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Diagnostics of children's influenza B

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Background: The purpose of the study was to identify the frequency of laboratory confirmation of influenza in children suspected of having influenza on the basis of clinical symptoms, assessing the peculiarities of the clinical picture of influenza and changes in blood.

Materials and methods: Forty-nine hospitalized children clinically suspected as having influenza were prospectively studied at the Paediatric Centre of Vilnius University Children's Hospital during the 2005–2006 influenza season. Clinical symptoms of the disease were assessed in a detailed manner, and laboratory and instrumental analyses were performed. The method of direct immunofluorescence (DIF) was used to find antigens of influenza A and B, respiratory syncytial, adenovirus and parainfluenza 1, 2 and 3 viruses in swabs taken from the pharynx and both nasal cavities of 48 patients. Specific IgM class antibodies of influenza A and B in blood serum of 36 patients were identified by the method of indirect immunofluorescence.

Results: The diagnosis of influenza B for 28 patients was confirmed by laboratory methods (for 15 DIF, for 9 DIF and serologic, and for 4 serologic only). Fever, cough, rhinitis, pharyngitis, head / eye ache and vomiting were the most common symptoms of influenza B. Complications of influenza B were rare (3.6%) and only in patients with underlying disease.

Conclusions: The clinical symptoms of influenza as well as general blood analysis were not specific. The influenza diagnosis can be wrong when it is not laboratory-confirmed. It is expedient to apply more frequently rapide confirmative laboratory methods for aetiological diagnostics of influenza.

Key words: influenza B, children, clinical picture, diagnostics

INTRODUCTION

Influenza, respiratory syncytial, rhinoviruses, adenoviruses, human metapneumoviruses, parainfluenza and other viruses can cause infections of respiratory organs during the winter season (1–3). The most common methods of laboratory diagnostics comprise identification in clinical samples of the virus infection agent, its antigens or nucleoacids, as well as identification of virus antibodies in a patient's blood serum (4). In every analysis, sensitivity and specificity may depend upon the method used, the samples analysed and the laboratory that is performing the analysis.

Influenza B dominated (62%) in Europe during the 2005–2006 influenza season, although influenza A was also recorded (5). The incidence of influenza and other respiratory diseases in Lithuania reached the base level in the eighth week of 2006. According to the data of the Laboratory of the AIDS Centre of Lithuania, influenza B virus was identified in 43, influenza A (H3N2) in 2, and respiratory syncytial virus in 2 patients. As a rule, influenza diagnosis in Lithuania is clinical and is confirmed by laboratory methods in very rare cases. Comprehensive studies in the diagnostics of children's influenza have not been carried out in Lithuania to date.

The purpose of the present study was to identify the frequency of laboratory confirmation of influenza in children suspected of having influenza on the basis of clinical symptoms, assessing the peculiarities of the clinical picture of influenza and changes in blood data.

MATERIALS AND METHODS

Forty-nine hospitalized children clinically suspected as having influenza were prospectively studied at the Paediatric Centre of Vilnius University Children's Hospital. The clinical case of influenza was defined in accordance with order No. V-344, dated May 10, 2004, by the Minister of Health of the Republic of Lithuania. The symptoms include a sudden onset of the disease, cough, fever in excess of 38 °C, muscular pain and / or headache.

Older children or parents of younger children were asked to answer an expressly drawn up questionnaire. The questions concerned the onset of the disease, its symptoms, contacts with persons suspected of having influenza, vaccination against influenza, etc. Clinical symptoms and their dynamics were assessed for each patient, a general blood test was taken and, in case of indications, CRP concentration in blood was determined and a chest X-ray was made.

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The patients studied were from 8 months to 17 years of age (median 9 years). There were 28 boys and 21 girls. Four of the children had underlying diseases, i. e. two bronchial asthma, one acute pyelonephritis and one acute glomerulonephritis. Swabs were taken from the pharynx and both nasal cavities of 48 patients during the first three days of hospitalization (i. e. on day 2–8 of disease) to determine the aetiology of respiratory tract infections. Antigens of influenza A and B, respiratory syncytial, adenovirus and parainfluenza 1, 2 and 3 viruses were sought at the Laboratory of the AIDS Centre of Lithuania by the method of direct immunofluorescence (DIF). To this end, *Bartels VRK* (from *Trinity Biotech*, Ireland) diagnostic kits were used. On the day of discharge from hospital (day 3-13 of the disease) venous blood samples were taken from 36 children for serological study. Specific IgM class antibodies of influenza A and B in blood serum were identified at the Microbiology Laboratory of Vilnius University Children's Hospital by the method of indirect immunofluorescence. *Pneumoslide IgM (Vircell, Spain)* or *Euroimmun (Germany)* kits were used for this purpose.

To assess the peculiarities of influenza in children, the patients were divided into two groups – group 1 whose clinical diagnosis of influenza was laboratory-confirmed (CI), and group 2 whose diagnosis was not laboratory-confirmed (UI).

The statistical reliability of the study data was assessed in terms of the Student criterion. A difference was considered statistically significant with a p value of less than 0.05.

RESULTS

In February–April 2006, the antigen of influenza B virus was identified for 24 patients (50%) and that of respiratory syncytial virus for 1 patient (2.1%). No other virus antigens were detected. Incidentally, out of the 20 patients' swabs taken during the first four days of disease, the antigen of influenza virus was found in 15 (75%), and only in 9 (32%) of the 28 swabs taken later than day 4 of the disease. The obtained data were statistically significant ($p < 0.001$).

IgM class antibodies of influenza B virus were identified in 13 patients (36.1%): 9 in whose smears the antigen of influenza B virus was found, 3 in whose smears the antigen of influenza B virus was absent, and 1 whose virus antigens were not studied. Thus, the diagnosis of influenza B for 28 patients was confirmed by laboratory methods (for 15 DIF, for 9 DIF and serologic, and for 4 serologic only), and for 21 patients the influenza diagnosis remained clinical, i. e. it was not confirmed by laboratory.

The patients' distribution by age with confirmed and unconfirmed influenza is shown in Table 1.

There were 17 boys and 11 girls in group 1, 7 boys and 14 girls in group 2. The patients' average age was 10 years in group 1 and 7 years two.

None of the children had been vaccinated with seasonal influenza vaccine.

Twenty-five patients (89%) of group 1 and 15 patients of group 2 (71%) noted a contact (equally often at school and at home) with a person ill with influenza that was not laboratory-confirmed.

The frequency of clinical symptoms in both groups of patients is shown in Table 2.

Head, eye, muscle, joint and abdominal pain was only taken into consideration beginning with four years of age, i. e. in 43 patients.

Table 1. **Patients' distribution by age**

Patient's age (years)	Total of patients n (%)	Confirmed influenza n (%)	Unconfirmed influenza n (%)
Under 1	3 (6.1)	2 (7.1)	1 (4.8)
1–3	3 (6.1)	1 (3.6)	2 (9.5)
4–6	10 (20.4)	3 (10.6)	7 (33.3)
7–9	10 (20.4)	7 (25)	3 (14.3)
10–12	13 (26.5)	5 (17.9)	8 (38.1)
13–15	5 (10.2)	5 (17.9)	0
16–17	5 (10.2)	5 (17.9)	0
Total	49 (100)	28 (100)	21 (100)

Table 2. **Frequency (%) of clinical symptoms in children ill with confirmed and unconfirmed influenza**

Symptom	Confirmed influenza n = 28	Unconfirmed influenza n = 21
Fever (>38.5 °C)	100	100
Rhinitis	82	86
Cough	100	81
Pharyngitis	82	90.5
Muscle / joint ache*	56	39
Head / eye ache*	84	72
Conjunctivitis	43	38
Bleeding from nose	28.5	33.5
Vomiting	50	48
Abdominal pain	36	44
Faint	3.5	0
Febrile seizures	0	14

* These symptoms were assessed in 43 patients (i. e. in 25 with CI and 18 with UI).

$p > 0.05$ for each symptom between CI and UI groups.

All the children were feverish. The average maximum temperature was 39.5 °C and 39.4 °C for patients of groups 1 and 2, respectively. Children from group 1 had fever for 2–8 days (4.6 days on the average, of which febrile fever for 3.3 days and subfebrile fever for 1.3 days), and those from group 2 for 1–7 days (5 days on the average, of which febrile fever for 4 days and subfebrile fever for 1 day). Cough and rhinitis appeared on day 1–5 of the disease (on day 2 on the average). Incidentally, a third of the patients had their noses stuffed and no rhinorrhoea. In addition to the abovementioned symptoms that are described in the clinical definition of influenza, patients in both groups manifested other symptoms such as pharyngitis, vomiting, conjunctivitis, bleeding from the nose and abdominal pain. There was no statistically significant difference in the frequency of clinical symptoms between patients from groups 1 and 2 ($p > 0.05$).

Blood samples for general analysis were taken on day 1–7 (3.3 on the average) of the disease.

The frequency of changes in blood data is summarized in Table 3.

Table 3. **Frequency (%) of changes in general blood data and CRP in children ill with confirmed and unconfirmed influenza**

Blood parameter	Confirmed influenza n = 28	Unconfirmed influenza n = 21
Leucocytopaenia	50	47.5
Normocytosis	50	47.5
Leucocyte formula:		
-shift to the right;	25	24
-shift to the left	25	24
-no shift	50	52
ESR ≤ 15mm/h	66.5	100
CRP ≤ 10mg/l	66.5	84

$p > 0.05$ for each blood parameter between CI and UI groups.

Normocytosis and leucopaenia in the patients were identified equally often. Only one patient whose influenza was not confirmed manifested faint leucocytosis. In half of the patients the leucocyte formula was within limits. A shift to the right was observed in one fourth of the patients and in the same number of cases a shift to the left was noted. The erythrocyte sedimentation rate (ESR) in four patients of group 1 was 16–41 mm/h. The CRP concentration was below 10 mg/l in most of the patients, and as high as 20–47 mg/l in only three of the patients from group 1. The difference in blood changes of the two groups of patients was statistically insignificant ($p > 0.05$).

Chest X-rays were made for 23 children, 13 in CI and 10 in UI. Pneumonia was diagnosed in two patients. Both had underlying illnesses, acute glomerulonephritis and bronchial asthma in CI and UI, respectively.

The duration of hospitalization of patients from both groups averaged 5 days.

DISCUSSION

Influenza B dominated in Lithuania during the 2005–2006 influenza season. Out of the 43 patients for whom influenza B virus was identified at the Laboratory of AIDS Centre, more than a half were patients of Vilnius University Children's Hospital.

The present study was the first of the kind in Lithuania. It enabled to determine the frequency of laboratory confirmation of influenza and the peculiarities of the clinical picture of influenza B in children. It is difficult to diagnose influenza clinically as the specific symptoms of the disease are absent (1, 6, 7). During the influenza season, not only influenza viruses but also other respiratory viruses can cause infections of respiratory organs leading to fever (1–3, 8). Therefore influenza is diagnosed in an unjustifiably large number of patients. Influenza hyperdiagnostics could be reduced and the aetiology of respiratory tract infections might be defined in a more accurate manner if specific methods of laboratory diagnostics were used more often. To date, the aetiological diagnostics of influenza and other respiratory infections has been extremely rarely applied in clinical practice in Lithuania.

Out of the 49 children hospitalized with suspicion of influenza, the diagnosis was confirmed in 28 (57.1%), more often than not by the fast method of immunofluorescence when the influenza B antigen was identified in smears taken from the pharynx and nose. Laboratory confirmation of influenza depends on different factors, such as the time and manner of sampling and the method of analysis. The influenza virus and its antigen are more often found in nasopharyngeal specimens taken with a catheter than in throat swab specimens (4, 9). The method of direct immunofluorescence, which was used also in the present study, is less sensitive than virus cultivation by standard methods (9, 10). However, it is considerably faster and cheaper.

It is maintained in literature (4, 11, 12) that children who contracted influenza for the first time and those ill for the first four days produce a larger number of viruses. According to our data, the antigen of influenza B was identified 2.5 times more often in smears taken during the first four days after the onset of the disease than in smears taken later. Incidentally, the antigen of influenza virus was found in 7 (29.1%) of our subjects as late as on day 6–7 of the disease, in other words, they could be the source of hospital influenza infection. Similar results were obtained by other scientists, too (11). Out of the 152 children ill with an acute infection of respiratory tract, 26 pharynx smears (17%) taken during the first four days of disease were positive in terms of influenza virus, versus only 4 smears (4.5%) from 88 patients taken later than day 4.

Fever, cough and rhinitis in our study, like in these by other authors (6, 10) were found to be the most frequent symptoms of influenza B. However, they are not specific, since they were also fairly common among children ill with influenza that was not confirmed by laboratory tests. We discovered that pharyngitis, conjunctivitis, vomiting and abdominal pain were more common than in other studies (1, 6, 10). Our subjects ill with influenza B did not manifest febrile seizures. In literature sources, febrile seizures and other CNS disorders are associated with influenza A rather than B (1, 6, 10, 13).

Pneumonia developed in only one of our subjects ill with influenza B and an accompanying disease. According to literature sources, complications caused by the influenza B virus are less frequent than those caused by the influenza A virus (1, 10). In immunocompromised patients, influenza B may result in a lethal outcome of the disease (7).

Changes in the general blood of children ill with influenza B identified in the present study were found to be similar to those

in other studies (1, 6, 10). As a rule, leucocyte count is normal, with leucopaenia less common. CRP concentration more often than not is normal or somewhat increased.

Several shortcomings of the present study should be mentioned. First of all, some children who were ill with influenza could probably be included in the UI group, as influenza was probably not diagnosed because clinical samples for antigen analysis were taken too late, or blood for serological analysis was taken too early. Furthermore, blood samples for serological analysis need to be taken twice and an increase in antibody titre measured (4). The children we treated were hospitalized for five days on the average, and it was impossible to subsequently obtain their blood samples for analysis.

CONCLUSIONS

During the 2005–2006 influenza season, influenza B prevailed both in Lithuania and in Europe as a whole. The prospective study we conducted at Vilnius University Children's Hospital showed that the influenza diagnosis could be laboratory-confirmed in only more than a half of the children hospitalized with suspicion of influenza. Clinical symptoms of influenza as well as the general blood analysis were not specific. Therefore the influenza diagnosis can be wrong when it is not confirmed by laboratory tests. The range of clinical symptoms of influenza B in children was identified. Complications of influenza B were rare and occurred only when an underlying disease was involved. It is expedient to apply more frequently rapide confirmative laboratory methods for the aetiological diagnostics of influenza. Rapid diagnosis might reduce the number of unnecessary laboratory tests, decrease the use of antibiotics and increase the use of antivirals.

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VAIKŲ B GRIPO DIAGNOSTIKA LIETUVOJE

Santrauka

Mūsų darbo tikslas buvo nustatyti gripo laboratorinio patvirtinimo dažnį vaikams, kuriems pagal klinikinius simptomus buvo įtartas gripas, įvertinti klinikinių simptomų bei kraujo rodiklių pokyčių ypatumus.

Pacientai ir metodai. 2005–2006 m. gripo sezono metu Vilniaus universiteto Vaikų ligoninės Pediatrijos centre prospektyviai ištyrėme 49 hospitalizuotus vaikus, kuriems kliniškai buvo įtartas gripas. Buvo nuodugniai įvertinti ligos klinikiniai simptomai, atlikti laboratoriniai ir instrumentiniai tyrimai. Tiesioginės imunofluorescencijos metodu 48 ligonių tepinėliuose iš ryklės ir abiejų nosies landų buvo ieškoma A ir B gripo, respiracinio sincitinio, adenovirusų ir paragripo 1, 2 ir 3 virusų antigenų. Netiesioginės imunofluorescencijos metodu 36 pacientų kraujo serume buvo nustatomi specifiniai A ir B gripo IgM klasės antikūnai.

Rezultatai. 28 ligoniams B gripo diagnozė buvo patvirtinta laboratoriniais metodais (15 virusologiniu, 9 virusologiniu ir serologiniu, 4 tik serologiniu). Dažniausi B gripo simptomai buvo karščiavimas, kosulys, rinitas, faringitas, galvos / akių skausmas, vėmimas. B gripo komplikacijos pasitaikė retai (3,6%) ir tik esant dar kitai ligai.

Išvados. Gripo klinikiniai simptomai, kaip ir bendrasis kraujo tyrimas, nėra specifiniai, todėl gripo diagnozė be laboratorinio patvirtinimo dažnai gali būti neteisinga. Kvėpavimo organų infekcijų etiologinei diagnostikai tikslinga dažniau naudoti greitus patvirtinančius laboratorinius metodus.

Raktažodžiai: B gripas, vaikai, klinika, diagnostika