

Evaluation of the frequency of *Pneumocystis jirovecii* occurrence in a group of children hospitalized for acute respiratory infections[1]

Elżbieta Gołąb[1], Wioletta Rożej-Bielicka[1], Katarzyna Pancer[2]

[1] Department of Medical Parasitology, [2] Department of Virology; National Institute of Public Health – National Institute of Hygiene, 24 Chocimska Street, 00-791 Warsaw, Poland

Corresponding author: Elżbieta Gołąb; E-mail: egolab@pzh.gov.pl

ABSTRACT. Primary infection with *Pneumocystis jirovecii* in small children may cause inflammation of the respiratory tract which requires hospitalization. Lack of characteristic clinical symptoms makes it impossible to recognize *P. jirovecii* infections without performing laboratory analyses. Nasopharyngeal swabs from 70 children with respiratory tract infections were screened for fragments of the *P. jirovecii* genome. *Pneumocystis* DNA was found in swabs taken from two (2.9%) of the tested children: a newborn who was infected in the hospital and a six month old baby admitted to hospital two days after pneumonia was diagnosed. The obtained results confirm that primary *P. jirovecii* infections may occur in the form of acute respiratory tract inflammations suggesting a viral infection. In differential diagnosis of *Pneumocystis* infections in children molecular methods are useful as their high sensitivity makes it possible to analyze samples obtained in a non-invasive way.

Key words: *P. jirovecii* infection, infants, nasopharyngeal swabs, nPCR

Introduction

Pneumocystis jirovecii is a human-specific atypical fungus exhibiting pulmonary tropism [1,2]. It is common; recently mild *P. jirovecii* infections have been found to occur periodically in over one-half of the general adult population [3]. Seroepidemiological studies have shown that primary *Pneumocystis* infections occur in childhood [4,5]. These infections are in general the cause of inflammations of the respiratory tract, which in most children have a mild or asymptomatic course and are self-limiting [6]. However, in premature, dystrophic or malnourished children or in those with primary immunodeficiencies, interstitial pneumonia (PCP) may develop, which is the consequence of an intensive, uncontrolled growth of the *P. jirovecii* population in lung alveoli.

The aim of the study was to determine the frequency of *Pneumocystis jirovecii* infections among children hospitalized for acute respiratory infections with etiology described as viral on the basis of clinical symptoms.

Material and methods

Samples from 70 children aged 11 days to 5 years (average age 9 months) were analyzed. The children were patients in 7 different wards of five large hospitals in Warsaw in the period from February 15, 2009 to February 18, 2010. Among the analyzed children there were 30 girls and 40 boys. The largest group were children with pneumonia and/or bronchitis (52). In the remaining patients upper respiratory tract inflammation symptoms were noted, in 2 of these children laryngitis was

[1]This study was presented during 50th Clinical Day of Medical Parasitology, 19–20 May 2011, Lodz, Poland

Table 1. Characteristics of examined patients

Clinical diagnosis	Patients#
Upper respiratory tract infections:	5
laryngitis	2
Lower respiratory tract infections:	53
bronchitis	12
pneumonitis	29
pneumonitis and bronchitis	6
No data	12

detected. For 12 patients no diagnosis was established (Table 1). Most of the children (71.8%) were newly admitted to the hospital ward, in 8.2% symptoms of infection of the respiratory tract occurred during their stay in the hospital. In 86% of the analyzed children infection with at least one RNA virus: RSV, hMPV, PIV 1-3, enteroviruses was noted.

Material for investigations of the presence of *P. jirovecii* DNA were swabs from the nasopharynx. DNA was isolated from samples using a QIAamp DNA Mini kit (Qiagen).

The purified DNA was used as a template to amplify the region containing mtLSU-rRNA by nested PCR (nPCR), as described elsewhere [7,8]. Briefly, in the amplification, the primers pair: pAZ102-E (5′-GAT GGC TGT TTC CAA GCC CA-3′) and pAZ102-H (5′-GTG TAC GTT GCA AAG TAC TC-3′), and then pair: pAZ102-X (5′-GTG AAA TAC AAATCG GAC TAG G-3′) and pAZ102-Y (5′-TCA CTTAATATTAATTGG GGAGC-3′) were used. The nPCR amplification yields a 263-bp fragment. Thirty five cycles of amplification were carried out for both rounds. The amplification products were analyzed by electrophoresis on a 2% agarose gel containing ethidium bromide, and the bands were visualized by UV light.

Results and discussion

Pneumocystis jirovecii infections were detected in two children (2.9%), in one of them infection with parainfluenza type 3 virus was also noted. The percentage of children infected during their stay in the hospital was 2.6%, whereas among children newly admitted into the hospital it was 2%.

P. jirovecii infections were detected in two (2.9%) children with acute respiratory infections with symptoms suggesting a viral etiology. One of the children was a newborn girl in whom an upper respiratory tract infection occurred during the hospital stay. A *P. jirovecii* infection was also detected in a six month old boy admitted to the hospital 2 days after occurrence of pneumonia symptoms. As a result of specialist analyses no respiratory viruses were detected in the newborn, while in the newly admitted boy parainfluenza type 3 virus was detected.

Investigations performed in Denmark on small children aged 7–265 days hospitalized because of acute respiratory tract infections indicated *P. jirovecii* infections in 16% of them. *Pneumocystis* was detected only in 2% of the youngest children aged 7–49 days, whereas in the group including children 50–112 and 113–265 days old the percentage of infected individuals was 48% and 13%, respectively. Primary *P. jirovecii* infection was found to be two times more common in cases of upper respiratory tract infection as compared to lower respiratory tract infection [9].

The high percentage of primary *Pneumocystis* infections among acute upper and lower respiratory tract infections in small children in Poland is indicated by the results of serological analysis. Specific class G antibodies were detected in 64% of the 1918 screened children up to 6 months of age [5]. According to available data, in Polish hospitals PCP may constitute up to 15% of postnatal infections in newborns requiring hospitalization in intensive therapy wards [10]. In 1999–2003 in one hospital *Pneumocystis* infections were detected in 18% of newborns requiring mechanical ventilation [11].

P. jirovecii is transmitted by droplet route from one person to another. In immunocompetent adults the infection is in general asymptomatic [3]. Periodic carrier status for *P. jirovecii* was found in 20% of Occupational Health Service employees, whose average age was 33.9±9.45 years, in investigations performed in Sevilla in southern Spain [12]. Among inhabitants of Warsaw of a similar age (20–45 years) 4% were found to be *Pneumocystis* carriers; the percentage was significantly higher (10%) in a group of persons over 60 years old [8]. Persons who are asymptomatically infected are suggested to be a *P. jirovecii* reservoir [3]. On the other hand, the results of epidemiological studies performed by Rivero indicate that asymptomatic carriers may transmit *Pneumocystis* to small children, who are susceptible to infection. Comparing the genotypes of *P. jirovecii* strains isolated from a child with PCP

and his family living together with him the author has found that the source of infection for a six month old child could have been his grandparents [13].

Recently the probability of detecting asymptomatic infections in material from the upper respiratory tract has been found to increase significantly when two samples taken from different sites are analyzed. Infection with *P. jiroveci* was detected in 10.6% (7/66) persons after analyzing and in 21.5% (14/65) persons when in parallel and nasal swabs were analyzed [14]. In our own investigations single samples of nasopharyngeal swabs were analyzed, thus it cannot be excluded that the prevalence of *P. jirovecii* among the screened children was higher than it was found. The used method could have been insufficiently sensitive to detect cases in which the *Pneumocystis* infection had not reached its peak.

A positive result for *Pneumocystis* in a newborn with an acute upper respiratory tract inflammation in whom no respiratory tract viruses were detected may suggest that the infection took place *in utero*. Montes-Cano et al. [15] so far have been the only group to show in 2009 that a vertical mode of infection is possible in humans. Investigations of *in utero* infections performed on animal models gave equivocal results [16,17]. In the light of the investigations of Montes-Cano et al., prenatal transmission can also be taken into consideration in cases of acute infections days described as postnatal found in children aged 7–49, or in the case of PCP, in a 3 week old baby of an HIV infected mother [9,18].

The obtained results confirmed that primary *P. jirovecii* infections may occur in the form of acute respiratory tract inflammations suggesting viral. In differential diagnosis of *Pneumocystis* infections in children molecular methods are useful as their high sensitivity makes it possible to analyze non-invasive samples.

References

[1] Durand-Joly I., Aliouat el M., Recourt C., Guyot K., François N., Wauquier M., Camus D., Dei-Cas E. 2002. *Pneumocystis carinii f. sp. hominis* is not infectious for SCID mice. *Journal of Clinical Microbiology* 40:1862-1865.

[2] Adl S.M., Simpson A.G., Farmer M.A., Andersen R.A., Anderson O.R., Barta J.R., Bowser S.S., Brugerolle G., Fensome R.A., Fredericq S., James T.Y., Karpov S., Kugrens P., Krug J., Lane C.E., Lewis L.A., Lodge J., Lynn D.H., Mann D.G., McCourt R.M., Mendoza L., Moestrup O., Mozley-Standridge S.E., Nerad T.A., Shearer C.A., Smirnov A.V., Spiegel F.W., Taylor M.F. 2005. The new higher level classification of Eukaryotes with emphasis on the taxonomy of Protists. *Journal of Eukaryotic Microbiology* 52: 399-451.

[3] Ponce C.A., Gallo M., Bustamante R., Vargas S.L. 2010. *Pneumocystis* colonization is highly prevalent in the autopsied lungs of the general population. *Clinical Infectious Diseases* 50: 347-353.

[4] Pifer L.L., Hughes W.T., Stagno S., Woods D. 1978. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. *Pediatrics* 61: 35-41.

[5] Sobolewska A., Gołąb E. Dzbeński T.H. 2009. Serological evaluation of the prevalence of *Pneumocystis jirovecii* infection in children with respiratory tract infections in Poland. *Przegląd Epidemiologiczny* 63: 359-362.

[6] Vargas S.L., Hughes W.T., Santolaya M.E., Ulloa A.V., Ponce C.A., Cabrera C.E., Cumsille F., Gigliotti F. 2001. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. *Clinical Infectious Diseases* 32: 855-861.

[7] Wakefield A.E., Pixley F.J., Banerji S., Sinclair K., Miller R.F., Moxon E.R., Hopkin J.M. 1990. Amplification of mitochondrial ribosomal RNA sequences from *Pneumocystis carinii* DNA of rat and human origin. *Molecular and Biochemical Parasitology* 43: 69-76.

[8] Gołąb E., Sadkowska-Todys M., Szkoda M.T., Dzbeński T.H. 2008. The occurrence of *Pneumocystis jirovecii* in people from three different age groups of Warsaw (Poland) community. *Acta Parasitologica* 53: 106-109.

[9] Larsen H.H., von Linstow M.L., Lundgren B., Hogh B., Westh H., Lundgren J.D. 2007. Primary *Pneumocystis* infection in infants hospitalized with acute respiratory tract infection. *Emerging Infectious Diseases* 13: 66-72.

[10] Jurczak A., Kordek A., Grochans E., Giedrys-Kalemba S. 2007. Clinical and microbiological characteristics of hospital infections in the neonatal intensive care unit. *Advances in Medical Science* 52: 30-33.

[11] Kordek A., Kołodziejczyk L., Adamska M., Skotarczak B., Łoniewska B., Pawlus B., Kuźna-Grygiel W., Rudnicki J., Czajka R. 2007. Prematurity and protracted mechanical ventilation as risk factors for *Pneumocystis jiroveci* infection in HIV-negative neonates in an intensive care unit. *The Turkish Journal of Pediatrics* 49: 158-164.

[12] Medrano F.J., Montes-Cano M., Conde M., de la Horra C., Respaldiza N., Gasch A., Perez-Lozano M.J., Varela J.M., Calderon E.J. 2005. *Pneumocystis jirovecii* in general population. *Emerging Infectious*

Diseases 11: 245-250.
[13] Rivero L. 2008. *Pneumocystis jirovecii* transmission from immunocompetent carriers to infant. *Emerging Infectious Diseases* 14: 1116-1118.
[14] Vargas S.L., Pizarro P., Lopez-Vieyra M., Neira-Aviles, Bustamente R., Ponce C. 2010. *Pneumocystis* colonization in older adults and diagnostic yield of single versus paired noninvasive respiratory sampling. *Clinical Infectious Diseases* 50e: 19-21.
[15] Montes-Cano M., Chabe M., Fontillon-Alberdi M., de la Horra C., Respaldiza N., Medrano F.J., Varela J.M., Dei-Cas E., Calderon E.J. 2009. Vertical transmission of *Pneumocystis jirovecii* in humans. *Emerging Infectious Diseases* 15: 125-127.
[16] Sanchez C.A., Chabé M., Aliouat el M., Durand-Joly I., Gantois N., Conseil V., López C., Duriez T., Dei-Cas E., Vargas S.L. 2007. Exploring transplacental transmission of *Pneumocystis oryctolagi* in first-time pregnant and multiparous rabbit does. *Medical Mycology* 45: 701-707.
[17] Icenhour C.R., Rebholz S.L., Collins M.S., Cushion M.T. 2002. Early acquisition of *Pneumocystis carinii* in neonatal rats as evidenced by PCR and oral swabs. *Eukaryotic Cell* 1: 414-19.
[18] Miller R.F., Ambrose H.E., Novelli V., Wakefield A.E. 2002. Probable mother-to-infant transmission of *Pneumocystis carinii f. sp. hominis* infection. *Journal of Clinical Microbiology* 40: 1555-1557.

Ocena częstości występowania *Pneumocystis jirovecii* w grupie hospitalizowanych dzieci z ostrym stanem zapalnym dróg oddechowych

E. Gołąb, W. Rożej-Bielicka, K. Pancer

Pierwotne zakażenie *Pneumocystis jirovecii* u małych dzieci może powodować stany zapalne dróg oddechowych wymagające hospitalizacji. Brak charakterystycznych objawów klinicznych uniemożliwia rozpoznanie infekcji *P. jirovecii* bez przeprowadzenia badań laboratoryjnych. Zbadano 70 dzieci z ostrymi infekcjami dróg oddechowych poszukując fragmentów genomu *P. jirovecii* w wymazach z nosogardzieli. DNA *Pneumocystis* wykryto w wymazach pobranych od dwojga (2,9%) zbadanych dzieci: noworodka, który uległ zakażeniu wewnątrzszpitalnemu i półrocznego dziecka przyjętego do szpitala dwa dni po zdiagnozowaniu zapalenia płuc. Uzyskane wyniki potwierdziły, że pierwotne zakażenia *P. jirovecii* mogą występować w postaci ostrych stanów zapalnych dróg oddechowych sugerujących infekcje wirusowe. W diagnostyce różnicowej zakażeń *Pneumocystis* u dzieci użyteczne są metody molekularne, których wysoka czułość umożliwia badania próbek materiału pobieranego nieinwazyjnie.

Received 1 March 2011
Accepted 30 March 2011