

Concentrations of Immunoglobulin G Antibodies Against Pertussis Toxin Does Not Decrease Over a Long Period of Time in Japan

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Abstract

Objective Adult patients with pertussis rarely show typical symptoms, such as paroxysmal coughing, inspiratory “whoop”, or post-tussive vomiting. While a culture is regarded as the gold standard for diagnosis, the sensitivity is very low. Therefore, the diagnosis of pertussis in adults in clinical practice is mostly based on single-sample serology using an enzyme-linked immunosorbent assay (ELISA) with the pertussis toxin antigen. Various cut-off values for the anti-pertussis toxin IgG (PT-IgG) have been proposed. It has been reported that concentrations of PT-IgG fall below the defined cut-off about 4.5 months after infection on average, and within 1 year in most patients. We investigated the distribution and time course of the PT-IgG levels.

Methods The data were collected from the medical records.

Patients The study retrospectively identified subjects who had visited Ikebukuro Otani Clinic, which is a specialized clinic for patients with cough. We retrospectively reviewed 406 patients with PT-IgG measurements to investigate the age distribution of PT-IgG levels. The changes in PT-IgG levels over time were assessed in the 205 patients who had more than one PT-IgG measurement.

Results PT-IgG levels were ≥ 100 EU/mL in more than 15% of subjects. The PT-IgG levels of a few subjects had diminished over a long period of time.

Conclusion A PT-IgG level greater than the defined cut-off value simply indicates past infection or immunization in most subjects. As such, a single measurement of PT-IgG using the cut-off values might lead to overdiagnosis of pertussis. Further data collection and analysis are required.

Key words: *Bordetella pertussis*, Bordetella, ELISA, pertussis toxin, PT-IgG

(Intern Med 55: 3257-3263, 2016)

(DOI: 10.2169/internalmedicine.55.6973)

Introduction

Cough is a common symptom for which medical attention is sought by patients. In the United States, “cough” was the third principal reason for office visits after “progress visit” and “general medical examination”; there were 31 million visits for cough in 2012 (1). In UK general practice, “cough” was the most common symptom in more than half

of all new referrals (2). In Japan, “cough” is also a common symptom among patients. Among the 35,768 patients who visited the Department of General Medicine of Juntendo University Hospital in 2012, 4,543 had a “cough”, which was the second most common symptom following a “fever” (3). Various guidelines for the diagnosis and management of cough have been published (4-9).

Pertussis is one of the important causes of cough, especially chronic cough (10). Pertussis remains one of the top

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Received for publication December 12, 2015; Accepted for publication April 11, 2016

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10 causes of childhood death worldwide, mainly in unvaccinated children, and it causes nearly 300,000 deaths in children every year (11). Pertussis is the most dangerous in infants, where the cough can be minimal or absent but can induce apnea, which leads to death. In unvaccinated children, the diagnosis of pertussis is easy from the clinical picture. Clinical suspicion of pertussis is made in patients with a cough-associated illness lasting at least two weeks with one of the following: paroxysms of coughing, inspiratory “whoop”, and post-tussive vomiting. In the initial catarrhal stage, the differentiation of pertussis from a common cold is difficult. The paroxysmal whooping cough sets in after one to two weeks and can persist for four weeks or longer. Adults and older children are less likely to have typical symptoms, instead showing only mild, non-specific symptoms like prolonged cough; therefore, the diagnosis of pertussis is difficult when it is based on symptoms alone. All studies assessing the relative proportion of pertussis in adults with longer-lasting coughs agree that a significant proportion of them have pertussis (12).

The best way to prevent pertussis is to get vaccinated. In Japan, the pertussis vaccine has been administered since 1950, and the number of patients with pertussis decreased from more than 100,000 in 1950 to 206 in 1971. The first vaccine against pertussis included whole-cell killed *Bordetella pertussis* (*B. pertussis*) bacteria and contained pertussis endotoxin (surface lipooligosaccharide), which had side effects. Severe neurologic disorders led to the suspension of pertussis vaccination in 1975, and the number of patients increased to about 13,000 in 1979 in Japan. New acellular pertussis vaccines, which include only a few selected pertussis antigens, were introduced in 1981 (13). Outbreaks occurred in university students in 2007, and it was suspected that many young people did not have immunity against *B. pertussis*, although recent studies have suggested another mechanism: asymptomatic transmission from individuals who were vaccinated with the currently used acellular *B. pertussis* vaccines (14).

Identification of the pathogen by culturing nasopharyngeal secretions has been considered the gold standard for diagnosis, but this method is unsuitable in clinical practice for young and adult patients, as *B. pertussis* loads are very low in these populations, and the isolation and identification of bacteria is very difficult, especially in previously vaccinated persons (15, 16). Compared with the culture method, nucleic acid amplification tests such as the IS481-based real-time polymerase chain reaction (PCR) and *B. pertussis*-specific loop-mediated isothermal amplification (LAMP) are highly sensitive for the detection of *B. pertussis* DNA (17-19). Therefore, these nucleic acid amplification tests have been recommended for the accurate diagnosis of pertussis (20). Nevertheless, the PCR and LAMP are virtually impossible to perform in clinical practice due to their cost and the fact that they are not covered by public health insurance in Japan.

Serology is a valuable aid in diagnosing pertussis in ado-

lescents. The most common pertussis serologic assays use enzyme-linked immunosorbent assay (ELISAs) with highly purified pertussis toxin (PT) antigen (21, 22). PT antigen is expressed only by *B. pertussis*, and no cross-reacting antigens have been described; furthermore, IgG responses occur in most patients with *B. pertussis* infection (23, 24). A two- to four-fold increase in the IgG titers against pertussis toxin antigen (PT-IgG) in paired acute and convalescent sera may be the most indicative of a recent pertussis infection. A review reported that a single measurement of PT-IgG can be indicative of current pertussis infection, with a sensitivity of 76% and a specificity of 99% for the diagnosis of acute pertussis (11). Some reports have proposed cut-off values for PT-IgG to indicate recent contact (25). In The Japan Respiratory Society “Guidelines for cough”, a cut-off value of 100 EU/mL was used for a definitive diagnosis of pertussis, and PT-IgG levels <10 EU/mL were used to indicate non-immunized status (26). It was reported that concentrations of PT-IgG fall below the defined cut-off about 4.5 months after infection on average, and in most patients (82%) within 1 year (27).

We herein investigated the effect of previous vaccination for pertussis on the levels of PT-IgG. We also examined the time course of the levels of PT-IgG in the Japanese population.

Materials and Methods

We retrospectively identified subjects who had visited Ikebukuro Otani Clinic between December 2012 and October 2014. In Japan, PT-IgG measurement was commercially available from December 2012. Ikebukuro Otani Clinic is a specialized clinic for patients with cough. About half of the patients were given a final diagnosis of bronchial asthma or cough-variant asthma, and one third were diagnosed with acute bronchitis by viral or bacterial infection. All patients who had their PT-IgG levels measured at multiple time points as of February 2015 were recruited for the present study, as well as those with measurements at a single time point (Fig. 1).

Information on sex, date of birth, and PT-IgG levels with the sampling date were collected from the medical records of each patient. The patients were followed up for one year, and their data were updated in February 2016.

We conducted epidemiologic studies of the age distribution for natural and vaccine-induced immunity against *B. pertussis* to evaluate the correlation between the age and PT-IgG levels. To study the natural time course of the PT-IgG levels, the average change in the logarithm of the PT-IgG level versus elapsed time was analyzed for patients who had their PT-IgG levels measured at multiple time points. We excluded the subjects who were deemed inappropriate for the natural time course analysis. To exclude subjects with active *B. pertussis* infection during the observation period, the data from subjects with more than a two-fold increase of PT-IgG level were excluded from the analysis. PT-IgG levels ex-

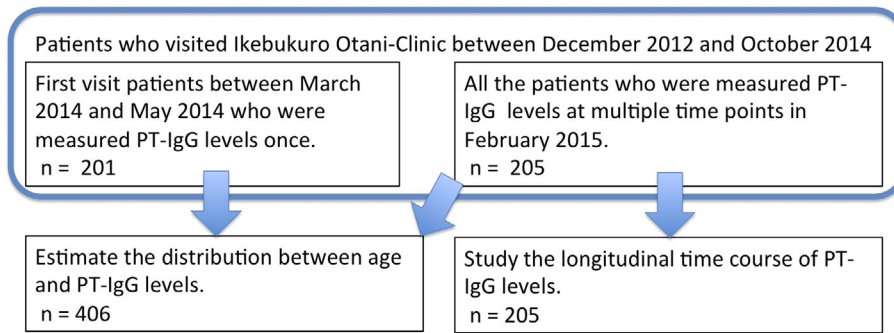


Figure 1. Patient recruitment.

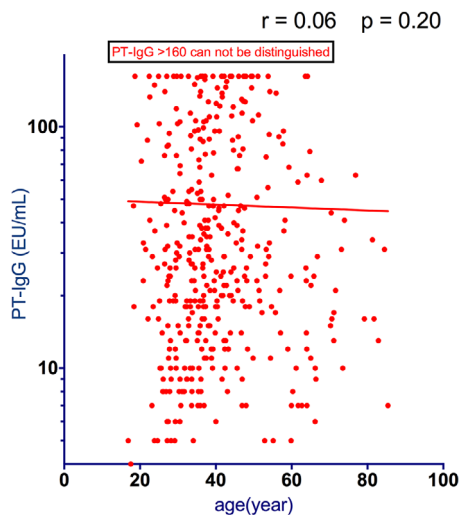


Figure 2. The age distribution of IgG antibodies against PT (PT-IgG) levels.

ceeding 160 EU/mL were reported as “PT-IgG >160”, and we were unable to obtain the exact values; therefore, these data were also excluded from the change rate analysis.

The levels of PT-IgG antibody were measured using commercially available enzyme immunoassays for pertussis (DENKA SEIKEN CO., LTD., Niigata, Japan. https://catalog.hardydiagnostics.com/cp_prod/product/324764-pertussis-antibody-eia-48-tests-per-kit-by-denka-seiken-research-use-only-antiser) at Showa Medical Science Corporation (Tokyo, Japan). Briefly, a plate coated with the pertussis toxin antigen was incubated with standard or test sera, washed, incubated with goat anti-human IgG conjugated with horseradish peroxidase, and then washed again. The peroxidase activity was then measured after color development. The PT-IgG titers were determined using calibration curves obtained from standard serum JNII-10 (28) from the National Institute of Infectious Diseases (NIID) in Japan. The measurements were conducted at the laboratories of the Showa Medical Science Corporation in accordance with the manual. PT-IgG levels exceeding 160 EU/mL were reported as “PT-IgG >160”; serum was not diluted, and no reexamination was performed to obtain the exact values.

The results are presented as the mean \pm standard deviation (SD). The robustness of our results was assessed using

the GraphPad PRISM 6 software program for Mac OS X (GraphPad Software, Inc., La Jolla, USA). The correlation coefficients between the age and PT-IgG levels were calculated by Spearman’s correlation, a method that makes no assumptions about Gaussian-like distributions. Differences in the PT-IgG levels between groups were estimated using the Kruskal-Wallis test. A p value <0.05 was considered significant.

This study was approved by the ethics committee of the Tokyo Medical and Dental University (approved number 1957). Given the retrospective nature of the study, informed consent from the participating patients was waived by the institutional ethics committee and not obtained, but the public was notified of the study by posters.

Results

A total of 205 (men, 66; mean age, 40.9 ± 13.8 ; women, 139; mean age, 39.8 ± 11.4) patients were recruited who had PT-IgG levels measured at multiple time points (Fig. 1). In addition, 201 (men, 88; mean age, 42.4 ± 12.8 ; women, 113; mean age, 39.7 ± 14.1) first-visit patients between March 2014 and May 2014 who had PT-IgG levels measured once were recruited. Thus, the age distribution of PT-IgG levels in a total of 406 patients was determined (Fig. 2). We found no correlation between the age and PT-IgG level ($r=0.06$, $p=0.20$). Fig. 3 shows the distribution of the PT-IgG levels in the three groups by date of birth. Only a small portion of babies who were born between 1975 and 1985 were vaccinated for pertussis. After more than 30 years, there was little difference in the levels between those born in or before 1974 (51.9 ± 49.5 EU/mL), those born between 1975 and 1984 (44.3 ± 43.6 EU/mL), and those born in or after 1985 (43.0 ± 46.7 EU/mL) ($p=0.15$).

More than 18% (37 out of 205) of recruited patients had PT-IgG levels exceeding 100 EU/mL. Because patients with a high PT-IgG level tend to be examined multiple times, we analyzed first-visit patients who had single measurements. Among the first-visit patients with single measurements, 15% (29 out of 201) had a PT-IgG level exceeding 100 EU/mL. The PT-IgG levels of very few subjects had diminished over a long period of time, and the average decay rate of PT-IgG levels per year was 0.97-fold. In the 37 patients

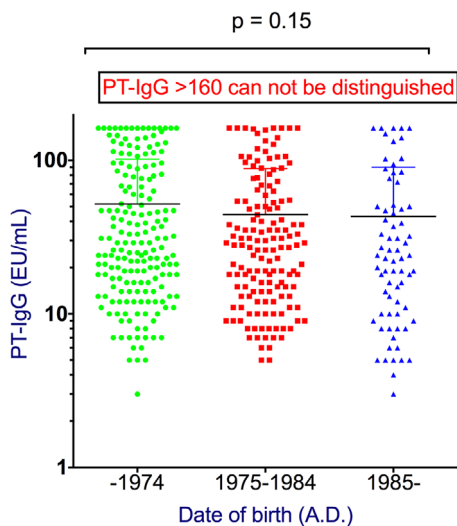


Figure 3. The distribution of PT-IgG levels in the three groups by date of birth. Pertussis vaccination was suspended in 1975, and new pertussis vaccines were introduced in 1981, but only a small number of babies were vaccinated.

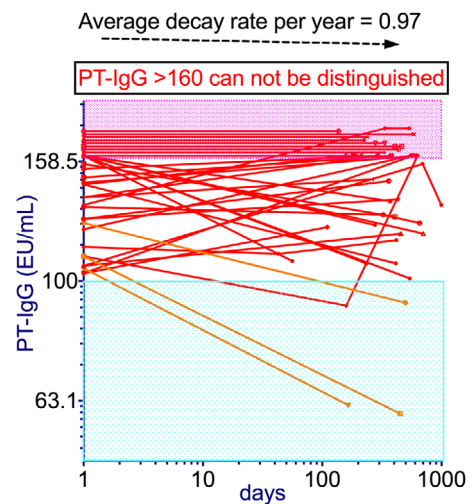


Figure 4. The temporal changes in the PT-IgG levels in patients with an initial PT-IgG level exceeding 100 EU/mL. The average annual decay rate of the PT-IgG levels was 0.97-fold. IgG-PT levels exceeding 160 EU/mL (upper shaded area) were not reported as exact values. Only a small portion of patients showed PT-IgG levels that had decreased to below 100 EU/mL (lower shaded area).

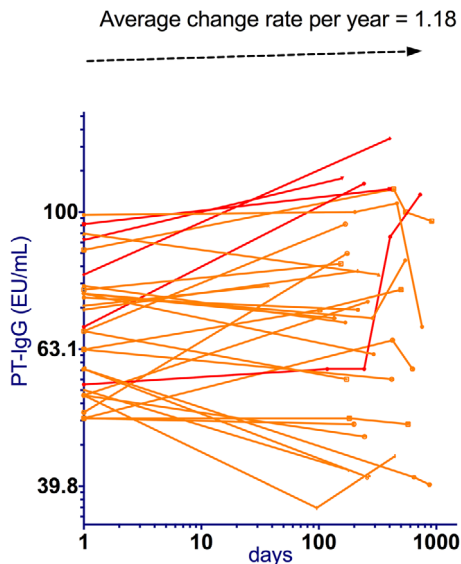


Figure 5. The temporal changes in the PT-IgG levels in patients with an initial PT-IgG level between 50 and 100 EU/mL. The average annual change rate of the PT-IgG levels was 1.18-fold.

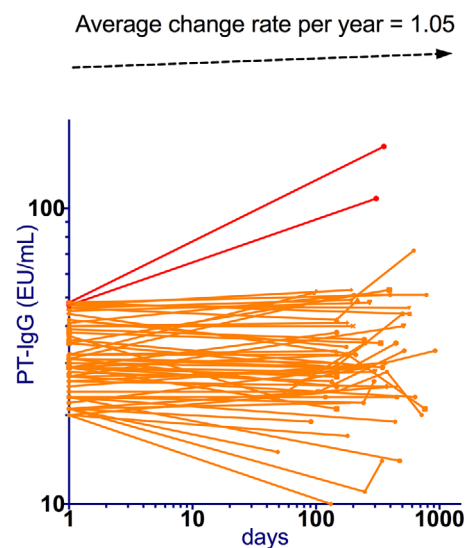


Figure 6. The temporal changes in the PT-IgG levels in patients with an initial PT-IgG level between 20 and 50 EU/mL. The average annual change rate of the PT-IgG levels was 1.05-fold.

with an initial PT-IgG level exceeding 100 EU/mL, only 3 had a PT-IgG level that had dropped below 100 EU/mL during follow-up. Most patients continued to show levels exceeding 100 EU/mL for at least several months (Fig. 4).

In patients with PT-IgG levels between 10 and 100 EU/mL, more than 6% (9 out of 135) showed a more than 2-fold increase in the PT-IgG level during long-term follow-up and were therefore regarded as having *B. pertussis* infection. Except for these subjects, the average change rate for the PT-IgG levels per year was 1.11-fold (Fig. 5-7).

In the patients with PT-IgG levels below 10 EU/mL, 9% (3 out of 32) showed a more than 2-fold increase in the PT-

IgG level during long-term follow-up, resulting in a level exceeding 10 EU/mL during follow-up. Except for these subjects, the average change rate for the PT-IgG levels per year was 1.15-fold (Fig. 8).

Discussion

We found no correlation between the age and PT-IgG levels. Because only a small portion of babies who were born between 1975 and 1984 had been vaccinated for pertussis,

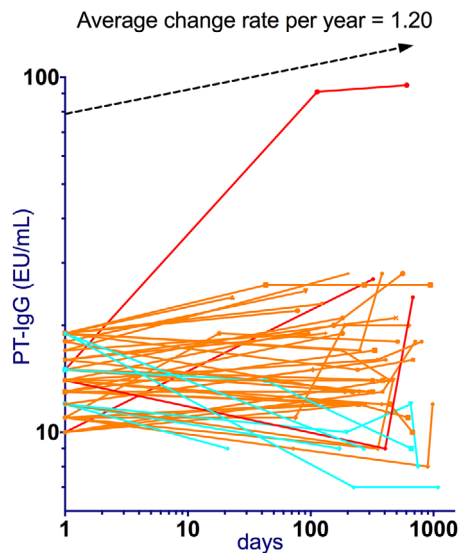


Figure 7. The temporal changes in the PT-IgG levels in patients with an initial PT-IgG level between 10 and 20 EU/mL. The average annual change rate of the PT-IgG levels was 1.20-fold.

we stratified subjects according to the date of birth, and the results showed that, after more than 30 years, there was little difference in the levels between the age groups. Using a cut-off value of <math><10</math> EU/mL as an indication of non-immunization for pertussis (26), the proportion of subjects without immunity for *B. pertussis* was 14% (55 out of 406), and 9% (3 out of 32) of these subjects were regarded as having *B. pertussis* infection subsequently. The patients with PT-IgG levels between 10 and 100 EU/mL are usually regarded as having immunity against pertussis. In the present study, more than 5% of subjects were regarded as having *B. pertussis* infection thereafter. These results reinforce those of past studies which concluded that, “The relation between antibody and protection is not straightforward, with no particular serological correlate of protection” (11).

We showed that the values of PT-IgG changed very little over a long period of time. It is unlikely that concentrations of PT-IgG had naturally fallen over time and increased again after another infection by *B. pertussis*. Because there is a very low possibility that many subjects would show almost equal concentrations of PT-IgG before naturally falling over time and then rising after *B. pertussis* reinfection. Because patients with a high PT-IgG level tend to be examined multiple times, we analyzed the patients who only had single measurements. Among them, 15% had a PT-IgG level exceeding 100 EU/mL. In other words, more than 15% of subjects could be definitively diagnosed with pertussis based on a single measurement of PT-IgG, according to The Japan Respiratory Society “Guidelines for cough”. However, only a small portion of them were clinically given a diagnosis of active pertussis. One reason for this is that the PT-IgG levels of very few subjects had diminished over a long period of time, and many subjects continued to have a value that exceeded the cut-off after infection or immunization.

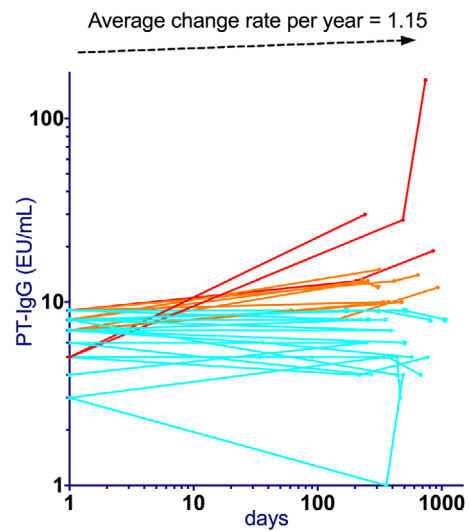


Figure 8. The temporal changes in the PT-IgG levels in patients with an initial PT-IgG level below 10 EU/mL. The average annual change rate of the PT-IgG levels was 1.15-fold.

Our finding that the PT-IgG levels of only a few subjects diminished over a long period of time was inconsistent with the results from previous reports (27). This discrepancy in findings may be due to differences in race/ethnicity or epidemic strains of *B. pertussis* in Japan. In the present study, the levels of PT-IgG antibody were measured using a commercially available enzyme immunoassay for pertussis (DENKA SEIKEN CO., LTD.), which is currently the only way to measure PT-IgG level in Japan. This technique is considered to allow the comparison of PT-IgG titer data obtained in the IU with values from other countries, as the IU value was approximately 70-80% of the EU value in this assay (28). Since serum antibodies are polyclonal, the composition rates of recognition epitopes may differ among sera. Subtle differences in the measurement methods for PT-IgG levels may also explain these discrepancies in findings. The purities of antigens, purification methods, strains, composition of buffer solutions, etc. used in the test differ among manufacturers. In the present study, we showed that a PT-IgG level exceeding 100 EU/mL might simply indicate past infection or immunization, at least in Japan. A single measurement of PT-IgG using cut-off values might therefore lead to the overdiagnosis of pertussis. Further data collection is required, and refined diagnostic criteria are needed. Commercially available measurement levels of PT-IgG range from 0 to 160 EU/mL in Japan. Exact values for subjects with a PT-IgG exceeding 160 EU/mL might be needed for detailed analyses.

The main limitation associated with this study was that the subjects were retrospectively identified at a single institute that specialized in treating patients with cough. Furthermore, we did not use culture, PCR, or LAMP methods to identify the pathogen. While we did perform culture and nucleic acid amplification tests, an accurate diagnosis of pertussis was impossible, as *B. pertussis* loads are very low in

adults (15, 16), creating the possibility of false-negative results. Only a small number of subjects were given a clinical diagnosis of active pertussis, although more than 15% of recruited patients had PT-IgG levels exceeding the cut-off value. About half of the patients were given a final diagnosis of bronchial asthma or cough variant asthma, and one third were diagnosed with acute bronchitis by viral or bacterial infection. In fact, only one subject was diagnosed with acute pertussis, and he was indistinguishable from the others because he did not present with typical symptoms such as paroxysmal coughing, inspiratory “whoop”, or post-tussive vomiting. Based on paired serum titers showing a more than 2-fold increase in IgG antibodies, about 7% of subjects were regarded as having *B. pertussis* infection in the long-term follow-up, which does not necessarily indicate an infection at the time of study entry.

In summary, a single measurement of PT-IgG level for the diagnosis of pertussis is insufficient, and using cut-off values for the diagnosis may run the risk of overdiagnosis. The guidelines (26) have three problems associated with diagnosing pertussis: (A) Patients with a PT-IgG level exceeding 100 EU/mL can be definitively diagnosed with pertussis; (B) Patients with a PT-IgG level exceeding 10 EU/mL who have no history of immunization can be definitively diagnosed with pertussis; and (C) Patients with a PT-IgG level exceeding 10 EU/mL who previously had a PT-IgG level less than 10 EU/mL can be definitively diagnosed with pertussis. With respect to (A), in actual clinical practice in Japan, the PT-IgG level rarely falls below the defined cut-off value (i.e. 100) (Fig. 4). With respect to (B), pertussis vaccinations were stopped from 1975 to 1981, and therefore, subjects born between 1975 and 1984 are at high risk of not having been vaccinated for pertussis. In this group, 86% (128 out of 149) of subjects showed a PT-IgG level exceeding 10 EU/mL (Fig. 3) and therefore should have been diagnosed with acute pertussis, according to the guideline (26). With respect to (C), we believe the variations in the PT-IgG level (Fig. 3. orange line) observed in the present study are within normal limits and not caused by pertussis. To make a definitive diagnosis, we recommend the use of the *B. pertussis*-specific LAMP or the confirmation of a two- to four-fold increase in the PT-IgG values in paired sera.

The authors state that they have no Conflict of Interest (COI).

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