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RESEARCH

Impact of influenza immunity on the mortality among older adults hospitalized with COVID-19: a retrospective cohort study

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Abstract

It has been suggested that the outcomes of coronavirus disease 2019 (COVID-19) are better in individuals having recently received an influenza vaccine than in non-vaccinated individuals. We hypothesized that this association depends on the humoral responses against influenza viruses. We aim to assess the relationship between the humoral immunity against influenza and the 3-month all-cause mortality among hospitalized older patients with COVID-19. We performed an exploratory retrospective study of older patients (aged 65 and over) hospitalized for confirmed COVID-19 between November 2020 and June 2021. Previous humoral responses to influenza viruses were assessed using a hemagglutination inhibition assay on routinely collected blood samples. The study's primary outcome was the 3-month all-cause mortality, and the secondary outcomes were severe COVID-19 (oxygen requirement ≥ 6 L/min or ventilatory support) and complications (kidney or heart failure, thrombosis and bacterial infection). In the cohort of 95 patients with COVID-19, immunity against influenza vaccine subtypes/lineages was not significantly associated with 3-month all-cause mortality, with an OR [95%CI] of 0.22 [0.02–1.95] ($p = 0.174$) for the H1N1pdm09 subtype, 0.21 [0.03–1.24] ($p = 0.081$) for A/Hong Kong/2671/2019 H3N2 subtype, 1.98 [0.51–8.24] ($p = 0.329$) for the B/Victoria lineage, and 1.82 [0.40–8.45] ($p = 0.437$) for the B/Yamagata lineage. Immunity against influenza vaccine subtypes/lineages was also not significantly associated with severity and complication. Immunity against influenza subtypes/lineages included in the 2020–2021 vaccine was not associated with a lower 3-month all-cause mortality among COVID-19 hospitalized patients.

Trial registration: The study was approved by a hospital committee with competency for research not requiring approval by an institutional review board (Tours University Medical Center, Tours, France: reference: 2021_015). All patients give the informed consent.

Keywords COVID-19 · Mortality · Influenza Human · Influenza vaccines · Aged

Abbreviations

ADL	Activities of daily living
CI	Confidence interval
COVID-19	Coronavirus disease 2019
HIA	Hemagglutination inhibition assay
IFN	Interferon

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IIV	Inactivated influenza vaccine
IQR	Interquartile range
OR	Odds ratio
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation

Background

Between March 2020 and August 2023, more than 249,150 million cases [1] of coronavirus disease 2019 (COVID-19) and over 2.080 million related deaths [2] were recorded in Europe. The influenza virus is a respiratory virus that causes seasonal epidemics (October to May in Europe), resulting in over 10,000 deaths in France every year [3]. Despite a decrease in the incidence of influenza infection since the beginning of the COVID-19 pandemic in France [3] and other countries [4–6], coinfections have been observed [7]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus are both enveloped RNA viruses with similar transmission routes, symptom profiles and severity risk factors [8]. In both cases, the main severity and mortality risk factors are age over 65 and the presence of comorbidities like obesity, immunodeficiency and chronic pulmonary disease [9, 10].

Due to the heightened risk of severe influenza and related infections in adults aged 65 and above, French Health Authorities recommends annual vaccination in this population which has been shown to reduce severe disease forms by 43% on average [11]. The most common circulating human influenza viruses are influenza A subtypes H1N1pdm09 and H3N2 and influenza B lineages (Victoria and Yamagata), with a seasonal epidemic in winter [12]. The vaccine's composition is updated every year, depending on the influenza virus strains circulating in the other hemisphere [13]. Influenza vaccination rates for the 65 and older were similar between Centre-Val-de-Loire region and national level for the 2020–2021 season (62.8% vs 59.9%, respectively) [14]. There have been no reports of active circulation of the influenza virus during the 2020–2021 epidemic winter period (October to April) in France [15].

Recent epidemiological data have indicated that inactivated influenza vaccine (IIV) may protect against COVID-19: IIV 2019–2020 was associated with a lower incidence of COVID-19 [16], hospital admission, use of mechanical ventilation [17] and, most importantly, mortality [18]. Lastly, the results of modeling studies have suggested that a 10% increase in influenza vaccination coverage is associated with a 5% decrease in COVID-19-related mortality [19].

The above-mentioned studies evaluated the influenza vaccine's effectiveness in preventing other infections on the basis of the person's vaccination history, but did not assess the level of immunity against influenza. Older patients have a lower humoral response to influenza vaccines [20] and so are unable to mount a sustained immune response to influenza virus infections. Furthermore, reliable data on vaccination in general are not always available for inpatients. Previous studies of immune responses (based on vaccination coverage) did not take these factors into account. For these reasons, we hypothesized that the level of humoral response against influenza viruses is associated with the degree of protection against the complications of COVID-19. The humoral response against influenza viruses can be explored by measuring hemagglutination inhibition activity [21]. Hence, the objective of the present study was to assess the all-cause mortality in patients aged 65 and over hospitalized with COVID-19, as a function of their humoral response against influenza virus.

Material and method

Type of study

We performed an exploratory, noninterventional, retrospective, single-center hospitalized cohort study at Tours University Medical Center (Tours, France) between November 1st, 2020, and June 30th, 2021. This period included the usual seasonal epidemic period (late October to April) and the influenza vaccination period (mid-October to late January), with a delay of 2 weeks after the start of the vaccination campaign. Recruitment was discontinued at the end of June 2021, due to the increase in SARS-CoV-2 vaccination campaign among the 65 years and older.

Participants

The main inclusion criteria were age 65 or over, admission to hospital during the study period with a positive RT-PCR test for SARS-CoV-2, and an available blood sample collected at some timepoint in the hospital stay (for blood cell counts, blood electrolyte assays, or serology assessments). The exclusion criteria were SARS-CoV-2 vaccination prior to inclusion, legal guardianship, an insufficient volume of blood for serum preparation, and refuse to participate in the study. Patients whose vital status 3 months after discharge could not be determined were excluded from the analysis of the primary endpoint.

Outcomes

The primary outcome in patients with COVID-19 was all-cause death within 3 months of inclusion in the study (in-hospital and after-discharge mortality). The secondary outcomes were (i) the occurrence of a severe form of COVID-19

(defined as the presence of at least one of the following criteria: the need for oxygen therapy ≥ 6 L/min and/or for ventilatory support (high-flow nasal oxygen therapy, non-invasive ventilation or orotracheal intubation for mechanical ventilation)) and (ii) the occurrence of complications (renal failure (KDIGO Stage 1 or more (increase of serum creatinine 1.5–1.9 times baseline OR ≥ 0.3 mg/dL (≥ 26.5 µmol/L)) [22]), heart failure (congestive signs de novo, elevated BNP > 400 ng/L or elevated NT-proBNP > 1800 ng/L [23]), bacterial superinfection (positive bacteriological culture), and thromboembolic events (confirmed by imaging)), based on medical records.

Data collection

Data extraction was performed manually by the principal investigator from the patients' electronic medical records for all clinical and biological data. The data included demographic variables, comorbidities (according to the Charlson Comorbidity Index and without taking account of age [24]), personal autonomy (according to the Activities of Daily Living (ADL) scale [25]), influenza vaccination status for the season 2020–2021, vaccination history, criteria for the diagnosis of COVID-19, the presence or absence of the severity criteria described above, and the presence or absence of renal, cardiac, infectious and thromboembolic complications.

Patients discharged alive were contacted by telephone 3 months later, in order to collect the following information: vital status and personal autonomy (on the ADL scale).

The hemagglutination inhibition assay (HIA)

Influenza viruses cause red blood cells to agglutinate. Hence, inhibition of hemagglutination by a patient's serum is a marker of anti-influenza virus antibodies. Sera were treated with receptor-destroying enzyme (to remove nonspecific inhibitors), decomplemented at 56 °C, and adsorbed with guinea pig red blood cells (to prevent auto-agglutination). In the HIA, dilutions of a patient's serum (first dilution: 1:10) are placed in contact with each influenza virus studied (4 HA units) and then with a 0.75% suspension of guinea pig red blood cells [26]. The inhibition titer for a given influenza virus corresponds to the greatest dilution that still inhibits hemagglutination. Based on the literature data on the influenza viruses used in the 2020–2021 IIV (A/Guangdong-Maonan/SWL1536/2019 H1N1pdm09 subtype, A/Hong Kong/2671/2019 H3N2 subtype, B/Washington/02/2019 Victoria lineage, and B/Phuket/3073/2013 Yamagata lineage), we defined immunity against influenza as an HIA titer of more than 40 [27–29].

The HIA was not part of routine care for the study participants. However, the assay was performed on serum samples initially collected in a dry tube or an EDTA tube as part of routine care during the hospital stay, and stored at -20 °C in the MYCO-PULM biological resource center (ethical approval was given by the French Ministry of Higher Education, Research and Innovation (authorization n° DC-2020–3961)). The HAIs were performed and managed by the National Reference Center for Respiratory Viruses (Molecular Genetics of RNA Viruses Unit, Institut Pasteur, Paris). Sample was shipped at – 20 °C.

Statistical analysis

Categorical variables were described with number of occurrences and relative (percentage), and continuous variables with median and interquartile range (IQR). Associations between variables were assessed in a univariate analysis, using chi-squared test, Fisher's test, Student's t-test or the Mann–Whitney tests depending on the variable's type and distribution. The threshold for statistical significance was set to $p < 0.05$. Variables with $p < 0.25$ in the univariate analysis were then included into a multivariate logistic regression analysis (complete final model) [30]. Immunity against the various influenza subtypes/lineages was forced into the model. The model with the best fit was selected by maximizing the Akaike information criterion (adjusted final model). For variable included in multivariate analysis, missing values were completed using multiple imputation by chained equations, using the MICE package [31] on R (for data with less than 5% missing value). For statistical analysis, we used software version: Excel 365 Microsoft suite, R version 4.2.3 and R studio Packages MICE [31], Tidyverse [32], finalfit [33], AIC [34].

Ethical Approval and Consent to Participate

In line with the French legislation on retrospective studies of laboratory samples (serum samples initially collected as part of routine care during the hospital stay and stored in the MYCO-PULM biological resource center in Tours University Medical Center, reference DC-2020–3961), the study protocol did not require the provision of informed consent or approval by an institutional review board. However, the study was approved by the local hospital committee with competency for this type of research, not requiring approval by an institutional review board (Tours University Medical Center reference: 2021_015). All patients received a study information document and were free to object to the use of their health data and laboratory samples for research purposes. Informed consent was obtained from all patients. The study data and laboratory samples were to be stored at Tours University Medical Center for a period of 15 years.

Results

Study population

A total of 101 patients were screened between November 1st, 2020, and June 30th, 2021, and 95 met the final inclusion criteria. Six patients (4.95%) were excluded due to subsequent refusal to participate (n = 1), legal guardianship (n = 1), the absence of sufficient serum for testing (n = 1), SARS-CoV-2 vaccination prior to inclusion (n = 1) or the absence of vital status data at 3 months (n = 2) (Fig. 1). The study population's median [IQR] age was 73 [70–83] years. Almost all the participants (n = 91, 95.8%) lived at home and had a median [IQR] ADL score of 6 [5.5–6]. The prevalence of a humoral response against the various influenza vaccine strains was as follows: 89 patients (93.7%) to the A/Guangdong-Maonan/SWL1536/2019 H1N1pdm09 subtype, 87 (91.6%) to the A/Hong Kong/2671/2019 H3N2 subtype, 46 (48.4%) to the B/Washington/02/2019 Victoria lineage, and 23 (24.2%) responded to the B/Phuket/3073/2013 Yamagata lineage (Fig. 2). Sixty study participants were screened with a multiplex PCR test: this revealed three other viral coinfections (SARS-CoV-2 plus adenovirus, rhinovirus or coronavirus NL63) but no SARS-CoV-2 + influenza virus coinfections.

During the 3-month follow-up period, 25 (26.3%) patients died (21 in hospital and 4 after discharge). Seventeen (68%) of the 25 deaths were directly related to COVID-19. Compared with survivors, deceased patients were significantly older (median [IQR] age: 73 [69–78] vs. 79 [73–87], respectively; $p = 0.013$) and had a lower ADL score (median [IQR] 6 [6–6] vs. 6 [4.5–6]; $p = 0.004$), a higher Charlson Comorbidity Index (median [IQR] 4 [3-6] vs. 6 [4–7]; $p = 0.002$) and a greater incidence of complications (in 43 patients (61.43%) vs. 23 patients (92.00%); $p = 0.009$) (Table 1).

Effect of influenza immunity on 3-month mortality

For univariate and multivariate analysis, only pre-hospital ADL (n = 3, 3%) need multiple imputation by chained equations. The other variables included (age, gender, Charlson index, severity, complications and immunization against viral strains) had no missing data. In the univariate analysis, the two main factors significantly associated with all-cause mortality (Table 2) were: age (OR [95%CI] 1.08 [1.01–1.17]; $p = 0.039$) and the presence of comorbidities (according to the Charlson Comorbidity Index) (OR [95%CI] 1.39 [1.02–1.91]; $p = 0.036$). Good autonomy was a protective factor of death with the ADL score (OR [95%CI] 0.40 [0.19–0.75]; ($p = 0.009$)). In univariate analysis, immunity against the various influenza vaccine subtypes/lineages and the occurrence of complications were not significantly associated with mortality. Influenza vaccination rates were similar between patients alive or dead at 3 months (59.7%, n = 40 out of 67 with available data vs. 70%, 7 out of 10 with available data, respectively).

In an Akaike-adjusted multivariate logistic regression analysis (Adjusted final model) (Table 2), immunity against influenza vaccine subtypes/lineages was not significantly

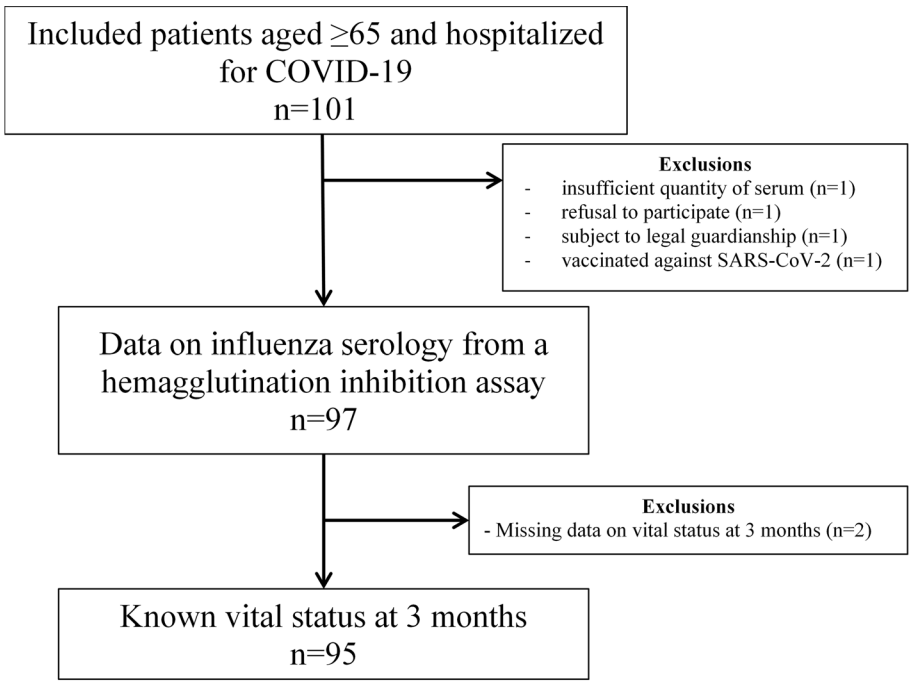

Fig. 1 Study flowchart

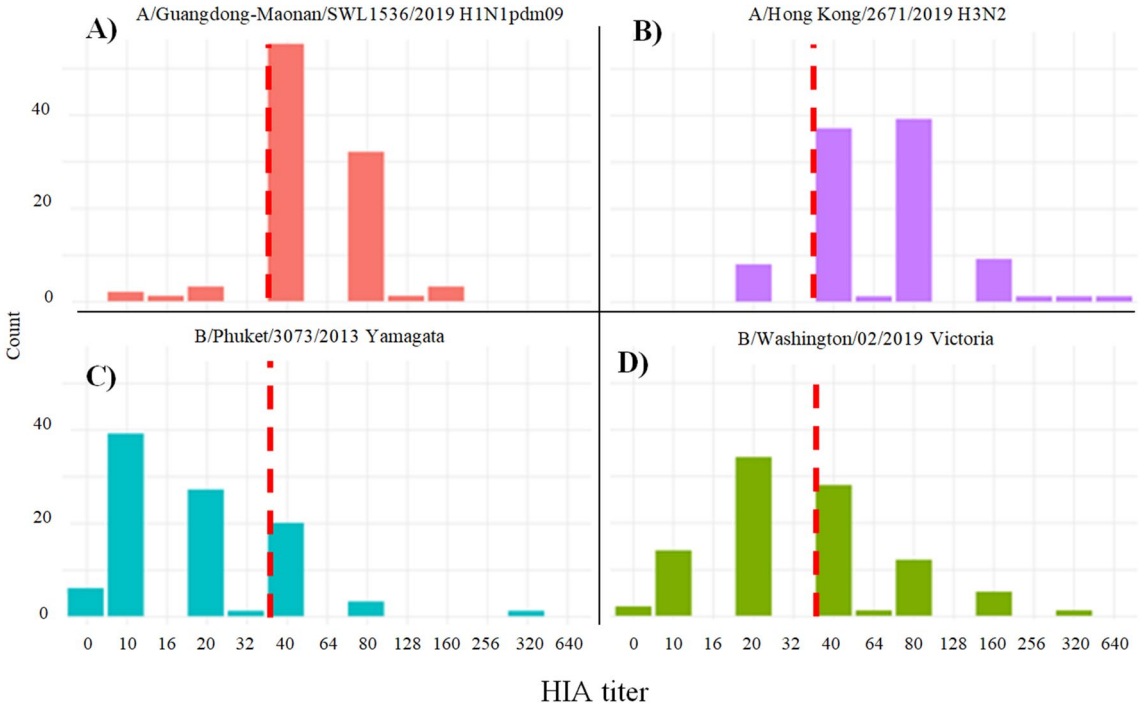

Fig. 2 Hemagglutination inhibition assay (HIA) data for the vaccine strains. The number of serum (count) with the corresponding dilution that inhibit hemagglutination (HIA titer) is shown for **a** the A/Guangdong-Maonan/SWL1536/2019 H1N1pdm09 subtype **b** A/Hong Kong/2671/2019 H3N2 subtype, **c** the B/Phuket/3073/2013 Yamagata lineage and **d** the B/Washington/02/2019 Victoria lineage. In HIA, serial dilutions of patient serum (first dilution: 1:10) are mixed with the influenza virus being tested and then with red blood cells. The HIA titer corresponds to the greatest serum dilution that still inhibits hemagglutination. A seroprotection is considered when the HIA titer is higher than 40 (represented by the red dotted line)

associated with mortality, with an OR [95%CI] of 0.22 [0.02–1.95] ($p=0.174$) for the H1N1pdm09 subtype, 0.21 [0.03–1.24] ($p=0.081$) for A/Hong Kong/2671/2019 H3N2 subtype, 1.98 [0.51–8.24] ($p=0.329$) for the B/Victoria lineage, and 1.82 [0.40–8.45] ($p=0.437$) for the B/Yamagata lineage. Age (OR [95%CI] 1.08 [1.01–1.17] ($p=0.039$)) and Charlson comorbidity Index (OR [95%CI] 1.39 [1.02–1.91] ($p=0.036$)) were associated with mortality whereas a low ADL score (reflecting a good autonomy) was a protective factor of all-cause mortality (OR [95%CI] 0.34 [0.13–0.79]; ($p=0.017$)) (Table 2).

Relationships between influenza immunity, COVID-19 severity, and the occurrence of complications

We performed a multivariate analysis of the impact of influenza immunity on the occurrence of severe COVID-19 and the occurrence of complications. Immunity against the various influenza vaccine subtypes/lineages was not significantly associated with the severe COVID-19 (Table 3); the OR [95%CI] in adjusted final model was 0.96 [0.07–11.02] for the H1N1pdm09 subtype ($p=0.972$), 0.52 [0.04–4.11] for the H3N2 subtype ($p=0.554$), 1.37 [0.37–5.49] for the B/Victoria lineage ($p=0.639$) and 0.75 [0.16–3.36] for the B/Yamagata lineage ($p=0.704$). Similarly, immunity against the various influenza vaccine strains was not significantly associated with the occurrence of complications (Table 3). The OR [95%CI] was 0.47 [0.02–7.96] for the H1N1pdm09 subtype ($p=0.604$), 1.23 [0.12–11.87] for the H3N2 subtype ($p=0.854$), 0.66 [0.18–2.41] for the B/Victoria lineage ($p=0.531$) and 0.75 [0.18–3.09] for the B/Yamagata lineage ($p=0.689$). The occurrence of severe COVID-19 was strongly associated with the occurrence of complications (OR [95%CI] 42.59 [10.43–298.59]; $p<0.001$).

Overall, immunity against influenza subtypes/lineages was not significantly associated with 3-month all-cause mortality, severe COVID-19, or the occurrence of complications.

Discussion

The present study is the first to have analyzed the impact of the level of humoral responses against various influenza subtypes/lineages on 3-month all-cause mortality in patients with COVID-19. Our results revealed no association between humoral immunity against influenza hemagglutinin from subtypes/lineages included in the 2020–2021 vaccine

Table 1 Characteristics of the study population overall and by vital status at 3 months

	Total (n = 95)	Deceased (n = 25)	Alive (n = 70)	p-value
Age (years) med[IQR] (min–max)	73 [70–83] (65–93)	79 [73–87] (66–93)	73 [69–78] (65–92)	0.013*
Sex n (%)				
Female	32 (33.7)	7 (28.0)	25 (35.7)	0.650
Male	63 (66.3)	18 (72.0)	45 (64.3)	
Living environment n(%)				
Own home	91 (95.8)	24 (96.0)	67 (95.7)	0.805
Residential home	3 (3.2)	1 (4.0)	2 (2.9)	
Nursing home	1 (1.1)	0 (0.0)	1 (1.4)	
Charlson Comorbidity Index med[IQR] (min–max)	4 [3–6] (2–10)	6 [4–7] (3–10)	4 [3–6] (2–10)	0.002*
ADL score before admission med[IQR] (min–max)	6 [5.5–6] (2–6)	6 [4.5–6] (2–6)	6 [6–6] (3–6)	0.004*
ADL score on admission med[IQR]	3.5 [2–5] (0–6)	2.5 [1.5–3.5] (0–6)	4 [2–5.5] (0–6)	0.003*
Received an IIV in 2020–2021 n(%)	47 (51)	7 (28)	40 (59.7)	0.73
Immune against the H1N1pdm09 subtype n(%)	89 (93.7)	22 (88.0)	67 (95.7)	0.378
Immune against the H3N2 subtype n(%)	87 (91.6)	21 (84.0)	66 (94.3)	0.242
Immune against the B/Victoria lineage n(%)	46 (48.4)	13 (52.0)	33 (47.1)	0.854
Immune against the B/Yamagata lineage n(%)	23 (24.2)	6 (24.0)	17 (24.3)	> 0.999
Immune status to the 2019–2020 vaccine subtypes/lineages n(%)				
Immune against 0 subtypes/lineages	1 (1.1)	1 (4.0)	0 (0.0)	0.323
Immune against 1 subtype/lineage	10 (10.5)	4 (16.0)	6 (8.6)	
Immune against 2 subtypes/lineages	36 (37.9)	7 (28.0)	29 (41.4)	
Immune against 3 subtypes/lineages	29 (30.5)	8 (32.0)	21 (30.0)	
Immune against 4 subtypes/lineages	19 (20.0)	5 (20.0)	14 (20.0)	
Length of hospital stay (days) med[IQR] (min–max)	11 [6–22] (1–93)	13 [9–25] (3–68)	10 [5–20.75] (1–93)	0.228
Severe COVID-19 n(%)	49 (51.6)	17 (68.0)	32 (45.7)	0.093
Oxygen therapy > 6 L/min	49 (51.6)	17 (68.0)	32 (45.7)	0.093
High-flow nasal oxygen therapy	34 (48.4)	8 (32.0)	38 (54.3)	0.088
Non-invasive ventilation	12 (12.6)	3 (12.0)	9 (12.9)	> 0.999
Mechanical ventilation	18 (18.9)	3 (12.0)	15 (21.4)	0.462
Complication	66 (69.5)	23 (92.0)	43 (61.4)	0.009*
Heart failure	16 (16.8)	6 (24.0)	10 (14.3)	0.422
Kidney failure	35 (36.8)	13 (52.0)	22 (31.4)	0.112
Bacterial infection	48 (50.5)	18 (72.0)	30 (42.9)	0.023*
Thromboembolic event	10 (10.5)	4 (16.0)	6 (8.6)	0.510

ADL activities of daily living. *IIV* inactivated influenza vaccine. *med[IQR]* median [interquartile range]. The Charlson Comorbidity Index did not take account of age. * $p < 0.05$

(A/Guangdong-Maonan/SWL1536/2019 H1N1pdm09 subtype, A/Hong Kong/2671/2019 H3N2 subtype, B/Washington/02/2019 Victoria lineage and B/Phuket/3073/2013/Yamagata lineage) and the COVID-19 outcomes.

Nevertheless, literature data showed that influenza vaccination is effective in reducing mortality in COVID-19 patients in general and in those aged 65 and over in particular [19, 35], even if the direct link with vaccine influenza subtype/lineage has not been demonstrated. For example, there is some evidence to suggest that immunity against SARS-CoV-2 is not associated with cross-immunity against the H3N2 subtype [36]. Our results are in line with the lack of cross-protection against COVID-19 observed after a recent influenza H3N2 outbreak in Cambodia [36]. Between August 2020 and September 2020 (i.e., during the COVID-19 pandemic), an epidemic of H3N2 subtype influenza (caused by A/Cambodia/VIR20080713/2020) occurred in a region of Cambodia [36]. This influenza outbreak was attributed to a decrease in the use of protective measures by the population, following the regression of the COVID-19 pandemic during this period. Moreover, no H3N2 + SARS-CoV-2 coinfections were found. In March 2021, Cambodia was affected by another wave of COVID-19 [37], with around 100,000 new cases and 2,000 deaths. This upsurge in SARS-CoV-2 infections and associated deaths just a few months after the epidemic of H3N2 influenza argues against

Table 2 Three-month all-cause mortality after COVID-19 as a function of immune status against influenza vaccine subtypes/lineages

	Alive	Deceased	OR [95%CI] (univariate)	OR [95%CI] (complete final model)	OR [95%CI] (adjusted final model)
Age (years) Mean (SD)	74.7 (7.3)	79.4 (8.3)	1.08 [1.02–1.15] ($p=0.011$)*	1.08 [1.00–1.17] ($p=0.051$)	1.08 [1.01–1.17] ($p=0.039$)*
Length of hospital stay (days) Mean (SD)	17.5 (18.9)	18.1 (14.9)	1.00 [0.97–1.03] ($p=0.889$)	–	–
ADL score before admission Mean (SD)	5.8 (0.5)	5.3 (1.0)	0.40 [0.19–0.75] ($p=0.009$)*	0.32 [0.12–0.76] ($p=0.016$)*	0.34 [0.13–0.79] ($p=0.017$)*
Charlson Comorbidity Index Mean (SD)	4.5 (1.8)	6.0 (2.0)	1.48 [1.16–1.94] ($p=0.002$)*	1.30 [0.93–1.82] ($p=0.125$)	1.39 [1.02–1.91] ($p=0.036$)*
Symptoms n(%)	69 (98.6)	24 (96.0)	0.35 [0.01–9.02] ($p=0.461$)	–	–
Immune against the H1N1pdm09 subtype n(%)	67 (95.7)	22 (88.0)	0.33 [0.06–1.88] ($p=0.192$)	0.21 [0.02–1.92] ($p=0.170$)	0.22 [0.02–1.95] ($p=0.174$)
Immune against the H3N2 subtype n(%)	66 (94.3)	21 (84.0)	0.32 [0.07–1.45] ($p=0.127$)	0.19 [0.03–1.22] ($p=0.081$)	0.21 [0.03–1.24] ($p=0.081$)
Immune against the B/Victoria lineage n(%)	33 (47.1)	13 (52.0)	1.21 [0.49–3.06] ($p=0.677$)	2.21 [0.54–9.79] ($p=0.276$)	1.98 [0.51–8.24] ($p=0.329$)
Immune against the B/Yamagata lineage n(%)	17 (24.3)	6 (24.0)	0.98 [0.32–2.77] ($p=0.977$)	1.92 [0.39–9.89] ($p=0.422$)	1.82 [0.40–8.45] ($p=0.437$)
Complications n(%)	43 (61.4)	23 (92.0)	7.22 [1.92–47.27] ($p=0.011$)*	5.14 [0.87–44.13] ($p=0.091$)	–
Severe COVID-19 n(%)	32 (45.7)	17 (68.0)	2.52 [0.99–6.90] ($p=0.060$)	1.41 [0.36–6.11] ($p=0.626$)	3.02 [0.98–10.48] ($p=0.064$)

The results of the univariate and multivariate analyses are expressed as the OR [95%CI] in a complete final model (fourth column), correspond to a logistic regression with inclusion of variable with $p<0.25$ in univariate analysis and adjusted final model correspond to the Akaike information criterion (AIC: 98.87). Severe COVID-19 correspond to oxygen therapy > 6 L/min and/or high-flow nasal oxygen therapy and/or non-invasive ventilation and/or mechanical ventilation. ADL: activities of daily living. SD: standard deviation. CI: confidence index. * $p<0.05$

cross-protection between the two viruses. These results must be considered with a degree of caution because successive epidemic increase the vulnerability of the population, which is not the case with vaccines.

Given that IIVs contain purified influenza virus proteins, specific cross-immunity against homologous SARS-CoV-2 sequences seems unlikely. However, there are other mechanistic explanations for lower mortality in COVID-19 patients vaccinated against influenza. Various mechanistic hypotheses have been put forward, such as an immunomodulatory effect of the vaccine via stimulation of the innate immune memory or a "trained immunity" that confers cross-protection against COVID-19 [38]. Influenza vaccination might stimulate the innate immune system (mediated by natural killer cells, dendritic cells, and monocytes) in addition to the adaptive immune system, and the former would probably respond more rapidly to new viral or bacterial infections [39, 39].

The study by Pawlowski C.et al. [40] highlighted a protective effect of recent vaccination with other vaccines (such as polio and hepatitis B) on the incidence of COVID-19. Trained innate immunity is defined as a more rapid innate immune response to contact with a known or unknown pathogen following stimulation by another pathogen [41]. This training involves several mechanisms: epigenetic and metabolic changes in innate immune cells, the release of inflammatory cytokines (interleukin-6, interleukin-1, and tumor necrosis factor alpha) and immunological stimulation of T cells [41]. Trained immunity might be one explanation for the protective effect of the influenza vaccine and other vaccines on COVID-19, but cannot alone explain the observed differences between the viral subtypes/lineages.

Indeed, the innate immune system is known to be stimulated by a trivalent IIV, with the overexpression of interferon-γ (IFN)-producing T lymphocytes and natural killer cells [42]. Similar results have been found in vitro experiments, after cells from individuals previously immunized with a quadrivalent IIV were stimulated with SARS-CoV-2; the level of IFN-γ release was higher, and pro-inflammatory cytokines (such as IL-1) were overexpressed [37]. The type 1 IFN pathway might also explain the link between innate and adaptive immunity because it increases antibody production by B cells [43]. Furthermore, Ye et al. [44] showed that the impairment of this mechanism in mice lacking type 1 IFN receptors was associated with less production of specific IgG2c and IgA antibodies after vaccination with an inactivated or live attenuated influenza vaccine. Bastard et al. [45] found that patients with severe COVID-19 had higher titers of autoantibodies against type 1 IFN, leading to inhibition of the type 1 IFN pathway; the prevalence of these antibodies was higher in patients aged 65 and over (13%, vs. 8.5% in younger patients).

Table 3 Severity and complications of COVID-19 as a function of immune status against influenza vaccine subtypes/lineages

	Non severe	Severe	OR [95%CI] (univariate)	OR [95%CI] (complete final model)	OR [95%CI] (adjusted final model)
Length of hospital stay (days) Mean (SD)	9.3 (7.8)	25.5 (20.9)	1.11 [1.06–1.18] ($p<0.001$)*	1.10 [1.04–1.18] ($p=0.003$)*	1.10 [1.04–1.18] ($p=0.003$)*
ADL score before admission Mean (SD)	5.8 (0.5)	5.6 (0.9)	0.67 [0.33–1.20] ($p=0.209$)	0.75 [0.29–1.54] ($p=0.476$)	–
Charlson Comorbidity Index Mean (SD)	5.0 (2.1)	4.8 (1.7)	0.97 [0.78–1.19] ($p=0.762$)	–	–
Immune against the H1N1pdm09 subtype n(%)	44 (95.7)	45 (91.8)	0.51 [0.07–2.76] ($p=0.452$)	1.01 [0.07–12.37] ($p=0.994$)	0.96 [0.07–11.02] ($p=0.972$)
Immune against the H3N2 subtype n(%)	43 (93.5)	44 (89.8)	0.61 [0.12–2.66] ($p=0.522$)	0.47 [0.04–3.77] ($p=0.501$)	0.52 [0.04–4.11] ($p=0.554$)
Immune against the B/Victoria lineage n(%)	24 (52.2)	22 (44.9)	0.75 [0.33–1.67] ($p=0.479$)	1.43 [0.38–5.86] ($p=0.601$)	1.37 [0.37–5.49] ($p=0.639$)
Immune against the B/Yamagata lineage n(%)	14 (30.4)	9 (18.4)	0.51 [0.19–1.32] ($p=0.174$)	0.80 [0.17–3.62] ($p=0.768$)	0.75 [0.16–3.36] ($p=0.704$)
Complications n(%)	19 (41.3)	47 (95.9)	33.39 [8.84–220.26] ($p<0.001$)*	26.34 [6.06–206.67] ($p<0.001$)*	26.07 [6.07–201.33] ($p<0.001$)*
	With complication	Without complication			
Sex (Male) n(%)	16 (55.2)	47 (71.2)	2.01 [0.81–5.00] ($p=0.131$)	2.12 [0.61–7.98] ($p=0.245$)	–
Length of hospital stay (days) Mean (SD)	9.5 (8.1)	21.2 (19.8)	1.08 [1.03–1.14] ($p=0.008$)*	1.01 [0.95–1.07] ($p=0.865$)	-
ADL score before admission Mean (SD)	5.8 (0.4)	5.6 (0.8)	0.71 [0.30–1.36] ($p=0.364$)	–	–
Charlson Comorbidity Index Mean (SD)	4.3 (1.8)	5.1 (2.0)	1.26 [0.99–1.65] ($p=0.069$)	1.44 [1.07–2.02] ($p=0.023$)*	1.44 [1.08–2.02] ($p=0.021$)*
Immune against the H1N1pdm09 subtype n(%)	28 (96.6)	61 (92.4)	0.44 [0.02–2.87] ($p=0.458$)	0.30 [0.01–6.56] ($p=0.438$)	0.47 [0.02–7.96] ($p=0.604$)
Immune against the H3N2 subtype n(%)	27 (93.1)	60 (90.9)	0.74 [0.10–3.46] ($p=0.724$)	1.01 [0.08–10.98] ($p=0.991$)	1.23 [0.12–11.87] ($p=0.854$)
Immune against the B/Victoria lineage n(%)	16 (55.2)	30 (45.5)	0.68 [0.28–1.62] ($p=0.384$)	0.73 [0.19–2.80] ($p=0.647$)	0.66 [0.18–2.41] ($p=0.531$)
Immune against the B/Yamagata lineage n(%)	10 (34.5)	13 (19.7)	0.47 [0.17–1.25] ($p=0.126$)	0.71 [0.16–2.94] ($p=0.633$)	0.75 [0.18–3.09] ($p=0.689$)
Severe COVID-19	2 (6.9)	47 (71.2)	33.39 [8.84–220.26] ($p<0.001$)*	40.73 [8.17–379.12] ($p<0.001$)*	42.59 [10.43–298.59] ($p<0.001$)*

Severe COVID-19 was defined as the need for oxygen therapy ≥ 6 L/min or for ventilatory support (high-flow nasal oxygen therapy, non-invasive ventilation, or orotracheal intubation for mechanical ventilation). The results of the univariate and multivariate analyses are expressed as the OR [95%CI] in a complete final model (fourth column), correspond to a logistic regression with inclusion of variable with $p<0.25$ in univariate analysis and adjusted final model correspond to the Akaike information criterion (AIC severity: 84.66; AIC complication 84.65). ADL: activities of daily living. SD: standard deviation. CI: confidence index. * $p<0.05$

The presence of autoantibodies against type 1 IFN is also associated with greater COVID-19-related mortality [46]. Given that we did not collect data on the levels of IFN or antitype 1 IFN antibodies, it would be interesting to assess if the presence of these autoantibodies could influence the titer of antibodies against influenza viruses in IIVs.

Lastly, it would have been interesting to evaluate the effect of immunosenescence, which is known to have an indirect impact on both the vaccine's induction of a robust response to the influenza virus and the body's response to SARS-CoV-2 infection [47, 48]. Unfortunately, the lack of data on influenza vaccination status and the small number of

deceased people prevented us from intergroup comparisons of antibody levels; we were therefore unable to explore this hypothesis.

Our study had several limitations. Firstly, it was an exploratory study and our null results may be due to the small number of patients and a lack of statistical power. Indeed, the lack of significance of COVID complications and severity with respect to mortality does not support the literature data [49]. Secondly, we did not have information on the etiology of the immunization against influenza (i.e., post-vaccination or post-infection immunity); this might have introduced confounding bias, with a potentially better vaccine response (and thus a lower risk of death) in the patients with less comorbidity and greater autonomy. These patients might also have been more aware of their health status, and patients vaccinated annually might have received better medical follow-up. As our population was initially hospitalized, we could expect that the incidence of severe COVID-19 was more important than in community patients. Thus, our results may not apply to all patients with COVID-19, especially non-hospitalized patients. Similarly, our patients were aged 65 and over, thus the results may not apply to the younger population at risk of severe disease. The retrospective nature of the study and manual data collection may also present a classification bias. Last, we only explored immune responses to influenza vaccines using a hemagglutination inhibition assay. Thus, we could not explore humoral responses against other influenza vaccine proteins [50] or the cellular immune responses.

The mechanisms of the immune response in older patients remain insufficiently understood, particularly the response to vaccinations. We recommend that in vivo studies be carried out to assess the impact of influenza vaccination on innate immunity, and in particular the link with the IFN type 1 pathway.

Conclusion

Our exploratory study revealed no association between humoral immunity against influenza hemagglutinin from subtypes/lineages included in the 2020–2021 vaccine and 3-month all-cause mortality among COVID-19 hospitalized patients. Further, more powerful studies are still needed to explore this hypothesis. Irrespective of the relation between influenza vaccination and COVID-19 mortality, we would like to emphasize that on the relevance of maintaining effective influenza vaccination programs in the older people is of value.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tours University Medical Center in 2021 (reference: 2021 015). In line with the French legislation on noninterventional studies of anonymized clinical data, the study database was registered at Tours University Medical Center (Tours, France; reference: 2021_034).

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