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Seroprevalence of anti-*Chlamydia trachomatis* IgM in neonatal respiratory tract infections in Hungary

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Abstract

Purpose. To determine the seroprevalence of specific IgM indicative of respiratory tract infection (RTI) due to *Chlamydia trachomatis* (CT) among symptomatic infants.

Methodology. A descriptive study was conducted on young infants up to 5 months old at the Bacterial Sexually Transmitted Infections Reference Laboratory, National Centre for Epidemiology, Budapest, covering the period 2008–2016. Serum samples from infants suffering from RTIs were screened with a micro-immunofluorescence test (Focus, Cypress, USA) for the presence of anti-*Chlamydia trachomatis*-specific IgM. A parallel *Bordetella pertussis* screening was performed by an indirect immunofluorescence test (Euroimmun, Lübeck, Germany) that detected specific IgM.

Results. The CT-specific serum IgM was highly reactive in 50 (19.1 %) of the 262 neonates with RTIs, while all proved negative for *Bordetella pertussis*-specific IgM.

Conclusion. Vertically transmitted *C. trachomatis* must be regarded as a common pathogen among symptomatic neonates with RTIs in Hungary. Routine screening and treatment of pregnant women could be one option to help prevent these conditions. Focused laboratory testing based on raised clinical awareness should enable early diagnosis and appropriate therapy for symptomatic infants.

INTRODUCTION

Respiratory tract infection (RTI) is one of the most common neonatal diseases. Its prevalence and mortality are strongly dependent on socioeconomic circumstances, and it particularly affects developing countries [1]. Other intrinsic risk factors, such as prematurity of infants, also predispose for RTIs [2]. Worldwide, pneumonia was the leading cause of death in 2015, with an estimated 12.8 % death rate among children between 1 and 59 months of age [3].

Neonatal RTIs may be caused by a wide range of pathogens and occur via different transmission routes. The infected birth canal itself may play an important aetiological role, as vertical transmission of frequently transmitted bacteria (e.g. Lancefield group B streptococci and enteric bacilli) and *Chlamydia trachomatis* (CT) can occur [2]. As one of the major pathogens causing sexually transmitted infections (STIs), CT is responsible for an estimated 146 million infections per year worldwide, and particularly affects young people aged 15–28 years [4, 5]. The likelihood of women becoming pregnant is increased in this age group and those that do become pregnant may become a maternal source of a potential CT infection in newborns.

The transmission rate from mother to child varies between 50–70 %, and the estimated prevalence rates of neonatal CT range from 4 to 60 per 1000 live births in developed countries [6].

Inclusion conjunctivitis, referred to as ophthalmia neonatorum (ON), affects about 30–50 % of infected newborns, while a more severe clinical manifestation, an RTI, develops in 10–20 % [7, 8]. Symptomatic chlamydial RTI is an invasive disease that elicits a systemic immune response producing CT-specific IgM, which can be used as a confirmatory test in infants with symptoms suggestive of CT-induced RTI [9].

Due to the lack of routine antenatal CT screening in Hungary we had no prevalence data from which we could deduce the actual risk of neonatal infections. Our aim was

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Abbreviations: CT, Chlamydia trachomatis; MIF, micro-immunofluorescence; NAAT, nucleic acid amplificationtest; ON, ophthalmia neonatorum; PCR, polymerase chain reaction; RTI, respiratory tract infection; STI, sexually transmitted infection.

to assess the potential role of this pathogen in neonatal RTIs, and discuss the potential value of installing such a systematic antenatal screening system to detect and treat CT. Azithromycin (1 g in a single dose) is the preferred treatment for maternal CT infection [5].

METHODS

Study population

From January 2008 until December 2016 serum samples were collected from 262 neonates with clinical RTIs. The age of the enrolled subjects varied between 1 and 20 weeks. Age, sex and hospital admission were recorded. The blood samples were collected by neonatologists after diagnosis of RTIs based on clinical (tachypnoea/persistent cough) and/or radiological signs (hyperinflation; diffuse pulmonary infiltrates). Forty-one per cent of the serum samples originated from medical institutes in the Hungarian capital, Budapest, while 59 % were referred from the countryside.

Laboratory methods

Serum samples from infants with RTIs were stored at −20 °C until further processing. A micro-immunofluorescence MIF test (Focus, Cypress, USA; catalogue number: IF1250M) was used according to the manufacturer's instructions to detect CT-specific IgM in these serum samples. The assay was performed from 10 µl serum diluted with 150 µl pretreatment diluent, and PBS was used to determine the endpoint titres. Purified elementary bodies of eight serotypes (D-K) of CT served as chlamydial antigens. In positive cases the fluorescein-labelled antihuman IgM is able to react with the antigen–human IgM complexes. Reactive samples show bright green fluorescence. The cut-off was defined at a serum dilution rate of 1 : 32, as this value is considered to be diagnostic for infection [10]. As a result, seronegative, weak seropositive (reactivity with dilution titre <32) and strong seropositive (dilution titre ≥32) results were obtained (Table 1).

Screening for *Bordetella pertussis*-specific IgM was performed using an indirect immunofluorescence test (IIFT) (Euroimmun, Lübeck, Germany; catalogue number: FI2050-1005M) with whole bacterial antigen. Serum samples were diluted to 1 : 100 as recommended in the manufacturer's instructions. Known cross-reactivity may be observed with other *Bordetella* spp., but there is no report about CT cross-reactivity. Further verification procedures, such as anti-pertussis toxin IgG determination, were not applied, as all these samples proved IgM-negative, i.e. did not show any fluorescence.

RESULTS

The male-to-female ratio of infants with RTIs was 149 male versus 113 female infants (1.3). Two hundred and thirteen out of 262 affected infants (81.3 %) were hospitalized (126 male and 87 female; ratio, 1.4). Seronegative infants represented 65.3 % (171/262) of the group, weak reactivity was detected in 15.6 % (41/262) of the group and 19.1 % (50/262) of the patients were found to be unequivocally seropositive.

The age of the IgM-positive patients ranged between 3–20 weeks, with the median age being 9 weeks. The gender distribution showed an even stronger male dominance: 32 males versus 18 females (ratio, 1.8). Eighty per cent of seropositive babies needed hospital care (40/50), again with a male dominance (25 males versus 15 females; ratio, 1.7). The age and sex distribution, together with the inpatient/outpatient rates, are outlined in Table 1.

Table 1. Age, gender-distribution and hospitalization rates for *C. trachomatis* IgM seropositive (SP) and weak reactive (WR)/nonreactive (NR) neonates (*N*=262)

Postpartum age (weeks)	Serostatus	No. of hospitalized patients (*N*=213)		No. of outpatient (*N*=49)		All
		Male	Female	Male	Female	
0–2	SP	0	0	0	0	16
	WR/NR	9	7	0	0	
3–4	SP	6	3	0	1	31
	WR/NR	7	9	2	3	
5–6	SP	5	4	1	0	38
	WR/NR	17	6	3	2	
7–10	SP	5	4	3	0	79
	WR/NR	30	25	4	8	
11–14	SP	5	1	1	2	60
	WR/NR	23	18	4	6	
15–20	SP	4	3	2	0	38
	WR/NR	15	7	3	4	
All	SP	25	15	7	3	50 (19 %)
	WR/NR	101	72	16	23	212 (81 %)
		126 (48 %)	87 (33 %)	23 (9 %)	26 (10 %)	262

From a differential diagnostic aspect, *B. pertussis* IgM serostatus was also investigated, but none of the serum samples was reported as positive in the laboratory database tested by anti-*Bordetella pertussis* IgM IIFT.

DISCUSSION

The 'classical' type of chlamydial infantile pneumonia is usually a late-onset disease, typically developing at 4–12 weeks of age, accompanied by a so-called 'staccato' cough. Rhinorrhoea may be a characteristic prodromal sign, but fever is infrequent [11, 12]. ON is present in about half of the cases, and is a valuable diagnostic clue, together with peripheral eosinophilia [13, 14]. As the majority of clinical symptoms and radiology signs are nonspecific, differential diagnosis should be extended to the most prevalent viruses (respiratory syncytial virus, adenovirus, cytomegalovirus, etc.) and bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus*, etc.), which can also predispose individuals to late-onset respiratory infections [1, 15, 16]. One should note that mixed infections with these respiratory pathogens may also occur, and viral co-infection has been reported in a higher proportion of severe cases compared to cases of mild pneumonia. [11, 17].

As clinical signs often resemble pertussis (whooping cough), this must also be considered, especially in unvaccinated young infants. Its real risk depends on the actual epidemiological situation, principally when similar cases are accumulating and/or no vaccination can be assumed among the contacts. In our series, we did not find any confirmed pertussis among study patients.

The gold standard for laboratory diagnosis of CT-RTI is PCR examination of the nasopharyngeal aspirates for the presence of CT, but only if these are collected before the start of antibiotic therapy, to prevent false-negative PCR results [5]. In this study most of the infants had already received some antibiotic treatment, rendering the negative results from PCR testing unreliable. For this reason, these types of clinical samples were not collected from the majority of our patients. In a prospective study focusing on different chlamydial clinical manifestations in infants with asymptomatic colonization versus symptomatic respiratory infection, the incidence per 1000 live birth was found to be 6.5 and 2.1, respectively [18].

In the absence of relevant respiratory samples, the specific IgM response can be detected by serological assays as a supportive test [14, 19, 20]. As the maternal IgM do not pass across the placenta, the presence of CT-specific IgM antibody in a newborn's blood sample is indicative of a systemic immune response from the neonate elicited by this invasive type of infection.

The seronegative or weak seropositive *C. trachomatis* IgM MIF titre results of <32 were not further investigated for any pathogens, so the aetiology of these cases remained unknown. In cases of weak seropositivity in clinically suspicious patients, we advise retesting 2–4 weeks later so as to observe any changes in IgM titres. We think that weak positive samples may refer to an early sampling and an early stage of immune response, rather than to a later stage of recovery.

Male infants appear to be more vulnerable to RTIs than females (ratio, 1.3), and also to severe infection requiring hospitalization (ratio, 1.4), especially in respect of CT infection (ratio, 1.7), suggesting that males are more vulnerable to severe CT RTI. This is very different in infants with ON, where the male-to-female ratio was 0.9 for all ON, 1.0 for CT-ON and 0.6 for severe ON requiring hospitalization [21]. Further, the time of onset for CT-related disease differed: the median age of patients suffering from early-onset CT ON was 2 weeks, while it was 9 weeks in CT RTI cases. These figures are similar to those in previously reported data [13]. Only 6 % of the RTI patients belonged to a younger age group than 4–12 weeks, which also corresponds to the clinical observations [11]. Compared to CT ON, the rate of hospitalization from CT RTI was high at 80 % (versus 6.7 % for CT ON). This is due to the need for a more detailed diagnosis, and for more prolonged therapy and follow-up. Furthermore, in premature babies, the increased risk of developing respiratory distress syndrome (RDS) may also favour hospital admission [2].

Based on the positive IgM MIF results (titre $\geq$32), 50 of 262 infants were diagnosed with CT infection, which means a seroprevalence of 19.1 % in this group. Similarly, among non-RSV infantile pneumonia cases, a CT prevalence of as high as 17.1 % was reported in the study of Khan and Potter [22]. These prevalence data are far higher than the recently published figure of 7 % that was based on the direct PCR detection of CT DNA [15]. This discrepancy may be attributed to the different detection methods, as specific IgM may persist up to 3 months in serum samples, while chlamydial DNA rapidly disappears after antibiotic treatment [9].

As the background for diagnosis, PCR detects the pathogen's DNA, while serology detects the specific IgM due to an active immune response. The onset and quality of the tests results are influenced by several extrinsic/intrinsic factors, for example antibiotic therapy, steroid treatment, transfusion, plasmaferesis, infective dose of CT, the timing of sample collection, etc., but in untreated cases with an intact immune system one can expect a positive PCR result first, which is then followed by massive IgM production. The question of whether the titre of the IgM is influenced by the infective dose, i.e. the copy number of the CT DNA, needs further research.

The clinical significance of CT is supported by the observation that it is still being detected as the second most frequent respiratory pathogen (after RSV) for infants less than 6 months old in the Netherlands [15]. Detailed surveillance reports are still lacking, but CT infection and testing should always be considered when viral aetiology is excluded or less likely [23].

Limitations

Optimal laboratory diagnosis of chlamydial neonatal infections relies on the detection of CT DNA from nasopharyngeal aspirates. However, due to previous antibiotic therapy, relevant samples of nasopharynx or conjunctivae were not available from all patients suffering from RTIs, which made the diagnosis of CT infection somewhat less definitive.

The maternal cervical samples were not available for parallel CT testing, however this investigation would have provided valuable additional data about CT prevalence in women, as well as a possible explanation of the potential source of infection in the seronegative or weak seroreactive infants. Furthermore, it would have been informative to know the distribution of the maternal chlamydial genotypes, as in a previous study we confirmed that certain genotypes are more likely to cause CT ON than others [21].

Conclusion

Neonatal STIs are still frequently undetected, misdiagnosed or treated inappropriately. Focused screening and therapeutic efforts are required to reduce the number of CT-infected infants, while routine screening of pregnant women should be implemented in order to potentially decrease the rate of vertical transmission.

Our data demonstrate that *C. trachomatis* is still a leading pathogen among respiratory neonatal infections in Hungary. Chlamydial etiology was found in one-fifth of the newborns with RTIs, in the first report of this kind in Hungary to date. As the neonatal clinical manifestations are the results of vertical transmission, this indicates CT infection of the mother and her sexual partner(s).

Summarizing our results, we would like to point out that serology may be a useful and reliable alternative diagnostic test when no respiratory samples are taken prior to antibiotic treatment for NAAT. Parallel testing of clinical samples for most frequent respiratory pathogens enable targeted therapy and recognition of a potentially mixed infection.

In addition to employing prenatal screening as a potential tool of prevention, neonatalogists and neonatal nurses should also be aware of the risks and prevalence of CT infection, and know about correct sampling and laboratory diagnosis. They have a crucial role in the early recognition of ocular and respiratory chlamydial infections.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study used the results of routine laboratory tests were performed exclusively for diagnostic and not for experimental purposes, and were clinically indicated for all patients. The patients' data were anonymized and consequently individual patient consent was not required.

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