Annals of Clinical Case Reports

Research Article
Published: 25 Feb, 2022

Additional Test of Anti-Pt IgA in the Samples with Detectable Anti-PT IgG can Improve Serodiagnosis of Recent Pertussis

Qinghong Meng[1], Huafeng Yu[2], Jing Sun[2], Yuling Han[2], Dandan Liu[1], Wei Gao[1], Xiang Ma[2#] and Kaihu Yao[1*#]*

[1]Beijing Key Laboratory of Pediatric Respiratory Infection Diseases, Key Laboratory of Major Diseases in Children, Ministry of Education, National Clinical Research Center for Respiratory Diseases, National Key Discipline of Pediatrics, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, China

[2]Department of Respiratory, Qilu Children's Hospital, Cheeloo College of Medicine, Shandong University, China

[#]These authors contributed equally to this work

OPEN ACCESS

Correspondence:
Xiang Ma, Department of Respiratory, Qilu Children's Hospital, Cheeloo College of Medicine, Shandong University, Ji'nan 250022, China, E-mail: maxiang0176@163.com
Kaihu Yao, Beijing Key Laboratory of Pediatric Respiratory Infection Diseases, Key Laboratory of Major Diseases in Children, Ministry of Education, National Clinical Research Center for Respiratory Diseases, National Key Discipline of Pediatrics, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No. 56 Nan-li-shi Road, 100045 Beijing, China, Tel: +86 010 59616981, E-mail: yaokaihu@bch.com.cn

Received Date: *18 Jan 2022*
Accepted Date: *08 Feb 2022*
Published Date: *25 Feb 2022*

Citation:
Meng Q, Yu H, Sun J, Han Y, Liu D, Gao W, et al. Additional Test of Anti-Pt IgA in the Samples with Detectable Anti-PT IgG can Improve Serodiagnosis of Recent Pertussis. Ann Clin Case Rep. 2022; 7: 2132.

ISSN: 2474-1655

Abstract

Objective: To evaluate the value of anti-PT IgA measurement for pertussis diagnosis.

Methods: A total of 302 sera of children including 84 pertussis cases and 218 non-pertussis cases were collected in 2018. Anti-PT IgG and IgA concentrations were measured by ELISA. The anti-PT IgG and IgA levels were categorized as positive when values obtained were ≥ 100 IU/ml and ≥ 20 IU/ml, respectively. Anti-PT IgA was determined when the anti-PT IgG was detectable (≥ 5 IU/ml).

Results: In non-pertussis patients, up to 73.9% of them had undetectable level. However, 34.5% of pertussis patients had anti-PT IgG level ≥ 100 IU/ml and only 32.1% were undetectable. A total of 23 patients had anti-PT IgA level ≥ 20 IU/ml, and 22 of them were found in pertussis patients. Among 29 pertussis patients who had anti-PT IgG levels of ≥ 100 IU/ml, only 8 unvaccinated and 7 vaccinated (>1 year) patients cloud be confirmed by only anti-PT IgG test in single samples. For 7 recently vaccinated patients and 7 children who may have been recently vaccinated (<1 year), eight of them cloud be confirmed by sequential testing of anti-PT IgA. Among 28 pertussis patients who had anti-PT IgG levels of 5- <100 IU/ml, 7 patients had anti-PT IgA level ≥ 20 IU/ml.

Conclusion: The sequential testing of anti-PT IgA in patients who had detectable and uncertain anti-PT improved the serological diagnosis of recent pertussis.

Keywords: Pertussis; Pertussis toxin; IgG; IgA; Children

Introduction

Recently, pertussis resurgence has been reported in China [1-3]. However, due to lack of laboratory tests [4], pertussis was still underestimated in China [5,6]. The laboratory-confirmed tests included culture, PCR and serodiagnosis [7]. The sensitivity of culture and PCR decreases over time, and culture and PCR for pertussis was not available in most community hospitals in China. Therefore, the use of serodiagnosis of pertussis is attractive in China.

The World Health Organization (WHO) criteria for a laboratory confirmed case include seroconversion observed in paired serology samples, but collection of serial samples is infrequent in practice [8]. For interpreting single samples, anti-PT IgG has repeatedly been shown to be the most robust serological marker for acute pertussis infection [9-11]. However, many of the problems associated with anti-PT IgG serodiagnosis still persist. It is difficult to interpret anti-PT IgG results in the following situation: (1) Positive anti-PT IgG results in recently vaccinated children (<1 year); (2) positive anti-PT IgG results in children with unknown vaccination history (at the age of pertussis vaccination); (3) equivocal or even lower anti-PT IgG results.

Anti-PT IgA assay was inferior to anti-PT IgG assay as the test of choice for pertussis diagnosis from a single sample [12]. The major interest in measuring anti-PT IgA, comes from the observation that anti-PT IgA responses are only weakly induced by pertussis vaccination [13-16]. The recently published study by Subissi et al. [17] suggested that reflex testing of anti-PT IgA improves pertussis

serodiagnosis in recently vaccinated symptomatic subjects with elevated anti-PT IgG levels. Therefore, anti-PT IgA may be an additional marker in pertussis serodiagnosis for uncertain anti-PT IgG results, when a second sample cannot be obtained.

In this present study we evaluated anti-PT IgG to assess the seroprevalence of pertussis in different groups of patients, and anti-PT IgA will be tested in patients with detectable anti-PT IgG levels. This study will help us better understand the value of anti-PT IgA for a single anti-PT IgG sample to diagnose pertussis.

Materials and Methods

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Qilu Children's Hospital affiliated to Shandong University. Written informed consent was obtained from all the parents or guardians, for these children's blood samples to be used for research on infectious diseases.

Samples collection

This is a cross-sectional and hospital-based study. From January 2018 through December 2018, 302 children diagnosed with respiratory tract infection (without pertussis history) or pertussis hospitalized in Qilu Children's Hospital, Shandong University was included in this study. It was discharge diagnosis. The clinical case criteria for pertussis diagnosis were referred to the WHO definition of pertussis [18]. A suspected case is a person of any age with a cough lasting ≥ 2 weeks, or of any duration in an infant or any person in an outbreak setting, without a more likely diagnosis and with at least one of the following symptoms, based on observation or parental report: Paroxysms (fits) of coughing; inspiratory whooping; post-tussive vomiting, or vomiting without other apparent cause; apnea (only in <1 year of age); OR clinician suspicion of pertussis. The confirmed cases of pertussis were determined by laboratory confirmation (culture, PCR or anti-PT IgG test), and all of them had at least one positive laboratory test. For recently vaccinated children (<1 year) or children who may have been recently vaccinated (unknown vaccination history), they were confirmed by culture or PCR. Those cases diagnosed with immune system related diseases were not enrolled. For all children, basic data about age, gender, date of sampling and medical histories were collected from hospital information systems and electronic medical records. The samples were not collected for this study if the informed consent could not get from the parents or guardians. All serum samples were frozen at −20°C until analysis.

Serological testing

Both anti-PT IgG (Euroimmun, Lübeck, Germany) and IgA (Institut Virion/Serion GmbH, Germany) were detected using the commercially available ELISA kit, according to the manufacturer's instructions. An absorbance of reaction was read using Infinite 200 Multi-Mode Microplate Reader (TECAN). The antibody results were expressed in international units per milliliter (IU/ml). The lower limit of detection for anti-PT IgG was 5 IU/ml and referred to the "First International Standard for Pertussis Antiserum", NIBSC code: 06/140, World Health Organization (WHO). The anti-PT IgG levels were categorized as negative, equivocal and positive when values obtained were <40, 40- <100 and ≥ 100 IU/ml. Anti-PT IgG ≥ 100 IU/ml (≥ 1 years post pertussis vaccination) was considered to be indicative of a recent pertussis infection, as recommended by the previous studies [19,20]. The lower limit of detection for anti-PT IgA was 10 IU/ml and referred to WHO Reference Reagent (06/142). The anti-PT IgA levels were categorized as negative, equivocal and positive when values obtained were <15, 15- <20 and ≥ 20 IU/ml.

Data analysis

Data were analyzed using the JMP (version 10.0). For statistical analysis, antibody concentrations below the lower limit for quantification were assigned as half the lower limit of quantification (2.5 IU/ml for anti-PT IgG, and 5 IU/ml for anti-PT IgA). χ^2 test was used to compare proportions of subjects with protective anti-PT IgG or IgA among different subgroup. $P \leq 0.05$ were considered statistically significant.

Results

Characteristics of study population

A total of 302 subjects were enrolled in the study with the age range of 1 month 4 days to 11 years 9 month (median: 4 years). The ratio of male to female was 1.6:1 (187:115). Among them, 218 subjects diagnosed with respiratory tract infection were considered 'non-pertussis', and the other 84 subjects meeting clinical case definition of pertussis were laboratory confirmed pertussis. Among non-pertussis group, the rate of at least 3 dose of DTP vaccination was 75.2% (164/218). Among pertussis group, 38.1% (32/84) of patients were not vaccinated against DTP, 41.7% (30/84) of patients had a confirmed history of DTP vaccination. A total of 17 pertussis patients were unknown for the history of DTP vaccination.

Distributions of anti-PT IgG and anti-PT IgA

The distributions of anti-PT level in the sera of 302 subjects are presented in Table 1. In non-pertussis patients, more than 70% of them had undetectable anti-PT IgG. In patients with pertussis, about 70% of them had detectable anti-PT IgG, and half of them have anti-PT IgG levels ≥ 100 IU/ml. There was no significant differences in rates of anti-PT IgG ≥ 100 IU/ml between unvaccinated and vaccinated children (P=0.7885). Among pertussis patients who had anti-PT IgG levels of ≥ 100 IU/ml, 63.2% of who have coughed more than 3 weeks.

The anti-PT IgA levels were tested in 114 patients who had detectable anti-PT IgG levels. The anti-PT IgA level distribution was summarized in Table 2. Anti-PT IgA concentration exceeded 20 U/ml in 23 subjects, and 22 of them were found in patients with pertussis. There was no significant differences in rates of anti-PT IgA ≥ 20 IU/ml between unvaccinated and vaccinated children (P=1.0). Among pertussis patients who had anti-PT IgA levels of ≥ 20 IU/ml, 76.5% of who have coughed more than 3 weeks.

The value of sequential testing of anti-PT IgA in single samples for pertussis diagnosis

Among 29 pertussis patients who had anti-PT IgG levels of ≥ 100 IU/ml, only 8 unvaccinated and 7 vaccinated (>1 year) patients cloud be confirmed by only anti-PT IgG test in single samples. For 7 recently vaccinated (<1 year) patients and 7 children who may have been recently vaccinated (unknown vaccination history), eight

Table 1: The distributions of anti-PT IgG in pediatric patients hospitalized in the respiratory department.

Anti-PT IgG level	Percentages (%, 95% CI)		*P*
	Non-pertussis	Pertussis	
<5 IU/ml	73.9 (67.6-79.2)	32.1 (23.1-42.7)	<0.0001
5- <40 IU/ml	18.8 (14.2-24.5)	17.9 (11.1-27.4)	0.849
40- <100 IU/ml	4.6 (2.5-8.2)	15.5 (9.3-24.7)	0.0014
≥ 100 IU/ml	2.8 (1.3-5.9)	34.5 (25.2-45.2)	<0.0001

Table 2: The distribution of anti-PT IgA in patients with detectable anti-PT IgG.

Diagnosis and anti-PT IgG level	Total	Distribution of anti-PT IgA (N)			
		<10 IU/ml	10- <15 IU/ml	15- < 20 IU/ml	≥ 20 IU/ml
Non-pertussis					
5- <40 IU/ml	41	41	0	0	0
40- <100 IU/ml	10	9	0	0	1
≥ 100 IU/ml	6	4	2	0	0
Pertussis					
5- <40 IU/ml	15	8	2	1	4
40- <100 IU/ml	13	10	0	0	3
≥ 100 IU/ml	29	9	3	2	15
Total	114	81	7	3	23

of them cloud be confirmed by sequential testing of anti-PT IgA. Among 18 pertussis patients who had equivocal or even lower anti-PT IgG results, eight of them cloud also be confirmed by sequential testing of anti-PT IgA.

For anti-PT IgG (100 IU/ml) or IgA alone (20 IU/ml) assay, the sensitivity was only 26.3%. However, when anti-PT IgG is used as the first indicator to confirm cases, and secondary confirmation is performed with anti-PT IgA, the sensitivity improved to 52.6%.

Discussion

The dilemma of anti-PT IgG measurement has been interpreting indeterminate anti-PT IgG levels, when a second sample cannot be obtained. Our results suggest that sequential testing of anti-PT IgA in the patients which had uncertain anti-PT IgG result would improve the pertussis diagnosis.

The major interest of measurement of IgA in pertussis serodiagnosis comes from the observations that, primary vaccinations with WCVs or ACVs in the first year of life induce IgM and IgG antibodies but do not induce IgA antibodies. Among 114 patients who had detectable anti-PT IgG levels, 23 of them had anti-PT IgA level ≥ 20 IU/ml, and 22 of them were found in patients with pertussis. Our result also suggested that anti-PT IgA would be highly specific for acute infection.

Prior vaccination history may theoretically confound interpretation of anti-PT IgG. Among 29 pertussis patients who had anti-PT IgG levels of ≥ 100 IU/ml, for 7 recently vaccinated (<1 year) patients and 7 children who may have been recently vaccinated (unknown vaccination history); the diagnosis could not be confirmed. For 8 of them, the diagnosis cloud is confirmed by sequential testing of anti-PT IgA.

Robson et al. [12] suggested that reflex testing of anti-PT IgA in the context of equivocal anti-PT IgG results may be worthwhile. For 13 pertussis patients with equivocal anti-PT IgG (40- <100 IU/ml), three of them cloud also be confirmed by sequential testing of anti-PT IgA. However, considering the relative low levels of anti-PT IgG in vaccinated children, we could not eliminate that those subjects who had detectable but negative anti-Ptx IgG (5- <40 IU/ml) did have a real pertussis infection. The sequential testing of anti-PT IgA in 4 patients had exactly increased the sensitivity of serological diagnosis of pertussis. The two studies by Guiso et al. [11] and May et al. [8] suggested that the likely cutoff is between 10 and 20 IU/ml, close to the minimum level of quantification. If the cutoff of 10 IU/ml was adopted, the diagnostic performance in another 3 patients which had anti-PT IgG levels of 5- <40 IU/ml was also changed. Our results suggest that anti-PT IgA should be tested for not only equivocal (40- <100 IU/ml), but also lower detectable anti-PT IgG level (5- <40 IU/ml).

In our study, nearly 90.0% (75/84) of the pertussis patients in our study are children aged <4 years. In patients with detectable anti-PT IgG, about half (30/57) of them have detectable anti-PT IgA. It was similar with previous study, which revealed the less frequent and less strong IgA levels for pertussis infection in children aged <4 years [21]. Even though there is some potential in IgA to improve the diagnosis, it is not applicable for routine diagnostics for infants and young children. However, it is reassuring to study this further in adolescents and adults, as already confirmed by other research [17]. In fact, serology overall is not recommended to children <2 years. The positive anti-PT IgA was also worthwhile for infants and young children with uncertain anti-PT IgG, when only serodiagnosis was available and a second sample cannot be obtained.

There were certain limitations in this study. Firstly, when clinical case definition of pertussis was used in the selection of subjects, the majority of (84.5%, 71/84) of the pertussis patients in our study are children aged <2 years. In the further research, more children older than 2 years and adolescents should be selected for serological analysis. Secondly, subjects not meeting clinical case definition were considered 'non- pertussis'. There is no exclusion from laboratory testing. Seven pertussis cases in non-pertussis group were missed in clinical work. The laboratory tests were performed in the future to give strong evidence for the select of non-pertussis patients. Thirdly, the anti-PT IgG ELISA kit from Euroimmun was used clinically, however, they had no commercially available anti-PT IgA ELISA kit, we used the kit from Institut Virion/Serion GmbH.

Conclusion

The sequential testing of anti-PT IgA in patients who had detectable and uncertain anti-PT improved the serological diagnosis of recent pertussis, when a second sample cannot be obtained.

Acknowledgement

This work was financially supported by Cultivation Fund Project of the National Natural Science Foundation in Beijing Children's Hospital, Capital Medical University (GPMS2020001) and Ji'nan Medical and Health Science Technology Innovation Plan (201805015 and 201907032).

References

1. Yang Y, Yao K, Ma X, Shi W, Yuan L, Yang Y. Variation in *Bordetella pertussis* susceptibility to erythromycin and virulence-related genotype changes in China (1970-2014). PLoS One. 2015;10(9):e0138941.
2. Huang H, Gao P, Gao Z, Wang L, Hao B, Liu Y, et al. A big pertussis outbreak in a primary school with high vaccination coverage in northern China: An evidence of the emerging of the disease in China. Vaccine. 2018;36(52):7950-5.
3. Fu P, Wang C, Tian H, Kang Z, Zeng M. *Bordetella pertussis* infection in infants and young children in Shanghai, China, 2016-2017: Clinical features, genotype variations of antigenic genes and macrolides resistance. Pediatr Infect Dis J. 2019;38(4):370-6.
4. Meng QH, Shi W, Li LJ, Yao KH. "Cleaved Lymphocytes" could be induced by pertussis toxin injection in mice, and are actually not lymphocytes. Clin Infect Dis. 2018;66(4):639-40.
5. Meng Q, Li L, Shi W, Wang Q, Ding M, Liu Y, et al. Seroprevalence of diphtheria and pertussis immunoglobulin G among children with pneumonia in Jinan, China. BMC Pediatr. 2018;18(1):383.
6. van den Brink G, Wishaupt JO, Douma JC, Hartwig NG, Versteegh FG. *Bordetella pertussis*: An underreported pathogen in pediatric respiratory infections, a prospective cohort study. BMC Infect Dis. 2014;14:526.
7. Cherry JD, Tan T, Wirsing von Konig CH, Forsyth KD, Thisyakorn U, Greenberg D, et al. Clinical definitions of pertussis: Summary of a global pertussis initiative roundtable meeting, February 2011. Clin Infect Dis. 2012;54(12):1756-64.
8. May ML, Doi SA, King D, Evans J, Robson JM. Prospective evaluation of an Australian pertussis toxin IgG and IgA enzyme immunoassay. Clin Vaccine Immunol. 2012;19(2):190-7.
9. Andre P, Caro V, Njamkepo E, Wendelboe AM, Van Rie A, Guiso N. Comparison of serological and real-time PCR assays to diagnose *Bordetella pertussis* infection in 2007. J Clin Microbiol. 2008;46(5):1672-7.
10. de Melker HE, Versteegh FG, Conyn-Van Spaendonck MA, Elvers LH, Berbers GA, van Der Zee A, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol. 2000;38(2):800-6.
11. Guiso N, Berbers G, Fry NK, He Q, Riffelmann M, Wirsing von Konig CH, et al. What to do and what not to do in serological diagnosis of pertussis: Recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis. 2011;30(3):307-12.
12. May ML, Evans J, Holgate T, Doi SA, Ross P, Robson JM. Pertussis toxin IgA testing over-diagnoses recent pertussis infection. Pathology. 2017;49(7):770-5.
13. Ward JI, Cherry JD, Chang SJ, Partridge S, Keitel W, Edwards K, et al. *Bordetella Pertussis* infections in vaccinated and unvaccinated adolescents and adults, as assessed in a national prospective randomized Acellular Pertussis Vaccine Trial (APERT). Clin Infect Dis. 2006;43(2):151-7.
14. Mertens PL, Stals FS, Steyerberg EW, Richardus JH. Sensitivity and specificity of single IgA and IgG antibody concentrations for early diagnosis of pertussis in adults: An evaluation for outbreak management in public health practice. BMC Infect Dis. 2007;7:53.
15. van der Zee A, Schellekens JF, Mooi FR. Laboratory diagnosis of pertussis. Clin Microbiol Rev. 2015;28(4):1005-26.
16. Thomas MG, Ashworth LA, Miller E, Lambert HP. Serum IgG, IgA, and IgM responses to pertussis toxin, filamentous hemagglutinin, and agglutinogens 2 and 3 after infection with *Bordetella pertussis* and immunization with whole-cell pertussis vaccine. J Infect Dis. 1989;160(5):838-45.
17. Subissi L, Rodeghiero C, Martini H, Litzroth A, Huygen K, Leroux-Roels G, et al. Assessment of IgA anti-PT and IgG anti-ACT reflex testing to improve *Bordetella pertussis* serodiagnosis in recently vaccinated subjects. Clin Microbiol Infect. 2020;26(5):645.e1-8.
18. World Health Organization. Vaccine Preventable Diseases Surveillance Standards.
19. Chen Z, Zhang J, Cao L, Zhang N, Zhu J, Ping G, et al. Seroprevalence of pertussis among adults in China where whole cell vaccines have been used for 50 years. J Infect. 2016;73(1):38-44.
20. Meng QH, Luo J, Yang F, Shen YJ, Li L, Li LJ, et al. A general lack of IgG against pertussis toxin in Chinese pregnant women and newborns. Pediatr Infect Dis J. 2018;37(9):934-8.
21. van der Zee A, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: Comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis. 1996;174(1):89-96.