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Assessing the temporal and cause-effect relationship between myocarditis and mRNA COVID-19 vaccines. A retrospective observational study

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A B S T R A C T

Objective: In 2021, the US Centers for Disease Control and Prevention reported increased cases of myocarditis and pericarditis in the United States after mRNA COVID-19 vaccines. Our study aims to estimate the incidence of myocarditis in Apulia (Southern Italy) and the cause-effect relationship between COVID-19 mRNA vaccines and the risk of myocarditis.
Methods: The Apulian regional archive of hospital discharge forms was used to define the cases of myocarditis in Apulia, considering data from 2017 to 2022. The overall vaccination status of patients was assessed via data collected from the Regional Immunization Database. The history of SARS-CoV-2 infection was extracted from the Italian Institute of Health platform.
Results: Since 2017, 5687 cases of myocarditis have been recorded in Apulian subjects; the overall incidence described a decreasing trend, with a slight increase in 0-40 years-old subjects. From 2021 to 2022, 2,930,276 doses of COVID-19 mRNA vaccines were administered; a diagnosis of myocarditis after the second dose of the mRNA vaccine was reported for 894 (0.03%) of Apulian inhabitants, with an incidence rate of 17.9 $\times$ 1,000,000 persons-month. The multivariate analysis, adjusted for age, sex, underlying medical conditions, and diagnosis of COVID-19, showed that mRNA vaccination is a protective factor for myocarditis even in younger subjects (aOR = 0.4; 95% CI = 0.3-0.5).
Conclusion: A temporal association between an exposure and an outcome is not equivalent to a causal association. Our study underlines how an approach that considers the other potential causes of myocarditis (primarily COVID-19) and a causality assessment must be prioritized in the study of the topic.

Introduction

Myocarditis is an inflammation of the heart muscle, and if it is accompanied by pericarditis, it is referred to as myopericarditis; in both cases, the body's immune system is causing inflammation in response to an infection or some other trigger. According to the US Centers for Disease Control and Prevention (CDC), the term "myocarditis" refers to myocarditis, pericarditis, or myopericarditis [1]. Myocarditis typically occurs more commonly in males than females, and the incidence is highest among infants, adolescents, and young adults [2–4]. The clinical presentation and severity of myocarditis vary among patients. Symptoms typically include chest pain, dyspnea, or palpitations, although other symptoms might be present, especially in younger children. Supportive therapy is a mainstay of treatment, with targeted cardiac medications or interventions as needed [4].

Historically, among other pharmaceutical drugs, smallpox and anthrax vaccines have been associated with the risk of acute myocarditis; a 2022 review evidenced that among 790 cases reported in the World Health Organization (WHO) pharmacovigilance database between 1967 and 2020, vaccine-associated myocarditis primarily affected young male adults (median age 24 years) [5]. In 2021, increased cases of myocarditis and pericardi-

Abbreviations: CDC, Centers for Disease Control and Prevention; SDO, hospital discharge forms; WHO, World Health Organization.

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tis were reported in the United States after mRNA COVID-19 vaccination (mRNA-1273 and BNT162b2 vaccines) [1]. Using the Vaccine Adverse Event Reporting System (VAERS), a long-standing national passive surveillance system, myocarditis reporting rates with onset within 7 days after dose 2 of an mRNA vaccine were 40.6 cases per 1,000,000 second doses of mRNA COVID-19 vaccines administered to males aged 12-29 years and 2.4 per 1,000,000 second doses administered to males aged ≥30 years; reporting rates among females in these age groups were 4.2 and 1.0 per 1,000,000 second doses, respectively. The highest reporting rates were among males aged 12-17 years and those aged 18-24 years (62.8 and 50.5 reported myocarditis cases per 1,000,000 second doses of mRNA COVID-19 vaccine administered, respectively) [4].

Oster et al. [6] performed a descriptive study monitoring the US VAERS; between December 14, 2020 and August 31, 2021, 192,405,448 individuals older than 12 years of age received a total of 354,100,845 COVID-19 mRNA vaccines. VAERS received 1991 reports of myocarditis; 1626 met the CDC's case definition for probable or confirmed myocarditis [1]. The rates of myocarditis cases were highest after the second vaccination dose in adolescent males aged 12-15 years (70.7 per 1,000,000 doses of the BNT162b2 vaccine), in adolescent males aged 16-17 years (105.9 per 1,000,000 doses of the BNT162b2 vaccine), and in young men aged 18-24 years (52.4 and 56.3 per 1,000,000 doses of the BNT162b2 vaccine and the mRNA-1273 vaccine, respectively). A further US study [7] retrospectively investigated patients within the US Military Health System who experienced myocarditis after COVID-19 vaccination between January and April 2021; 23 male patients (median age: 25 years) presented with acute onset of marked chest pain within 4 days after receipt of an mRNA COVID-19 vaccine. All military members were previously healthy with a high level of fitness. A 2021 study [8] reported seven cases of acute myocarditis in healthy male adolescents who presented with chest pain, all within 4 days after the second dose of Pfizer-BioNTech COVID-19 vaccination. Straus et al. [9] assessed the potential risk by examining the Moderna global safety database for reports of myocarditis/myopericarditis among recipients of the mRNA-1273 vaccine worldwide (December 2020-February 2022). The database revealed 3017 cases of myocarditis/myopericarditis among 252 million individuals who received at least one dose of mRNA-1273. The observed rates were most elevated among males under 40 years of age, particularly in the 18-24 age group (53.76 per 100,000 person-years). The overall reporting rate was 9.23 per 100,000 person-years. None of the above-reported studies performed a causality assessment conducted in compliance with WHO recommendations [10]; therefore, a cause-effect relationship between COVID-19 mRNA vaccines and myocarditis has never been demonstrated. It is not coincidental that the potential mechanisms of COVID-19 mRNA vaccination-related myocarditis have yet to be definitively demonstrated. Potential explanations include underlying mRNA immune reactivity, the possibility of antibodies against SARS-CoV-2 spike glycoproteins cross-reacting with myocardial proteins, and the potential occurrence of a multisystem inflammatory syndrome. Furthermore, mRNA vaccines induce a more robust and prolonged immune response compared to non-mRNA vaccines, potentially serving as a catalyst in individuals with a heightened immune reactivity. The prevalence of young males in these cases suggests hormonal factors, with testosterone possibly contributing to a more aggressive immune response, while estrogen may exert inhibitory effects on pro-inflammatory T cells [11].

In this context, this study aims to estimate the incidence of myocarditis in Apulia (Southern Italy, ∼4,000,000 inhabitants) and the risk-incidence of myocarditis following mRNA COVID-19 vaccines; moreover, a cause-effect relationship between COVID-19 mRNA vaccines and the risk of myocarditis has been evaluated.

Methods

This is a retrospective observational study. The study population was identified via the Apulian regional archive of hospital discharge forms (SDO), an online database containing all information on hospital and inpatient procedures in the whole region [12]. We considered all records referring to ICD9 codes of myocarditis [391.2, 398.0, 422.91, 429.0] and of pericarditis [391.0, 393, 420.90, 324.1, 423.2, and 423.9] (according to Massari et al. [13]), extending our search to all diagnosis performed from 2017 to 2022; the choice of this time-span is due to evaluate the incidence rate of myocarditis in Apulian inhabitants, comparing 3 years before COVID-19 pandemic with 3 years during the pandemic. Only subjects living in Apulia were considered. All records were independently reviewed by two epidemiologists specialized in health informative flows to mitigate the risk of misclassification and erroneous diagnosis. Following this examination, the reviewers reached a consensus that none of the records exhibited a high risk of misclassification. Therefore, all records were considered for the final data analysis.

The COVID-19 vaccination status was assessed using the Regional Immunization Database (GIAVA) [12] and/or the Italian COVID-19 Immunization Database. Data relevant to the COVID-19 cases recorded (March 2020-December 2022) were extracted from the Italian Institute of Health (ISS) platform "Integrated surveillance of COVID-19 cases in Italy." This platform, processed by the ISS, integrates the microbiological and epidemiological data provided by all Italian regions, Autonomous Provinces, and the ISS SARS-CoV-2 national reference laboratory. All COVID-19 cases diagnosed by the regional and national reference laboratories fall within the scope of the surveillance; COVID-19-related hospitalizations and deaths are also reported [14].

These data sources were extracted and matched using the Patients' unique Identification Numbers (PINs). We chose not to include foreign subjects with a temporary unique identification number in our study, considering that after hospitalization, they could have returned to their countries or settled in Italy, obtaining a non-temporary PIN, but we cannot trace it. Our investigation ended in December 2022.

The final dataset was created as an Excel spreadsheet that included sex, age at hospitalization, date of hospitalization, length of hospitalization, discharge mode, diagnosis of COVID-19, vaccine prophylaxis (YES/NO), date of vaccination, and the type of vaccine. An anonymized data analysis was performed using the STATA MP17 software.

Continuous variables are reported as the mean ± standard deviation (SD) and range or median and interquartile (IQR) range, and categorical variables as proportions. The incidence rates (IRs) (×100,000 inhabitants) of myocarditis per year and age class were estimated; the ISTAT archives were used to estimate the population as the denominator.

The IRs (×1,000,000 immunized with the second dose of mRNA vaccine persons-month) were calculated to estimate the overall risk of myocarditis after the second dose of mRNA vaccines, the risk of myocarditis after 7 days of the second mRNA vaccines dose (according to CDC definition [1,4]), and the risk of myocarditis after 21 days the second mRNA vaccines' dose (according to the definition of several authors in the literature [13,15]) in Apulian population. The incidence rate ratio (IRR) was estimated to compare the IRs between mRNA vaccines type (mRNA-1273 vs. BNT162b2) and sex (male vs. female), indicating 95% confidence intervals (95% CIs).

Subsequently, a case-control study model was set up; the cases of myocarditis recorded since January 1, 2021 (case group) were matched with Apulian residents without a diagnosis of myocarditis (control group), with an allocation ratio of 1:4, to empower the statistical analysis. To establish the control group population, iden-

Table 1
Characteristic of myocarditis, years 2017-2021.

Variables	2017	2018	2019	2020	2021	2022
n	1,013	1,053	1,076	712	907	926
Males; n (%)	539 (53.2)	565 (53.7)	594 (55.2)	368 (51.7)	474 (52.3)	490 (52.9)
Age at hospitalization (yrs); mean ± SD (range)	62.8 ± 20.4 (0-99)	62.8 ± 20.2 (0-98)	64.1 ± 20.4 (0-98)	64.6 ± 19.0 (0-99)	62.7 ± 20.6 (1-96)	60.8 ±2.2 (0-97)
Age class; n (%)						
• 0-17	33 (3.3)	36 (3.4)	26 (2.4)	15 (2.1)	34 (3.8)	52 (5.6)
• 18-39	119 (11.8)	105 (10.0)	122 (11.3)	64 (9.0)	99 (10.9)	122 (13.2)
• 40-64	287 (28.3)	337 (32.0)	301 (28.0)	208 (29.2)	274 (30.2)	259 (28.0)
• 65+	574 (56.7)	575 (54.6)	627 (58.3)	425 (59.7)	500 (55.1)	493 (53.2)
Intrahospital death; n (%)	40 (4.0)	43 (4.1)	55 (5.1)	36 (5.1)	43 (4.7)	48 (5.2)
Death for age class; n (%)						
• 0-17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
• 18-39	1 (2.5)	0 (0.0)	2 (3.6)	0 (0.0)	1 (2.3)	2 (4.2)
• 40-64	9 (22.5)	6 (14.0)	7 (12.7)	3 (8.3)	8 (18.6)	9 (18.8)
• 65+	30 (75.0)	37 (86.0)	48 (83.6)	33 (91.7)	34 (79.1)	36 (75.0)
Length of hospitalization; median (range IQR)	8 (5-13)	8 (4-12)	7 (4-13)	6 (5-9)	7 (4-12)	7 (4-13)

tical data records and temporal spans (2017-2022) as those of the case group population were utilized. Furthermore, only subjects residing in Apulia were included, and foreign individuals with a temporary unique identification number were excluded from control group based on the considerations mentioned earlier. The assignment to the groups has been performed by randomization, with the homogeneity of the two groups for the covariates age at the second dose of COVID-19 mRNA vaccine or, if not immunized, age at the start of the vaccination campaign, sex, previous diagnosis of COVID-19 (for case group, it was evaluated the COVID-19 status before the diagnosis of myocarditis); the choice of these determinants was not causal, considering that male sex and younger age are known risk factors for myocarditis [4], and the scientific literature showed that the risk of myocarditis is high in persons infected with the SARS-CoV-2 [16]. Randomization has been performed using the STATA MP17 software.

To refine our sample characterization, we cross-referenced information on chronic diseases using the Edotto platform, identifying user-fee exemption codes [17]. Additionally, these data were integrated with information from the Italian Institute of Health platform "Integrated surveillance of COVID-19 cases in Italy" and the archive of hospital discharge forms. Eleven comorbidities were deciphered, encompassing chronic lung diseases, cardiopathies, diabetes mellitus, and other metabolic diseases, chronic renal failure/adrenal insufficiency, hematopathies and hemoglobinopathies, tumors, HIV and immunodepression, chronic inflammatory diseases and bowel malabsorption syndromes, chronic liver diseases, multiple pathologies, and dementia.

The chi-square test was used to compare the proportions between groups. The skewness and kurtosis test was conducted to evaluate the normality of the continuous variables; any variable was normally distributed, and it was impossible to set a normalization model; therefore, the Wilcoxon rank sum test was performed to compare continuous variables between groups.

To analyze the determinants of myocarditis, a multivariate logistic regression model was built; the administration of the second dose of COVID-19 mRNA vaccine was considered as the main determinant, adjusted for sex (male vs. female), age at the vaccination (years), and previous COVID-19 diagnosis (as defined per subgroup population). This model was repeated considering only subjects aged <40 years and per mRNA vaccine type (BNT162b2 mRNA vaccine or mRNA-1273 vaccine). The adjusted odds ratios (aORs)

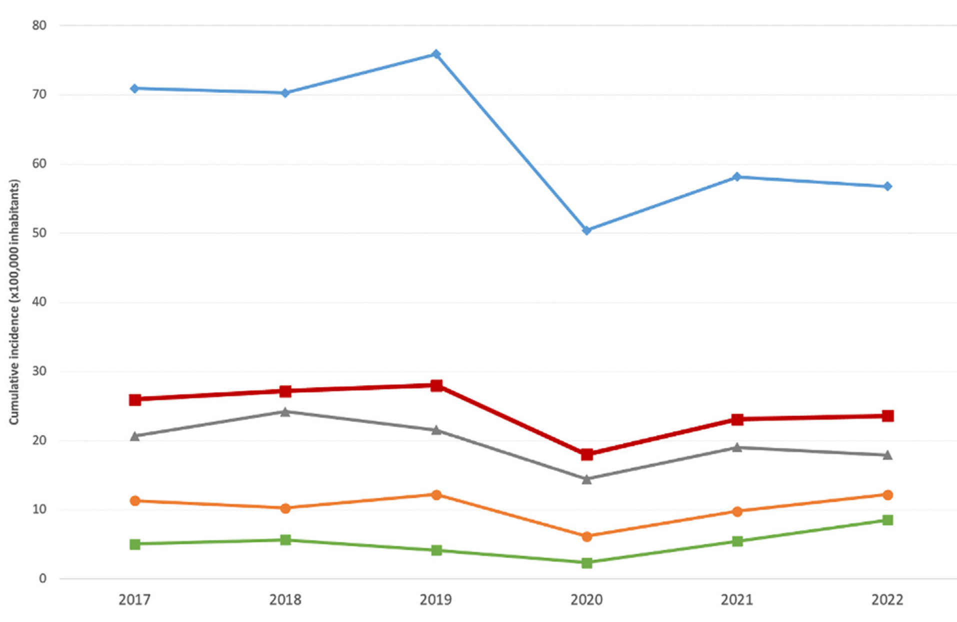

Figure 1. Cumulative incidence of myocarditis (×100,000 inhabitants) per age class and year.

Table 2
Number of myocarditis in the Apulian vaccinated population and incidence rates per vaccine type, age class, and days of onset.

Age class	mRNA vaccine's type	Onset until 7 days after immunization			Onset until 21 days after immunization			Total		
		n	IR	IRR	n	IR	IRR	n	IR	IRR
5-17	BNT162b2	0	0.0	–	2	0.50 (0.12-1.99)	0.0 (0.0-57.8)	29	8.2 (5.2-10.4)	0.8 (0.1-3.0)
	mRNA-1273	0	0.0		0	0.0		2	5.4 (1.2-21.6)	
	Total	0	0.0		2	0.45 (0.01-0.18)		31	7.0 (4.9-10.1)	
18-39	BNT162b2	4	0.4 (0.1-1.0)	3.0 (0.4-17.7)	4	0.38 (0.14-1.01)	4.0 (0.7-21.4)	95	8.8 (7.3-11.0)	0.7 (0.4-1.2)
	mRNA-1273	3	1.1 (0.4-3.5)		4	1.51 (0.57-4.01)		16	6.1 (3.6-9.7)	
	Total	7	0.53 (0.25-1.11)		8	0.60 (0.30-1.21)		111	8.5 (7.0-10.1)	
40-64	BNT162b2	2	0.12 (0.03-0.48)	0.0 (0.0-25.3)	3	0.18 (0.06-0.56)	0.0 (0.0-11.5)	215	13.1 (11.2-14.0)	0.8 (0.5-1.1)
	mRNA-1273	0	–		0	–		36	10.3 (7.3-14.3)	
	Total	2	0.10 (0.02-0.40)		3	0.15 (0.05-0.46)		251	12.4 (10.9-14.9)	
65+	BNT162b2	2	0.19 (0.05-0.76)	0.0 (0.0-32.5)	11	1.05 (0.58-1.89)	1.7 (0.3-6.3)	447	42.6 (38.6-46.5)	0.7 (0.5-1.0)
	mRNA-1273	0	–		3	1.73 (0.56-5.38)		54	31.3 (24.0-40.7)	
	Total	2	0.16 (0.04-0.65)		14	1.14 (0.68-1.93)		501	41.0 (37.4-44.7)	
Total	BNT162b2	8	0.19 (0.10-0.38)	1.9 (0.3-7.9)	20	0.48 (0.31-0.65)	1.8 (0.6-4.4)	786	18.7 (17.5-20.2)	0.7 (0.6-0.9)
	mRNA-1273	3	0.36 (0.12-1.13)		7	0.85 (0.40-1.78)		108	13.1 (10.9-15.8)	
	Total	11	0.22 (0.12-0.40)		27	0.54 (0.37-0.79)		894	17.9 (16.8-19.1)	

IR: incidence rate x1,000,000 immunized with a second dose of mRNA vaccine persons-month; IRR: incidence rate ratio (mRNA-1273 vs. BNT162b2).

were calculated, as well as 95% CIs. The Hosmer-Lemeshow's chi-squared test was used to evaluate the goodness-of-fit of multivariate logistic regression models. To check for multicollinearity in the independent variables, the variance inflation factor (VIF) technique was used.

A two-sided *P*-value < 0.05 was considered an indicator of statistical significance for all tests.

The study was conducted according to the principles of the Helsinki Declaration; as this study constituted public health surveillance, ethical approval from the institutional review board was not required.

Results

Since 2017, 5,687 cases of myocarditis have been recorded in subjects living in Apulia; the characteristics of these subjects per year in analysis are reported in Table 1

The incidence (×100,000 inhabitants) of myocarditis per age class and year is described in Figure 1; the overall incidence describes a decreasing trend, as well as the one in older subjects, while a slight increase is observed in 0-40 years-old subjects.

From January 2021 to December 2022, 2,930,276 doses of COVID-19 mRNA vaccines were administered to Apulian inhabitants, of which 2,429,084 (82.9%) of the BNT162b2 mRNA vaccine and 501,192 (17.1%) of the mRNA-1273 vaccine. A diagnosis of myocarditis after the second dose of mRNA vaccine was reported for 894 (0.03%) subjects, with an incidence rate of 17.9 × 1,000,000 immunized with a second dose of mRNA vaccine persons-month (95% CI = 16.8-19.1). The incidence rate was higher for vaccinated with the BNT162b2 mRNA vaccine (18.7; 95% CI = 17.5-20.2 × 1,000,000 persons-month) than with the mRNA-1273 vaccine (13.1; 95% CI = 10.9-15.8 × 1,000,000 persons-month), with an IRR of 0.7 (95% CI = 0.56-0.85). The number of myocarditis and the IRs per mRNA vaccine type, age class, and days of onset are reported in Table 2. Overall, males seem more prone to develop myocarditis, especially considering younger ages (Table S1).

The characteristics of subjects diagnosed with myocarditis 7 or 21 days after the second dose of COVID-19 mRNA vaccines are reported in Table 3.

The characteristics of subjects included in the case-control study are reported in Table S2.

The analyses of the determinants of myocarditis are described in Table 4. A statistically significant aOR < 1 was observed when

Table 3
Characteristics of Apulian inhabitants diagnosed with myocarditis 7 or 21 days after the second dose of COVID-19 mRNA vaccines.

Variable	Onset until 7 days after immunization ($n = 11$)	Onset until 21 days after immunization ($n = 27$)
Males; n (%)	9 (81.8)	16 (59.3)
Age; mean	38.8 ± 21.4 (18-74)	54.9 ± 26.8 (12-92)
Age class; n (%)		
• 0-17	0 (0.0)	2 (7.4)
• 18-39	7 (63.6)	8 (29.6)
• 40-64	2 (18.2)	3 (11.1)
• 65+	2 (18.2)	14 (51.9)
Intra-hospital death; n (%)	0 (0.0)	1 (3.7)
Booster dose after myocarditis; n (%)	11 (100.0)	26 (100.0)
Days of recovery; median (range IQR)	4 (3-8)	6 (4-8)

Table 4
Analysis of the determinants of myocarditis in a multivariate logistic regression model.

Determinant	Total		Age <40 years-old	
	aOR (95%CI)	*P*-value	aOR (95%CI)	*P*-value
Sex (male vs. female)	1.03 (0.92-1.16)	0.570	1.12 (0.83-1.53)	0.459
Age (years)	1.00 (0.99-1.01)	0.656	1.01 (0.99-1.02)	0.228
Comorbidities				
• 1 vs. none	1.28 (1.03-1.59)	0.026	1.09 (0.69-0.74)	0.707
• 2 vs. none	1.37 (1.09-1.72)	0.007	1.15 (0.31-4.30)	0.833
• ≥3 vs. none	1.58 (1.19-2.09)	0.001	1	-
Diagnosis of COVID-19	0.98 (0.84-1.15)	0.838	0.95 (0.69-1.30)	0.731
Second dose of mRNA vaccine	0.28 (0.24-0.32)	<0.0001	0.36 (0.27-0.48)	<0.0001

Hosmer-Lemeshow *P*-value = 0.813; Hosmer-Lemeshow *P*-value = 0.666.

considering the mRNA vaccine type (Tables S3 and S4). No evidence of multicollinearity was found among the independent variables (Table S5).

Conclusions

Our study highlighted several considerations; the incidence of myocarditis in the Apulian population showed a slight decrease, and any values significantly higher than expected were observed in any age class. Indeed, only 0-17 years-old people showed an increase in incidence, with the value reached in 2022 higher than the previous years; for the other age classes, the higher values were recorded in 2019, before the COVID-19 pandemic. However, the assessment of myocarditis incidence over the years under analysis is significantly influenced by the COVID-19 pandemic. Initially, the pandemic led to a reduction in hospital admissions and outpatient services. Subsequently, with the initiation of the vaccination campaign, there was heightened attention to the issue of myocarditis, driven by media attention and scientific studies on the subject. Therefore, when comparing the years during the pandemic with those pre-COVID, it is essential to take the aforementioned factors into consideration.

The overall risk of myocarditis after the mRNA vaccine is very low (18 × 1,000,000 persons-year), in accordance with the absolute risk of 10-50 per 1,000,000 vaccinated individuals reported in the literature [11], making it a rare event; the highest value was observed in 65+ years-old (41.0 × 1,000,000 persons-year) and the lowest in 5-17 years-old subjects (7.0 × 1,000,000 persons-year). The low incidence rate was also reported by a 2022 study [18] that focused on 35 million individuals in four European countries; the authors concluded that mRNA-based COVID-19 vaccines are associated with increased myocarditis risk in younger individuals, although absolute incidence remains low. Moreover, our study showed that, compared to the BNT162b2 vaccine, the mRNA-1273 vaccine seems less temporally associated with myocarditis considering the overall population (IRR = 0.7; 95% CI = 0.6-0.9). Any case of myocarditis was recorded in 5-17 years-old in the 7 days after immunization, with only two cases in the 21 days after immunization; the higher IRs of myocarditis diagnosticated until 7 days the immunization were recorded in 18-39 years-old (IR = 0.53 × 1,000,000 persons-year), as well as myocarditis diagnosticated until 21 days the immunization (IR = 0.60 × 1,000,000 persons-year), without differences per mRNA vaccine's type (IRR = 3.0; 95% CI = 0.4-17.7 and IRR = 4.0; 95% CI = 0.7-21.4, respectively); the higher risk of myocarditis following immunization in subjects younger than 40 years old was evidenced in a 2022 self-controlled Italian case series study [13].

Regarding sex, a higher overall risk is recorded in males (IRR = 1.3; 95% CI = 1.1-1.4), with the younger males that showed a higher IRR value (2.3 in 5-17 years-old and 3.1 in 18-39 years-old), compared to older persons. Considering the onset until 7 and 21 days, a higher risk is evidenced in younger males, while for older ages, the risk between males and females seems to be similar. This evidence is confirmed by other studies in the literature [4,13].

The fundamental concept in commenting on this type of evidence is that a temporal association between an exposure and an outcome is not equivalent to a causal association. The risk of myocarditis after vaccination, as described in our study (and in all the studies on the subject in the literature), refers to the temporal occurrence after immunization. As already reported by other authors, establishing a causal link between adverse events following anti-COVID-19 vaccination is not easy, particularly in the case of relatively frequent conditions such as cardiovascular events [19]. Moreover, most of the study models (case series or postmarketing passive surveillance) of the above-cited studies do not allow to evaluate a cause-effect relationship between the vaccination and the diagnosis of myocarditis, considering that any of them performed a causality assessment in compliance with the WHO recommendations [10]. Moreover, none of the above-reported studies considered a prior diagnosis of COVID-19 as a risk factor for myocarditis; the literature clearly showed that the incidences of cardiac complications after SARS-CoV-2 infection of almost sevenfold higher than after mRNA COVID-19 vaccination [16,20], and therefore it appears to be a strong confounding when evaluating the role of vaccination in the insurgence of acute myocarditis.

For all these reasons, we estimated the statistical cause-effect relationship between administering the second dose of mRNA COVID-19 vaccines and diagnosing myocarditis through a case-control study. Our multivariate regression models, adjusted for age, sex, underlying medical conditions, and diagnosis of COVID-19, estimated a statistically significant inverse association between mRNA COVID-19 vaccines and diagnosis of myocarditis; in other words, mRNA COVID-19 vaccines seem to be a protective factor for myocarditis, even considering younger subjects and vaccines' type. To our knowledge, this is the first study that evaluates the role of COVID-19 vaccines by an adjusted multivariate model. Nevertheless, this statistical evidence needs confirmation from dedicated studies that assess the causal relationship, particularly through clinical trials. Moreover, studies highlighting the biological mechanism of perimyocarditis and the vaccine's role, whether in terms of harm or protection, are crucial. Without further scientific evidences and the discovery of the biological mechanism, our evidence is more of a hypothesis than a certain assertion. In any case, the findings of our study may be a starting point to shed a different light on the vaccine's role in myocarditis, although further investigations are essential to delineate its precise role.

An intriguing result, warranting further investigation, is the evidence suggesting that individuals with more chronic conditions appear to be at a higher risk of developing myocarditis, although this does not seem to be confirmed in younger subjects. Some literature evidence indicates a heightened susceptibility to cardiovascular diseases in individuals with chronic conditions [21–23], but dedicated studies need to be conducted to examine this phenomenon.

The strengths of our study are the long study period (6 years), the large population we addressed, and the comparison with the pre- and postpandemic time. Moreover, we estimated the IRs, IRRs, and aORs stratified per age class and mRNA vaccine type. A further strong point is the case-control analysis, which allowed us to estimate the hypothetical cause-effect relationship between vaccine and myocarditis; to our knowledge, no other studies in the literature used this methodology. However, this methodology has several limitations, including potential biases such as selection and classification biases. Despite employing rigorous methods to mitigate these biases, it cannot be ruled out that our study is entirely unaffected. However, our multivariate regression models were unable to evaluate the association between our outcomes and community care determinants, medications, and lifestyle factors (e.g., smoking, drugs, and alcohol abuse) due to a lack of available data. Another significant limitation is that some of our data sources (i.e., the Edotto platform) are built for administrative and nonepidemiological purposes. It is essential to acknowledge that hospital discharge forms are primarily designed for administrative purposes, intending to secure economic reimbursement for the provided medical services. For this reason, healthcare personnel are motivated to meticulously and promptly compile these records, and such documentation has been validated by both the Apulia Region and the Italian Ministry of Health. Moreover, all records have been double checked and validated by two epidemiologists specialized in health informative flows. Therefore, it can be considered a trustworthy source of data. Finally, the study's methodology, limited to inpatient records, poses a potential oversight by not encompassing subclinical or outpatient-managed instances of myocarditis. It must be considered that our study focused on an Italian region with a population of 4,000,000 inhabitants, while other studies on the topic review the data of entire countries [1,4–6,9,18]. In this light, it should not be surprising to find a small number of absolute cases among the younger age classes. Milder cases, especially in younger individuals, may go unnoticed within the scope of hospital-based data collection. However, the media hype surrounding (often unfounded) vaccine risks has effectively heightened awareness of potential adverse effects [24]. Therefore, it is plausible that in the postvaccination campaign period, which is particularly relevant to the study's objectives, even mild symptoms might have prompted individuals to seek access to hospital services. However, our results must be interpreted in terms of their relative frequencies (i.e., IRs), allowing for a comparison with the results of studies that investigated a larger sample. Future studies should perform ad hoc analysis to evaluate the cause-effect relationship between vaccines and myocarditis, especially considering the episodes temporally close to vaccination. The pharmacovigilance studies should not only describe the temporal association but also perform a causality assessment in compliance with the World Health Organization [10] to define the cause-effect relationship.

The topic under study is still controversial and needs further scientific evidence; as already stated, all studies only established a temporal relationship between vaccination and myocarditis, without a definite causality. Therefore, is not known the mechanism for which mRNA should cause the disease, and it is not clear why mainly young males are affected [11]; not by chance, the CDC determined that the benefits (such as prevention of COVID-19 cases and its severe outcomes) outweigh the risks of myocarditis and pericarditis after receipt of mRNA COVID-19 vaccines, recommending COVID-19 vaccination for everyone 6 months of age and older [1]. Moreover, the CDC released specific guidelines to manage additional vaccine doses if myocarditis or pericarditis is diagnosed after a dose of mRNA COVID-19 vaccine [25]. Even the European Medicines Agency confirmed that the benefits of all authorized COVID-19 vaccines outweigh their risks, as scientific evidence shows that they reduce deaths and hospitalizations due to COVID-19 [26].

Our study confirms the existence of a temporal relationship between the vaccine and myocarditis, as highlighted by other studies on the subject [1,4–9,11,13]. Unlike other studies, our model allows for a statistical assessment of the cause-effect relationship, yielding results contrary to the hypothesis of a causal risk of myocarditis, even though our results should be interpreted in light of the described methodological limitations. Naturally, our evidence alone is not sufficient to conclude this matter; indeed, a biological mechanism needs to be studied in order to establish the protective effective of the vaccine on myocarditis. Further studies need to be designed to assess the causal link, considering the established existence of the temporal association. However, we believe that the evidence reported in our study is a starting point to question what in the scientific literature now seems to be a certainty, rather than a hypothesis under study. In scientific literature, it is now common to find definitions such as "vaccine-induced myocarditis" [27] or "the vaccine causes myocarditis" [28]. These assertions, in addition to being scientifically incorrect, risk increasing vaccine hesitancy in the general population and subgroups at risk; in 2019, the WHO listed vaccine hesitancy as a significant health threat that year [29], and one of the main determinants of vaccine hesitancy is the fear of adverse effects after immunization. Therefore, the task of scientists is also to correctly frame the phenomena that are observed and know how to communicate them in a scientifically correct way. In this sense, our study underlines how an approach that considers the other potential causes of myocarditis (primarily COVID-19) and a causality assessment must be prioritized in the study of the topic.

Declarations of competing interest

The authors declare that they have no competing interests.

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Supplementary materials

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