vaccinated was 561 days while the post-vaccination follow-up of those vaccinated was 399 days. Therefore, it can be estimated that the vaccinated persons lived on average 561-399 = 162 days when still unvaccinated. These 162 days belong to the denominator of the unvaccinated mortality rate.

The following Table 1 shows the person-months of observation of those vaccinated (after vaccination), of those never vaccinated, of those vaccinated before vaccination, and the total person-months of the unvaccinated (person-months of never vaccinated + person-months before vaccination of the vaccinated). Person-months were calculated as: number of persons x average follow-up days/30. The Table also shows, for each category of vaccinated and unvaccinated, the number of deaths and the death rate per 1,000 person-months, both for overall deaths and non-COVID-19 deaths. The last line shows the COVID-19 mortality.

Flacco et al. concluded that "the overall all-cause mortality was significantly higher among unvaccinated individuals than those who received at least one vaccine dose. The average monthly death rate was 2.26 x 1000 individuals among the unvaccinated and 0.97 x 1000 among the vaccinated (with at least one dose)". Unfortunately, they have not considered the person-months experienced by vaccinated subjects before vaccination (1,403,033 person-months), which must be included in the denominator of the unvaccinated cohort; therefore, they overestimated the mortality of the unvaccinated. After correction the crude death rate of vaccinated and unvaccinated persons is the same.

Moreover, the cohort from the province of Pescara shows an extraordinary effect of vaccination on non-covid mortality. Vaccination seems to have halved it: 0.88/1,000 person-months among vaccinated vs. 1.72/1,000 person months among the unvaccinated. Such a high mortality in the Pescara province population seems surprising. Indeed, before the COVID-19 pandemic, the 2015-2019 mortality data of the National Institute of Statistics for the province of Pescara[6] show a mortality rate of 10.8 per 1,000 person-years (= 0.9 x 1,000 person-months), about half the observed mortality of unvaccinated people (1.72/1,000 person-months). After correction, the non-COVID-19 mortality of the unvaccinated becomes 0.74, slightly lower than the pre-COVID-19 mortality of the province of Pescara and lower than the non-covid mortality of vaccinated (0.88). Flacco et al. overestimated the overall mortality of the unvaccinated by 2.26/0.97 = 2.33 times and the non-COVID mortality by 1.72/0.74 = 2.32 times. These calculations indicate that the conclusions drawn in the article based on the univariate analysis need to be radically modified. However, all the above computations should be adjusted at least for age and comorbidity. Flacco et al. did such an adjustment in Table 3, but these analyses are also affected by the same immortal bias of missed person-time in the denominators. The results, in fact, show an unbelievable 81% lower mortality (95% CI 80-82) in vaccinated per-

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	Vaccinated (after vaccination)	Never vaccinated	Vaccinated (before vaccination)	Unvaccinated (total)
No.	259,821	56,494	259,821	
Mean follow-up days	399	561	162	
Total person-month	3,455,619	1,056,438	1,403,033	2,459,471
Total number of deaths	3,351	2,392	0	2,392
Death rate/1,000 person-month	0.97	2.26	0	0.97
Non-COVID-19 deaths	3,058	1,815	0	1,815
Non-COVID-19 death rate / 1,000 person-month	0.88	1.72	0	0.74
COVID-19 death rate/1,000 person-month	0.08			0.23

Table 1. Distribution of population, person-time of observation, number of total and non COVID-19 deaths, and mortality rates, by vaccination status.
Tabella 1. Distribuzione della popolazione, dei tempi di osservazione, del numero di morti totali e non COVID-19, e dei tassi di mortalità in funzione dello stato vaccinale.

sons. Such a result should have raisen the suspect of a wrong computation.

Flacco et al. also separately analysed the mortality of those who received only one dose of vaccine, those who received 2 doses, and those who received also the third dose, as recommended by the vaccination campaign. The results are quite impressive, because they suggest that the COVID-19 vaccination increases mortality after the first and the second injection, indeed a very high mortality (2.55 and 3.78 per thousand per month). However, the computation is wrong, because of the same kind of bias above mentioned: in fact, with regard to those with a single vaccination, the authors forgot to include in the denominator the experience of life with a single vaccination of those who got further vaccinations: 51,684 person-months of those with two doses and 192,305 person-months of those with three doses (assuming that the second dose was administered one month after the first dose, on average). By the same token, the denominator of those who received two doses must include the population experience between the second and third dose of the fully vaccinated subject. The following Table 2 shows the correct computation. The corrected results look more reasonable: those vaccinated with only one dose have a mean monthly rate of non-COVID-19 deaths almost equal to that of the unvaccinated, while those vaccinated with only two doses and with three or more doses have monthly non-COVID-19 death rates slightly or moderately higher than unvaccinated. In any case this latest evidence should be adjusted for age and other confounding factors. It must be clear that these computations are just an exercise to illustrate the direction of the bias and do not pretend to provide true estimates of the effect. They only suggest that the immortal bias may turn round the conclusions.

In conclusion, in the crude analysis, there is no evidence that "the overall all-cause mortality was significantly higher among unvaccinated individuals", as stated in the paper, and there is conflicting evidence of increased non-COVID-19 mortality depending on the number of doses received.

Also, PVR-SAEs data (Table 2 B) may be affected by the same bias, because the authors underestimated the denominators of unvaccinated individuals and of people vaccinated with only one or two doses, but the published data do not allow a correction. The correction made here for mortality does not apply, because before receiving one, two or three doses several people may have experienced events classifiable as PVR-SAEs, nevertheless continuing to undergo vaccination. Most likely the dramatically high risk of miocardial infarction, stroke, miocarditis/pericarditis and deep venous thrombosis registered after one or two doses are wrong, but deserve further investigation.

A further methodological issue is that Flacco et al. have followed up vaccinated and unvaccinated people in different calendar and seasonal periods. The follow-up of the unvaccinated starts on 2 January, 2021, during the second wave of the pandemic, when the winter season implies a higher mortality also for non-COVID-19 causes (as shown by the National Institute of Statistics mortality data also for the province of Pescara). Instead, the follow-up of the vaccinated groups begins at different times (characterized by different mortality risks both for COVID-19 and for all-cause mortality), distributed over the entire study period. For example, the follow-up of those vaccinated with three doses begins on 1 July 2021, when the second wave of the pandemic was over and all-cause mortality was significantly lower than in winter (see data from the National Institute of Statistics).[14] This may have contributed

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	Vaccinated one dose	Vaccinated two doses	Vaccinated three doses	Unvaccinated (total)
No.	15,832	51,684	192,305	
Mean follow-up days	204	279	209	
Person-month of observation*	107,658	480,661	1,339,725	
Corrected Person-months of observation**	107,658+51,684+ +192,305 = = 351,647	480,661+ 305 x 6 = = 1,634,491		2,459,471
Number of deaths	275	1,819	1,257	2,392
Mean monthly rate/1,000 Computed by Flacco et al	2.55	3.78	0.94	2.26
Corrected mean monthly rate/1,000	0.78	1.11	0.94	0.97
Non-COVID-19 deaths	256	1,715	1,087	1,815
Corrected Non-COVID-19 death rate/1,000 person- month	0.73	1.05	0.81	0.74

* Obtained by multiplying the number of vaccinated with a single dose by the average follow-up days divided by 30 / *Ottenuto moltiplicando il numero di vaccinati con una singola dose per i giorni medi di follow-up divisi per 30.*
** Obtained by adding to the denominator of the rate for those who received a single vaccination the person-months experienced before the second vaccination by those who received two or three vaccinations (we assumed that the second dose was administered, on average, one month after the first dose), and, respectively, adding to the denominator of those who received two vaccinations the hypothetical person months experienced between the second and third vaccination by those who received three doses (we assumed tentatively that the third vaccination occurred six months after the second) / *Ottenuto aggiungendo al denominatore del tasso per coloro che hanno ricevuto una singola vaccinazione i mesi-persona vissuti prima della seconda vaccinazione da coloro che hanno ricevuto due o tre vaccinazioni (si è ipotizzato che la seconda dose sia stata somministrata, in media, un mese dopo la prima dose) e, rispettivamente, aggiungendo al denominatore di coloro che hanno ricevuto due vaccinazioni gli ipotetici mesi-persona vissuti tra la seconda e la terza vaccinazione da coloro che hanno ricevuto tre dosi (si è ipotizzato provvisoriamente che la terza vaccinazione sia avvenuta sei mesi dopo la seconda).*

Table 2. Distribution of population, person-time of observation, number of total and non COVID-19 deaths, and mortality rates, by number of vaccine doses.
Tabella 2. Distribuzione della popolazione, dei tempi di osservazione, del numero di morti totali e non COVID-19, e dei tassi di mortalità in funzione del numero di dosi di vaccino.

to the gap between the monthly COVID-19 mortality rate in the unvaccinated compared to the vaccinated (0.23 vs 0.08), possibly only partly due to the protection offered by the vaccine. A more appropriate analysis would have been a month-by-month comparison of outcomes for different vaccination states, based on person-times, similar to the analyses enabled by the data of the United Kingdom Office for National Statistics (UK ONS).[16]

The monthly data published in the UK ONS bulletin show a progressive increase over time of the relative risk of total mortality in the vaccinated vs the unvaccinated groups. Such an increase might also have occurred in Pescara. As Flacco et al. did not consider the evolution of risks over time, they may have missed it. The calculation of average mortality rates over the entire follow-up period, in fact, may have masked such a trend. It would be advisable to make the input data available, to enable such analysis.

An alternative method to address the issue of people initiating treatment at various time points during follow-up would have been, as suggested by the references 4 and 5, to match each person receiving vaccination 1:1 (or 1: n) with one or more unvaccinated people up to the same point in time, chosen to be as similar as possible to the vaccinated person for any potential confounder, thus trying to emulate the randomized trial process.

In the discussion, Flacco et al. cite a population-based study conducted in the United States to assess mortality not associated with COVID-19 in a general population setting (approximately 11 million persons enrolled in seven sites and followed up from December 2020 to July 2021).[8] Correctly, in this study, to avoid *immortal bias*, "Person-time for unvaccinated persons included unvaccinated person-time before COVID-19 vaccination among COVID-19 vaccinees, and unvaccinated person-time of persons who did not receive a COVID-19 vaccine". After standardizing for age and sex, also this study found that COVID-19 vaccine recipients had lower non-COVID-19 mortality than did unvaccinated persons. This result does not seem logical, because the anti-COVID-19 vaccination can protect against mortality from COVID-19, but it is unlikely that it may protect against deaths from other causes The Authors had no information on comorbidity, but suggested that the lower mortality of vaccinated was due to *healthy-vaccinee effect* (i.e. vaccinated persons tend to be healthier than unvaccinated persons). Flacco et al., adjusting for comorbidity, can at least partly avoid the bias of *confounding by indication*. However, it would be difficult, if not impossible, to correct for the *healthy-vaccinee bias*,[6] which can be very powerful. In fact, voluntary

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adherence to a treatment can be associated with a nearly halved mortality,[9-11] and even with a reduction in mortality of 2.5 to 3 times or more[12,13,15] compared to the mortality of those who do not adhere. Moreover, this effect can last for years.[11,12,15] Adjusting for social factors, such as education, income and civil status might help to reduce the possibility of a *healthy-vaccinee bias*, but would not be sufficient to control it.

It is well known that postmarketing pharmacoepidemiology is a difficult science with many traps. It would have been better to plan studies of vaccine side effects and mortality at the time of implementation of the vaccination plan, in order to prospectively collect all the relevant information and to profit of the fact that vaccines were not immediately available for millions of people to schedule the implementation of the vaccination plan, creating comparable groups of vaccinated and unvaccinated people.

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