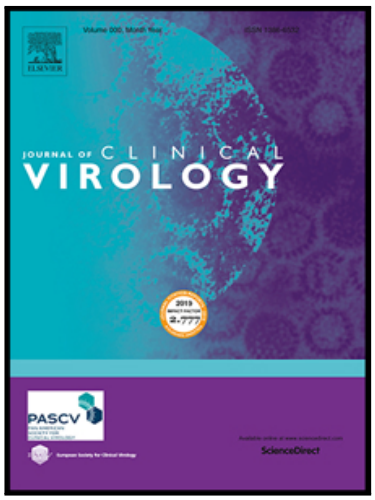

Clinical and Phylogenetic Influenza Dynamics for the 2019-20 Season in the Global Influenza Hospital Surveillance Network (GIHSN) – pilot study

Grégory Quéromès , Emilie Frobert , Elena Burtseva , Anca Drăgănescu , Paravaiz A. Koul , Andrey Komissarov , V Alberto Laguna-Torres , Jason Leblanc , F-Xavier López-Labrador , Snežana Medić , Alla Mironenko , Nancy A. Otieno , Guillermo M. Ruiz-Palacios , Tanriover MD , NGS team - Lyon , GIHSN collaborators , Laurence Josset , Bruno Lina

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Conflict of interest

The funders had no role in the data analysis, interpretation or writing of this report. More details on FIE and its governance can be found on www.gihsn.org. SSC is an employee of Sanofi Pasteur who is part-time seconded to the FIE where she contributes to the GIHSN scientific activities. AD has received grants or contracts from the EU/EFPIA Innovative medicine Initiative 2 Joint Undertaking. AK has received grants or contracts from Sanofi Pasteur. JS has received grants or contracts from Merck Inc, Pfizer Canada, Glaxo Smith Kline, and Sanofi Pasteur. FXL has received grants or contracts from Sanofi Pasteur and Instituto de Salud Carlos III. TMD has received grants or contracts from Sanofi Pasteur. SM serves on the Republic Commission for Infectious Diseases of Republic of Serbia. BL serves as chair of the scientific committee of Immuniser Lyon and co-chair of the Global Influenza Initiative. GQ, EF, EB, PK, VLT, AM, NO, GRP, and LJ have no conflicts of interest to declare.

Ethics approval and patient consent

The core protocol used by the GIHSN in this study was conducted in accordance with the Declaration of Helsinki and approved by all ethics committees from each contributing site. Informed consent was obtained from all subjects involved in the study or from their legal representatives.

Authors' contribution

Conceptualization, GIHSN principal investigators; Investigation, all authors; Formal analysis, G.Q., E.F., and B.L.; Writing-original draft preparation, G.Q., E.F., and B.L.; Writing-review and editing, all authors; Supervision, B.L. All authors have read and agreed to the published version of the manuscript.

HIGHLIGHTS

- To date, the GIHSN counts the collaboration of hospital sites in over 19 countries.
- For the 2019-20 flu season, influenza B infected a higher proportion of younger individuals <50 years.
- Hospitalized cases show large A(H1N1)pdm09, A(H3N2), and B/Victoria-like clade diversity.

ABSTRACT (239 words)

Background

The Global Influenza Hospital Surveillance Network (GIHSN) has operated with the aim of investigating epidemiological and clinical factors related to severe influenza-related hospitalisations.

Study design

A common GIHSN core protocol for prospective patient enrolment was implemented. Hospital personnel completed a standardized questionnaire regarding the included patients' medical history, compiled a hospitalisation summary, collected an upper respiratory swab sample for laboratory diagnosis, and genome sequencing was performed for a subset of samples. Patient data were compared according to influenza subtype, lineage, and phylogenetic groups using the Fisher's exact test.

Results

From September 2019 to May 2020, 8,791 patients aged ≥5 years were included. Among them, 3,021 (34.4%) had a laboratory-confirmed influenza diagnosis. Influenza A(H1N1)pdm09 dominated the season among all age groups, while the B/Victoria-like lineage accounted for over half of the infections among younger age groups (5-49 years). Sequencing of the hemagglutinin segment was possible for 623 samples and revealed an influenza A and B clade frequency among severe influenza hospitalisations similar to other medically attended surveillance networks, such as the WHO GISRS. No phylogenetic clustering was observed among hemagglutinin substitutions depending on the administration of supplemental oxygen or vaccine failure.

Conclusions

The GIHSN confirms its ability as an international hospital-based active surveillance network to provide valuable information on influenza infection dynamics in hospital settings. Increasing the number of participating sites and compiling more complete data, such as genome sequencing, will allow the exploration of associations between viral factors, vaccine protection, and disease severity.

Keywords

influenza virus; surveillance; epidemiology; hospitalization; genome sequencing

MANUSCRIPT (2,501 words)

Background

Influenza infection is ubiquitous and human viruses (less commonly derived from zoonotic spillovers) provoke predictable annual epidemics [1,2]. Affecting approximately 5-15% of the world population annually, seasonal influenza infections are of great public health interest [3,4]. The viruses responsible for these epidemics are continuously monitored in order to understand their evolution regarding the clinical presentation, vaccine effectiveness, and antiviral resistance. Local, regional, and global surveillance networks have greatly contributed to the description of virus circulation and are instrumental for the implementation and monitoring of tools to mitigate their impact.

Since its first reports in 2013, the Global Influenza Hospital Surveillance Network (GIHSN) has strived to investigate a thorough assessment of influenza epidemiology and vaccine effectiveness in a multicentre hospital-based setting [5]. This public-private collaboration system maintains an active surveillance network of hospital cases that has continued to expand since its foundation, as 13 of the 18 World Health Organization (WHO) influenza transmission zones are represented so far [6]. Every year, the GIHSN reviews new site applications and evaluates their eligibility to join according to their experience in hospital-based surveillance, geographical representativeness, and ability to collect and share patient and respiratory sample data.

Using a standardized protocol that allows for collaborative data pooling from multiple hospital networks, the GIHSN has been able to report annually the patterns of influenza-related hospitalisations at a global scale. Past studies from the GIHSN have described the

epidemiological distribution of influenza virus depending on patient age, chronic conditions, vaccination status, and hospital care [5,7,8].

Objectives

We first aimed to report the epidemiological dynamics of the 2019-20 season at the GIHSN scale for the influenza-associated hospitalisation of patients aged over 5 years. The second objective was to describe the phylogenetic characterization of the viruses responsible for these hospitalisations for the first time within our network, with a specific focus on the representativeness of these cases compared to other medically attended influenza surveillance networks.

Study Design

Coordinating GIHSN sites (n=19; Supplementary Data 1), each being part of the GIHSN and managing the participation of a regional network of hospitals and medical institutions, share a common core protocol for patient eligibility and data collection that was reviewed by the ethics committee of each institution [5]. Patients aged over 5 years admitted to hospital for acute respiratory illness in the previous 72 hours between September 2019 and May 2020 were considered eligible. Inclusion criteria were the presentation of influenza-like illness (ILI) within the 7 days prior to hospital admission and informed consent signed by the patient, family member, or legal representative. An ILI clinical case was characterized by at least one systemic symptom (fever, malaise, headache, myalgia) and at least one respiratory symptom (cough, sore throat, shortness of breath) [9,10]. Hospital personnel completed a standardised questionnaire regarding patient history, compiled a hospitalisation summary, and collected a nasopharyngeal swab sample. Categorical variables (sex, age group, comorbidities, and clinical parameters) were compared between flu-positive and flu-negative patients, as well as between influenza subtype or lineage, using the Fisher's exact test.

Quantitative variables were compared using Mann-Whitney test. The patient factors considered were the vaccination status and supplemental oxygen administration. A patient was considered fully vaccinated if the vaccine was administered over 14 days before symptom onset.

Each site submitted the respiratory samples to their reference laboratories for influenza diagnosis by reverse transcription polymerase chain reaction. Influenza A subtype or B lineage classification was documented when available. When possible, next-generation sequencing (NGS) was then performed using local standardised protocols. The coordinating sites without NGS capacity sent RNA extracts on dry ice to the *Centre national de référence des virus des infections respiratoires (dont la grippe)* in France (Lyon) for genome sequencing to be performed with Illumina reagents, as previously described [11].

After sequencing, nucleic acid alignment of the hemagglutinin (HA) segment was performed with the muscle alignment program and UPGMA cluster method on MEGAX software (version 10.1.8) [12]. Phylogenetic tree construction was performed using optimized substitution models according to Bayesian Information Criterion (K3Pu+F+G4 for A(H1N1)pdm09, TVM+F+I for A(H3N2), and HKY+F+G4 for B/Victoria) followed by ultrafast bootstrap analysis (10,000 replicates) on the IQ-TREE web server [13]. Tree annotation was then completed with iTOL (version 6.3) and Inkscape (version 1.1) [14]. Comparative frequency distribution of influenza clades, subclades, and genetic groups between the GIHSN samples and the wider WHO Global Influenza Surveillance and Response System (GISRS) via GISAID [15] was analysed using Fisher's exact test. In addition, the phylogenetic trees of the GIHSN cases were compared to those provided by the WHO Collaborating Centres to identify differences between the genetic profiles of the WHO (GISRS) and those of the GIHSN surveillance. Vaccine failure cases and/or those requiring oxygen were reported for potential identification of specific substitutions/genetic profiles.

The comparative occurrence of vaccination status or oxygen administration against known subclade/genetic groups was performed using the Fisher's exact test.

Results

Patients

A total of 8,791 patients across the 19 coordinating sites were included (Figure 1; Supplementary Data 2). Regardless of age, 5,001 (56.9%) patients had at least one underlying chronic condition, but the proportion of hospitalised patients having multiple conditions seemed to increase with age. A total of 4,229 (48.1%) patients were aged >50, 2,870 (32.6%) had a cardiovascular disease, 1,169 (13.3%) had a chronic obstructive pulmonary disease, and 1,239 (14.1%) had diabetes (Supplementary Data 3).

Overall, 3,021 (34.4%) patients had a laboratory-confirmed influenza diagnosis; among them, 852 (28.2%) had a cardiovascular disease, 355 (11.8%) had diabetes, 279 (9.2%) had a chronic obstructive pulmonary disease, and 210 (7.0%) had asthma, and these proportions were similar regardless of A subtypes or B/Victoria-like lineage. Vaccination status was known for 2,905 influenza-positive patients and was complete for the current season for 399/2,905 (13.7%) of them. Supplemental oxygen without mechanical ventilation was administered to 712/2,850 (25.0%) influenza-positive patients and 1,308/4,566 (28.6%) influenza-negative patients ($p<0.05$). The use of supplemental oxygen was less frequently observed among B/Victoria-like lineage cases (105/551, 19.1%; $p<0.05$) compared to other A sub-typed influenza-positive cases, but this population was also significantly younger. Death during hospitalisation was reported in 83/3,003 (2.8%) influenza-positive cases and 251/5,730 (4.4%) influenza-negative cases ($p<0.05$) and was similar regardless of A subtypes or B/Victoria-like lineage ($p>0.05$; Table 1). Considering influenza-positive patients, death

was more frequent among patients requiring the use of supplemental oxygen compared to those who did not ($p<0.0001$).

Epidemiological analysis

The first cases were reported at week 36 (September 2019) and remained rare until week 48 (end of November). From week 49 (December 2019), a sharp increase in the number of cases was observed, reaching a peak by week 5 (February 2020), and subsequently declining from week 8 until the last detections by week 16 (Figure 2A).

Influenza A dominated the 2019-20 season: there were 1,814 (60.0%) influenza A cases, 1,153 (38.2%) influenza B cases, and 54 (1.8%) not typed influenza A or B cases (Figure 2A). Within type A influenza, there were 1,088/1,814 (60.0%) A(H1N1)pdm09 cases, 245/1,814 (13.5%) A(H3N2) cases, and 481/1,814 (26.5%) not subtyped cases. Within type B influenza, there were 559/1,153 (48.5%) B/Victoria-like cases, 4/1,153 (0.3%) B/Yamagata-like cases, and 590/1,153 (51.2%) unclassified cases (Table 1). The distribution of influenza viruses varied between geographic regions, but most sites in the Northern hemisphere, representing 2,588/3,021 (85.7%) cases, observed similar patterns of influenza A and B co-circulation, and the median [IQR] A/B ratio was 4.2 [1.3-5.8] for the Northern hemisphere. Furthermore, A(H1N1)pdm09 and A(H3N2) also co-circulated in most sites, and the median [IQR] H1/H3 ratio was 5.6 [2.1-11.1] for the Northern hemisphere. In the inter-tropical region, representing 415/3,021 (13.7%) cases, there also was influenza A/B co-circulation, and the median [IQR] H1/H3 ratio was 2.1 [0.8-6.9] for this region. There were 18/3,021 (0.6%) positive cases from the Southern Hemisphere, with mostly A(H1N1)pdm09 (Supplementary Data 2 and 4). Overall, influenza B accounted for 965/1,914 (50.4%) cases within the younger population (aged 5 to 49 years), as compared to 188/1,107 (17.0%, $p<0.05$) cases from patients aged $\geq$50 (Figure 2B). The large absence of type A subtyping in

the older groups (up to 43% among the ≥85 years) hampers the description of H1/H3 distribution.

Genome sequencing

As of 25 May 2021, 623 HA sequences (20.6% of influenza-positive) were generated (GISAID accession numbers available in Supplementary Data 5), corresponding to 343 (55.1%) A(H1N1)pdm09, 93 (14.9%) A(H3N2), 187 (30.0%) B/Victoria-like, and 1 (0.1%) B/Yamagata-like viruses, all originating from the Northern hemisphere.

Influenza A(H1N1)pdm09

Phylogenetic analysis of the HA genes from A(H1N1)pdm09 viruses showed that all belonged to the 6B.1A clade and harboured the S183P substitution (Figure 3). The distribution of A(H1N1)pdm09 clades was similar to the publicly available GISAID data submitted mostly by GISRS labs. More specifically, within the 6B.1A clade, 5 subclades were identified: 6B.1A.5a, 6B.1A.5a.1, 6B.1A.5a.2 (the latter two nested within the 6B.1A.5a subclade), 6B.1A.5b, and 6B.1A.7, representing 95.0% vs 88.6%, 38.8% vs 38.4%, 14.3% vs 15.6%, 3.8% vs 2.8%, and 1.1% vs 2.0% (GIHSN vs GISAID) of the viruses sequenced, respectively (Supplementary Data 6). Only the clade 6B.1A.5a was slightly more represented in the GIHSN population ($p<0.05$).

Influenza A(H3N2)

Six major A(H3N2) genetic groups could be detected in the GIHSN population (Figure 4). More specifically, the distribution of clades 3C.2a1b.1, 3C.2a1b.2a, and 3C.2a1b.2b was similar to the publicly available GISAID data, representing 39.8% vs 32.7%, 14.0% vs 18.0%, and 5.4% vs 9.7% (GIHSN vs GISAID) of A(H3N2) sequences, respectively. Yet, the clades 3C.3a and 3C.2a1b.1b were over-represented in the GIHSN samples (40.9% vs 12.9% and

33.3% vs 3.2%, respectively; p<0.05). In contrast, the clade 3C.2a1b.1a was under-represented in the GIHSN population (6.5% vs 16.0%; p<0.05; Supplementary Data 6).

Influenza B

The phylogenetic analysis of the HA genes from B/Victoria-like sequences showed that all circulating viruses belonged to the genetic clade 1A (Figure 5). More specifically, they all fell in the genetic group 1A(Δ3)B (Δ162-164 triple deletion). The V1A.3/220M genetic group detected in the present study represented 3.7% vs 3.2% (GIHSN vs GISAID) of B/Victoria-like sequences, while the V1A.3/133R and V1A.3/126K were over-represented in the GIHSN population (83.7% vs 66.4% and 9.1% vs 4.0%, respectively; p<0.05; Supplementary Data 6). The ancestral V1A.3 and V1A.3/150K genetic groups, as well as the more recent V1A.3/144L group, were not observed among GIHSN sequenced samples but represented 17.3%, 7.0%, and 2.0% of GISAID B/Victoria-like sequences, respectively, during the same period.

For the B/Yamagata-like lineage, the single sample sequenced belonged to clade 3 (172Q substitution).

Antigenic escape and vaccine failure

The vaccination status of patients was related to their respective influenza HA sequence, allowing for vaccine escape analysis. Within the subset of sequenced samples, 31/332 (9.3%), 9/91 (9.9%), and 18/186 (9.7%) patients infected by A(H1N1)pdm09, A(H3N2), and B/Victoria-like viruses, respectively, were considered fully vaccinated before hospitalization. These cases were distributed across all subclades and no particular clustering was observed (Figures 3, 4, and 5).

Disease severity and supplemental oxygen

Among the sequenced samples, there were 56/290 (26.8%), 23/84 (27.4%), and 21/179 (11.7%) patients infected by A(H1N1)pdm09, A(H3N2), or B/Victoria-like virus, respectively, who required the use of supplemental oxygen. Furthermore, these cases were also distributed across all subclades with no particular clustering (Figures 3, 4, and 5).

Discussion

Data of the 2019-20 season constitute a continuation of the GIHSN efforts from previous seasons, effectively illustrating the dynamics of severe cases of influenza infection in hospital settings. The emergence of SARS-CoV-2 in China by late 2019, its exponential spread in most GIHSN sites by early 2020, regional lockdowns, and the use of non-pharmaceutical interventions undeniably influenced the dynamics of influenza infection for the end of the 2019-20 season. Indeed, the detection of influenza cases was abruptly stopped in some GIHSN sites, as well as in the WHO European Region [16], and traditional seasonal flu surveillance and hospital resources were disrupted; as such, it is important to acknowledge that the data presented herein for the 2019-20 season may be subject to collection bias.

Moreover, the present study took place during the first season of sequencing data collection from virus samples within the GIHSN, after a feasibility study was conducted in 2018-19 [17]. Technical and operational constraints were encountered, such as the requirement for high-quality samples for sequencing and GIHSN sites that were still in the early stages of establishing their genome sequencing facilities. This is reflected by the low proportion of patient samples actually sequenced in this study. In addition, the wide spectrum of resources and practices across the geographically diverse network led to the exclusion of some data from the analysis and to a heterogeneity in patient care. Also, data are incomplete for some sites that began patient screening and data collection relatively late during their influenza period. Even so, the diversity of the institutions participating in both surveillance and

sequencing, and the richness of the linked patient data, constitute strengths built on the established infrastructure of the GIHSN. For the first time, we were able to report the frequency of clade-specific viruses from influenza-positive hospitalised patients related to patient data, allowing viral factors to be included in the analysis of clinical progression, as well as in the challenges of vaccine strain selection for the upcoming season.

Indeed, vaccine failure cases did not seem to be found exclusively within specific genetic groups for which poorer antigenic recognition against the 2019-20 vaccine strain selections has been documented, such as 6B.1A.5a.2 against the A/H1N1/Brisbane/02/2018-like strain or 3C.2a1b against the A/H3N2/Kansas/14/2017-like strain [18]. Regarding influenza B circulation, the trend observed was similar to real-world observations reporting a majority of B/Victoria-like viruses [19,20]. In recent years, influenza B viruses have become more evolutionarily diverse and epidemiologically present, and therefore also contribute to the growing challenge of vaccine selection [21].

Surveillance networks, such as the US CDC, European Influenza Surveillance Network via ECDC, and GISRS via WHO, monitor medically attended influenza-associated illness both in out-patient and in-patient settings [22,23]. The viral frequencies reported herein were similar to those observed by different surveillance networks. In addition, supplemental oxygen requirement, considered as a proxy for disease severity, did not seem to be specifically related to any of the influenza A or B hemagglutinin genetic groups. The tendency observed for the A(H1N1) 6B.1A.5b and the A(H3N2) 3C.3a clades could be discussed, but the relatively small number of sequenced samples and the limited contribution of sites impeded any robust extrapolation to be drawn. Overall, it could be argued that disease severity is mainly driven by innate host factors [24], as reported in about 20% of COVID-19-related severe respiratory distress cases with underlying host immune anomalies, such as the presence of anti-interferon autoantibodies [25,26].

Reinforcing the GIHSN network with more thorough and standardised data from each participating site, such as the newly included whole genome sequencing, offers the opportunity to obtain a more complete understanding of influenza-associated hospitalisations. Influenza-infection-related hospitalisations will keep on occurring for the years to come, especially considering the inconsistent yet generally modest vaccination coverage rate achieved worldwide. More immediately, we now face a unique challenge in responding to the reestablishment of influenza after a disrupted 2019-20 season and an astonishingly low 2020-21 activity, and thus the GIHSN presents a formidable operational platform to evaluate influenza evolution and the interventions necessary to better prepare and treat severe outcomes.

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Table 1. Characteristics of patients included in the GIHSN study for the 2019-20 season (02 Sep 2019-23 May 2020), according to the influenza laboratory diagnosis (total negative, total positive, by influenza A subtype and B lineage)

	Influenza laboratory diagnosis			Influenza type A						Influenza type B					
	Negative	**Positive**		**A(H1N1)pdm09**		**A(H3N2)**		**A not subtyped**		**B/Victoria-like**		**B/Yamagata-like**		**B not classified**	
	N=5,770	N=3,021	p-value	N=1,088	p-value	N=245	p-value	N=481	p-value	N=559	p-value	N=4	p-value	N=590	p-value
Sex, n (%)															
Male	2,777 (48.1)	1,411 (46.7)	0.1828	532 (48.9)	0.6993	127 (51.8)	0.2069	228 (47.4)	0.7056	259 (46.3)	0.3241	2 (50.0)	0.9403	243 (41.2)	0.0008
Female	2,993 (51.9)	1,610 (53.3)		556 (51.1)		118 (48.2)		253 (52.6)		300 (53.7)		2 (50.0)		347 (58.8)	
Age group (years) [a], n (%)															
5-17	707 (12.3)	682 (22.6)	<0.0001	220 (20.2)	<0.0001	98 (40.0)	<0.0001	27 (5.6)	<0.0001	162 (29.0)	<0.0001	1 (25.0)	0.4363	159 (26.9)	<0.0001
18-49	1,941 (33.6)	1,232 (40.8)	<0.0001	427 (39.3)	<0.0001	61 (24.9)	0.0049	95 (19.8)	<0.0001	290 (51.9)	<0.0001	1 (25.0)	0.7255	352 (59.7)	<0.0001
50-64	997 (17.3)	392 (13.0)	<0.0001	193 (17.8)	0.7066	33 (13.5)	0.1093	91 (18.9)	0.3302	44 (7.9)	<0.0001	0 (0.0)	0.3067	19 (3.2)	<0.0001
65-74	838 (14.5)	298 (9.9)	<0.0001	119 (10.9)	0.0015	24 (9.8)	0.0533	97 (20.2)	0.001	33 (5.9)	<0.0001	0 (0.0)	0.4097	19 (3.2)	<0.0001
75-84	744 (12.9)	231 (7.6)	<0.0001	79 (7.3)	<0.0001	18 (7.3)	0.0124	94 (19.5)	<0.0001	16 (2.9)	0.2276	1 (25.0)	0.0336	16 (2.7)	0.294
≥85	543 (9.4)	186 (6.2)	<0.0001	49 (4.5)	<0.0001	11 (4.5)	0.0083	77 (16.0)	<0.0001	14 (2.5)	<0.0001	1 (25.0)	0.286	25 (4.2)	<0.0001
Chronic conditions, n (%)															
0	2,325 (40.3)	1,465 (48.5)	<0.0001	486 (44.7)	0.0073	123 (50.2)	0.0012	95 (19.8)	<0.0001	346 (61.9)	<0.0001	0 (0.0)	0.1005	406 (68.8)	<0.0001
1	1,308 (22.7)	627(20.7)	0.0385	245 (22.5)	0.9592	52 (21.2)	0.552	95 (19.8)	0.0989	124 (22.2)	0.825	2 (50.0)	0.192	96 (16.3)	0.0002
≥2	2,137 (37.0)	929 (30.7)	<0.0001	357 (32.8)	0.007	70 (28.6)	0.0001	291 (60.5)	<0.0001	89 (15.9)	<0.0001	2 (50.0)	0.5915	88 (14.9)	<0.0001
Cardiovascular disease	2,018 (35.0)	852 (28.2)	<0.0001	311 (28.6)	<0.0001	70 (28.6)	0.032	282 (58.6)	<0.0001	87 (15.6)	<0.0001	3 (75.0)	0.0934	71 (12.0)	<0.0001
Chronic obstructive pulmonary disease	890 (15.4)	279 (9.2)	<0.0001	99 (9.1)	<0.0001	11 (4.5)	<0.0001	99 (20.6)	0.005	34 (6.1)	<0.0001	0 (0.0)	0.3931	27 (4.6)	<0.0001
Asthma	332 (5.8)	210 (7.0)	0.0255	65 (6.0)	0.7121	18 (7.3)	0.3133	64 (13.3)	<0.0001	11 (2.0)	<0.0001	0 (0.0)	0.6212	38 (6.4)	0.4638
Diabetes	884 (15.3)	355 (11.8)	<0.0001	128 (11.8)	0.002	29 (11.8)	0.1246	112 (23.3)	<0.0001	44 (7.9)	<0.0001	1 (25.0)	0.5911	32 (5.4)	<0.0001
Immunodeficiency (except HIV)/transplant	93 (1.6)	91 (3.0)	<0.0001	25 (2.3)	0.1152	9 (3.7)	0.0154	33 (6.9)	<0.0001	7 (1.3)	0.4945	1 (25.0)	0.0002	11 (1.9)	0.6875
Renal impairment	482 (8.4)	208 (6.9)	0.0132	66 (6.1)	0.0096	16 (6.5)	0.2947	70 (14.6)	<0.0001	29 (5.2)	0.0071	0 (0.0)	0.546	21 (3.6)	<0.0001
Rheumatologic disease/Autoimmune disease	0 (0.0)	0 (0.0)	NA	42 (3.9)	<0.0001	11 (4.5)	<0.0001	24 (5.0)	<0.0001	6 (1.1)	<0.0001	0 (0.0)	NA	12 (2.0)	<0.0001
Neurological or neuromuscular disease	255 (4.4)	171 (5.7)	0.0112	43 (4.0)	0.4704	13 (5.3)	0.529	73 (15.2)	<0.0001	14 (2.5)	0.0284	0 (0.0)	0.6672	21 (3.6)	0.289
Cirrhosis / Liver disease	0 (0.0)	0 (0.0)	NA	28 (2.6)	<0.0001	15 (6.1)	<0.0001	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA
Neoplasm (active)	0 (0.0)	0 (0.0)	NA	85 (7.8)	<0.0001	15 (6.1)	<0.0001	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA
Obesity	523 (9.1)	264 (8.7)	0.577	95 (8.7)	0.6933	17 (6.9)	0.2401	104 (21.6)	<0.0001	11 (2.0)	<0.0001	1 (25.0)	0.2674	27 (4.6)	<0.0001
Malnutrition	0 (0.0)	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA
Tuberculosis (active)	6 (0.1)	2 (0.1)	0.5737	0 (0.0)	0.2862	1 (0.4)	0.1729	0 (0.0)	0.4756	1 (0.2)	0.6193	0 (0.0)	0.9486	0 (0.0)	0.429
HIV infection	48 (0.8)	22 (0.7)	0.5937	9 (0.8)	0.9774	5 (2.0)	0.0495	0 (0.0)	0.0429	1 (0.2)	0.0895	0 (0.0)	0.8547	6 (1.0)	0.6719
HIV exposure	0 (0.0)	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA	0 (0.0)	NA
Other	1,006 (17.4)	506 (16.7)	0.3993	195 (17.9)	0.7461	38 (15.5)	0.4047	118 (24.5)	0.0001	68 (12.2)	0.001	0 (0.0)	0.3581	74 (12.5)	0.0021
Influenza vaccination ≥14 days from symptom onset [b], n (%)	1,032 (18.8)	399 (13.7)	<0.0001	105 (10.1)	<0.0001	29 (12.3)	0.0131	140 (29.4)	<0.0001	43 (8.0)	<0.0001	1 (25.0)	0.7106	64 (11.4)	<0.0001
Intensive Care Unit Admission [c], n (%)	382 (6.6)	175 (5.8)	0.1302	71 (6.6)	0.9452	9 (3.7)	0.0629	51 (10.7)	0.0011	20 (3.6)	0.0041	1 (25.0)	0.1412	18 (3.1)	0.0006
Supplemental oxygen [d], n (%)	1,308 (28.6)	712 (25.0)	0.3694	237 (23.6)	0.5165	52 (24.1)	0.552	215 (49.0)	<0.0001	105 (19.1)	0.0247	2 (50.0)	0.192	75 (12.9)	<0.0001
Hospitalization stay (days), median [IQR]	6 [4-10]	5 [3-8]	<0.0001	6 [4-8]	<0.0001	5 [3-8]	<0.0001	5 [3-8]	0.0186	6 [4-8]	<0.0001	6 [6-10]	<0.0001	5 [3-7]	<0.0001
Death [e], n (%)	251 (4.4)	83 (2.8)	0.0002	32 (3.0)	0.0325	5 (2.1)	0.0761	21 (4.4)	0.9685	19 (3.4)	0.2776	0 (0.0)	0.6697	5 (0.8)	<0.0001

The p-values denote the statistics result between each categorical or continuous variable compared to its negative influenza laboratory diagnosis homologue. There were 54 influenza-positive patients for whom type A or B was not determined. ǂ Percentage adjusted for missing data; (a) Age missing for 1 A(H1N1)pdm09 patient; (b) Vaccination status missing for 269 influenza-negative patients, and 51 A(H1N1)pdm09, 8 A(H3N2), 4 A not subtyped, 24 B/Victoria-like, and 27 B not classified patients; (c) Intensive care unit admission missing for 25 influenza-negative patients, and 16 A(H1N1)pdm09, 1 A(H3N2), 3 A not subtyped, and 3 B/Victoria-like patients; (d) Supplemental oxygen administration data missing for 1,204 influenza-negative patients, and 83 A(H1N1)pdm09, 29 A(H3N2), 42 A not subtyped, 8 B/Victoria-like, and 7 B not classified patients; (e) Patient outcome (death) was missing for 40 influenza-negative patients, and 6 A(H1N1)pdm09, 5 A(H3N2), 2 A not subtyped, and 4 B/Victoria-like patients. Abbreviations: HIV, Human immunodeficiency virus; IQR, interquartile range.

FIGURE LEGENDS

Figure 1. The GIHSN study flow diagram. Abbreviations: NGS, next-generation sequencing.

Figure 2. A) Epidemiological distribution of influenza-associated hospitalisations from coordinating GIHSN sites for the 2019-20 season. The inclusion period spans from September 2019 to May 2020. The overall proportion of each influenza subtype/lineage detected during the inclusion period is also depicted. B) Proportion of influenza-associated hospital admissions by influenza A subtype or influenza B lineage within age groups.

Figure 3. Phylogenetic tree of the influenza hemagglutinin gene for a subset of GIHSN patients infected by A(H1N1)pdm09 viruses. Grouped amino acid substitutions in the HA1 are indicated on the left of the sequence names, while individual substitutions are on the right. The use of supplemental oxygen without ventilation is indicated by a filled blue circle and the complete vaccination status for the current influenza season is indicated by a filled purple circle. The selected vaccine strain for the current season of the Northern hemisphere is labelled in orange and non-vaccine reference strains are labelled in red. Symbols: § = loss of glycosylation site; > = amino acid reversion.

Figure 4. Phylogenetic tree of the influenza hemagglutinin gene for a subset of GIHSN patients infected by A(H3N2) viruses. Grouped amino acid substitutions in the HA1 are indicated on the left of the sequence names, while individual substitutions are on the right. The use of supplemental oxygen without ventilation is indicated by a filled blue circle and the complete vaccination status for the current influenza season is indicated by a filled purple

circle. The selected vaccine strain for the current season of the Northern hemisphere is labelled in orange, and non-vaccine reference strains are labelled red. Symbols: § = loss of glycosylation site; # = gain of glycosylation site; > = amino acid reversion.

Figure 5. Phylogenetic tree of the influenza hemagglutinin gene for a subset of GIHSN patients infected by B/Victoria-like viruses. Grouped amino acid substitutions in the HA1 are indicated on the left of the sequence names, while individual substitutions are on the right. The use of supplemental oxygen without ventilation is indicated by a filled blue circle and the complete vaccination status for the current influenza season is indicated by a filled purple circle. The selected vaccine strain for the current season of the Northern hemisphere is labelled in orange, and non-vaccine reference strains are labelled red. Symbols: § = loss of glycosylation site; Δ = amino acid deletion; > = amino acid reversion.

FIGURES

FIGURE 1

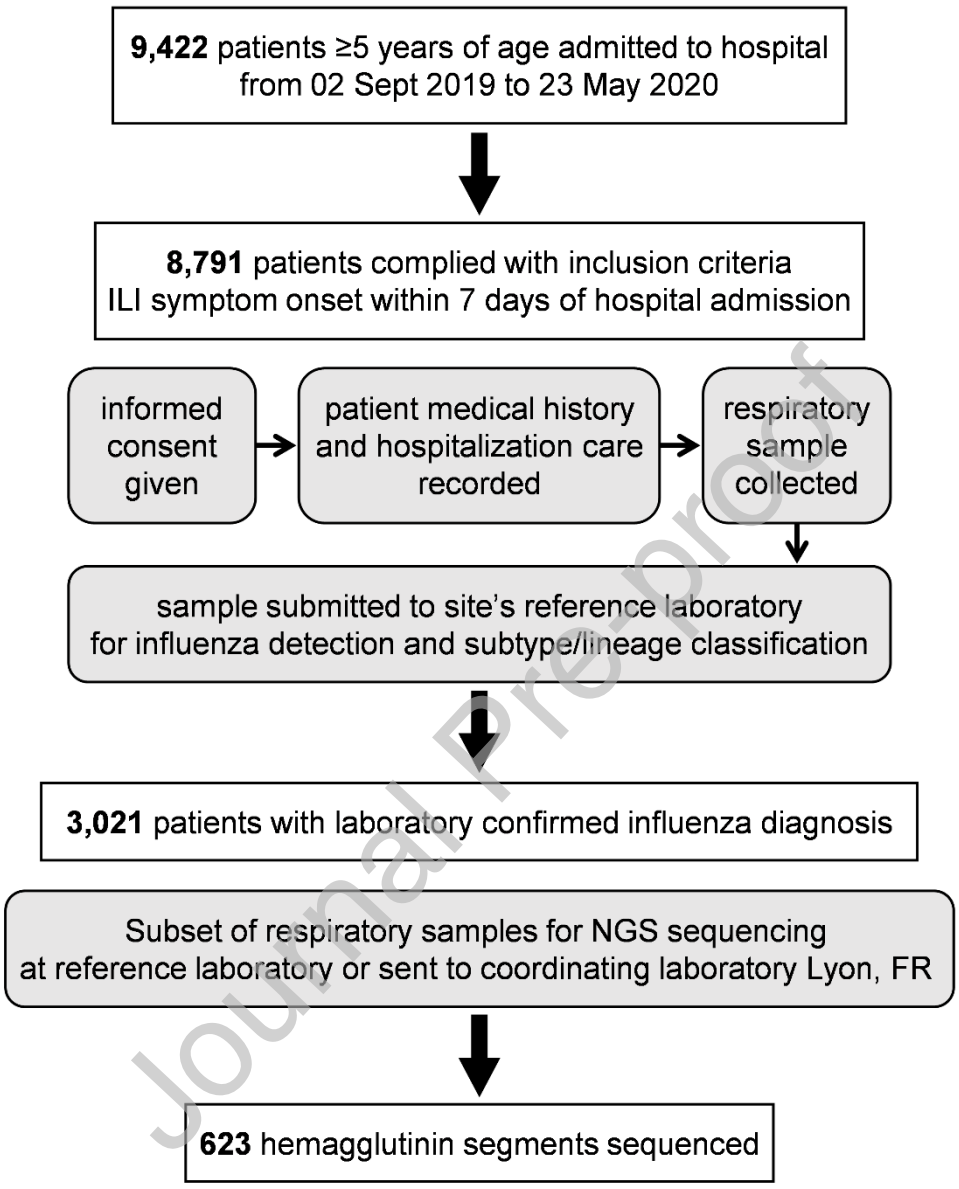


FIGURE 2 (Color should be used in print)

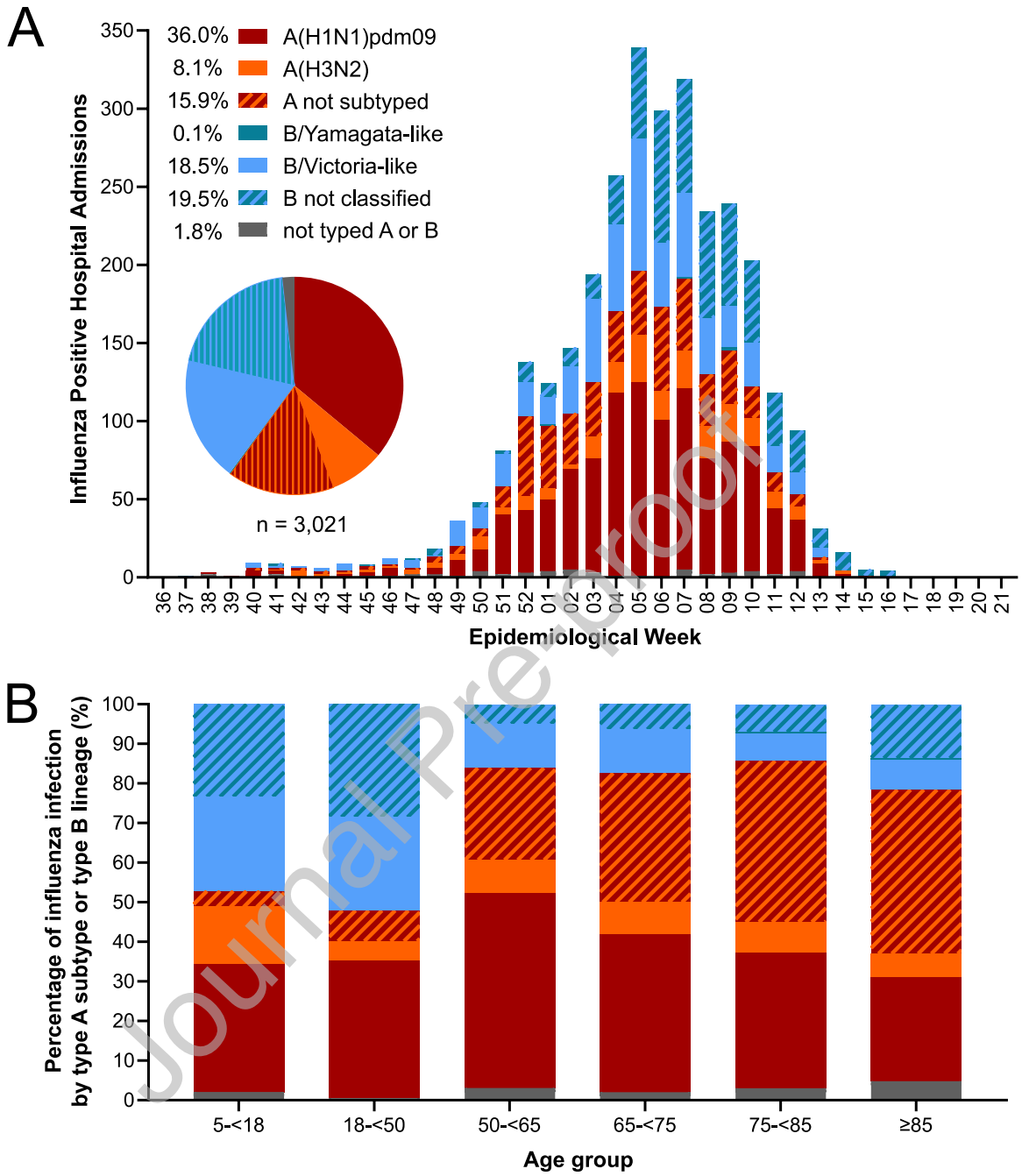


FIGURE 3 (.eps format submitted separately)

(** Full phylogenetic tree without split is available; color should be used in print)

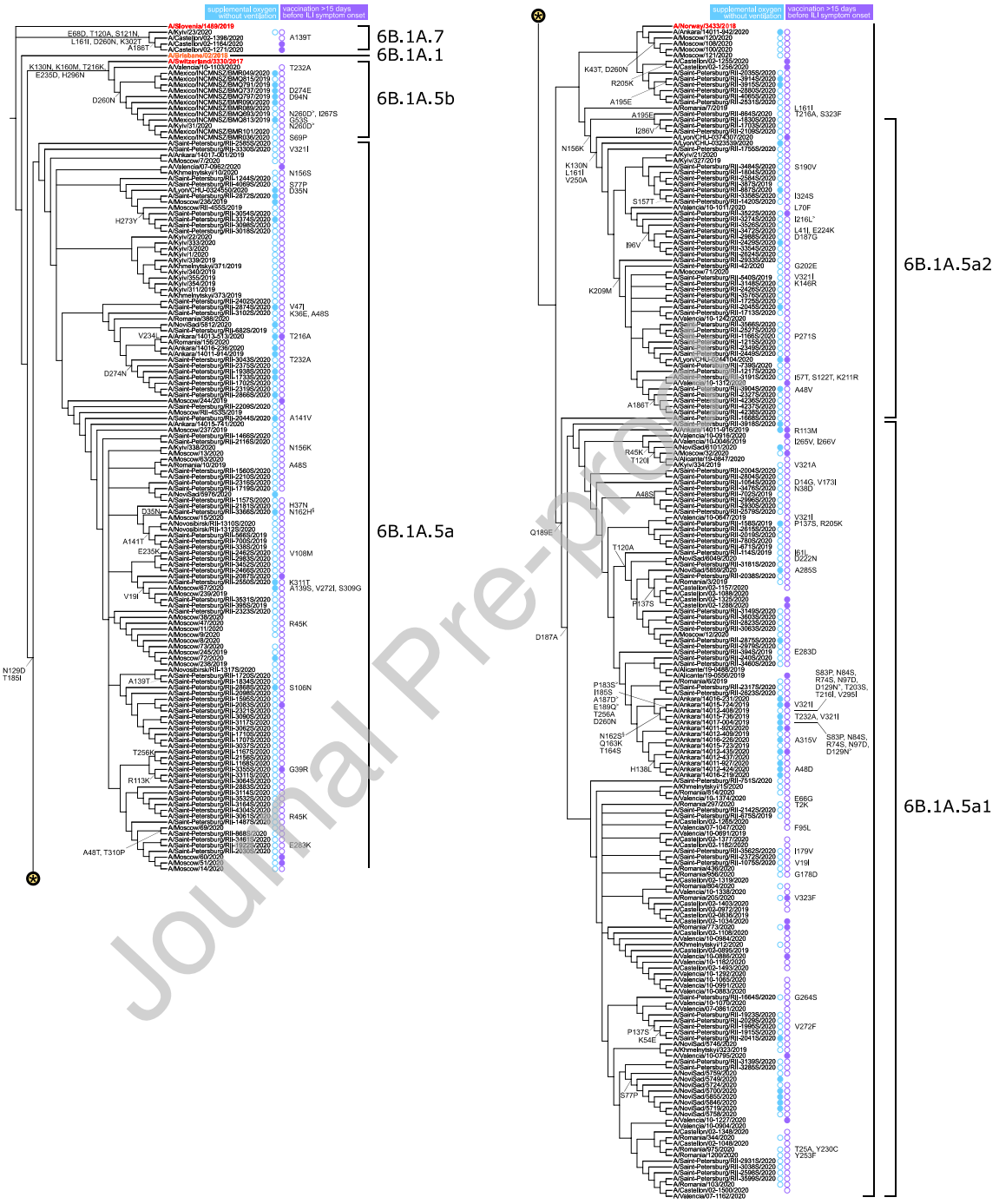


FIGURE 4 (.eps format submitted separately)

(Color should be used in print)

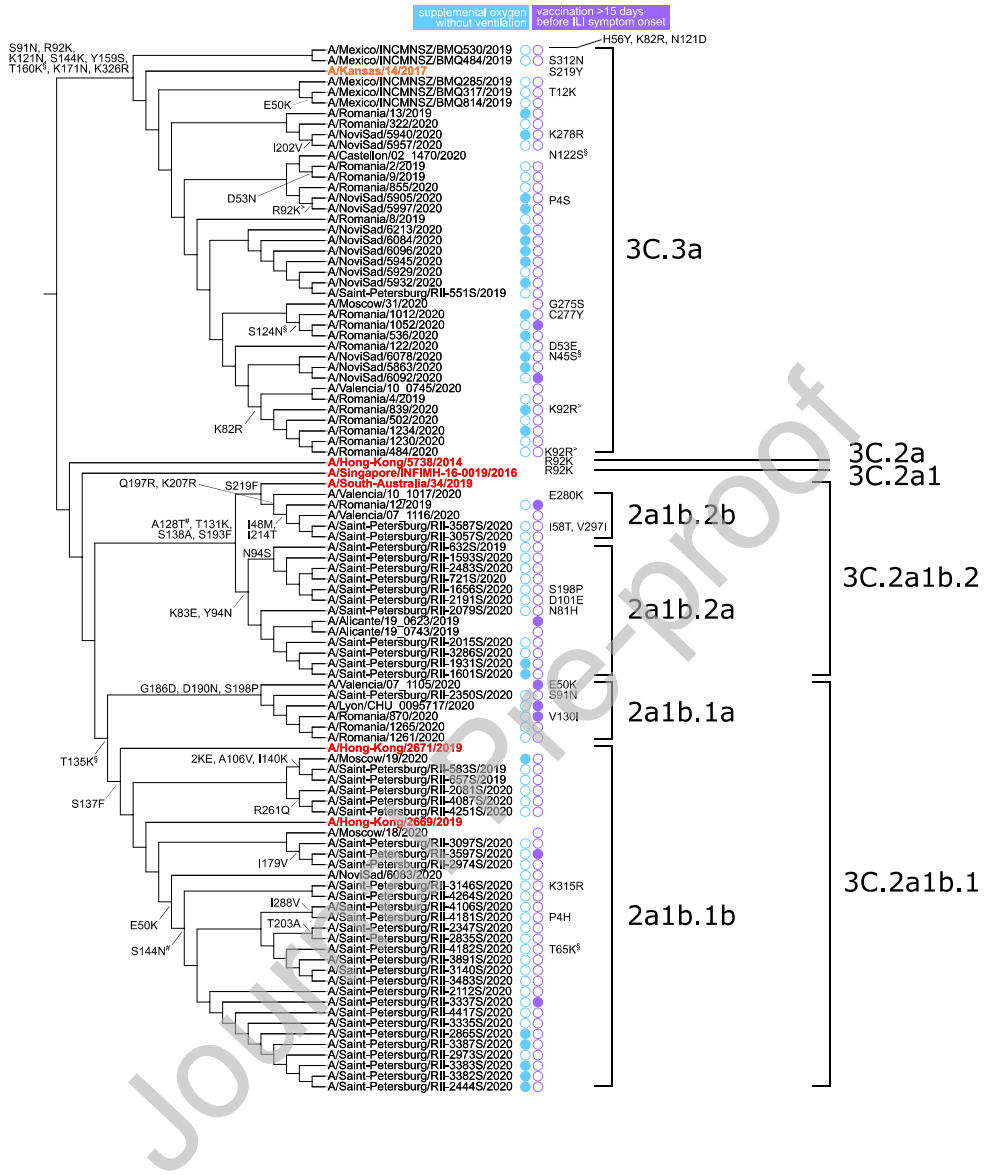
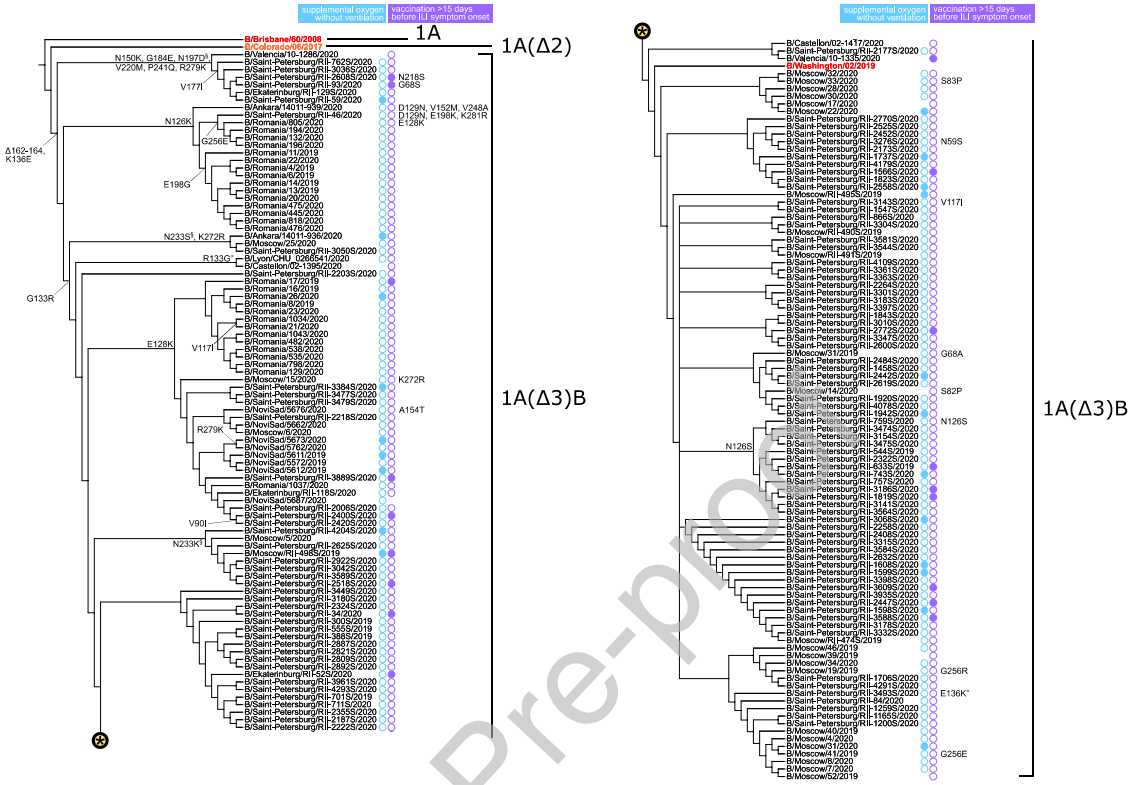


FIGURE 5 (.eps format submitted separately)

(** Full phylogenetic tree without split is available; color should be used in print)



Conflict of interest

The funders had no role in the data analysis, interpretation or writing of this report. More details on FIE and its governance can be found on www.gihsn.org. SSC is an employee of Sanofi Pasteur who is part-time seconded to the FIE where she contributes to the GIHSN scientific activities. AD has received grants or contracts from the EU/EFPIA Innovative medicine Initiative 2 Joint Undertaking. AK has received grants or contracts from Sanofi Pasteur. JS has received grants or contracts from Merck Inc, Pfizer Canada, Glaxo Smith Kline, and Sanofi Pasteur. FXL has received grants or contracts from Sanofi Pasteur and Instituto de Salud Carlos III. TMD has received grants or contracts from Sanofi Pasteur. SM

serves on the Republic Commission for Infectious Diseases of Republic of Serbia. BL serves as chair of the scientific committee of Immuniser Lyon and co-chair of the Global Influenza Initiative. GQ, EF, EB, PK, VLT, AM, NO, GRP, and LJ have no conflicts of interest to declare.