GENETIC AND CLINICAL RESULTS OF 2018-2019 GIHSN STUDY IN CITIES OF NORTH-WESTERN, URAL AND SIBERIAN REGIONS OF RUSSIA

Global Influenza Hospital Surveillance Network

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Site presentation

Nine Hospitals from 3 cities of Russia (St. Petersburg, Novosibirsk and Ekaterinburg) with population 5.35 mln, 1.61mln and 1.5 mln inhabitants, respectively, participated in GIHSN study in the season 2018-2019.

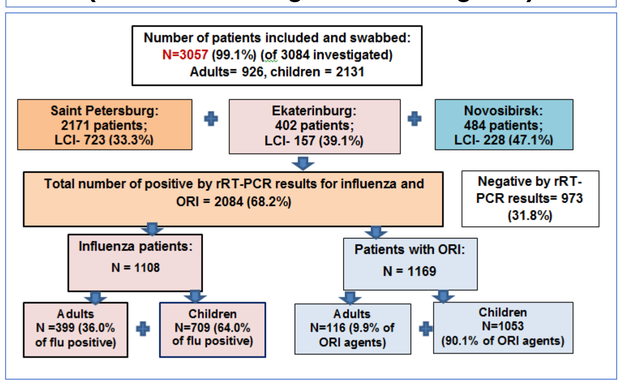
St. Petersburg site as WHO recognized National Influenza Centre (Smorodintsev Research Institute of Influenza of the Ministry of Health of the Russian Federation) was a Coordination Centre where all the data accumulated in Electronic Database to be exported in GIHSN database. All genetic and antigenic investigation of influenza viruses were performed in St. Petersburg site which worked in close cooperation with Novosibirsk and Ekaterinburg Institutions.

Methods

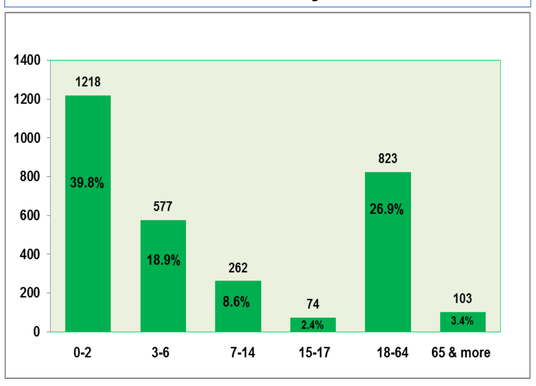
ILI patients of all age groups were selected by criteria of inclusion/exclusion in study. All procedures were performed according to GIHSN standardized protocol, Version 7.0, October 2018. Core questionnaires for patients <5 years and ≥ 5 years were applied across all hospital sites. The study was approved by the Local Ethics Committees and was conducted in accordance with the principles of GCP. Clinical specimens were tested by rRT-PCR using "AmpliSense" kits (Interlabservice, Russia) for influenza A&B as well as for subtyping of H1N1pdm09, H3N2 and ORV viruses; Influenza B lineage was specified using CDC&P (USA) primers & probes. Virus isolation, antigenic cartography and genetic analysis of HA and NA was performed to determine matching of vaccine and circulating influenza viruses. NGS was performed for 86 samples according to Zhou et al (2009). Libraries for next-generation sequencing were obtained using Nextera XT sample preparation kit, Illumina MiSeq was used for sequencing. All sequences were submitted to Epiflu GISAID database. Significance of differences was determined using the program "Statistic 10.0".

Results

Flowchart of patients included in GIHSN study (Saint Petersburg Coordinating Site)



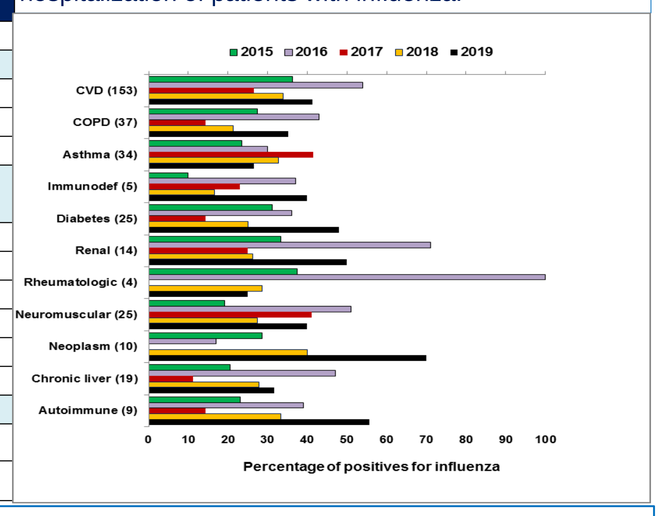
Age distribution of patients included in the study



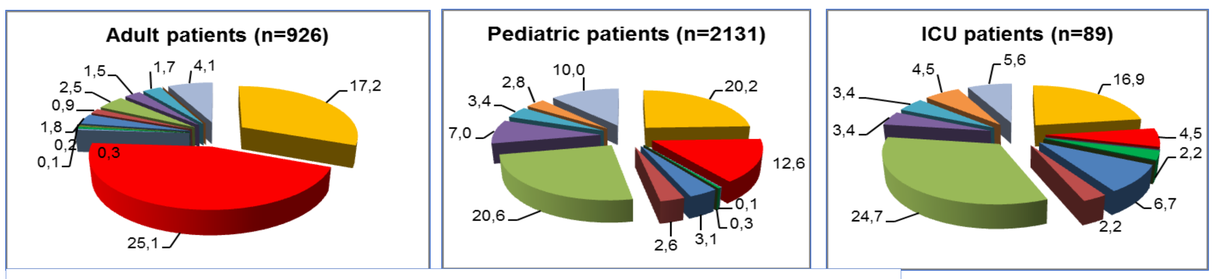
Pregnancy as a risk factor. A total of 129 pregnant women were included in the study in 2019. Percent of positives for influenza A(H3N2) was 1.3 times higher among pregnant woman this season compared to the control group. However ORI agents and co-morbidity were 1.4 times less often among pregnant women. The differences were not statistically significant.

Index	Pregnant woman	Not pregnant (adjusted control)
Number of women	129	75
Influenza positives:	80 (62,0%)	35 (46.7%)
A(H1N1)pdm09	32 (24.8%)	16 (21,3%)
A(H3N2)	48 (37.2%)	18 (24.0%)
A n/t	0	1 (1.3%)
ORI agents positives:	11 (8.5%)	9 (12.0%)
Parainfluenza virus	3 (2.3%)	0
Adenovirus	0	2 (2.7%)
RSV	0	1 (1.3%)
Metapneumovirus	3 (2.3%)	0
Corona	2 (1.6%)	2 (2.7%)
Rhinovirus	3 (2.3%)	4 (5.3%)
Co-morbidity cases:	17 (13.2%)	15 (20.0%)
Co-morbidity cases + FLU	10 (7.8%)	7 (9.3%)
Co-morbidity cases + ORI	3 (2.3%)	3 (4.0%)

The risk groups for influenza. Percentage of influenza positive patients with different chronic co-morbidity in 2018-2019 season increased compared to previous two seasons however did not reach the level of season 2015-2016 when influenza A(H1N1)pdm09 dominated. CVD, COPD, asthma, neuromuscular and diabetes were the most often co-morbidity leading to hospitalization of patients with influenza.



Comparative data on percentage of influenza, RSV, MpV, PIV, CoV, RhV, AdV and BoV in adults, children and ICU patients. Influenza A(H1N1)pdm09 and A(H3N2) viruses co-circulated in 2018-2019 season. The percent of influenza A viruses was high both in adult (43.2%) and pediatric (33.3%) patients. Influenza B cases were very rare. The ORI agents prevailed in children. RSV was the dominating causative agent (20.6%) of admission in pediatric patients. A total 89 (2.9%) patients were placed in ICU and most of them (48 patients) were young children. The dominating agent in ICU patients was RSV (24.7%), then followed by the influenza A(H1N1)pdm09 virus (16.9%) and PIV (6.7%).

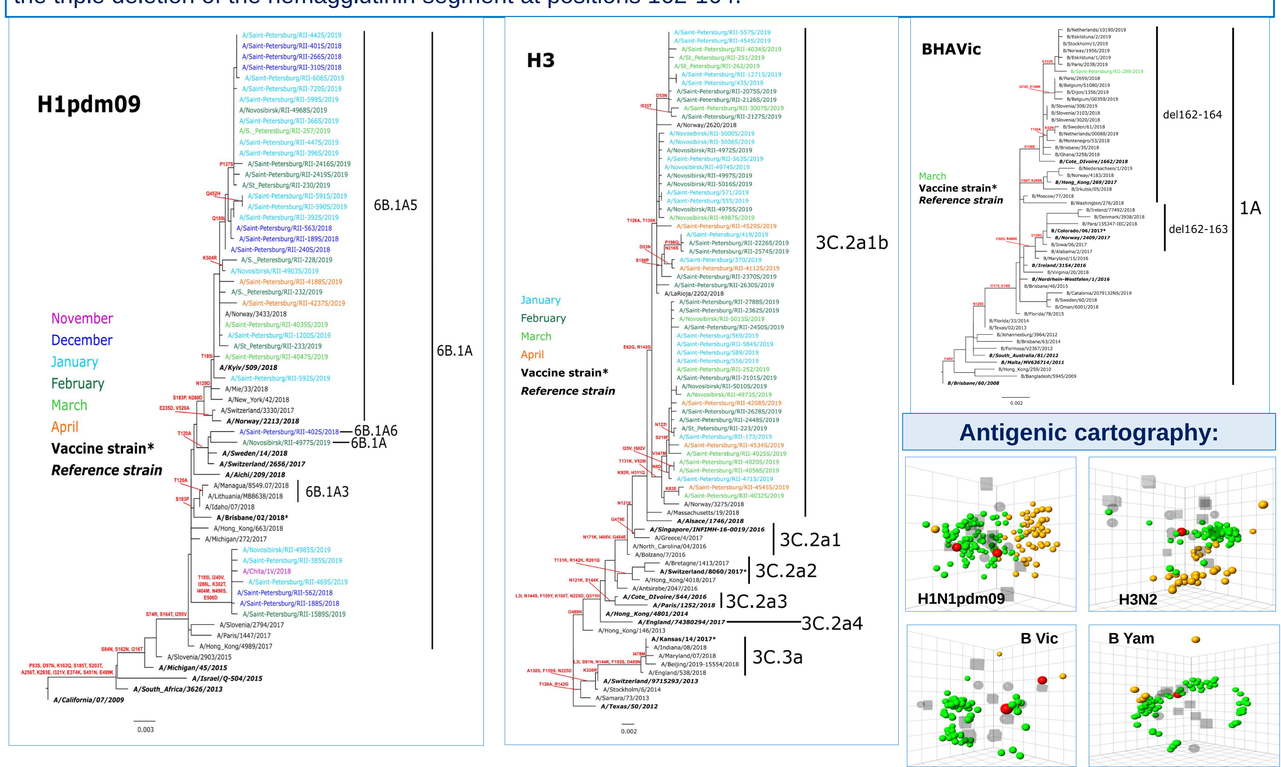


Genetic and antigenic analysis of influenza viruses

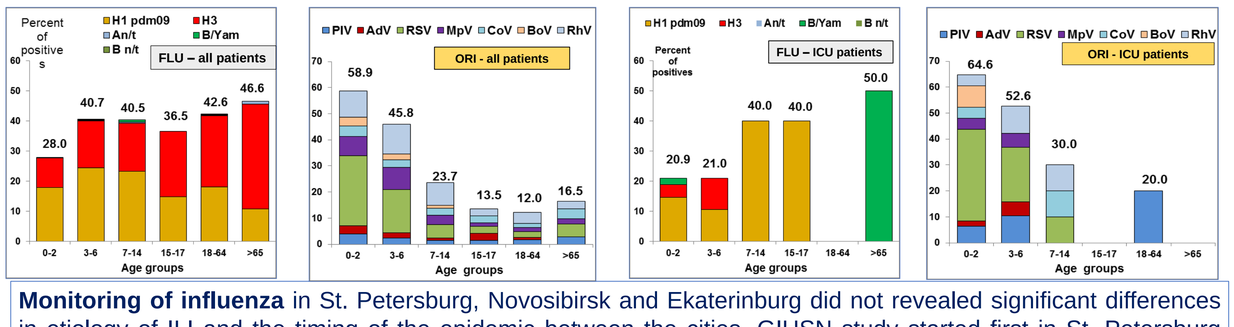
A(H1N1)pdm09. All 33 A(H1N1)pdm09 viruses belonged to genetic group 6B.1 (A/Michigan/45/2015-like viruses). All sequenced influenza A(H1N1)pdm09 viruses carried additional amino acid substitutions in the Cb (S74R 1295V) and Sa (5164T) antigenic sites. Genetic subgroup 6B.1A5 strains were most abundant in all sites. Despite a significant number of substitutions residing in antigenic sites in comparison to A/Michigan/45/2015 (two in Cb, one in Sa, one in Sb), these viruses preserve antigenic and genetic similarity to the vaccine strain according to HI assay.

A(H3N2). Most of 52 influenza A(H3N2) viruses belonged to subclade 3C.2a1b. Viruses of subclade 3.2a1b from Russia can be divided into three genetic subgroups. Subgroup 1 is defined by amino acid substitution T135N in 130-loop (antigenic site A). Subgroup 2 and 3 originate from one cluster of viruses with substitutions in antigenic sites E and A. Subgroup 2 is defined by amino acid substitutions T131K in HA1 and V1841 in HA2. Viruses of this subgroup bear truncated NS1. Subgroup 3 is defined by amino acid substitutions T128A and T135K in antigenic site A resulting in the loss of two potential N-glycosylation sites (in positions 126 and 133).

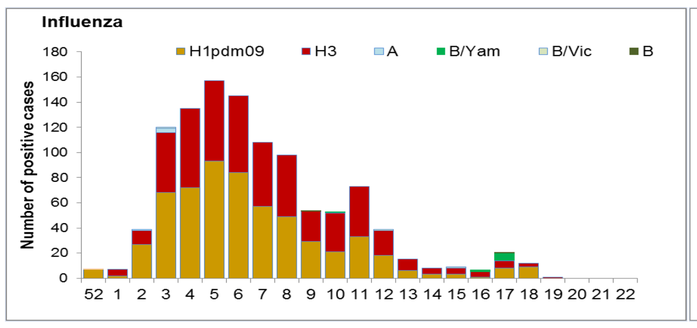
Influenza B viruses. Only one strain of the influenza B Victoria lineage was genetically characterized - B/St. Petersburg/RII-289/2019. This strain belongs to clade Δ1A, the genetic subgroup Δ162-164 (B/Cote d'Ivoire/1662/2018-like viruses), and also has additional substitutions G133R, K136E. This group is characterized by the triple deletion of the hemagglutinin segment at positions 162-164.



Age specific admission with influenza, RSV, MpV, PIV, CoV, RhV, AdV and BoV infections. RSV was the main causative agent for admission of young children ≤2 yrs (p<0.05) both among all hospitalized patients and among ICU patients, decreasing with the age, in contrast to influenza viruses which were detected more often in older age groups. RhV, CoV and MpV and ORI agents affected more pediatric patients (p<0.05). A total 89 patients were placed in ICU(82 children and 7 adult patients), 6 and 3 of whom needed mechanical ventilation.



Monitoring of influenza in St. Petersburg, Novosibirsk and Ekaterinburg did not revealed significant differences in etiology of ILI and the timing of the epidemic between the cities. GIHSN study started first in St. Petersburg (week 52.2018), 1-2 later weeks in other two cities. Influenza activity peaked on weeks 4-6.2019 with following decrease and finished on week 20, when no influenza cases were revealed in 9 hospitals participated in the study. As in previous years high RSV activity was observed throughout the influenza epidemic. MpV and RhV substantially complemented the etiological picture of diseases.



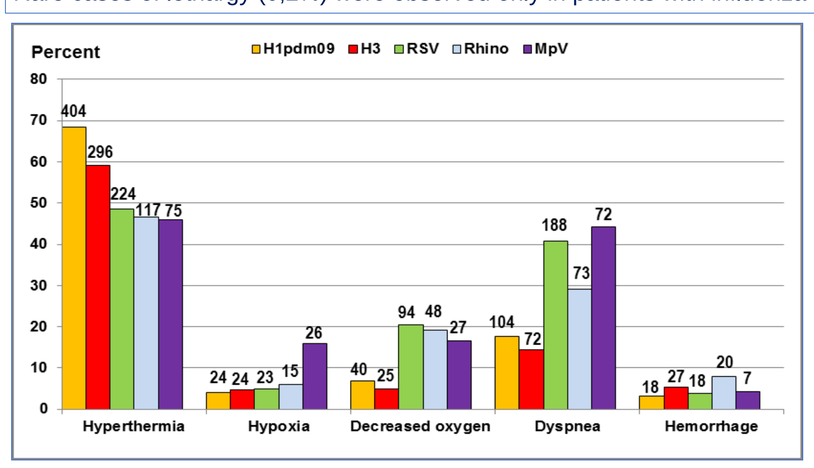
IVE against admission of patients with influenza (by age groups and virus subtypes). Trivalent IIV "Sovigrip" sponsored by the Federal Budget was used mainly for immunization in Russia. Two quadrivalent IIVs "Grippol-Quadrivalent" and "Ultrix-Quadri" were licensed and applied for the first time in 2018. IIV "Vaxigrip" was available as well. A total 70.8 mln people were vaccinated in the country. In GIHSN study of 3057 enrollees 590 and 500 patients were positive for influenza A(H1N1)pdm09 and A(H3N2), respectively; only 9 and 3 patients were positive for influenza B/Yamagata and B Victoria. A total 95 patients aged ≥ 3 years, included in GIHSN study, were vaccinated. The overall IVE was 60% (62% in adults and 57% in children). IIV offered moderate protection against influenza A(H1N1)pdm09 virus (60.3%), slightly less regarding influenza A(H3N2) virus (45.8%).

Age group (years)	Number of vacci-nated patients	Vaccinated #		Not vaccinated#		Odds ratio	IVE adjusted
		Flu (+)	Flu (-)	Flu (+)	Flu (-)		
3-6	31	9	22	225	318	0,58	42,0%
7-14	23	5	18	100	138	0,38	62,0%
15-17	6	0	6	27	41	0	100%
Subtotal children	60	14	46	352	497	0.43	57,0%
Adults	35	8	27	391	499	0,38	62.0%
Total*	95	22	73	743	996	0,40	60,0%

Virus subtype detected	Vaccinated		Not vaccinated		Odds ratio	IVE
	Flu (+)	Flu (-)	Flu (+)	Flu (-)		
A(H1N1)pdm09	9	86	363	1376	0,40	60,3%
A(H3N2)	12	83	366	1373	0,54	45,8%
B/Yam	1	94	6	1733	3,07	No

** Note: Age group 0-2 years was excluded from analysis*

Application **of new GIHSN criteria in evaluation of influenza and ORI severity.** Hyperthermia (>39oC) was the most often sign and was registered in 59.2% - 68.5% in influenza patients and in 46% - 48% in patients with ORI. Rare cases of lethargy (0,2%) were observed only in patients with influenza (both subtypes).



Hypoxia, dyspnea and decreased oxygen concentration in blood were more regular (up to 16,0%, 44,2% and 19,1%, respectively) in RSV, rhinovirus and metapneumovirus infections compared to influenza (not more than 4,8%, 17,6% and 6,8%, correspondingly). Hemorrhagic syndrome manifested in 3.1% - 8.0% of patients.

Key aspects :

1. Influenza epidemic in Russia in 2018-2019 was caused by co-circulation of A(H1N1)pdm09 and A(H3N2) viruses, it was of moderate intensity and duration of virus detection in hospitalized patients was estimated as 19 weeks;
2. RSV was dominating agent of admission in young patients. It circulated actively throughout the influenza epidemic.
3. Hyperthermia was the most often sign in influenza patients. Hypoxia, dyspnea and decreased oxygen concentration in blood were more regular in RSV, rhinovirus and metapneumovirus infections.
4. CVD, COPD, asthma, neuromuscular diseases and diabetes were the main co-morbidity leading to hospitalization of patients with influenza.
5. Influenza vaccine effectiveness was high against influenza A(H1N1)pdm09 virus (60.3%) and less effective (45.8%) against heterogeneous influenza A(H3N2) population, which was confirmed by genetic and antigenic analysis.
6. Most of the strains investigated matched vaccine strains; however, a number of strain-specific mutations were revealed, significance of which for virus pathogenicity are currently being analyzed. Additionally, in-host virus genetic diversity is under evaluation.

Challenges:

Full genome analysis of influenza viruses using NGS is important for the recognition of pathogenicity determinants of influenza viruses and is able to provide valuable additional information to address the composition of influenza vaccines for the upcoming seasons.

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