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HUMAN CYTOMEGALOVIRUS SEROLOGICAL STATUS IN PATIENTS WITH INTERSTITIAL LUNG DISEASES

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Recent data, especially from immunocompromised patients, suggest that human cytomegalovirus (HCMV) plays an important role in lung pathology. In this study the serological status of HCMV infection and characteristic of the HCMV infected patients with interstitial lung diseases were determined. The subjects were enrolled prior to immunosuppression therapy introduction.

Słowa kluczowe: ludzki wirus cytomegalii, idiopatyczne włóknienie płuc, sarkoidoza
Key words: HCMV, serology, idiopathic pulmonary fibrosis, sarcoidosis

BACKGROUND

Many interstitial lung diseases (ILD, syn. diffused parenchymal lung disease) are still of unknown etiology. Infectious agents are suggested triggering or modifying agents, which can stimulate development or accelerate ILD in permissive human organism.

Recent data, specially from immunocompromised patients suggest that HCMV plays an important role in lung pathology (1–3).

Because many patients with ILD need temporary or continuous immunosuppressive therapy and sometimes progression of the disease is seen in spite of intensification of immunosuppression, we decided to analyze HCMV serological status of patients with newly diagnosed, different ILD before administration of steroid therapy.

MATERIALS AND METHODS

The blood was drawn from the patients and from persons donating blood after the informed consent was signed. The experimental purpose and text of patient's information was approved by the Local Ethical Committee – document number NN-013-167/00.

Control blood samples included in this study were obtained from healthy persons who donate blood at the Regional Center for Blood Donation and Blood Therapy in Katowice, mean age 39.52 +/– 10.21 (M=35; F=15).

The study group consisted of 52 patients, mean age 47.32 +/– 13.49 (M=32; F=20) admitted to the Department of Phtisiopneumonology because of diffuse changes or hilar adenopathy seen on CXR (chest X-ray) and confirmed by HRCT (high resolution computed tomography) for further diagnostics. None of them was treated from pulmonary pathology before. They had not previously received immunosupression.

The patients, included in the study, were divided into subgroups representing different interstitial lung diseases.

The first group comprised 15 patients with idiopathic pulmonary fibrosis (IPF), mean age 56.10 +/– 10.20 (M=10, F=5). Diagnosis was confirmed by histopathology – usual interstitial pneumonia (UIP) (4) and HRCT findings typical for UIP (5, 6).

The second group included 37 patients diagnosed as sarcoidosis (M=22; F=15), mean age 43.47 +/– 11.16, with diagnosis confirmed by histopathology examination of lymph--nodes and clinical syndrome as detailed in (7). This group consisted of:

- patients in stage I of sarcoidosis – only hilar adenopathy on CXR with exclusion of parenchymal changes by HRCT: 17 patients (mean age 42.09 +/– 11.95), male 11, female 6;
- patients in stage II, III, IV – bilateral hilar lymphadenopathy with parenchymal infiltrates, parenchymal infiltrates without bilateral hilar adenopathy and parenchymal infiltrates with upper zone shrinkage on CXR and HRCT findings typical for sarcoidosis: 20 patients, (mean age 39.80 +/– 9.53), male 11, female 9;.

All serological tests were performed with new generation ELISA test containing recombinant virus antigens (8–11). Along with IgG and IgM qualitative assays anti-gB-IgG tests were performed to distinguish primary HCMV infection from reactivations.

Specific IgG and IgM antibodies determination using HCMV recombinant antigens pp150 (UL (unique sequence of long region of genome) 32), pp52 (UL44), p130 (UL57), glycoprotein B gp150 (UL55) was performed by the immunoenzymatic method of sandwich ELISA (*enzyme-linked immunosorbent assay*), applying Biotest ELISA tests. Microplate reader ELX 800 Bio-Tek Instruments Inc. (USA) linked to a computer was used in the investigations. The following serological criteria for infection activity were applied: IgG (–) negative and IgM (–) negative – sero-negative patient who never met with HCMV (Sero (–)); IgG (+) positive and IgM (–) negative – a sero-positive patient, HCMV infected in the past (Sero (+)); IgG (+) positive and IgM (+) positive – sero-positive patient assumed to have active HCMV infection (Active). All patients with active HCMV infection underwent EIA test for presence of IgG anti-gB as a marker of a new infection or reactivation of infection. Cut-off values were established according to manufacturer's protocol and were as follows: IgG (+) OD (optical density) = 0,35; IgM (+) OD= 0,35; IgG anti-gB OD= 0,30.

All calculations were performed using STATISTICA®-software. Differences between means were estimated using non-parametric tests (Chi square). Correlations were calculated with Spearman rank order test. The level of significance was estimated at $p < 0,05$.

RESULTS

In the group of 15 patients with IPF, 4 were sero-negative (26%), 9 sero-positive (60%) and 2 had serologically active infection (14%). Of the 37 sarcoidosis patients 7 were sero-

-negative (19%), 29 sero-positive (78%) and 1 had serologically active infection (3%); In 17 patients with sarcoidosis stage I (no interstitial changes) 3 were sero-negative (18%), 14 sero-positive (82%) none with serologically active infection and from 20 patients with sarcoidosis and concomitant lung interstitial changes – 4 were sero-negative (20%), 15 sero-positive (75%) and 1 had serologically active infection (5%).

The control group consisted of 50 blood donors where 11 were sero-negative (22%), 38 sero-positive (76%) and 1 had serologically active infection (2%). Serological data are summarized in Figure 1.

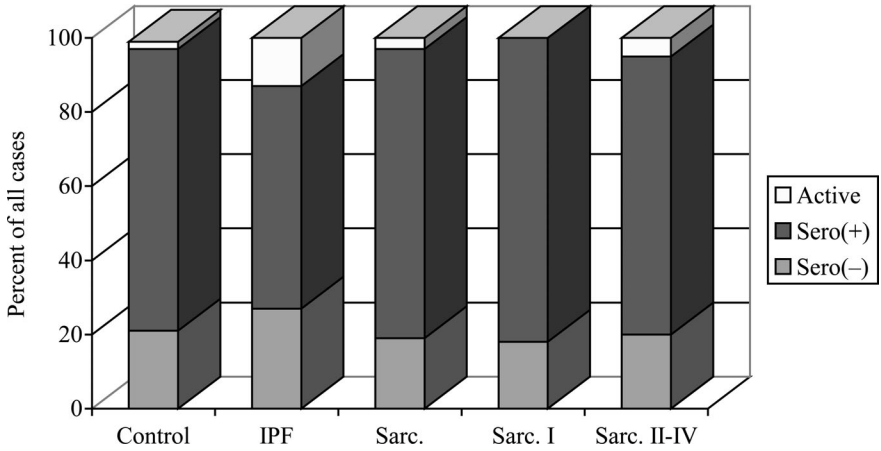

Fig. 1. Comparison of serostatus of the control and study groups (IPF: idiopathic pulmonary fibrosis; Sarc.: sarcoidosis; Sarc. I: sarcoidosis stage I; Sarc. II–IV: sarcoidosis stage II–IV; Active: IgG (+) positive and IgM (+) positive; Sero (+): IgG (+) positive and IgM (–) negative; Sero (–): IgG (-) negative and IgM (–) negative)

Ryc. 1. Porównanie statusu serologicznego grupy kontrolnej I grup badanych (IPF: idiopatyczne włóknienie płuc; Sarc.: sarkoidoza; Sarc. I: sarkoidoza stopień I; Sarc. II–IV: sarkoidoza stopień II–IV; Active: IgG (+) pozytywny i IgM (+) pozytywny; Sero (+): IgG (+) pozytywny i IgM (–) negatywny; Sero (–): IgG (–) negatywny i IgM (–) negatywny)

One case of active HCMV infection in the control group had not detectable level of IgG-gB. This confirmed primary infection. All three patients with active HCMV infection belonging to IPF and sarcoidosis II-IV groups were positive for IgG-gB, which confirmed reactivation of infection.

Statistical analysis using non-parametric tests did not reveal any significant differences between means of subsequent sero-groups. Obtained results suggest that frequency of HCMV infection as evaluated by specific antibody presence, is similar in patients with ILD and in the control group.

Noteworthy, a very similar pattern of serostatus was observed in IPF and sarcoidosis II-IV group; also a similar pattern was observed in the control as in all cases of sarcoidosis group (see Figure 1).

Because age is one independent, important factor which influences HCMV serostatus –age increases the number of sero-positive patients (12), we performed Spearman rang or-

der analysis of serostatus *vs.* age, weighing case number in each group. (Figure 2) This analysis revealed a very similar pattern (coefficient of correlation R) in the control group and in sarcoidosis group stage I and stages II-IV i. e. in younger age groups more sero-negative patients were seen and serologically confirmed activity was observed in older men. In IPF patients we observed quite an opposite trend – reactivation of infection was observed more frequently in younger people and seronegativity was seen in older men. These correlations were very weak but statistically significant.

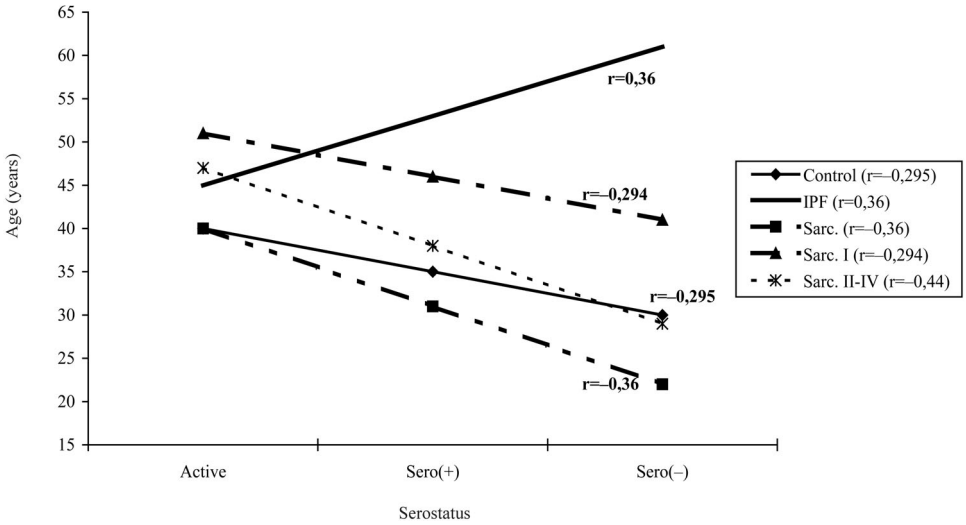

Fig. 2. Rank order correlation Spearman test – serostatus vs. age weighing case number in each group. All correlations were statistically significant $p<0,05$ (IPF: idiopathic pulmonary fibrosis; Sarc.: sarcoidosis; Sarc. I: sarcoidosis stage I; Sarc. II-IV: sarcoidosis stage II-IV; Active: IgG (+) positive and IgM (+) positive; Sero (+): IgG (+) positive and IgM (–) negative; Sero (–): IgG (–) negative and IgM (–) negative)

Ryc. 2. Test korelacji rang Speramn'a – status serologiczny względem wieku przypisując wagę do liczby pacjentów w każdej grupie. Wszystkie korelacji były istotne statystycznie przy założeniu $p<0,05$. (IPF: idiopatyczne włóknienie płuc; Sarc.: sarkoidoza; Sarc. I: sarkoidoza stopień I; Sarc. II–IV: sarkoidoza stopień II-IV; Active: IgG (+) pozytywny i IgM (+) pozytywny; Sero (+): IgG (+) pozytywny i IgM (–) negatywny; Sero (–): IgG (–) negatywny i IgM (–) negatywny)

DISCUSSION

Our results obtained in the investigated groups were very similar to those presented by others (13-17). The mean percentage of sero-positive patients observed by other authors ranged from 25% to 73% in the control groups, depending on age, gender and method used. The percentage of sero-positivity was slightly higher in specific populations: medical workers, haemophiliacs, alcoholics, drug abusers, prostitutes, ranging from 44% to 98% (12, 17–19). This indicates that the intensity of contacts between people and specific behaviours have an influence on HCMV infection and contact frequency and virus spreading. Literature about HCMV serological status in patients with

IPF or sarcoidosis is relatively obscure. There are some publications exploring a possible pathogenic role of herpesviruses in interstitial lung diseases (20). However, only Yonemaru et al. (21) have noticed an elevated level of anti-HCMV IgG antibodies in patients with idiopathic pulmonary fibrosis indicating a possible role of HCMV in pathogenesis of IPF. Animal models of interstitial lung diseases and viral infection are more often used (22).

We observed a correlation between age and serostatus in patients with sarcoidosis and the control group; also noticed by others (14,15) in some populations – an increasing incidence of HCMV infection with age.

In IPF patients we observed a different situation – serologically active HCMV infection could be noticed in younger patients whereas older patients were more frequently sero-negative. This may suggest that older patients with IPF are more susceptible to acute infection with a fatal outcome when immunosuppressed. In the study of Szepietowski T. et al. (12) different temporal trends in HCMV serostatus were also observed in a special group of medical workers, which may suggest different ways of spreading infection.

Noteworthy is the fact, that the clinical status of the patients with IPF or sarcoidosis with serologically active HCMV infection did not differ from the clinical status of the HCMV seropositive or seronegative patients with IPF or sarcoidosis.

CONCLUSIONS

High prevalence of HCMV sero-positivity in patients with sarcoidosis or IPF should be taken into account before planning immunosuppressive therapy because of emerging reactivation of infection.

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STAN SEROLOGICZNY ZAKAŻENIA LUDZKIM WIRUSEM CYTOMEGALII U PACJENTÓW Z CHOROBAMI ŚRÓDMIĄŻSZOWYMI PŁUC

STRESZCZENIE

Pewne dane z piśmiennictwa sugerują istotną rolę ludzkiego wirusa cytomegalii w patogenezie chorób śródmiąższowych płuc, głównie u pacjentów poddanych immunosupresji.

W pracy podjęto próbę oceny statusu serologicznego w kierunku zakażenia wirusem cytomegalii oraz charakterystykę populacji zakażonych pacjentów ze zmianami śródmiąższowymi płuc przed wdrożeniem terapii.

Wszystkie badania serologiczne (IgG, IgM, IgG-gB) wykonano przy użyciu zestawów typu ELISA zawierających rekombinowane antygeny wirusa cytomegalii. Grupę kontrolną stanowiło 50 dawców krwi. Grupa badana składała się z 52 pacjentów: 15 z idiopatycznym włóknieniem płuc (IPF) i 37 z sarkoidozą płuc.

W grupie 15 pacjentów z IPF czterech było serologicznie ujemnych (26%), 9 serologicznie dodatnich (60%) i dwóch z serologicznie aktywną infekcją (14%). Wśród pacjentów z sarkoidozą siedmiu było serologicznie ujemnych (19%), 29 serologicznie dodatnich (78%) i jeden z serologicznie aktywną infekcją (3%). Analiza statystyczna nie wykazała istotnych różnic w liczebności poszczególnych grup serologicznych pomiędzy grupą kontrolną oraz badaną. Uzyskane wyniki sugerują, że częstość infekcji HCMV oceniana na podstawie statusu serologicznego jest podobna w grupie kontrolnej jak i w grupie pacjentów z chorobami śródmiąższowymi płuc.

Stosunkowo wysoko odsetek osób HCMV serologicznie dodatnich wśród pacjentów z chorobami śródmiąższowymi płuc oraz niejednokrotnie współistniejąca immunosupresja sugeruje możliwość częstszej reaktywacji tego zakażenia w tej grupie chorych.

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