Abstract No: 1527

Presentation at ESCV 2015: Poster 1

Serological diagnosis of mumps – The benefit from IgA antibody screening and IgG antibody avidity testing in the population of the Czech Republic with a high vaccination coverage

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Backround: Although the Czech Republic is among the countries with a high vaccination coverage, mumps cases have recently been on the rise, even in the vaccinated population. The present study addresses the question of whether the increase in post-vaccination mumps cases is the result of primary or secondary vaccine failure – by comparing IgG antibody avidity and IgM and IgA antibody levels in acute sera in vaccinated versus naturally immunized patients.

Methods: Sixty-four patients with laboratory confirmed mumps (by direct detection of the virus and/or significant increase in anti-mumps antibodies) were included in the study. Of this cohort, 38 were vaccinated (36 acute sera were analyzed) and 26 were non-vaccinated (23 acute sera were analyzed). Altogether 26 convalescent sera were available from vaccinated and non-vaccinated subjects. The control groups were 30 healthy, naturally immunized adults and 22 vaccinated, healthy children aged 2–4 years. To measure the avidity index, the Siemens Enzygnost Anti-Mumps/IgG kit with 6 M urea was used. IgM, IgG, and IgA antibodies were detected using the Siemens Enzygnost Anti-Mumps/IgM and/IgG and Mast Diagnostica Mastazyme Mumps IgA kits.

Results: High avidity antibodies were only detected in convalescent sera from vaccinated subjects or in those who became naturally immunized as a result of recent infection. In contrast, none of the controls had high avidity antibodies. Based on the screening of acute sera, the infection was laboratory confirmed by the detection of IgA antibodies in 56% (20/36) of cases and by the detection of IgM antibodies in 36% (13/36) of cases only. Of 23 non-vaccinated patients, 87% (20/23) showed IgA positivity and 74% (17/23) IgM positivity.

Conclusion: High incidence of post-vaccination mumps cases is very probably the result of secondary vaccine failure. Testing both convalescent and acute sera for IgG antibody avidity is of diagnostic benefit. Nevertheless, the acute sample of blood should be collected not earlier than on day six of the onset of symptoms when significant increase in antibody avidity is observed in both vaccinated and naturally immunized subjects. The highest detection rate of mumps virus infection was achieved by testing IgA antibodies. The addition of this serological method to the standard detection of IgM antibodies improved the mumps diagnosis rate, particularly in vaccinated patients. Supported by grant NT 14059-3/2013 from the Internal Grant Agency of the Ministry of Health of the Czech Republic

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Is there a relationship between of viral load and co-infections of respiratory viruses with disease severity in patients with acute respiratory tract infection?

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Background: In this study, we aimed to investigate viral load of single or multiple respiratory viruses in patients with upper and lower respiratory tract infections and also whether there is a relationship between viral load and patient clinical characteristics.

Methods: Between February 2014 and March 2015, nasopharyngeal swab specimens were collected from 930 [524 (56.3%) male, 406 (43.7%) female] patients with upper (521, %56.0) and lower (409, 44.0%) respiratory tract infections. The age range of patients is between 20 days to 87 years (median: 2 year). Nucleic acid was extracted (Ribospin vRD viral RNA/DNA Extraction Kit, GeneAll, Seegene, South Korea) and cDNA was synthesized (cDNA Synthesis Premix, Seegene, South Korea) according to the manufacturer's protocol. Multiplex amplification of nucleic acid was performed using DPO primers [Influenza A&B virus (INF), RSV A&B, human adenovirus (ADV), human metapneumovirus (HMPV), human coronavirus (229E, NL63, OC43)(HCoV), parainfluenza virus type 1-4(PIV), human rhinovirus A/B/C(HRV), human enterovirus (HEV), and human bocavirus 1/2/3/4(HBoV)] and Anyplex II RV16 Detection kit (Seegene, South Korea). PCR product was detected by semi-quantitative real-time PCR on Bio-Rad.

Results: Of the 930 patients, 606 (65.2%) were positive and 324 (34.8%) were negative for respiratory viruses. Viral single infections were detected in 435 (46.8%) patients and co-infections in 171 (18.4%) patients. HMPV, INF, HCoV, ADV, HBoV, and HEV were detected in over 50% co-infections. In the group of 521 patients with upper respiratory tract infection (URTI), 349 (67.0%) of them were positive, in the group of 409 patients with lower respiratory tract infection (LRTI), 257 (62.8%) patients were positive for respiratory viruses. Viral load ratios of respiratory viruses for URTI and LRTI were found %51.6 and %48.4 for >105, %70.0 and %30.0 for 102–105, %49.5 and %50.5 for <102, respectively ($p = 0.0003$). The difference between viral load of respiratory viruses detected in single and co-infections was not significant statistically ($p > 0.05$) except human rhinovirus ($p = 0.0002$).

Conclusion: Respiratory viruses were identified approximately 66% in patients with acute respiratory tract infection and viral co-infections were detected in 19% of the patients. HMPV, INF, HCoV, ADV, HBoV, and HEV have been detected co-infections more than single infection. Although respiratory viruses have been detected similar percentages in LRTI and URTI, the difference between viral loads of respiratory viruses was significant statistically. Viral load of HRV was found mostly less than 10^2 in co-infections and mostly more than 10^2 in single respiratory infec-

tion. The difference between viral load of other respiratory viruses detected in single and co-infections was not significant statistically.

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Epidemiological characteristics of multiple respiratory virus infections

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Background: In this study, we aimed to investigate the prevalence and seasonal distribution of multiple respiratory viruses in patients with the upper and lower respiratory tract infections of outpatient or inpatient monitoring who admitted to hospital.

Methods: Patients population. Respiratory tract specimens were collected from 4084 patients [1809(44.3%) female, 2275(55.7%) male] with upper and lower respiratory tract infections. The age range of patients [1040(24.4%) adult, 3044(75.6%) pediatric] is between one day to 93 years (median: 5 years). Extraction, cDNA analysis and Multiplex PCR Tests. Respiratuvar RealAccurate^{TM} (Pathofinder, Netherlands). Extraction of nucleic acids was done by RTP DNA/RNA Virus Mini Kit (InvitekGmbH, Germany). Multiplex amplification and cDNA synthesis were performed using Taqman primers and probs (INF-A&B, RSV, ADV HMPV, HCoV, PIV1-4, HRV), enzyms, dNTP, QiagenOne Step RT-PCR solution, dH2O mix and PCR product was detected by real-time PCR on ABI 7500 (Applied Biosystems, ABD). Seeplex RV15 ACE Detection (Seegene, South Korea). Extraction of nucleic acids and cDNA synthesis with revers transcription enzymes were performed by Viral DNA/RNA Extraction Kit (iNtRON, South Korea' and 'RevertAid First Strand cDNA Synthesis Kits, Fermentas, USA'). Multiplex amplification of nucleic acids was done by DPO primers (INF-A&B, RSV, ADV, HMPV, HCoV, PIV1-4, HRV, HBoV) and Seeplex RV15 ACE Detection kit (Seegene, South Korea). It's analyzed using Screen tape automated system, which allows ultra-rapid migration and analysis of the PCR products in small polyacrylamide gels. Anyplex II RV16 Detection (Seegene, South Korea). Extraction and cDNA synthesis with reverse transcription enzymes were performed using Ribospin vRD viral RNA/DNA Extraction Kit (GeneAll, Seegene, South Korea) and cDNA Synthesis Premix (Seegene, South Korea). Multiplex amplification of nucleic acid was done by DPO primers [INF-A&B, RSV-A&B, ADV, HMPV, HCoV (229E, NL63, OC43), PIV type 1-4, HRV A&B&C, HBoV 1/2/3/4, HEV] and Anyplex II RV16 Detection kit. PCR product was detected by semi-quantitative real-time PCR on BioRad.

Results: Three hundred and five (7.5%) of these patients were infected by multiple respiratory viruses, 1174 (28.7%) patient were infected by single virus. Of the multiple virus infections, 129 (42.3%) were female, 176 (57.7%) were male ($p = 0.465$). The age range of multiple virus infections is between one day to 87 years (median: 1 years). A total of 38(12.5%) multiple infections were adult, 267 (87.5%) were pediatric patients ($p = 0.000$). Of the 305 specimens, 33(10.8%) were obtained from outpatient and 272(89.2%) were inpatient ($p = 0.000$). A total of 266 (87.2%) multiple infections were dual infection, 36 (11.5%) were triple infection, 4 (1.3%) were quadruple infection. Of the all multiple virus infections, 38(14.2%) were found RSV/influenza A virus and 30(11.2%) were found RSV/rhinovirus. Mixed infection was observed in January–February (19.3%) at the rates specified ($p = 0.006$).

Conclusion: Multiple respiratory viruses were detected approximately 7.5% in patients with upper and lower respiratory tract infection. Multiple infections were detected more frequently in male sex, pediatric group, inpatients and winter months. The most prevalent viruses in dual infections were RSV and influenza A virus. Human metapneumovirus and human bocavirus were more detected in multiple infections than single infections.

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Comparison of virusneutralization and hemagglutination inhibition assays using whole or tween-ether split antigens for the specific detection of influenza A(H7) antibodies

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Background: In March 2013, a novel avian Influenza A(H7N9) virus causing severe respiratory infections with a case fatality rate of 36% was discovered in China. Zoonotic influenza viruses exhibit a high pandemic potential, and accurate diagnostic and surveillance activities are crucial for proper disease management. Serological assays such as virusneutralization test (VNT) and haemagglutination inhibition test (HIT) play a fundamental role in this regard. VNTs are inherently complex and require strict adherence to BSL-3 safety precautions for the work with highly pathogenic influenza viruses. HIT can be performed under BSL-2 conditions. However, for emerging viruses, a lower sensitivity has been observed compared to VNT. HIT using tween-diethyl-ether split viral antigen (TW/E-HIT) demonstrated increased sensitivity compared to standard HIT, but with lower specificity. This study compared the specificity of a TW/E-HIT, standard HIT, and VNT by determining potential cross-reactive antibodies to influenza A/H7 viruses in human sera.

Methods: In total, 1028 paired serum samples from 544 Czech patients (average age: 26y) presenting with respiratory symptoms between 1998 and 2013 were used for the specificity evaluation. One hundred patients had confirmed Influenza A infection. HIT and TW/E-HIT were performed following WHO standard procedures, using whole virion and TW/E split antigen of two viral strains (A/Anhui1/2013/H7N9 and A/Oregon/1971/H7N3). TW/E antigen was prepared as described previously (Kendal and Cate, 1983). Assays were performed using 0.5% suspensions of turkey and horse red blood cells (RBCs). VNTs were performed according to WHO recommendations, with hemagglutination reactions based on a described in-house protocol (Havlickova et al., 2012). Animal sera from guinea pigs and hens, immunised with inactivated whole viral antigen, were used as positive controls. Geometric mean titres (GMT) were calculated.

Results Three sera revealed cross-reactivity in HIT and TW/E-HIT assays with low titres (<1:20). A titre of 1:80 was observed for one serum in assays using horse RBCs. Five sera showed titres of 1:20 in VNT. Animal control sera demonstrated the highest sensitivity in TW/E-HIT assays performed with horse RBCs, reflected by overall GMTs of 1:276 and 1:645.