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Systematic Molecular Influenza A/B Screening Upon Hospital Admission in Belgium, January–April 2022: Positivity Ratios and Viral Loads According to Symptomatology, Age, and Vaccination Status

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ABSTRACT

Three hospitals implemented molecular point-of-care tests (POCTs) to screen patients for SARS-CoV-2 infection upon admission during the 2021/2022 influenza season, which in Belgium lasted from January to April 2022. The samples were simultaneously tested for influenza A/B. Influenza positivity at admission was examined in relation to patient characteristics and symptomatology. Influenza POCTs were performed on all patients requiring urgent hospitalization, regardless of the admission reason. A total of 9327 patients were included in the study, of which 411 (4.4%) tested positive for influenza A/B. Asymptomatic infection and mild illness accounted for respectively 11.2% (95% CI: 8.5%–14.6%), and 43.3% (95% CI: 38.6%–48.1%) of the cases. A total of 66% (95% CI: 60%–72%) of all patients in these symptom categories (asymptomatic and mild illness) showed a high viral load (cycle threshold [Ct] < 24). Only in 30 (7.3%, 95% CI: 5.2%–10.2%) of all cases and in two (4.4%, 95% CI: 1.2%–14.5%) of the asymptomatic cases, the symptomatology worsened during hospital stay. Coinfections with both influenza and SARS-CoV-2 occurred in 35 patients (8.5% of all influenza positive patients). There was no difference in symptomatology between patients with co-infections and those with an influenza mono-infection. Patients could not be reliably categorized into carriers with low versus high viral loads based on symptomatology, age, and vaccination status. More than half of the influenza-positive individuals were either asymptomatic or had mild symptoms upon admission, while often carrying high viral loads. Our results show that without screening of patients at hospital admission, a considerable number of patients with a high viral load may be incorrectly classified as being not infectious.

1 | Introduction

Influenza accounts for an estimated mortality of 13.8 per 100.000 person-years in Europe [1], making it one of the infectious diseases with the highest impact on population health [2–4]. The acquisition of influenza within healthcare facilities is a significant concern due to the high proportion of patients at risk of severe influenza. Hospital outbreaks, being associated with substantial morbidity and mortality, have been extensively documented in literature [5–10].

While asymptomatic influenza asymptomatic carriership is a well-known phenomenon [11, 12], it remains uncertain to which extent hospitals face undiagnosed influenza cases among hospital admissions during an epidemic wave. Even in the aftermath of SARS-CoV-2, monitoring of viral respiratory pathogens in humans continues to focus on symptomatic individuals, thereby potentially underestimating or neglecting the role of asymptomatic transmission [13].

In Belgium, hospitals systematically screened all admitted patients for the presence of SARS-CoV-2 infection throughout the COVID-19 pandemic regardless of symptoms at the time of admission. The participating centers in this study utilized the Roche cobas Liat Influenza A/B and SARS-CoV-2 POC RT-PCR test (Roche Diagnostics, Basel, Switzerland) to screen hospitalized patients at the emergency department. During the 2021–2022 seasonal influenza epidemic, the samples collected for SARS-CoV-2 were tested simultaneously for the presence of influenza A/B. The primary objective of this study was to inventory asymptomatic influenza A/B infections amongst hospitalized patients in view of infection control. This includes the positivity ratio, viral load, symptomatology at admission, evolution of symptoms during the period of hospitalization, age distribution, and influenza vaccination status.

Additionally, the study examined the characteristics of SARS-CoV-2 and influenza co-infections. Since this study was executed during the COVID-19 pandemic, the authors were able to investigate the effect of SARS-CoV-2 co-infection on the symptoms of influenza patients. Previous studies showed more severe symptoms with more complications in patients with such co-infections [14–16]. Given that these studies were conducted during pandemic periods in which build-up of vaccine-induced and natural immunity in the population was more limited, and other virus strains were circulating, it is unpredictable whether this finding will be confirmed here.

2 | Materials and Methods

2.1 | Study Population

Three Belgian hospitals using the Roche cobas Liat Influenza A/B and SARS-CoV-2 POC RT-PCR test, participated in the study. Data were collected from Ziekenhuis Netwerk Antwerpen (ZNA), a 2202-bed hospital with different campuses across the city of Antwerp, the Jessa Hospital, a 981-bed hospital located in Hasselt, Limburg, and the University Hospitals Leuven (UZ Leuven), a 1764-bed hospital located in Leuven, Flemish Brabant.

2.2 | Data Collection and Definitions

Patients tested for influenza on the Roche cobas Liat Influenza A/B and SARS-CoV-2 POC RT-PCR test between January 1 and April 30, 2022 were included. Only patients that were tested on hospital admission or within 24 h after admission were included in the study. Patients admitted for less than or equal to 1 day were excluded as they were not routinely screened.

For ZNA, the systematic use of the multiplex tests detecting both SARS-CoV-2 and influenza was terminated on April 7, 2022, after which patients were no longer included. UZ Leuven and Jessa Hospital continued systematic screening until the end of the study period (April 30, 2022).

Influenza positive patients were subdivided into categories according to their symptoms at the time of hospital admission. Data were retrieved from the medical records.

Since there is no standardized severity score for influenza, and the US Food and Drug Administration recommends considering endpoints that include clinical signs and symptoms, vital signs, oxygenation, and mortality, we implemented the following case symptom categories [17–19], which were based on SARS-CoV-2 symptom categories [20].

1. Asymptomatic (or presymptomatic) infection **(**i.e., patients who tested positive for influenza but who had no symptoms consistent with an influenza infection as described below).
2. Mild illness (i.e., patients presenting with signs and symptoms of influenza (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea) but without clinical or radiological evidence for lower respiratory disease).
3. Moderate illness (i.e., patients with clinical or radiological signs of lower respiratory disease and with oxygen saturation (SpO_2) ≥ 94% on room air).
4. Severe illness (i.e., patients with SpO_2 < 94% on room air, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mmHg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50%).
5. Critical illness (i.e., patients who suffered from respiratory failure, septic shock, and/or multiple organ dysfunction).
6. Deceased (i.e., patients deceased as a result of influenza infection).

To assess disease evolution, data on most severe symptoms during hospitalization were collected for each patient and classified as described above.

The vaccination status of influenza positive patients was recorded, if available (registration of influenza vaccination is not mandatory in Flanders, Belgium) in the governmental database (Vaccinet). Only EMA approved vaccines targeting the 2021–2022 influenza season were considered. In Belgium, vulnerable groups are recommended to be vaccinated between October and mid-December [21]. Consequently, the vaccination

campaign had already been completed before the start of the study period.

In this study, a distinction between high viral load and no high viral load was made. Since there is to date no scientific basis to define high viral load in terms of Ct-values, most of the studies use an ad-hoc cut-off value determined based on expert opinion [22]. In this study, high viral load was defined as a Ct-value smaller than 24.

2.3 | Statistical Analyses

Descriptive statistics were calculated for both categorical, expressed as absolute and relative frequencies, and continuous variables, summarized in terms of means and standard deviations or medians and interquartile ranges. Positivity ratios were defined as the ratio of the number of positive influenza tests and the total number of tested patients. Wilson score confidence intervals are reported for proportions.

A generalized additive model using a Bernoulli distribution for the binary endpoint defining influenza positivity and with a logit-link function was employed to describe the evolution of the influenza positivity over time, while correcting for day of testing, age (using 10-year age groups) and sex. Age- or sex-related differences in evolution of positivity were assessed using interaction terms. The nonlinear time effect was modeled using a cubic regression spline. Akaike's Information Criterion (AIC) was used to decide whether interaction terms improved the model fit. Furthermore, the relation between Ct-values and age (in 10 years groups), sex, vaccination status, symptomatology and hospital was studied using multiple linear regression after performing multiple imputation by chained equations (i.e., MICE with $M = 30$ imputations) to account for missing vaccination status data [23]. The observed Ct-values were log10-transformed to satisfy the conditional normality assumption. A stepwise selection procedure was used to select all significant fixed main and interaction effects.

Based on a logistic regression model including symptomatology at hospital admission, age and vaccination status, we assessed the predictive ability when classifying subjects into high (≥ 24) or low (< 24) Ct-value groups. To evaluate the performance of the classification rule, we calculated pooled optimism-adjusted performance measures across the different imputed datasets. More specifically, for each of the M imputation sets we non-parametrically bootstrapped the data 500 times to obtain estimates with regard to the optimism of different metrics when training and evaluating the classifier based on the same data. Consequently, optimism-adjusted measures were derived.

Multiple testing was corrected for using a Bonferroni-Holm multiplicity correction to obtain adjusted p-values. Two-sided tests were performed at a prespecified significance level of 5%. All statistical analyses were performed using the statistical software R (version 4.1.2) [24].

2.4 | Ethical Committee

This study was approved by the ethical committees of the three contributing centers (approval number 5695; approved by the UZ/KU Leuven Ethical committee for research (S67104). ZNA acted as the main investigator. A data transfer agreement was concluded with UZ Leuven and Jessa Hospital. Data were pseudonymised for statistical analysis. No specific funding was attributed to the study as testing was reimbursed by Belgian National Institute of Health and Disability Insurance in the context of the SARS-CoV-2 pandemic.

3 | Results

3.1 | General Description of Patient Characteristics

A total of 9327 patients were tested for influenza before hospitalization using the Roche cobas Liat Influenza A/B and SARS-CoV-2 POC RT-PCR test ($n = 4766$ (51.1%) in ZNA, $n = 1790$ [19.2%] in Jessa Hospital, $n = 2771$ [29.7%] in UZ Leuven). In total, 4.4% ($n = 411$, 95% confidence interval [CI]: 4.0%–4.8%) of patients tested positive for influenza The positivity ratio increased to 8.0% ($n = 341$ out of 4262 individuals, 95% CI: 7.2%–8.9%) during the period of the official influenza epidemic in Belgium, as defined by the national scientific institute for public health, Sciensano [25] (March 7 to April 17 [week 10–15]). Here, the following definition is used to define the official influenza epidemic: the number of consultations at the general practitioner due to a flu syndrome is higher than 140 consultations per 100,000 inhabitants per week; and this threshold is exceeded for at least two consecutive weeks. Furthermore, at least 20% of the respiratory samples analysed are positive for influenza viruses. Amongst all positive influenza cases, only one patient tested positive for the influenza B virus. Consequently, in subsequent analyses we did not distinguish between influenza A and B positive patients. Consultation of Vaccinnet (the national platform for vaccine registration) was possible for 293 patients (out of 411 patients that tested positive for influenza), of which 40 patients (13.7%) had a registered vaccine. Actually, as vaccination registration is not mandatory, vaccination status cannot be ascertained for the 118 patients for which Vaccinnet could not be accessed, as well as for the 253 patients for which Vaccinnet could be accessed but did not show a registered vaccine administration. The median age was 70.5 years (IQR: 42.3–84.5 years) in the vaccinated group and 38.0 years (IQR: 3.4–71.0 years). 22 patients (55% of vaccinated) had a Ct-value < 24.

Patient characteristics for all tested patients and influenza positive patients are presented in Table 1. Median age of tested patients was 65 years (IQR: 37.0–79.0 years). Age in influenza positive patients had a bimodal distribution with a large share of patients aged less than 10 years (i.e., 10.1%) while the remaining part of the age distribution was left skewed with higher ages being more frequently observed. The proportion of influenza positive patients was significantly different across different age groups (chi-square test two-sided p-value $< 2.2e-16$) with the proportion of positive cases in the –9 year age group being the highest (33.1% of the positive cases; see Figure 1).

TABLE 1 | Demographic variables and patient characteristics of all tested patients and influenza positive patients in the study, stratified by symptomatology at hospital admission. Relative frequencies are computed within symptom categories and are expressed as percentages between brackets. SD: standard deviation; IQR: interquartile range shown as the first and third quartile.

		Influenza positive patients					
	All tested patients	**Asymptomatic**	**Mild symptoms**	**Moderate symptoms**	**Severe symptoms**	**Critical symptoms**	**All**
N (%)	9327	46 (11.2%)	178 (43.3%)	90 (21.9%)	87 (21.2%)	10 (2.4%)	411 (100%)
Age							
Mean (SD)	57.0 (27.7)	34.5 (24.2)	28.1 (33.6)	55.7 (28.8)	55.6 (29.3)	59.7 (32.2)	41.4 (33.3)
Median (IQR)	65.0 (37.0–79.0)	32.0 (18.3–46.5)	5.5 (2.0–64.5)	66.5 (38.0–73.8)	63.0 (38.0–78.5)	67.0 (50.8–86.3)	43 (4.0–72.0)
Sex: Male	4795 (51.4%)	20 (43.5%)	90 (50.6%)	49 (54.4%)	51 (58.6%)	6 (60.0%)	216 (52.6%)
SARS-CoV-2 co-infections (%)		3 (8.6%, 95% CI: 3.0%–22.4%)	12 (34.3%, 95% CI: 20.8%–50.8%)	11 (31.4%, 95% CI: 18.6%–48.0%)	8 (22.9% 95% CI 12.1%–39.0%)	1 (2.9% 95% CI: 0.5%–14.5%)	35 (100%)
Vaccinated (%)		1 (2.5%, 95% CI: 0.4%–12.9%)*	12 (30.0%, 95% CI: 18.1%–45.4%)*	11 (27.5%, 95% CI: 16.1%–42.8%)*	16 (40.0%, 95% CI: 26.3%–55.4%)*	0 (0.0%)*	40 (100%)*
Viral load							
Mean (SD)		24.3 (7.0)	21.4 (5.3)	23.9 (6.4)	23.2 (6.5)	19.7 (4.2)	22.6 (6.1)
Median (IQR)		24.0 (18.0–30.5)	20.2 (17.3–25.0)	23.2 (18.5–28.6)	22.6 (17.7–28.2)	18.5 (17.1–21.2)	21.5 (17.6–27.1)
High viral load (Ct < 24) (%)		23 (9.1%, 95% CI: 6.1%*–13.3%)	125 (49.4%, 95% CI: 43.3%–55.5%)	48 (19.0%, 95% CI: 14.6%–24.3%)	49 (19.4%, 95% CI: 15.0%–24.7%)	8 (3.2%, 95% CI: 1.6%–6.1%)	253 (100%)

*Note that there is underreporting of influenza vaccination, Vaccinnet could be consulted in 293 cases.

3.2 | Description of the Influenza Epidemic Over Time

In Figure 2, the number of influenza positive individuals and the influenza positivity ratio over time (by ISO week number) by 10-year age groups is depicted. Both the number of influenza positive cases and the overall positivity ratio increased after ISO week 8 (February 21, 2022–February 27, 2022) with the highest number of positive tests and highest positivity ratio (i.e., 78 positive cases resulting in a positivity ratio of about 12%) observed in week 14 (April 4, 2022–April 10, 2022).

In Figure 3, we show the estimated influenza positivity over time for different age groups based on the best fitting generalized

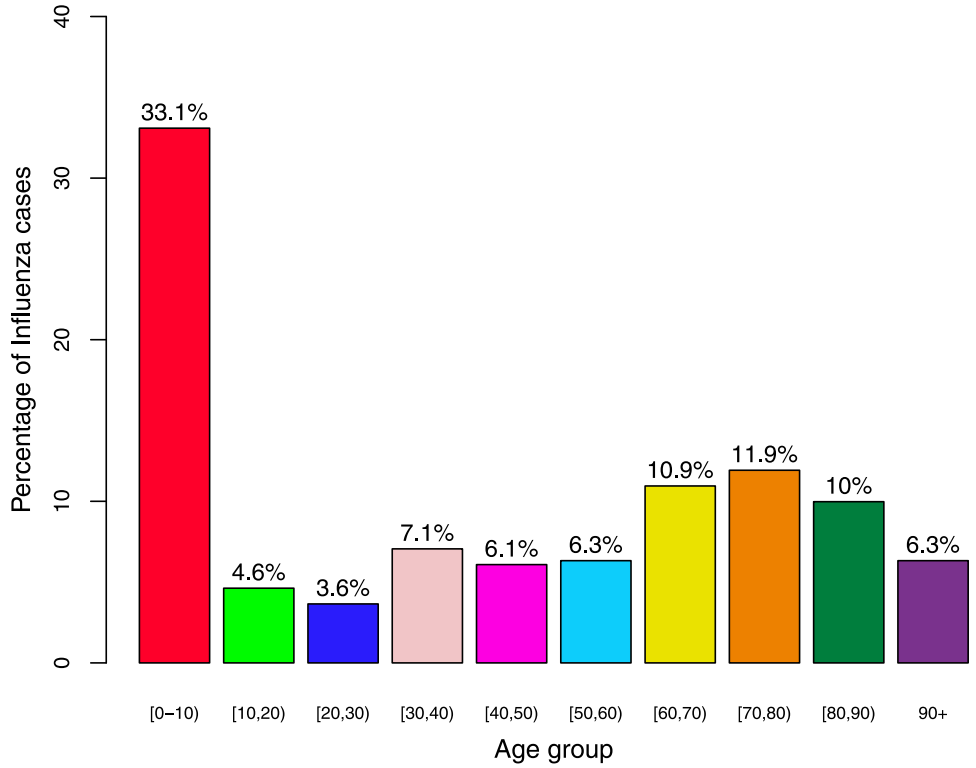

FIGURE 1 | The percentage of confirmed influenza positive cases by age group.

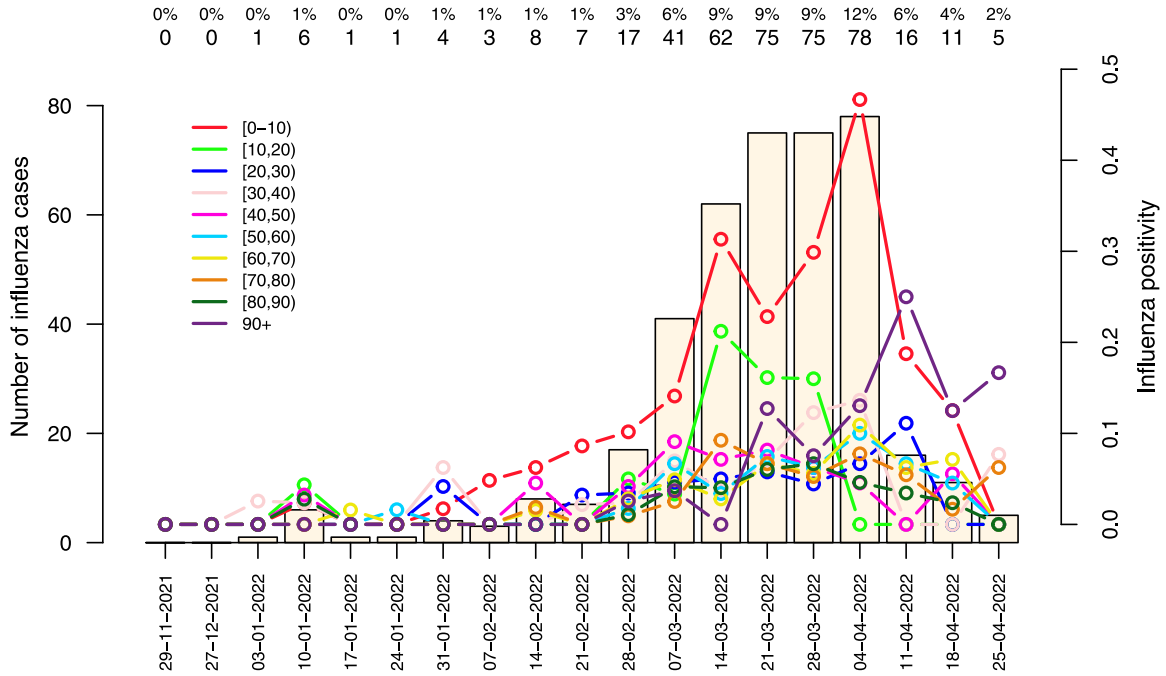

FIGURE 2 | Number of influenza positive patients over time (orange bars) and evolution of the influenza positivity ratio over time by 10-year age group.

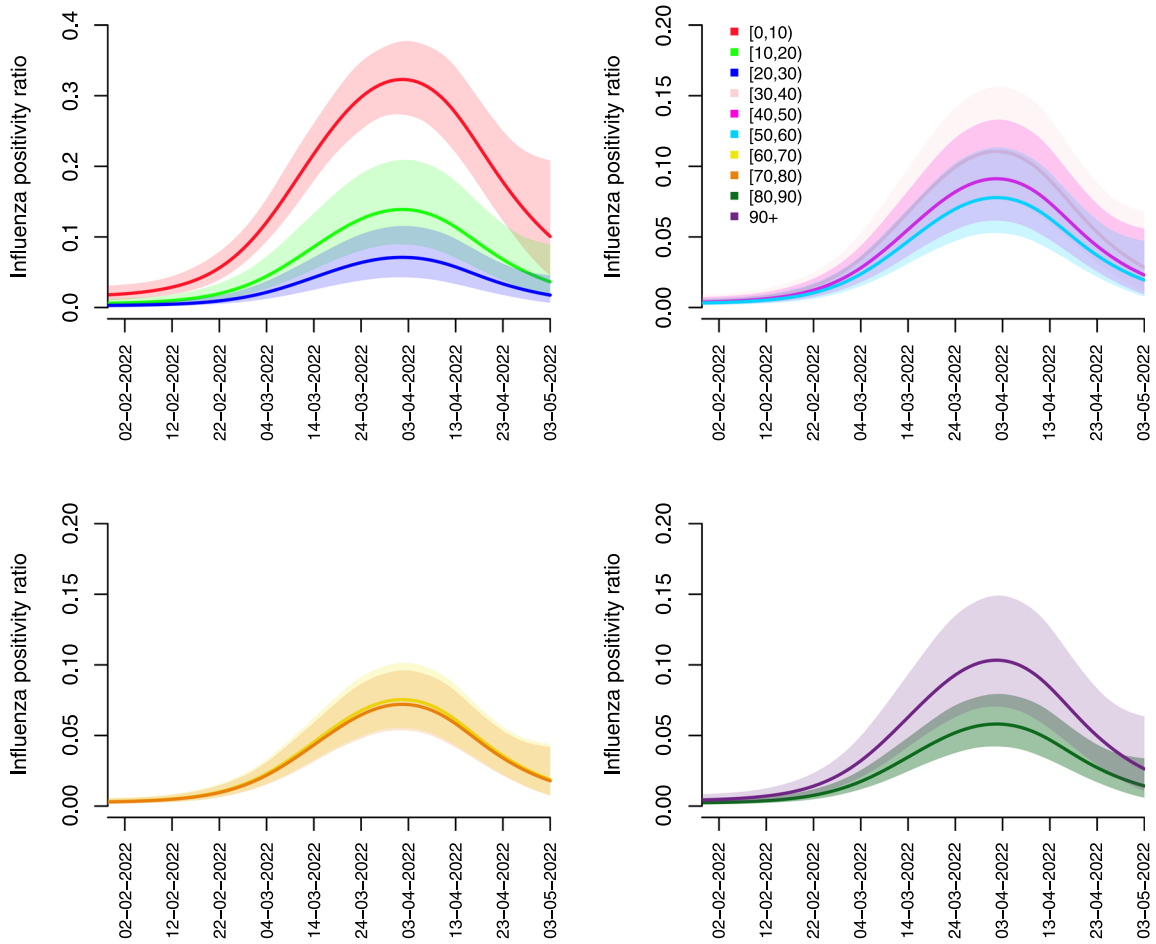

FIGURE 3 | Estimated influenza positivity over time for different age groups based on a generalized additive model fitted to the observed data. (Age groups [0, 10], [10, 20], and [20, 30]) in the upper left panel, age groups ([30, 40], [40, 50], and [50, 60]) in the upper right panel, age groups ([60, 70) and [70, 80]) in the lower left panel and age groups ([80, 90] and 90+ in the lower right panel).

additive model (including time and age as fixed effects). From the graph, it is clear that the youngest age group (i.e., 0–9 years old) experienced the highest influenza positivity over time, followed by the 10-19 year age group (left upper panel). Differences between age group 0–9 and all other age groups were significant at an overall 5% significance level. Furthermore, the positivity ratio was significantly different between 10 and 19 year olds and 80-89 year olds (chi-square test two-sided adjusted p-value = 0.034), which was not the case when pairwise comparing other age groups after multiplicity adjustment. Allowing for age-dependent differences in the evolution of the positivity ratio over time did not outperform a model without such an interaction effect between age and time (AIC = 2820.59 vs. 2829.75); thus, a more rapid increase in positivity ratio in the younger age groups, although visually apparent from Figure 2, is not statistically significant. Furthermore, no significant differences in time-dependent positivity ratio between males and females (chi-square test two-sided p-value = 0.793) nor between different hospitals (chi-square test two-sided p-value = 0.484) were observed.

3.3 | Symptomatology of Influenza Cases

The highest number of influenza cases displayed mild symptoms (i.e., 178 cases; 43.3%, 95% CI: 38.6%–48.1%) at the time of hospital admission while only 10 out of 411 influenza positive cases were critically ill at admission (see Table 1). The age-dependent proportion of influenza cases by symptom category is presented in Figure 4. Individuals in older age groups have more severe symptoms at hospital admission as compared to younger individuals (one-sided p-value < 0.001 with Jonckheere-Terpstra trend test). In Supplementary Figure S4, we graphically depict the proportion of influenza cases of each symptom severity class in each of the age groups considered.

The individual disease progression in terms of symptoms is visualized using a contingency table showing symptoms at the time of admission versus the worst clinical symptoms during hospital stay (Table 2). Most patients (381 out of 411; 92.7%, 95% CI: 89.7%–94.8%) did not progress to a more severe symptom category throughout the hospitalization period, although 4 out of 13 patients (30.8%, 95% CI: 12.7%–57.6%) who died in the hospital initially displayed mild or moderate symptoms.

In the supplementary data (Supplementary Figure S3), the evolution of symptomatology over time is presented. Some fluctuations in symptomatology are observed which appear to indicate an aggravation of symptoms during the epidemic, however, these differences were not significant.

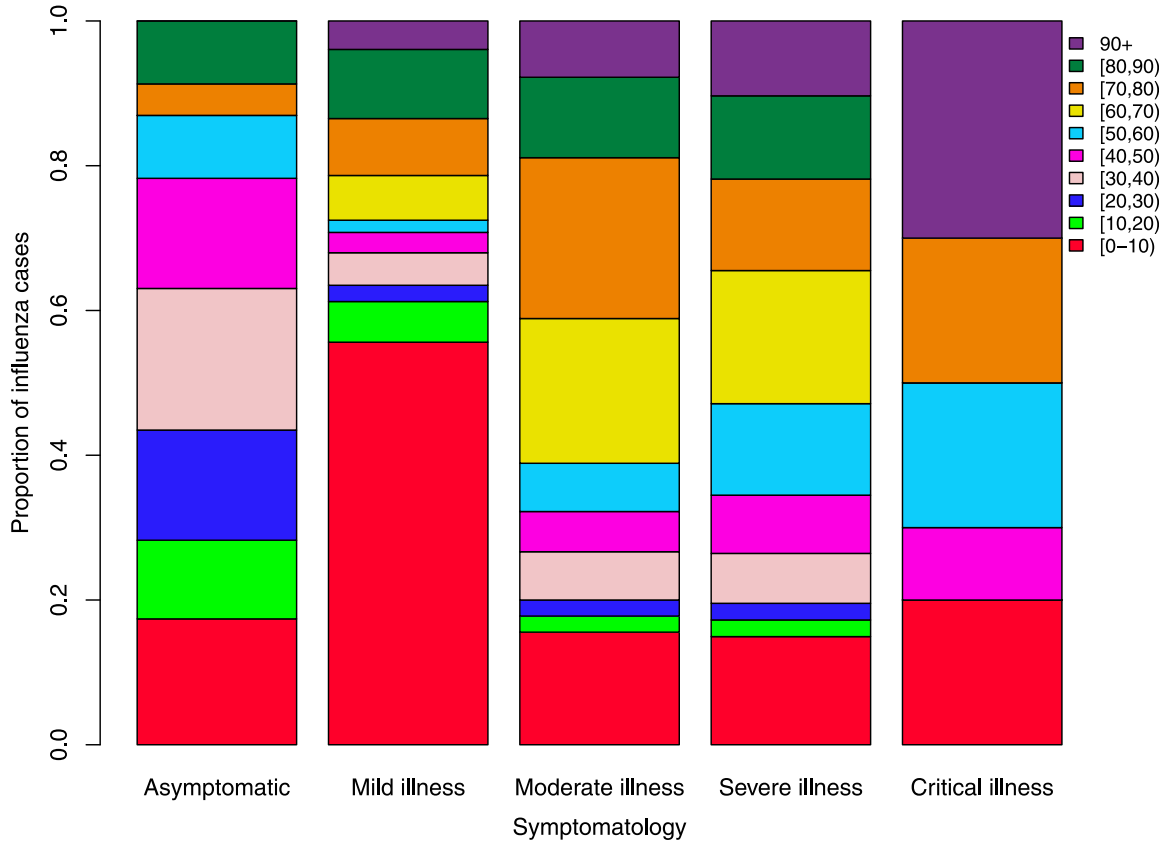

FIGURE 4 | Age-dependent proportions of influenza cases by symptomatology at hospital admission and age group.

TABLE 2 | Displaying the number of patients within each symptom category, with the accompanying number of patients who experienced no worsening of their influenza symptoms, experienced worsening of their influenza symptoms but survived and patients who deceased during hospital stay.

Symptom category at admission	Total	No worsening of symptoms during hospital stay		Worsening of symptoms during hospital stay, but no decease		Deceased during hospital stay	
	N	*n*	%	*n*	%	*n*	%
Asymptomatic	46	44	95.65	2	4.35	0	0.00
Mild symptoms	178	171	96.07	5	2.81	2	1.12
Moderate symptoms	90	83	92.22	5	5.56	2	2.22
Severe symptoms	87	77	88.51	5	5.75	5	5.75
Critical symptoms	10	6	60.00	0	0.00	4	40.00
Total	411	381	92.70	17	4.14	13	3.16

3.4 | Ct-Values in Relation to Age, Sex, Vaccination Status, and Symptomatology

In Figure 5, we present boxplots of the observed Ct-values by symptomatology at hospital admission and by vaccination status (i.e., no registered vaccine, vaccinated, or unknown). In total, for 118 out of 411 patients (i.e., 28.7%) the national vaccination platform could not be consulted, while 40 patients (i.e., 9.7% of all positive patients) were vaccinated. For patients without a registered vaccine or for whom the vaccination status could not be consulted the mean Ct-values were 24 (95% CI: 22–26) for asymptomatic, 21 (95% CI: 20–22) for the mild symptomatic, 24 (95% CI: 22–25) for the moderate symptomatic patients, as compared to 30 (only 1 case) for the asymptomatic, 23 (95% CI: 10–28) for the mild symptomatic and 25 (95% CI: 22–28) for the moderate symptomatic patients in the vaccinated group. Given the low number of individuals with a registered vaccine in this study, the observed differences in mean (and median) Ct-value between vaccinated and patients without a registered vaccine were not statistically significant.

Studying Ct-values in relation to the symptomatology at admission (Supporting Information S1: Figure S1), no monotonically decreasing pattern in median and mean Ct-value is observed for increasing symptom severity (Jonckheere-Terpstra trend test p-value = 0.801). Despite a nonsignificant decreasing evolution in the median

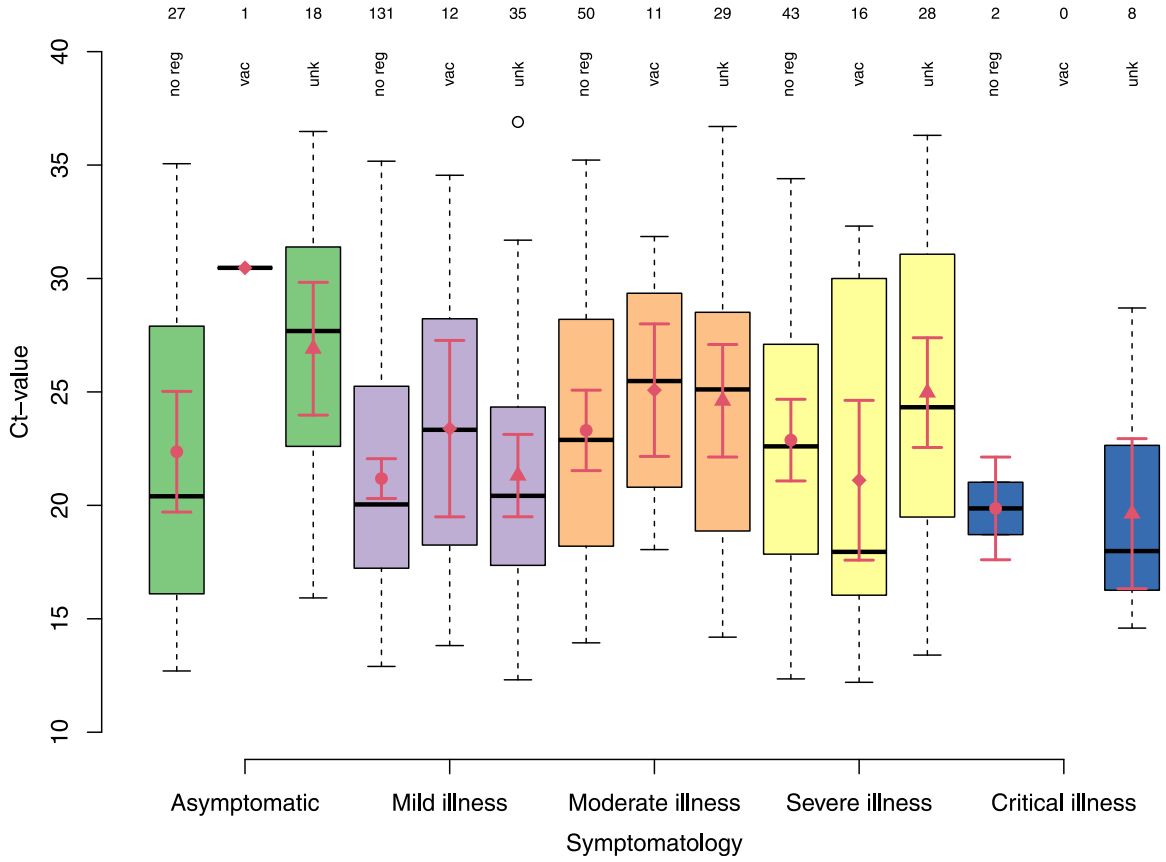

FIGURE 5 | Boxplots of the observed Ct-values by symptomatology category at hospital admission and vaccination status (i.e., no registered vaccine, vaccinated or unknown patient). Mean Ct-values are represented by red dots ("no reg": no registered vaccine), red squares ("vac": vaccinated patients) or red triangles ("unk": unknown patient) with corresponding 95% Wald-based confidence intervals (red error bars). (Green boxes: asymptomatic patients, purple boxes: patients with mild illness, orange boxes: patients with moderate illness and blue boxes patients with critical illness at admission).

Ct-value, a non-parametric Kruskal-Wallis test (given deviations from normality for Ct-values conditional on symptomatology) signals significant differences (at 5% significance level) among median Ct-values in different symptomatology groups. Pairwise comparisons using the Wilcoxon rank sum test, with Holm-Bonferroni correction for multiple testing, only indicated a significant difference in median Ct-value between patients with mild and moderate symptoms (two-sided adjusted p-value = 0.016) at an overall 5% significance level. The relation between observed Ct-values and most severe symptomatology during hospital stay is also depicted in Supporting Information S1: Figure S2.

In a multivariable linear regression model used to assess the association between mean log10-transformed Ct-values on the one hand, and age (groups), sex, vaccination status, and symptomatology on the other hand, while correcting for potential differences between hospitals, no significant interaction effect between hospital and vaccination status (F-test two-sided p-value = 0.809) and between sex and vaccination status (F-test two-sided p-value = 0.472) was seen. Furthermore, in the reduced model (i.e., the model without these two interaction effects), no significant association between mean (log10-transformed) Ct-value and vaccination was observed (F-test two-sided p-value = 0.686). Furthermore, no conclusive evidence was obtained for any structural differences in mean log10-transformed Ct-value across different symptom groups, even when correcting for differences between hospitals.

Among male patients, estimated mean (log10-transformed) Ct-values were significantly different in patients aged between 40 and 50 years of age (t-test two-sided p-value = 0.042) and patients between 70 and 80 years of age (t-test two-sided p-value = 0.012) as compared to the mean Ct-value in boys below 10 years of age. For females, however, only the age group between 80 and 90 years of age showed a higher mean Ct-value as compared to girls less than 10 years of age (t-test two-sided p-value < 0.001).

3.5 | Influenza SARS-CoV-2 Co-Infections

In total, 35 patients tested positive for both influenza and SARS-CoV-2 (Table 1). The ratio of asymptomatic and mild symptomatic patients in this group was 8.6% (95% CI: 3.0%–22.4%) and 34.3% (95% CI: 20.8%–50.8%). These patients did not have more severe symptoms compared to symptoms of other influenza positive patients (p-value = 0.603).

3.6 | Classification in Low or High Ct-Value Based on Symptomatology, Age and Vaccination Status

In total, 253 influenza positive cases (61.6%, 95% CI: 56.8%–66.1%) had a low Ct-value (i.e., a Ct-value < 24) (see

Table 1) of which 125 (49.4%, 95% CI: 26.2%–35.0% of all positive cases) showed mild symptoms. Amongst all symptom categories, patients with a low Ct-value were most prevalent as compared to those with high Ct-values (Table 1).

Looking at the predictive ability to classify subjects into high (≥ 24) or low (< 24) Ct-values (based on the logistic regression model) the optimism-adjusted area under the ROC curve (AUC) equals 0.655 (95% CI: 0.612–0.698) and accuracy was 0.645 (95% CI: 0.608–0.684). Moreover, the probability of correctly classifying a patient with low Ct-value (optimism-adjusted sensitivity) based on a cut-off value of 0.5 (for the model predictions) was equal to 0.759 (95% CI: 0.711–0.803). Optimism-adjusted specificity using the same threshold was found to be suboptimal with a value equal to 0.466 (95% CI: 0.394–0.537). Consequently, about 24.1% (95% CI: 20.0%–28.9%) of the highly infectious patients (with Ct-value < 24) would be incorrectly classified as having a high Ct-value based on this model. Optimal threshold values which balance sensitivity and specificity would lead to higher specificity, however we decided to focus on optimal sensitivity to correctly detect patients with low Ct values. This analysis shows that without screening of patients at the time of hospital admission, a considerable number of individuals could incorrectly be classified as being not infectious.

4 | Discussion

To the best of our knowledge, this is the first study to investigate asymptomatic influenza A/B infections amongst hospitalized patients in view of infection control.

In the weeks preceding the 2021–2022 epidemic, which was categorized by the European Center of disease Prevention and Control (ECDC) as mild, the positivity ratio of systematic screenings in our study cohort was low and below 1% [26]. In the period near the epidemic threshold, the ratio rapidly increased ending up at a positivity ratio of 12% at the peak of the epidemic (week 14). In the same period, the influenza positivity ratio in ECDC sentinel specimens of patients with influenza like illness, evolved from 10% at the epidemic threshold, toward 30%–40% at the peak [27].

In total 9327 subjects were included. As the mean age of the study population is 57.0 years, the included patients are older compared to the Belgian citizens where the mean age is 42.0 years [28]. With 51.4% men in the study, the male/female ratio is higher than in the general Belgian population where 49.3% population is male [29]. Since patients were included based on their visit to the emergency department with hospitalization regardless the reason for hospitalization, it could be stated that the results are representative of hospitalized patients during the influenza epidemic in Belgium.

Interestingly, the temporal positivity ratio in our study cohort appeared to be, although not statistically significant, age-dependent with younger age groups (infants, children, and adolescents) presenting at the hospital earlier in the epidemic course as compared to the elderly (90+ age group). From our data, we cannot conclude whether these findings reflect differences in population positivity ratios, or whether it predominantly reflects a different pattern of disease evolution and admission indication in the different age groups. One might hypothesize that the pediatric population is more prone to admission earlier in the disease course because of presentation with more acute symptoms of a viral infection (e.g., feverish child with convulsions). Elderly people might present later after a prior viral infection because of admission reasons related to more posterior infectious sequelae, overall deterioration, and comorbidities.

Importantly, the results of our study suggest a substantial underestimation of positive cases by following a symptom-based testing strategy [11, 17]: significant numbers of influenza cases were either asymptomatic ($n = 44$; 11.2% of total number of positive cases) or mildly symptomatic ($n = 173$; 43.3% of total number of positive cases). Only 2.4% of cases ($n = 10$) were critically ill upon hospital admission. Since reimbursement of influenza testing in Belgium is based on a symptomatic approach and directed by individual patient care, patients are not routinely screened [30]. When only patients presenting with moderate or severe disease (corresponding to the severe acute respiratory infection definition of ECDC) are tested, more than half of the patients presenting with influenza asymptomatic carriership upon hospital admission will be missed, thereby leading to suboptimal hospital quarantining and isolation strategies. To the best of our knowledge, a screening strategy for influenza is also not part of testing protocols and guidelines in other European countries.

When looking at disease progression, only a minority of the asymptomatic patients at admission developed symptoms during hospitalization, thereby thus not pointing towards substantial presence of presymptomatic asymptomatic carriership. Similar conclusions were found by Hermes et al [31]. Moreover, in 93% (381 out of 411 cases), the symptoms reported at the time of hospital admission were indicative of the most severe symptoms observed during the entire hospitalization period.

Further, our results show that symptomatology at hospital admission (or during hospital stay) does not provide a good indication of the individual-specific Ct-value, used as a proxy of viral load: we only found an indication of a significant difference ($p < 0.05$) in median Ct-value between patients with mild and moderate symptoms. This finding is in contrast with earlier published findings which showed that viral loads of influenza in mild symptomatic patients were lower as compared to more severely ill patients in both hospitals and ambulatory settings [32–34]. When making an arbitrary distinction of high viral loads at a Ct-value < 24, we found overall high proportions ($> 50\%$) of high viral loads of positive cases as per symptom category. In the absence of general screening of (asymptomatic) patients, patients with low Ct-values admitted to the hospital potentially might lead to a substantial number of nosocomial infections as their infectiousness would be unknown.

Even in the hypothetical case that asymptomatic and paucisymptomatic cases are less infectious, it remains doubtful that detecting (and isolating, thereby following international guidelines) only about half of all patients is effectively prohibiting nosocomial transmission and preventing outbreaks. Since isolation of positive cases represents a high economical and psychological burden, we

argue for setting up rigorous prospective trials establishing transmission dynamics in settings of vulnerable patients and taking into account the relevance of the asymptomatic, presymptomatic and pauci-symptomatic carriers.

Interestingly and of importance for public health measures, the impact of vaccination status on influenza infections was investigated in this study. Since only 40 registered vaccines were found, no firm conclusions could be made based on vaccination status. In Belgium vaccination registration is not mandatory, leading to a possible underreporting. Notably, 22 patients (55% of the vaccinated) had a Ct-value < 24 and 27 patients (67.5% of the vaccinated) showed up with moderate or severe symptoms. Based on this, a conclusion that can be made is that vaccines do not protect subjects from having a high viral load, nor having moderate or severe symptoms. A remark to be made about the conclusion on symptomatology is that the median age in the vaccinated group was 70.5 years (IQR: 42.3–84.5 years), while in the unvaccinated group the median age was 38.0 years (IQR: 3.4–71.0 years). Given the fact that the vaccination status in the influenza negative group is not investigated in this study, it is not known to what extent influenza infections in vaccinated patients are due to immunosenescence or the vaccine itself. The reduced vaccine efficacy in vulnerable groups such as the elderly has been documented previously [35].

Based upon our data, we were not able to establish a successful classification rule for patients with low versus high Ct-values based on symptomatology, age and vaccination status. This underlines the inability to adequately differentiate between patients with and without high viral load at hospital admission when focusing on symptomatology only. Therefore, this finding is important for directing testing strategies in the hospitals or even public health settings. Since point-of-care testing is becoming more and more accessible and affordable, we propose to seriously consider the role of point-of-care screening in terms of preventing nosocomial transmission in a hospital setting [36, 37].

Since the study was performed during the SARS-CoV-2 pandemic, we were able to study characteristics of co-infections with SARS-CoV-2: 8.5% of the positive influenza patients in our study also tested positive for SARS-CoV-2. Although various studies [14, 16] found an increase of complications and mortality rates, we did not find any significant correlation between the severity of the symptoms and a positive test for both SARS-CoV-2 and influenza. A possible contribution of SARS-CoV-2 to influenza symptoms was not executed since only 7.3% of all patients suffered from symptoms that worsened during hospital stay. As this study was performed in a patient population with high background SARS-CoV-2 immunity, symptomatology of SARS-CoV-2 infections was likely to be milder in our study than in previous studies, reported in the earlier days of the SARS-CoV-2 pandemic [15].

5 | Strengths and Limitations

The strength of this study is that this is the first time that patients were systematically screened by PCR upon hospital admission. Hereby, we got a clear insight on influenza positivity of hospitalized patients during flu season. Furthermore, more than 9000 patients were tested for influenza in this study, with a wide range of age groups and reasons for hospitalization. Hereby, a reliable cross-section of hospitalized patients was included. An important limitation is the suboptimal registry of influenza vaccinations (registration coverage of influenza vaccination not available upon request) and the lack of subtyping of influenza strains, precluding strong conclusions about the protective effect of vaccination. Another limitation is the lack of information about the symptomatology in patients that tested negative for influenza. Furthermore, this is a retrospective study which possibly leads to an underestimation of symptomatology in pauci-symptomatic patients compared to a prospective study design where patients are actively surveyed about influenza-related symptoms. On the other hand, from an infection control point of view, this retrospective study design probably gives a better estimation of the real-life hospital setting, in which patients are also not routinely asked about influenza symptoms at admission. Our results cannot be extrapolated to a population level, as the population presenting for admission to the hospital is not representative for the overall population. In addition, this study is limited to the detection of influenza and SARS-CoV-2. It could be possible that other infectious agents contributed to the symptomatology with misclassification of patients. This could underestimate the number of asymptomatic and mild symptomatic patients. As no peak of other respiratory infections was seen in Belgium during the study period, this effect can be suspected to be limited. A last limitation is the use of symptom categories defined to be applied in SARS-CoV-2 patients due to the lack of a categorization of influenza symptomatology, to the best of our knowledge.

6 | Conclusion

During the Influenza wave of 2021–2022, more than half of all influenza positive patients admitted to hospital were either asymptomatic or pauci-symptomatic. Viral loads were not significantly different between the different symptomatic groups. Being vaccinated did not exclude the possibility of asymptomatic carriership. Our findings might be useful to take into consideration for further optimizing the testing policy and the non-pharmaceutical infection prevention measures in a hospital setting.

Author Contributions

Study conception: Evelyne Huyghe and Reinout Naesens. Data collection and project administration: Emmanuel André, Kurt Anseeuw, Eva Bernaert, Peggy Bruynseels, Lize Cuypers, Pieter De Schouwer, Petra Hilkens, Els Keyaerts, Lies Laenen, Justine Maes, Koen Magerman, Otto Van de gaer, Ann Verdonck, and Walter Verstrepen. Data analysis: Steven Abrams and Sien Ombelet. Manuscript writing: Evelyne Huyghe, Steven Abrams, Sien Ombelet, and Reinout Naesens. Manuscript review: Emmanuel André, Kurt Anseeuw, Eva Bernaert, Peggy Bruynseels, Lize Cuypers, Pieter De Schouwer, Petra Hilkens, Els Keyaerts, Lies Laenen, Justine Maes, Koen Magerman, Otto Van de gaer, Ann Verdonck, and Walter Verstrepen.

Ethics Statement

This study was approved by the ethical committees of the three contributing centers (approval number 5695; approved by the UZ/KU

Leuven Ethical committee for research [S67104]). ZNA acted as the main investigator. A data transfer agreement was concluded with UZ Leuven and Jessa Hospital.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data is available and can be requested from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.