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A Reanalysis of an Italian Study on the Effectiveness of COVID-19 Vaccination Suggests That It Might Have Unintended Effects on Total Mortality

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Abstract: Immortal-time bias (ITB) is known to be common in cohort studies and distorts the association estimates between treated and untreated groups. We used data from the last of two large studies in an Italian province on COVID-19 vaccines safety and effectiveness incurred this bias, and aligned the entire population on a single index date, to correct the ITB. We considered the "all-cause deaths" outcome to compare the survival curves between the unvaccinated group and the various vaccination statuses. The all-cause deaths Hazard Ratios in univariate analysis for unvaccinated (reference) versus vaccinated with 1, 2, 3/4 doses were 0.88 (CI95: 0.78 –1.00; p-value 0.044), 1.23 (1.16–1.32; p-value ≤0.001) and 1.21 (1.14–1.29; p-value ≤0.001), respectively. The multivariate values were 2.40 (2.00–2.88; p-value <0.0001), 1.98 (1.75–2.24; p-value <0.0001), 0.99 (0.90–1.09; ns). The possible explanations of the trend of the Hazard Ratios as vaccinations increase could be a *harvesting effect*; a *calendar-time bias*, accounting for seasonality and pandemic waves; a *case-counting windows* bias; a *healthy-vaccinee bias*; or some their combination. With two and even with 3/4 doses the calculated Restricted Mean Survival Time and Restricted Mean Time Lost have shown a small but significant downside for the vaccinated populations

Keywords: COVID-19 vaccines; immortal-time bias; healthy-vaccinee bias; all causes mortality

1. Introduction

The SARS-CoV-2 pandemic has led to an unprecedented effort to generate real evidence on the safety and effectiveness of various treatments, mRNA vaccines included. Already in articles published during the pandemic [1,2] it has been argued that, in observational cohort studies, an incorrect management of follow-up times may introduce the so-called Immortal-Time Bias (ITB) in favor of the exposed group (for studies on mRNA vaccines the exposed are vaccinated people).

Nevertheless, the ITB still appears to be present in several cohort studies. As highlighted in a recently published paper [3], a study on the safety of COVID-19 vaccines in the population of an Italian province is no exception [4]. A possible explanation for why the ITB is still largely prevalent in such cohort studies may be that the structure of the ITB is still poorly understood [2].

Furthermore, it has been noted that observational studies on the effectiveness of COVID-19 vaccines are subject to inherent biases, including differences in testing strategies or doubtful attribution of causes of death between vaccinated and unvaccinated [5–7]. Therefore, "all-cause deaths" seems the outcome less affected by misclassification.

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We welcomed with great interest the publication of another article on mortality in the general population of the Italian province of Pescara, divided by vaccination status, with a follow-up of two years [8] instead of the 18 months of the previous study in the same province [3], because of the large size of the sample analysed and the longer follow-up. By the way, the new study showed that people receiving only one or two vaccine doses had a significantly higher risk of all-cause death (HRs 1.40 and 1.36, respectively; both $p < 0.001$), while the subjects receiving three or more vaccine doses showed a substantially lower risk of death (HR 0.22; 95% CI: 0.20–0.23). Unfortunately, also this new study falls into the same fallacies that were already highlighted in our previous paper [4]. In consideration of the interest of this study, we asked the authors for the original dataset on which the study was based and they kindly provided it to us.

Starting from the same population of the province of Pescara, Italy, studied by Rosso et al. [8] the aim of this study was to compare the "all-cause deaths" outcome in the cohorts of people vaccinated with 1, 2 and 3/4 doses respectively, with the same outcome in the cohort of unvaccinated people, collected from the population of that province, and correcting for the ITB. Therefore, we have aligned the entire population to a single index date, in order to avoid the ITB.

Considering that overall COVID-related deaths represent a minority portion of total deaths, in Italy equal to 9,0% according to the last available data from ISTAT [9], even assuming that vaccination leads very high reductions in the risk of COVID-related deaths [10], this will affect the risk of all-cause deaths only marginally. Therefore, we hypothesize that the correction of the ITB can push the estimates of the hazard ratio for the "all-cause deaths" outcome towards a null, or at least to a limited effect, differently from the result obtained by Rosso et al [8].

2. Materials and Methods

This retrospective cohort study used information collected from the dataset kindly provided by Rosso et al. (8). Any information contained in this dataset come from the Italian National Healthcare System. The population considered is that of residents or domiciled in the province of Pescara on January 1st, 2021, aged 10 years and older, without a positive SARS-CoV-2 swab at the date of the follow-up start.

Furthermore, in the dataset provided by Rosso et al. [8], subjects who died before January 15, 2021 were excluded.

Vaccination data were acquired from the official regional SARS-CoV-2 vaccination dataset, up to December 31st, 2022.

The follow-up considered ranges between January 1st, 2021 and February 15, 2023.

The dataset was restructured in order to correct the ITB recognized by Berrino et al. [4] in a previous paper using the same dataset [8].

2.1. Immortal Time Bias Correction

To correct the ITB we aligned the entire population on a single index date (1st January 2021), calculating the time spent as an Unvaccinated for the 1 – dose population, the time spent as an Unvaccinated and as 1 – dose for the 2 – doses population and, finally, the time spent as an Unvaccinated, 1 – dose and 2 – doses for the 3/4 doses population.

The time spent by each individual in their respective cohorts was calculated as person-day according to following formula: $person{-}days = \Sigma fwi$

where fwi is the follow-up of the *i-th* subject of the cohort.

In this way, the cohort was divided as follows:

(a) "Unvaccinated": in this group were inserted never vaccinated individuals, and vaccinated individuals before receiving 1 or more doses;
(b) "1–dose": in this group were inserted all individuals vaccinated with 1 dose and all individuals vaccinated before they received 2 or more doses;
(c) "2–doses": in this group were inserted all individuals vaccinated with 2 dose and all vaccinated individuals before they received 3 or more doses;
(d) "3/4 doses": In this group were inserted all vaccinated individuals with 3 or more doses.

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Accordingly, the follow-up obtained were:

(a) "Unvaccinated": the follow-up starts on January 1st, 2021 and ends the day of death, or of the 1st dose, or on 15 February 2023;
(b) "1–dose": the follow-up starts on the 15th day after 1st dose and ends the day of death, or of the 2nd dose, or on February 15, 2023;
(c) "2–doses": the follow-up starts on the 15th day after 2nd dose and ends the day of death, or of the 3rd dose, or on February 15, 2023;
(d) "3/4 doses": the follow-up starts on the 15th day after the 3rd dose and ends the day of death, or on February 15, 2023.

2.2. Follow-Up

The entire period of observation lasted 775 days considering the start and end dates above indicated (January 1st, 2021 and February 15, 2023). Average follow – up of each cohort obtained from the restructuring of the dataset are:

(a) "Unvaccinated": 258 days
(b) "1 dose": 61 days
(c) "2 doses": 247 days
(d) "3/4 doses": 400 days

2.3. Outcome

The only outcome considered was "All-cause deaths". In fact, the outcome "Covid-19-related death" presented some questionable relations during the exploration of the dataset and, in our opinion, it does not allow a reliable estimate of the statistical parameters. The questionable relations of the outcome "Covid-19-related death" can be summarized as follows: Covid-19-related death without severe Covid–19, Covid-19-related death occurring more than 90 days after the last positive swab, Covid-19-related death occurring on the same day as the positive swab (Table 1).

In several cases the first two relations can find some reasonable explanation. On the contrary, the third, which involves over 30% of deaths related to COVID-19, appears very difficult to justify.

Table 1. Questionable Covid-19-related death classifications; 1 Percentage compared to All–cause death; 2 Percentage compared to Total Covid–19 related death.

	Unvaccinated	1 Dose	2 Doses	3/4 Doses	Total Sample
Total COVID-19 related deaths, n (%)[1]	573 (28.9)	66 (20.1)	225 (11.5)	658 (25.8)	1522 (22.3)
Deaths without severe COVID-19, n (%)[2]	78 (13.6)	34 (51.5)	68 (30.2)	267 (40.6)	447 (29.4)
Same day for swab and death, n (%)[2]	18 (3.1)	0 (0)	8 (3.6)	22(3.3)	48 (3.2)
Deaths > 90 days from swab, n (%)[2]	107 (18.7)	43 (65.2)	83 (36.9)	244 (37.1)	477 (31.3)
Cumulative questionable classifications, n (%)[2]	142 (24.8)	47 (71.2)	100 (44.4)	330 (50.2)	619 (40.7)

2.4. Statistical Analysis

Cox proportional hazards analysis was used to compute the adjusted Hazard Ratio (HR) of all covariates used in model. In order to compare survival distributions between Unvaccinated group and the several vaccination statuses considered, Log-rank test was used and α level was fixed to 0,05. The exposure time was defined in days.

We considered "All-cause deaths" as dependent variable in model, and "Groups" as independent variable that it compares Unvaccinated population and "1-dose" population or "2-doses" population or "3/4 doses" population. To adjust the estimated HRs we considered nine

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covariates: gender, age, hypertension, diabetes, chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), kidney diseases, cancer, and infection.

With the aim to verify the validity of assumption of the model Schoenfeld's test was used considering both value of global test and for each covariate, so we can use the appropriate stratification if the assumptions of the model were not satisfied. Furthermore, we plotted log cumulative hazard in order to ensure graphically the validity of the assumptions of the model.

For covariates, the HRs must be interpreted by comparing the population with the disease to the population without the disease at the same vaccine dose.

Finally, we used Restricted Mean Survival Time (RMST) and Restricted Mean Time Lost (RMTL) to estimate the difference and the ratio between groups, inasmuch the construction of a time-dependent variable did not allow us to correct the model assumptions for the 2-dose and 3-dose vaccination status. The RMST is the best index of 'life expectancy' in those comparison where the assumptions of the model were not respected [11], while the RMTL can approximate the HR in absence of proportional hazard assumption [12]. The truncation time, tau (τ), was fixed to be equal to the minimum of the largest observed times on each of the two groups.

Data was processed using R studio (version 2023.09.0).

3. Results

3.1. Population Distribution after ITB Correction

Following the alignment the composition of the populations compared was different from those used by Rosso et al.[8]: in the Unvaccinated population we inserted never vaccinated and not vaccinated individuals prior to receiving 1 or more doses; in the 1-dose population group were inserted all individuals vaccinated with 1 dose and all individuals vaccinated prior to receiving 2 or more doses; in the 2-doses population were inserted all individuals vaccinated with 2 doses and all individuals vaccinated prior to receiving 2 or more doses and, finally, in the 3 or 4 doses we inserted all individuals vaccinated with 3 or more doses.

The size of each population, the demographic characteristics and the comorbidity are summarized in Table 2.

Table 2. Characteristics of the sample - SD = Standard deviation. [a] In this group were inserted never vaccinated and not vaccinated individuals before to receive 1 or more doses; [b] In this group were inserted all individuals vaccinated with 1 dose and all individuals vaccinated before to receive 2 or more doses; [c] In this group were inserted all individuals vaccinated with 2 dose and all individuals vaccinated before to receive 2 or more doses; [d] In this group were inserted all individuals vaccinated with 3 or more doses.

	Unvaccinated[a] (n = 290,727)	1 Dose[b] (n = 245,741)	2 Dose[c] (n = 234,287)	3/4 Dose[d] (n = 186,684)
Age in years (Mean, SD)	48.9 (20.8)	49.7 (20.7)	50.1 (20.7)	52.5 (20.2)
Gender (n,%)				
Females	148,770 (51.2)	127,121 (51.7)	121,516 (51.9)	97,440 (52.2)
Males	141,957 (48.8)	118,620 (48.3)	112,771 (48.1)	89,244 (47.8)
Risk factors and comorbidities (n,%)				
Hypertension	40,255 (13.8)	37,003 (15.1)	36,159 (15.4)	32,264 (17.3)
Diabetes	15,599 (5.4)	14,224 (5.8)	13,837 (5.9)	12,282 (6.6)
CVD	23,252 (8)	20,940 (8.5)	19,991 (8.5)	17,321 (9.3)
Kidney disease	5,431 (1.9)	4,718 (1.9)	4,568 (1.9)	3,793 (2)
Cancer	16,580 (5.7)	15,065 (6.1)	14,676 (6.3)	12,810 (6.9)
Infection	117,559 (40.4)	104,397 (42.5)	97,102 (41.4)	69,637 (37.3)
COPD	11,035 (3.8)	9,802 (4)	9,391 (4)	7,683 (4.1)

3.2. 1-Dose versus Unvaccinated

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In the Log-rank test of the univariate analysis the 1-dose population show an HR of 0.88 (CI_{95}=0.78 – 1.00; p-value=0.044) versus unvaccinated; the hypertensive population shows a HR of 12.59 (CI_{95}=11.58 – 13.69; p<0.001) compared to non-hypertensive population; the diabetic population shows a HR of 8.07 (CI_{95}=7.31 – 8.90; p<0.001) compared to non-diabetic population; the CVD population show an HR of 11.56 (CI_{95}=10.63 – 12.57; p<0,001) compared to non-CVD population; the population with kidney disease show an HR of 17.89 (CI_{95}=16.08 – 19.90; p<0.001) compared to population with no kidney disease; the cancer population shows a HR of 9.34 (CI_{95}=8.51 – 10.25; p<0.001); the infected population show an HR of 0.58 (CI_{95}=0.53 – 0.63; p<0.001) compared to uninfected population; the HR of the covariate Age is 1.11 (CI_{95}=1.11 – 1.11; p<0,001); male population show an HR of 0.87 (CI_{95}=0.81 – 0.95; p<0.001) compared to female population; finally, the COPD population show an HR of 7.11 (CI_{95}=6.40 – 7.91; p<0.001) compared to non-COPD population (Table 5).

In the multivariable analysis the HRs were obtained by a stratification of the covariates cancer, infection, and age: this allowed us to correct the significance of Global Schoenfeld's test. The Log-rank test of this analysis gave the following results: the HR for "All-cause deaths" between 1-dose versus Unvaccinated is 2.40 (CI_{95}=2.00 – 2.88; p<0.0001). The hypertensive population show an HR of 1.49 (CI_{95}=1.23 – 1.82; p<0.0001) compared to non-hypertensive population; the diabetic population show an HR of 2.00 (CI_{95}=1.60 – 2.49; p<0.0001) compared to non-diabetic population; the CVD population show an HR of 1.60 (CI_{95}=1.31 – 1.96; p<0,0001) compared to non-CVD population; the population with kidney disease show an HR of 1.77 (CI_{95}=1.35 – 2.34; p<0.0001) compared to population with no kidney disease; the male population show an HR of 1.50 (CI_{95}=1.27 – 1.78; p<0.0001) compared to female population and, finally, the COPD population show an HR of 2.01 (CI_{95}=1.56 – 2.60; p<0.0001) compared to non-COPD population (Table 5).

3.3. 2-Doses versus Unvaccinated

In the Log – rank test of the univariate analysis, the 2-doses population show a HR of 1.23 (CI_{95}=1.16 – 1.32; p<0.001) versus Unvaccinated people; the hypertensive population show an HR of 11.47 (CI_{95}=10.76 – 12.23; p<0.001) compared to non-hypertensive population; the diabetic population show an HR of 6.71 (CI_{95}=6.23 – 7.23; p<0.001) compared to non-diabetic population; the CVD population show an HR of 10.88 (CI_{95}=10.21 – 11.60; p<0,001) compared to non-CVD population; the population with kidney disease show an HR of 16.83 (CI_{95}=15.56 – 18.20; p<0.001) compared to population with no kidney disease; the cancer population show an HR of 8.65 (CI_{95}=8.07 – 8.27; p<0.001); the infected population show an HR of 0.35 (CI_{95}=0.32 – 0.38; p<0.001) compared to uninfected population; the covariate Age shows an HR of 1.11 (CI_{95}= 1.11 – 1.12; p<0,001); male population show an HR of 0.95 (CI_{95}= 0.89 – 1.01; ns); the COPD population show an HR of 6.28 (CI_{95}=5.79 – 6.82; p<0.001) compared to non-COPD population (Table 5).

The HRs obtained in the multivariable analysis represent the best fit of the model achieved by stratification of the covariates hypertension, cancer, infection, sex and age. Despite this the Global Schoenfeld's test remained significant whereby the estimate of the HRs could be inaccurate. In addition to these estimates the Restricted Mean Survival Time and the Between-group contrast were calculated (Table 4). At the multivariable analysis, the HR for "All-cause deaths" between 2-dose versus Unvaccinated is 1.98 (CI_{95}=1.75 – 2.24; p<0.0001). The diabetic population show an HR of 1.74 (CI_{95}=1.38 – 2.20; p<0.0001) compared to non-diabetic population; the CVD population show an HR of 1.78 (CI_{95}=1.44 – 2.20; p<0.0001) compared to non-CVD population; the population with kidney disease show an HR of 2.44 (CI_{95}=1.84 – 3.24; p<0.0001) compared to population with no kidney disease; the COPD population show an HR of 2.89 (CI_{95}=2.18 – 3.84; p<0.0001) compared to non-COPD population (Table 5).

Restricted Mean Survival Time for 2-doses population (τ=739 days) is 728.92 (CI_{95}=728.32 – 729.51) days compared to Unvaccinated population 731.62 (CI_{95}=731.27 – 731.98), while the Between-group contrast is -2.70 days (CI_{95}= -3.40 – -2.01; p<0.0001). Restricted Mean Time Lost ratio is 1.37 (CI_{95}=1.27 – 1.48; p<0,0001) (Table 3).

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Table 3. Estimate of Restricted Mean Survival Time and Between-group contrast in 2-doses versus Unvaccinated.

Restricted Mean Survival Time (RMTS) (τ=739 days)				
Groups	Estimate	SE	95% CI	
RMST 2-doses (arm1)	728.92	0.30	728.32 – 729.51	
RMST Unvaccianted (arm0)	731.62	0.18	731.27 – 731.98	
Restricted Mean Time Lost (RMTL)				
RMTL 2-doses (arm1)	10.08	0.30	9.49 – 10.67	
RMTL Unvaccianted (arm0)	7.37	0.18	7.01 – 7.73	
Between-group contrast				p-value
RMST (arm1-arm0)	-2.7		-3.40 – -2.01	<0.0001
RMTL (arm1/arm0)	1.37		1.27 – 1.48	<0.0001

3.4. 3/4-Doses versus Unvaccinated

In the Log-rank test of the univariate analysis the 3/4-doses population show an HR of 1.21 (CI_{95}=1.14 – 1.29; p<0.0001) versus unvaccinated; the hypertensive population show an HR of 9.65 (CI_{95}=9.09 – 10.24; p<0.0001) compared to non-hypertensive population; the diabetic population show an HR of 5.90 (CI_{95}=5.51 – 6.31; p<0.0001) compared to non-diabetic population; the CVD population show an HR of 10.03 (CI_{95}=9.45 – 10.63; p<0,0001) compared to non-CVD population; the population with kidney disease shows a HR of 15.89 (CI_{95}=14.78 – 17.08; p<0.0001) compared to population with no kidney disease; the cancer population show an HR of 7.62 (CI_{95}=7.15 – 8.12; p<0.0001); the infected population show an HR of 0.61 (CI_{95}=0.58 – 0.66; p<0.0001) compared to uninfected population; the HR of the covariate Age is 1.12 (CI_{95}= 1.11 – 1.12; p<0.001); male population show an HR of 0.98 (CI95=0.93 – 1.04; ns) compared to female population; finally the COPD population show an HR of 5.96 (CI_{95}=5.52 – 6.43; p<0.0001) compared to non-COPD population (Table 5).

Even in this case the HRs obtained in the multivariable analysis represent the best fit of the model achieved by stratification of the covariates cancer, infection and age. The Global Schoenfeld's test remained significant whereby the estimate of the HRs could be inaccurate. Also, in this case the estimates of the Restricted Mean Survival Time and the Between-group contrast are added (Table 5). At multivariable analysis, the HR for "All-cause deaths" between 3/4-doses versus Unvaccinated is 0.99 (CI_{95}=0.90 – 1.09; ns). The hypertensive population show an HR of 1.24 (CI_{95}=1.11 – 1.39; p<0.0001) compared to non-hypertensive population; the diabetic population show an HR of 1.68 (CI_{95}=1.48 – 1.90; p<0.0001) compared to non-diabetic population; the CVD population show an HR of 1.86 (CI_{95}=1.65 – 2.09; p<0.0001) compared to non-CVD population; the population with kidney disease show an HR of 2.47 (CI_{95}=2.11 – 2.89; p<0.0001) compared to population with no kidney disease; the male population show an HR of 1.37 (CI_{95}=1.24 – 1.51; p<0.0001) compared to female population; finally, the COPD population show an HR of 1.85 (CI_{95}=1.59 – 2.15; p<00001) compared to non-COPD population (Table 5).

Restricted Mean Survival Time for 3-doses population (τ=579 days) is 573.68 (CI_{95}=573.46 – 573.89) days compared to Unvaccinated population 574.44 (CI_{95}=574.22 – 574.66), while the Between-group contrast is -0.764 days (CI_{95}= -1.07 – -0.46; p<0.0001). Restricted Mean Time Lost ratio is 1.17 (CI_{95}=1.10 – 1.24; p<0.0001) (Table 4).

Table 4. Estimate of Restricted Mean Survival Time and Between-group contrast in 3-doses versus Unvaccinated.

Restricted Mean Survival Time (RMTS) (τ=579 days)			
Groups	Estimate	SE	95% CI
RMST 3-doses (arm1)	573.68	0.11	573.46 – 573.89
RMST Unvaccianted (arm0)	574.44	0.11	574.22 – 574.66
Restricted Mean Time Lost (RMTL)			
RMTL 3-doses (arm1)	5.33	0.11	5.11 – 5.54
RMTL Unvaccianted (arm0)	4.56	0.11	4.34 – 4.78

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	Between-group contrast		p-value
RMST (arm1-arm0)	-0.764	-1.07 – -0.46	<0.0001
RMTL (arm1/arm0)	1.17	1.10 – 1.24	<0.0001

Table 5. All-cause deaths - HR = Hazards ratios; CI = Confidence Interval; ‡ p-value=0,044; * significance with p-value ≤0,001; ** significance with p-value <0,0001. The HRs indicated with "/" are the covariate stratified in order to correct the assumptions of the Proportional Cox Model.

Covariate	1 Dose		2 Doses		3/4 Doses	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
	HR (95% CI)	HR (95% CI)		HR (95% CI)		HR (95% CI)
Groups	0.88 (0.78 – 1.00)‡	2.40 (2.00 – 2.88)**	1.23 (1.16 – 1.32)*	1.98 (1.75 – 2.24)**	1.21 (1.14 – 1.29)*	0.99 (0.90 – 1.09)
Hypertension	12.59 (11.58 – 13.69)*	1.49 (1.23 – 1.82)**	11.47 (10.76 – 12.23)*	/	9.65 (9.09 – 10.24)*	1.24 (1.11 – 1.39)**
Diabetes	8.07 (7.31 – 8.90)*	2.00 (1.60 – 2.49)**	6.71 (6.23 – 7.23)*	1.74 (1.38 – 2.20)**	5.90 (5.51 – 6.31)*	1.68 (1.48 – 1.90)**
CVD	11.56 (10.63 – 12.57)*	1.60 (1.31 – 1.96)**	10.88 (10.21 – 11.60)*	1.78 (1.44 – 2.20)**	10.03 (9.45 – 10.63)*	1.86 (1.65 – 2.09)**
Kidney disease	17.89 (16.08 – 19.90)*	1.77 (1.35 – 2.34)**	16.83 (15.56 – 18.20)*	2.44 (1.84 – 3.24)**	15.89 (14.78 – 17.08)*	2.47 (2.11 – 2.89)**
Cancer	9.34 (8.51 – 10.25)*	/	8.65 (8.07 – 9.27)*	/	7.62 (7.15 – 8.12)*	/
Infection	0.58 (0.53 – 0.63)*	/	0.35 (0.32 – 0.38)*	/	0.61 (0.58 – 0.66)*	/
Age	1.11 (1.11 – 1.11)*	/	1.11 (1.11 – 1.12)*	/	1.12 (1.11 – 1.12)*	/
Sex	0.87 (0.81 – 0.95)*	1.50 (1.27 – 1.78)**	0.95 (0.89 – 1.01)	/	0.98 (0.93 – 1.04)	1.37 (1.24 – 1.51)**
COPD	7.11 (6.40 – 7.91)*	2.01 (1.56 – 2.60)**	6.28 (5.79 – 6.82)*	2.89 (2.18 – 3.84)**	5.96 (5.52 – 6.43)*	1.85 (1.59 – 2.15)**

4. Discussion

The aim of this retrospective cohort study was to correct for the ITB from the dataset kindly provided by Rosso et al [8], which refers to the population of the province of Pescara, and to compare the survival curves for the "all-cause deaths" outcome between the cohorts of people vaccinated with 1, 2 and 3/4 doses and the cohort of unvaccinated people.

We have focalized only on all-cause deaths variable because we observed an unreliability of attributions of deaths to COVID-19: incorrect classification of deaths, a critical factor for the evaluation of vaccine effectiveness (VE) with respect to fatal outcomes, together with disparities in hospitalizations between vaccinated and non-vaccinated people, as well as in the execution of tests [7]. To prevent such biases from significantly altering the results in observational studies, it would be necessary to have information not always available [5].

In the study by Rosso et al. [8] COVID-19 deaths appear to be overestimated: for example, over 30% of COVID-19 deaths occurred more than 90 days after the last positive swab (in some cases even more than a year later), which makes such attributions particularly unreliable.

Also other features indicate that COVID-19 deaths are overestimated: in fact, in the dataset that the Authors provided to us, COVID-19 deaths represent 22.3% of deaths from all-causes (see Table 1), a percentage more than twice that the one available from ISTAT data for the province of Pescara (the last update is for the year 2021, with a total of 10% COVID-19-related deaths; n=398/3978) and 2.5 times higher than the national one, i.e. 9.0%% [9]. This indicates that a portion of the other COVID-19 deaths of doubtful attribution indicated in Table 1 are also to be considered incorrect classification, in addition to the deaths attributed to COVID-19 that occurred more than 90 days after the last positive swab.

Therefore, the choice of disregarding this output proves to be valid, as the consequent choice to ignore the non-COVID deaths.

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The univariate analysis, carried out using the Cox proportional hazards model, shows an increase in the risk of the vaccinated compared to the unvaccinated, as one moves from the first to the subsequent doses. This confirms what has already been highlighted in our previous article [4]. In fact, the HR was slightly lower than 1 with the first dose, while with the second and third doses the risk for the vaccinated resulted significantly higher (more than 20%) than that of the unvaccinated.

However, age, sex and previous pathologies are confounding factors affecting the HR of all-cause deaths of the vaccinated compared to the unvaccinated. Therefore, only a multivariate analysis allows a more reliable estimate of the HRs for the different vaccination status compared to the unvaccinated.

Our starting hypothesis was that the ITB correction would have brought to the null (HR=1) the HRs of the different vaccination statuses compared to the unvaccinated, unlike the estimates by Rosso et al. [8]. On the contrary, the HR of the individuals vaccinated with only 1 dose is almost double compared to that of Rosso et al. (2.40 vs 1.26). Moreover, the HR of the subjects vaccinated with 2 doses is approximately reduced by 20% (1.98 vs 2.41). The HR of those vaccinated with 3 or more doses is not significantly different from 1, showing an absence of effect of the booster vaccination.

There are several possible interpretations of these results. The interpretation of Rosso et al. is incorrect, as they only consider as vaccinated with one and two doses the individuals who have afterwards interrupted their vaccinations, whereas also those receiving subsequent doses must be included in that number. Moreover, they hypothesize that, as a consequence of the green-pass policies, the sickest subjects were concentrated among the vaccinated with 1 and 2 doses. However, the figures in the dataset do not confirm such a hypothesis. Indeed, if anything, the population of those vaccinated with 3 or more doses displays the highest percentage of subjects with pathologies (28% versus 16% of those vaccinated with only 2 doses, and 17% of those with only 1 dose). It should also be considered that in Italy the release of exemptions was very limited, while the subjects with pathologies were vaccinated with a priority indication and induced to continue with vaccination with 1 or 2 booster doses.

Instead, among the possible explanations of the HR trend as vaccinations increase, one must consider the so-called harvesting effect, taking into account also the vaccine adverse effects, including some fatal outcomes. Indeed, if for the individuals vaccinated with three or more doses the HR shows no effect on all-cause deaths, assuming that vaccination against COVID-19 reduces the COVID-19 related deaths, it must be assumed that this reduction is counterbalanced by an increase in deaths from other causes. We must therefore admit that vaccination increases the risk of death from causes other than COVID-19, or by direct damage (adverse effects), or indirect damage, e.g. to the immune system [13–15]. Therefore, may be that the risk of death is greater for one dose than for two, and for two than for three, because individuals more liable to harm are already dead after the first and second doses. So, it will be important to continue the follow-up of the cohort, to capture any long-term damage. To understand the impact of the harvesting effect, deaths in vaccinated with one or two doses represent about half of all deaths occurred among vaccinated (47.2%, the majority of them among vaccinated with two doses).

Another specific explanation could lie in the so called calendar-time bias: it consists in not taking into account either seasonality [16] or pandemic waves. In the present study, such a bias may have affected particularly the results of the third doses, which began in July 2021, in the summer, when the pandemic wave was over. The follow-up of the unvaccinated, as well as that of the first doses, began in January 2021, in winter and during the second pandemic wave, that is when the risk of death from COVID-19 and of all-cause deaths were significantly higher. This leads to underestimate the HR of all-cause deaths for individuals vaccinated with three or more doses.

There is also another common bias in studies of COVID-19 vaccines effectiveness, that can explain the HR trend: the so-called Case-Counting Windows Bias, which consists in considering those vaccinated in the 14 days following vaccination as if they had not yet received the corresponding dose.

As regards the Case-counting Windows Bias, we can cite a study [17] showing, with data taken from the authorization trial of the Pfizer-BioNTech vaccine, that an ineffective vaccine could appear

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effective at 48%, due to the above mentioned 14-day shift. In the first 14 days (from 1 to 15 January 2021) of the Rosso's trial, deaths were counted neither for the vaccinated nor for the unvaccinated individuals. For the entire remaining duration of the study, the deaths of individuals vaccinated in the first 14 days after the dose administration were not counted (as explicitly stated in the materials and methods section of the Rosso's study). However, we cannot exclude that the deaths not counted for individuals vaccinated with 1 dose were attributed to the unvaccinated and, in cascade, the deaths of individuals vaccinated with 2 doses to those vaccinated with 1 dose, and so on.

Note that the Italian Superior Institute of Health, in an answer [18] to a specific question about the 14 days shift, stated:

«The Italian Superior Institute of Health, both in the scientific publications and in its reports published in the last two years, for the purposes of evaluating the effectiveness of anti-COVID-19 vaccines, considers people who are diagnosed in the first 14 days after administration of the first dose, as "not vaccinated" (regardless of whether they have developed a serious disease or if they died).

There are mainly two reasons for this choice: 1. the protection of the vaccine requires approximately two weeks for the immune response to be developed against the virus; 2. the incubation period of the disease, i.e. the time from infection to the development of symptoms, varies from 2 to 14 days; it is emphasized that the diagnosis (in the pharmacy or in authorized laboratories) usually takes a few additional days. Therefore, a good part of the cases diagnosed within 14 days contracted the infection before the first dose was administered. In the case of evaluating adverse events from vaccines (e.g. anaphylactic shock) the choice to consider the date of administration of the vaccination as the moment of onset of exposure is clearly shared. In both cases what has been done is in accordance with scientific knowledge and in line with what is suggested by national and international health bodies» [18].

In light of the ISS declaration reported above, we can assume that in Pescara the health institutions have conformed to the national indications. It would even appear that the authors of the Rosso's dataset have gone further: in fact, despite the follow-up starting 15 days after the vaccine administration, some deaths in the dataset (4 for the first dose, 1 for the second, 8 for the third) occurring within 14 days of the start of follow up (therefore within 28 days of administration of the dose), were assigned to the previous vaccination group.

However, even if the deaths have not been moved (apart from the few above reported) but only cancelled, the described effect would still exist, even if to a lesser extent.

Another bias likely influencing the results is the healthy-adherer bias, or healthy-vaccinee bias in the vaccination field. It is true that priority was to vaccinate the so-called "fragile". However, even before the obligation came into force, were also prioritized categories whose good health is an essential requirement, such as healthcare workers and the police. In addition, the voluntary adhesion of the population not subject to obligations (direct or indirect, through the conditioning of the so-called green pass) can contribute to the aforementioned bias, as highlighted in the literature [19–33].

This bias is much more powerful than commonly thought, and independent of the type of treatment to which one adheres voluntarily, being also found in randomized controlled trials in placebo adherers (compared with placebo non-adherers). It is a bias more difficult to correct than the opposite effect of confounding by indication (subjects in worse health conditions are vaccinated first) [31], because the healthy-adherer bias can be linked also to features not captured by typical pharmaco-epidemiological databases. E.g. subjects more adherent to preventive therapies are often more likely to engage in behaviors consistent with a healthy lifestyle, including diet, exercise, moderation of alcohol intake, avoidance of illegal drugs or risky behaviors, seeking better quality health assistance, and to be more confident in the benefits of a treatment, with a greater placebo effect. These unmeasured characteristics may be associated with mortality outcomes in observational studies. Anyway, the healthy-vaccinee bias has shown huge effects in a national study linking mortality to COVID-19 vaccination status [33]. Indeed, it is plausible that, in observational studies, it also matters that the most fragile people, in terminal stages of their diseases, could choose not to be vaccinated, or that the doctor does not think to vaccinate them.

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It is likely that the healthy-vaccinee bias continued to operate to varying degrees in 2022, throughout the follow-up of the analyzed study [8].

Moreover, the third dose or booster was not foreseen at all either by the authorization trials or by the vaccination protocols. It was introduced following the observation of the loss of the primary cycle vaccine effectiveness (VE), foremost highlighted in Israel, a state that had signed an agreement with Pfizer, to monitor the VE on the general population. Therefore, even before confirmation by studies in other countries, in the summer of 2021 Pfizer requested to the FDA an emergency authorization for the third dose, then extended to other vaccines. This was a clear admission of the vaccines inability to guarantee a stable and long-lasting protection against COVID-19.

Another aspect to consider very carefully are the HRs obtained for the covariates included in the model. The HRs of the covariates in the Cox proportional model represent the comparison between subjects who have a specific comorbidity with subjects who do not have it, for the same vaccination doses. The results obtained from the model indicate that, for any dose, the HRs are significantly higher than the reference value. The unexpected result obtained in this analysis indicates that vaccinated subjects who present at least one comorbidity have a higher risk of death from all causes than subjects vaccinated with the same doses, but without comorbidities. Considering these results, it appears necessary to make appropriate assessments regarding public health and to reevaluate opportunity to reserve vaccination priority for specific categories of fragile subjects.

Finally, the HRs for the persons vaccinated with 2, or 3 or more doses might not be accurate, given that the Schoenfeld tests remained significant despite the stratifications carried out. Therefore, for these two vaccination statuses we calculated also the RMSTs and the RMTL, comparing them with the same indexes for the unvaccinated. The differences in RMSTs between vaccinated and unvaccinated are significant for both the 2-dose and the 3-or-more-dose groups. They may appear irrelevant (of the order of a few days), but they refer to a limited period of time (739 days for those vaccinated with 2 doses and 579 days for those vaccinated with 3 or more doses). They could be compared with the entire life expectancy of an individual, which in the province of Pescara has an average value of 82.6 years [34] (corresponding to 30,149 days).

For those vaccinated with two doses, the loss of life expectancy (RMTL) in 739 days is 1.37 (CI 95 =1.27 – 1.48; $p<0.0001$) times that of the unvaccinated. The difference between the life expectancy (RMST) of the vaccinated and that of the unvaccinated limited to the period considered is -2.71 (CI 95 = -3.40 to -2.01; $p<0.0001$) days. However, to have an easily understandable comparison, if we extrapolated this result to the entire life expectancy of the Pescara population, we would obtain a loss of life expectancy difference of about -3.6 months. Obviously, this is an extrapolation made for the sole purpose of giving the reader an idea of the order of magnitude of the RMT. It may not constitute a realistic prediction, as it would presuppose health conditions invariant over time, an assumption very difficult to realize.

For those vaccinated with 3 or more doses, the RMTL in 579 days is 1.17 times (CI 95 =1.10 – 1.24; $p<0.0001$) the one of the unvaccinated. The difference between the vaccinated and unvaccinated RMST in the considered period is 0.764 days (CI 95 = -1.07 to -0.46; $p<0.0001$). With the above extrapolation, it would correspond to a loss of life expectancy of -1.31 months.

For comparison, between 2019 and 2022 the life expectancy in the province of Pescara fell by 1.0 year, from 83.6 to 82.6 years [34], indeed corresponding to an annual loss of 4.0 months.

5. Conclusions

A previous article about the safety of COVID-19 vaccination in the cohort of the Italian province of Pescara managed incorrectly the of follow-up times, introducing the immortal-time bias (ITB) in favor of the vaccinated group. A study about the same province with a longer follow-up re-proposed the same bias, by the way showing that the group who received at least a booster dose had a significantly lower risk of all-cause death versus unvaccinated.

Comparing the most reliable “all-cause deaths” outcome in the cohorts of people vaccinated with 1, 2 and 3/4 doses with the unvaccinated cohort, we corrected the immortal-time bias aligning the entire population on a unique index date and calculate the time spent as unvaccinated for each of

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the 1, 2 and 3/4 doses populations. Our hypothesis was that correcting the ITB could push the estimates of the HR for all-cause deaths towards the null.

On the contrary, the HR for all-cause deaths in univariate analysis for unvaccinated (reference) versus vaccinated with 1, 2, 3/4 doses were 0.88 (CI95: 0.78–1.00; p-value 0.044), 1.23 (1.16–1.32; p-value ≤0.001) and 1.21 (1.14–1.29; p-value ≤0.001), respectively; the multivariate values were 2.40 (2.00–2.88; p-value <0.0001), 1.98 (1.75–2.24; p-value <0.0001), and 0.99 (0.90–1.09; ns). Some possible explanations of the trend of the Hazard Ratios as vaccinations increase could be: a harvesting effect; a calendar-time bias, accounting for seasonality and pandemic waves; a case-counting windows bias; a healthy-vaccinee bias; or some of their combinations. Anyhow, with two and even with 3/4 doses the calculated Restricted Mean Survival Time and Restricted Mean Time Lost have shown a small but significant downside for the vaccinated populations.

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References

1. Renoux, C.; Azoulay, L.; Suissa S. Biases in Evaluating the Safety and Effectiveness of Drugs for the Treatment of COVID-19: Designing Real-World Evidence Studies. Am J Epidemiol. 2021 Aug 1;190(8):1452-56. doi: 10.1093/aje/kwab028.
2. Mansournia, M.A.; Nazemipour, M.; Etminan, M. Causal diagrams for immortal time bias. Int J Epidemiol. 2021 Nov 10;50(5):1405-09. doi: 10.1093/ije/dyab157.
3. Flacco, M.E.; Acuti Martellucci, C.; Soldato, G.; Di Martino, G.; Carota, R.; De Benedictis M.; Di Marco, G.; Parruti, G.; Di Luzio, R.; Caponetti, A.; Manzoli, L. COVID-19 Vaccination Did Not Increase the Risk of Potentially Related Serious Adverse Events: 18-Month Cohort Study in an Italian Province. Vaccines (Basel). 2022 Dec 23;11(1):31. doi: 10.3390/vaccines11010031.
4. Berrino, F.; Donzelli, A.; Bellavite, P.; Malatesta, G. COVID-19 vaccination and all-cause and non-COVID-19 mortality. A revaluation of a study carried out in an Italian Province. Epidemiol Prev. 2023 Nov-Dec;47(6):374-378. English. doi: 10.19191/EP23.6.A643.075.
5. Ioannidis, J.P.A. Over- and under-estimation of COVID-19 deaths. Eur J Epidemiol 36, 581–588 (2021). https://doi.org/10.1007/s10654-021-00787-9.
6. Fung, K.; Jones, M.; Doshi, P. Sources of bias in observational studies of covid-19 vaccine effectiveness. J Eval Clin Pract. 2023; 1-7. doi:10.1111/jep.13839.
7. Ioannidis, J.P.A. Factors influencing estimated effectiveness of COVID-19 vaccines in non-randomised studies. BMJ Evidence-Based Medicine 2022;27:324-329.
8. Rosso, A.; Flacco, M.E.; Soldato, G.; Di Martino, G.; Acuti Martellucci, C.; Carota, R.; De Benedictis, M.; Di Marco, G.; Di Luzio, R.; Fiore, M.; Caponetti, A.; Manzoli, L. COVID-19 Vaccination Effectiveness in the General Population of an Italian Province: Two Years of Follow-Up. Vaccines (Basel). 2023 Aug 4;11(8):1325. doi: 10.3390/vaccines11081325.
9. https://www.istat.it/it/dati-analisi-e-prodotti/banche-dati/statbase, accessed on 25/05/2024.
10. Polack, F.P.; Thomas, S.J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J.L.; Pérez, G.; Moreira, E.D.; Zerbini, C.; Bailey, R.; et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. N Engl J Med. 2020;383(27):2603–2615. doi: 10.1056/NEJMoa2034577.
11. Rulli, E.; Ghilotti, F.; Biagioli, E.; Porcu, L.; Marabese, M.; D'Incalci, M.; Bellocco, R.; Torri, V. Assessment of proportional hazard assumption in aggregate data: a systematic review on statistical methodology in clinical trials using time-to-event endpoint. Br J Cancer. 2018 Dec;119(12):1456-1463. doi: 10.1038/s41416-018-0302-8.
12. Uno, H.; Claggett, B.; Tian, L.; Fu, H.; Huang, B.; Kim, D.H.; Wei, LJ. Adding a new analytical procedure with clinical interpretation in the tool box of survival analysis. Ann Oncol. 2018 May 1;29(5):1092-1094.
13. Seneff, S.; Nigh, G.; Kyriakopoulos, A.M.; McCullough, P.A. Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs. Food Chem Toxicol. 2022 Jun;164:113008. doi: 10.1016/j.fct.2022.113008.

 doi:10.20944/preprints202406.0384.v1

14. Uversky, V.N.; Redwan, E.M.; Makis, W.; Rubio-Casillas, A. IgG4 Antibodies Induced by Repeated Vaccination May Generate Immune Tolerance to the SARS-CoV-2 Spike Protein. Vaccines (Basel). 2023 May 17;11(5):991. doi: 10.3390/vaccines11050991.
15. Pillai, S. Is it bad, is it good, or is IgG4 just misunderstood? Sci Immunol. 2023 Mar 31;8(81):eadg7327. doi: 10.1126/sciimmunol.adg7327.
16. Quinn, G.A.; Connolly, M.; Fenton, N.E.; Hatfill, S.J.; Hynds, P.; ÓhAiseadha, C.; Sikora, K.; Soon, W.; Connolly, R. Influence of Seasonality and Public-Health Interventions on the COVID-19 Pandemic in Northern Europe. J Clin Med. 2024 Jan 6;13(2):334. doi: 10.3390/jcm13020334.
17. Fung, K.; Jones, M.; Doshi, P. Sources of bias in observational studies of COVID-19 vaccine effectiveness. J Eval Clin Pract. 2024;30:30-36.
18. https://www.iss.it/documents/20126/0/Risposta+_Eccesso+Mortalita%CC%80+Umanita%CC%80+e+Ragione_ultimo_rev31_07.pdf/34ae7793-32f1-7d7d-57e3-4f0b16833cd1?t=1696589930009
19. Coronary Drug Project Research Group. Influence of adherence to treatment and response of cholesterol on mortality in the Coronary Drug Project. N. Engl. J. Med. 1980, 303, 1038-1041.
20. Horwitz, R.I.; Viscoli, C.M.; Berkman, L.; Donaldson, R.M.; Horwitz, S.M.; Murray, C.J.; Ransohoff, D.F.; Sindelar, J. Treatment adherence and risk of death after a myocardial infarction. The Lancet 1990, 336, 542-545.
21. Simpson, S.H.; Eurich, D.T.; Majumdar, S.R.; Padwal, R.S.; Tsuyuki, R.T.; Varney, J.; Johnson J.A. A meta-analysis of the association between adherence to drug therapy and mortality. BMJ 2006, 333, 15.
22. Granger, B.B.; Swedberg, K.; Ekman, I.; Granger, C.B.; Olofsson, B.; McMurray, J.J.; Yusuf, S.; Michelson, E.L.; Pfeffer, M.A.; CHARM investigators. Adherence to candesartan and placebo and outcomes in chronic heart failure in the CHARM programme: double-blind, randomised, controlled clinical trial. The Lancet 2005, 366, 2005-2011.
23. Jackson, L.A.; Jackson, M.L.; Nelson, J.C.; Neuzil, K.M.; Weiss, N.S. Evidence of bias in estimates of influenza vaccine effectiveness in seniors. Int. J. Epidemiol. 2006, 35, 337-344.
24. Pinsky, P.F.; Miller, A.; Kramer, B.S.; Church, T.; Reding, D.; Prorok, P.; Gelmann, E.; Schoen, R.E.; Buys, S.; Hayes, R.B.; Berg, C.D. Evidence of a healthy volunteer effect in the prostate, lung, colorectal, and ovarian cancer screening trial. Am. J. Epidemiol. 2007, 165, 874-881.
25. Brookhart, M.A.; Patrick, A.R.; Dormuth, C. Adherence to lipid-lowering therapy and the use of preventive health services: an investigation of the healthy user effect. Am. J. Epidemiol. 2007, 166, 348-354.
26. Dormuth, C.R.; Patrick, A.R.; Shrank, W.H. Statin adherence and risk of accidents: a cautionary tale. Circulation 2009, 119, 2051-2057.
27. Vestbo, J.; Anderson, J.A.; Calverley, P.M.; Celli, B.; Ferguson, G.T.; Jenkins, C.; Knobil, K.; Willits, L.R.; Yates, J.C.; Jones, P.W. Adherence to inhaled therapy, mortality and hospital admission in COPD. Thorax 2009, 64, 939-943.
28. Ladova, K.; Vlcek, J.; Vytrisalova, M.; Maly, J. Healthy adherer effect - the pitfall in the interpretation of the effect of medication adherence on health outcomes. J. Eval. Clin. Pract. 2014, 20, 111-116.
29. Tielemans, S.M.A.J.; de Melker, H.E.; Hahné, S.J.M.; Boef, A.G.C.; van der Klis, F.R.M.; Sanders, E.A.M.; van der Sande, M.A.B.; Knol, M.J. Non-specific effects of measles, mumps, and rubella (MMR) vaccination in high income setting: population based cohort study in the Netherlands. BMJ 2017, 358, j3862.
30. Howard, J.; Huang, A.; Li, Z.; Tufekci, Z.; Zdimal, V; van der Westhuizen, H.M.; von Delft, A.; Price, A.; Fridman, L.; Tang, L.H.; Tang, V.; Watson, G.L.; Bax, C.E.; Shaikh, R.; Questier, F.; Hernandez, D.; Chu, L.F.; Ramirez, C.M.; Rimoin, A.W. An evidence review of face masks against COVID-19. Proc. Natl. Acad. Sci. U.S.A. 2021, 118, e2014564118.
31. Remschmidt, C.; Wichmann, O.; Harder, T. Frequency and impact of confounding by indication and healthy vaccinee bias in observational studies assessing influenza vaccine effectiveness: A systematic review. BMC Infect. Dis. 2015, 15, 429.
32. Furst, T.; Straka, R.; Janosek, J. Healthy vaccinee effect: a bias not to be forgotten in observational studies on COVID-19 vaccine effectiveness. Pol Arch Intern Med. 2024 Feb 28;134(2):16634. doi: 10.20452/pamw.16634.
33. Fürst T, Bazalová A, Fryčák T, Janošek J. Does the healthy vaccinee bias rule them all? Association of COVID-19 vaccination status and all-cause mortality from an analysis of data from 2.2 million individual health records. Int J Infect Dis. 2024 May;142:106976. doi: 10.1016/j.ijid.2024.02.019.
34. https://www.istat.it/it/files//2023/10/BesT-Abruzzo-2023-VSP.pdf