Original article

Title: A nationwide epidemiological survey of adolescent patients with diverse symptoms similar to those following human papillomavirus vaccination: background prevalence and incidence for considering vaccine safety in Japan

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Abstract

Background: Since June 2013, Japan has suspended proactive recommendation of human papillomavirus (HPV) vaccination due to self-reported diverse symptoms, including pain and motor dysfunction, as possible serious adverse events following immunization. Although these symptoms may be seen in adolescents without HPV vaccination, their frequency taking into account disease severity has not been examined.

Methods: A two-stage, descriptive, nationwide epidemiological survey was conducted in 2016 with a 6-month targeted period from July 1 to December 31, 2015 to estimate the prevalence and incidence of diverse symptoms among Japanese adolescents without HPV vaccination. Participants were 11,037 medical departments in hospitals selected nationwide by stratified random sampling. Eligible patients had to satisfy four criteria: (1) aged 12-18 years upon visiting hospital; (2) having at least one of four symptoms/disorders (pain or sensory dysfunction, motor dysfunction, autonomic dysfunction, or cognitive impairment); (3) symptoms/disorders persisting for at least three months; and (4) both criteria (2) and (3) influence attendance at school or work. We further extracted patients with diverse symptoms similar to those after HPV vaccination while considering opinions of doctors in charge.

Results: Estimated 6-month period prevalence of diverse symptoms among girls aged 12-18 years without HPV vaccination was 20.2 per 100,000. Annual incidence was estimated to be 7.3 per 100,000.

Conclusion: Adolescent Japanese girls without HPV vaccination also visited hospitals with diverse symptoms similar to those following HPV vaccination. Our findings predicted the
medical demands for diverse symptoms that are temporally associated with but not caused by vaccination of Japanese adolescents.

29  **Keywords:** human papillomavirus vaccine; safety; adverse event; diverse symptoms; adolescents
Introduction

Human papillomavirus (HPV) vaccines have been globally available as a primary prevention tool against cervical cancer since their first licensure in 2006. In addition to the well-known efficacy/effectiveness of HPV vaccines in reducing pre-cancerous lesions, several reports have indicated herd immunity with decreased prevalence of vaccine-targeted HPV genotypes among unvaccinated women. A recent modelling study suggested that increased HPV vaccination coverage, together with intensive cervical cancer screening, could achieve elimination of cervical cancer by the end of the century.

In Japan, HPV vaccination for girls aged 13-16 years started through a governmental urgent project from November 2010, followed by designation as a national immunization program (NIP) from April 1, 2013, with high vaccine coverage of around 70%. After that, however, “diverse symptoms including pain and motor dysfunction” (hereafter referred to as “diverse symptoms”), lasting several months with influence on attendance at school or work in some cases, were reported as adverse events following immunization (AEFI). The Ministry of Health, Labour and Welfare (MHLW) Japan announced suspension of proactive recommendation on June 14, 2013 until they could provide sufficient information about the symptoms. Despite the fact that HPV vaccination is currently still available under NIP, vaccine coverage has severely decreased to around 1%.

Safety of HPV vaccines is well documented by numerous studies that compared vaccinated and unvaccinated individuals and targeted well-known reactions or diseases that were already recognized in medical practice. However, these reports have not directly answered the claim in Japan that “there have been no diverse symptoms before the introduction of HPV vaccine”. Alternatives may be newly designed comparative studies with prospective/retrospective information collection on self-reported diverse symptoms. However, unbiased identification of symptoms irrespective of vaccination status would not be achieved.
because HPV vaccine safety is already a social issue in Japan. Besides, prospective cohort studies are no longer feasible in Japan due to very low vaccination coverage. These considerations cause reluctance to choose comparative study designs for evaluating HPV vaccine safety in Japan.

A lingering key issue is whether or not diverse symptoms can be attributed to diseases that already exist. Although some clinicians mentioned that a certain proportion of adolescents presented diverse symptoms even before HPV vaccines, few studies have provided quantitative data. A population-based questionnaire survey including 29,846 female residents in Nagoya, Japan, targeted 24 non-specific diverse symptoms rather than specific disease entities, and indicated that the cumulative incidence of diverse symptoms among non-vaccinated adolescent girls ranged between 0.2% for loss of ability to walk in a normal way or becoming dependent on a walking stick/wheelchair and 25.6% for menstrual irregularity.\textsuperscript{14} In contrast, according to AEFI data managed by the MHLW Japan, as of November 2014, a total of 2,584 cases (0.08%) were reported among 338 million girls with HPV vaccination, and 186 cases (0.005%) were additionally found to be “not recovered”, with various status including hospitalization, assistant requirement for daily life, or influence on attendance at school or work.\textsuperscript{15} This suggests that the two surveys may have observed different events in terms of disease severity and frequency. In order to reveal the whole picture of more severe (i.e., very rare) diverse symptoms that have received social attention in Japan, a nationwide survey is required. Our objective was to conduct a nationwide epidemiological survey to estimate prevalence and incidence of diverse symptoms in Japanese adolescents without history of HPV vaccination, and to obtain background data for considering HPV vaccine safety. Despite not being our original purpose, we also calculated frequency of diverse symptoms among vaccinated adolescents to demonstrate the challenge of comparing frequencies between those with and without HPV vaccination.
Material and methods

Overview of the survey

This was a descriptive nationwide survey with a two-stage procedure: a first-stage survey estimated the number of patients visiting hospitals, and a second-stage survey revealed patients’ clinical characteristics. We followed “a protocol for a nationwide epidemiological survey on intractable diseases”16-18 which targeted “rare” diseases.

Sampling frames for participants were 25,325 medical departments in hospitals across Japan including pediatrics, neurology, anesthesiology (pain clinic), obstetrics/gynecology, orthopedic surgery, gastroenterology, rheumatology, general practice, neurosurgery, and psychiatry/psychosomatic medicine, where adolescents with diverse symptoms were likely to visit. A nationwide hospital database (WELLNESS Co., Ltd., Tokyo, Japan) was used. Additionally, 88 special departments from 83 hospitals that were designated by the MHLW Japan were included in the sampling frames because, as of January 2016, they had to offer clinical management for patients with diverse symptoms after HPV vaccination. From these 25,413 departments, 18,302 (72.0%) were selected by stratified random sampling according to inpatient bed numbers and hospital characteristics (Table 1). Sampling fractions were 100% for departments in hospitals with ≥200 beds, departments in university hospitals, and special departments, and 50% for departments in hospitals with <200 beds. We modified the sampling fractions in the original protocol (i.e., gradual increase from 5% to 100%) to include many departments because patients with diverse symptoms were expected to be very rare.

The first-stage survey started in January 2016. Departments were asked whether patients satisfying criteria visited them during July 1, 2015 to December 31, 2015. Eligible patients had to satisfy four criteria: (1) aged 12-18 years when visiting the departments; (2) having at least one of the following symptoms/disorders: (a) pain or sensory dysfunction, (b) motor dysfunction, (c) autonomic dysfunction, or (d) cognitive impairment; (3) symptoms/disorders
persisting for at least three months; and (4) both criteria (2) and (3) influence attendance at school or work. These criteria were applied uniformly regardless of the department that was invited for the survey. Survey explanation forms are shown in Supplementary Material 1. If departments had one or more patients, they were asked to report the number of patients by gender and age (1-year intervals). The numbers of eligible boys and girls were reported. As of 2015, that is, at the time of this survey development, gender neutral HPV vaccination had already been recommended in the USA, Canada, Austria, and Australia. We thought it would be meaningful to evaluate the background frequency of diverse symptoms for both genders in Japan.

If departments that responded in the first-stage survey had one or more patients that satisfied the criteria, the doctors in charge were invited to the second-stage survey to provide the following clinical characteristics for each patient based on pre-existing medical charts: previous medical history, date of/age at symptom onset, date of the first/last visit, history of HPV vaccination, presence/absence of each symptom (during July to December 2015), duration of the symptoms, status of schooling/working attendance (during July to December 2015), and diagnoses that were identified/recognized by departments (up to 10 diagnoses, regardless of the cause for the present symptoms, but not providing any diagnoses where examinations were only requested). We also solicited the opinions of doctors in charge about whether they were able to adequately explain the patient’s symptoms during July to December 2015 by their diagnoses (no/yes/unknown), and, if yes, which diagnosis was the most explicable. Informed consent was waived because anonymity was maintained during information collection. The study protocol was approved by the ethics committee at Osaka University, Graduate School of Medicine (No. 15320) and Osaka City University, Graduate School of Medicine (No. 3276).

Do reported symptoms correspond to “diverse symptoms after HPV vaccination”?: decision
At the time of information collection, symptoms of the reported patients did not necessarily correspond to those after HPV vaccination. Thus, we further defined whether those symptoms corresponded to “diverse symptoms after HPV vaccination” based on two kinds of information in the second-stage survey: “opinions of doctors in charge” whether or not they were able to adequately explain patients’ symptoms by the diagnoses that were identified/recognized by the department, and “a diagnosis” that was designated as the most explicable by the doctor in charge (Table 2). Our decision harmonized with a claim in Japan that “there have been no diverse symptoms before the introduction of HPV vaccine”, and a consensus of the National Expert Committee for Vaccine Safety by the MHLW Japan that diverse symptoms were possibly attributed to a certain mental and physical reaction (referred to as “functional somatic disorder” thereafter20). Detailed explanations of the decision process are provided in Supplementary Material 2. Supplementary Tables 1 to 5 show the complete list of diagnoses of patients in the second-stage survey.

Estimation of prevalence and incidence of diverse symptoms
Using information from the first and second stage survey, we calculated the period prevalence (from July 2015 to December 2015) of patients aged 12-18 years with diverse symptoms by gender and HPV vaccination history (Figure 1). We limited our estimation to patients whose age at onset was ≥12 years because HPV vaccine is designated as NIP for girls aged 12-16 years in Japan. HPV vaccination history was classified into five categories: “never vaccinated (Group A)”; “vaccinated, symptom occurred before vaccination (Group B)”; “vaccinated, symptom occurred after vaccination (Group C)”; “vaccinated, the time relation between vaccination and symptom onset was unknown (Group D)”; and “unknown vaccination status (Group E)”. All boys were considered “never vaccinated (Group A)”, since HPV vaccine is not designated as
NIP for Japanese boys. The data on cumulative number of the vaccinated population aged 12-18 years with at least one dose of HPV vaccine, by gender, was provided by the MHLW. The number of non-vaccinated individuals aged 12-18 years was calculated by subtracting the cumulative number of the vaccinated population from the national population, based on census data (as of October 1, 2015). While we used preliminary national data in reporting our prevalence for the first time at the meeting of National Expert Committee for Vaccine Safety in 2016 and 2017, finalized national data were used in this report.

We defined “incident patients” as those who experienced onset of diverse symptoms between July and December 2015 (i.e., the period identical to the period surveyed). For incidence estimation, we calculated the proportion of incident patients among reported patients in the second-stage survey and multiplied that proportion by the 6-month period prevalence of diverse symptoms.

All calculations were independently performed by two epidemiologists (WF and MH) using SAS software version 9.3 (SAS Institute, Inc., Cary, NC) and cross-checked to guarantee accuracy.

Results

Of the 18,302 departments selected for the first-stage survey, 11,037 (60.3%) responded (Table 1 and Supplementary Table 6). A total of 508 departments reported one or more patients who satisfied inclusion criteria. The reported number of patients was 903 boys and 1,652 girls.

Estimated number of patients with diverse symptoms and prevalence

The estimated number of patients aged 12-18 years with diverse symptoms, whose age at onset was ≥12 years during the period of July to December 2015, was 829 boys and 1,590 girls (Table 3). The corresponding 6-month period prevalence of diverse symptoms was 19.8 per 100,000
for boys and 40.1 per 100,000 for girls (Table 4). The prevalence for girls was further calculated separately for five categories of vaccination history (Table 5). The estimated number of patients with diverse symptoms and the 6-month period prevalence in Japan were, respectively, 477 and 20.2 per 100,000 for Group A, and 445 and 27.8 per 100,000 for Group C. The estimated number of patients with diverse symptoms for Group E was 604, and prevalence was undetermined because an appropriate denominator was not available.

**Patient characteristics**

Of 508 departments that reported one or more patients who satisfied criteria in the first-stage survey, 324 (63.8%) departments responded to the second-stage survey with clinical information on 1,418 patients. The number of patients with diverse symptoms aged 12-18 years whose age at onset was $\geq$12 years was 183 boys (all were Group A) and 365 girls (Group A: 110, Group B: 2, Group C: 103, Group D: 13, Group E: 137) (Supplementary Figure 1). Among girls in Group A, Group C, and Group E, in which the number of patients was not sparse, the proportion having each symptom, the number of symptoms, and the status of schooling/working was evaluated (Supplementary Figure 2). Group C showed higher proportions/numbers for most items, whereas Group A and Group E indicated lower proportions/numbers, but were similar to each other.

**Estimated prevalence of diverse symptoms according to number of symptoms**

We calculated period prevalence of diverse symptoms according to the number of symptoms among girls separately for Group A and Group C (Figure 2) by using period prevalence (Table 5) and distribution of number of symptoms (Supplementary Figure 2-(v)). Lower prevalence of diverse symptoms was shown among those with a higher number of symptoms. When patients were limited to those whose number of symptoms was $\geq$10, the 6-month period prevalence
among unvaccinated girls (Group A) was 5.3 per 100,000. The most frequent diagnoses among
their girls were orthostatic dysregulation for Group A (n=5, reported from five departments,
including one patient with postural orthostatic tachycardia syndrome [POTS]), and autoimmune
encephalopathy due to HPV vaccine for Group C (n=18, reported from one department).

Estimated incidence of diverse symptoms
Among 110 girls in Group A that were reported to the second-stage survey, 18% (n=20)
experienced onset of diverse symptoms between July and December 2015. When we multiplied
this proportion (0.18) by the 6-month period prevalence of diverse symptoms in Group A (20.2
per 100,000), the incidence of diverse symptoms among unvaccinated girls was 3.6 per 100,000
during the 6-month period or 7.3 per 100,000 annually. Using the same calculation, the annual
incidence of diverse symptoms among unvaccinated girls with ≥10 symptoms was 1.6 per
100,000.

Discussion
This was the first nationwide survey to estimate prevalence of severe diverse symptoms among
adolescents without a history of HPV vaccination in Japan. We confirmed that adolescents with
no history of HPV vaccination also visited hospitals due to diverse symptoms and a wide
spectrum of diagnoses, demonstrating that diverse symptoms include elusive and heterogeneous
disease entities. We also estimated the annual incidence of diverse symptoms among
unvaccinated girls, which predicts the medical demand for diverse symptoms that
coincidentally occur following vaccination of Japanese female adolescents. A response rate
greater than 60% in each stage of the survey would ensure a certain degree of generalizability.
There may be some criticism that our survey did not accurately focus on unvaccinated girls
with diverse symptoms in “a multilayered way”, since one eligibility criteria for reported
patients was “having at least one of the symptoms”. However, even girls with a single symptom have been reported to the MHLW as “not recovered” cases of adverse events following HPV vaccination.\textsuperscript{15} We also found that both 6-month period prevalence and annual incidence among unvaccinated girls with \( \geq 10 \) symptoms were “not zero” when number of symptoms was considered as a surrogate variable of multilayered symptoms.

In addition to the prevalence of diverse symptoms among girls without a history of HPV vaccination (Group A), we estimated the prevalence among vaccinated girls whose symptoms occurred after vaccination (Group C). However, these estimates cannot be directly compared between groups because suspension of the proactive recommendation for HPV vaccination in Japan led to a smaller vaccinated population among girls aged 12-14 years (Table 5). Some unstable age-specific prevalence due to a sparse denominator (e.g., for aged 14 years in Group C) prevented us from obtaining reliable age-standardized estimates. Furthermore, there were many potential biases that could have led to underestimation or overestimation of prevalence in each group (Table 6). Nonetheless, it is noteworthy that the prevalence in Group A seemed to be underestimated overall because situation No. 1 in Table 6 (i.e., if patients already have diverse symptoms, they will not receive HPV vaccine), which results in overestimation of prevalence, would be very unlikely due to low HPV vaccination coverage at the time of the survey.

There are other reasons why we cannot compare prevalence between unvaccinated and vaccinated girls. First, there were 604 girls with diverse symptoms in Group E whose vaccination status was unknown (Table 5) because information collection in the second-stage survey relied on pre-existing medical charts. In a hypothesized situation in which all patients in Group E were “never vaccinated” (i.e., vaccine coverage: 0\%), period prevalence in Group A was greater than that in Group C (Supplementary Figure 3). This situation may be plausible when considering the similar characteristics between Groups A and E in the second-stage.
survey (Supplementary Figure 2). Second, patients in Group C included girls whose diverse symptoms occurred more than several months after last HPV vaccination (maximum: 48 months in the second-stage survey). When we limited Group C to those who had shorter durations, the period prevalence was lower than that in Group A (Supplementary Table 7). The proportion/number of each symptom between groups in the second-stage survey (Supplementary Figure 2) also cannot be compared due to potential bias, as shown in Table 6. Despite these biases, we believe that providing proportions/numbers, with careful interpretation, is meaningful for a better understanding of HPV vaccine issues in Japan.

Reports similar to ours have not yet been published in terms of severity of diverse symptoms. The previous study in Nagoya, Japan, which targeted diverse symptoms among Japanese adolescent girls, differed from our survey not only because it compared the odds of various symptoms in vaccinated and non-vaccinated girls, but also because it evaluated milder symptoms: a cumulative incidence in unvaccinated girls of 0.2 to 25.6%. While that study showed no association between HPV vaccination and 24 symptoms by comparing vaccinated and unvaccinated girls, another study re-analyzed the data and showed possible associations for several symptoms, such as cognitive impairment and movement disorders. That study used the same dataset but applied different methodology, in terms of study period, interaction term, age adjustment, and selection of unvaccinated controls. Such discrepancies may indicate the difficulty of controlling confounding and reducing selection bias in the dataset. However, since our study targeted different levels of symptoms, which were more severe and less frequent, findings from that previous study are not directly relevant to our findings.

While our study demonstrated how challenging it was to compare the frequency of diverse symptoms between adolescents with and without HPV vaccination in observational studies, comparability between treatment groups may be retained in pre-licensure clinical trials. Supplementary Table 8 summarizes results from phase III, double-blind, randomized controlled
trials of HPV vaccine, which included adolescents aged 12-18 years and safety data on serious adverse events during the follow-up period, as compared to placebo vaccines or other equivalent vaccines (e.g., hepatitis A virus vaccine) as controls. Overall, the safety profile was similar between the HPV vaccine and control groups. These reports also consistently showed that the most frequently reported solicited adverse reactions following vaccination were injection site symptoms (pain, redness, and swelling), which were reported significantly more often in the HPV vaccine group than in the control group, but were generally transient.

Orthostatic dysregulation and its associated disorders were the most frequent diagnoses, and could not be clearly distinguished from diverse symptoms after HPV vaccination. Our findings were in line with the fact that POTS has been frequently evaluated as a possible adverse event following HPV vaccination, although the majority of reports found no significant safety concern or supportive evidence for a causal relationship. The annual incidence of POTS among unvaccinated girls was estimated to be 6.49 per 100,000 in Finland, which is consistent with our findings in terms of disease rarity.

The underlying mechanisms of diverse symptoms that can occur among unvaccinated girls are complex and remain incompletely understood. Although generalizability is controversial, some of the recent findings are worth noting. Regarding frequently reported diagnoses in the present survey, pathogenesis for orthostatic dysregulation or POTS may have included impairment of circulatory adjustment against gravitational stress, altered central blood volume, abnormal autonomic reflexes and elevated sympathetic tone, damaged skeletal muscle pump activity, local vascular tension regulation dysfunction, iron insufficiency, mast cell activation, and autoimmune dysfunction. In patients with somatoform disorders, morphological alterations that encompass motor, limbic and somatosensory circuits were observed in neuroimaging research. Although not a frequently reported diagnosis in this study, fibromyalgia can present symptoms similar to diverse symptoms after HPV vaccination because the clinical features of
fibromyalgia and persistent somatoform pain disorder or somatization disorder overlap in patients with chronic widespread pain without specific somatic disease factors.\textsuperscript{44} There was a report of an increased inflammatory response in glia cells associated with abnormal brain function in patients with fibromyalgia.\textsuperscript{45} A phase II clinical trial that showed clinical improvement of myalgic encephalopathy/chronic fatigue syndrome after treatment with rituximab suggested that the syndrome may be a variant of an autoimmune disease,\textsuperscript{46} while a subsequent phase III clinical trial failed to detect a significant improvement.\textsuperscript{47} However, considering that a bio-psycho-social model is now widely accepted as the most heuristic approach to chronic pain, it may be impossible to explain diverse symptoms simply by biological mechanisms.\textsuperscript{48}

Some limitations in our survey should be mentioned. First, our sampling frame included all hospitals in Japan, but did not include clinics because the number of clinics was tenfold or more than that of hospitals. Although severe or difficult to diagnose cases are referred to hospitals, and patients can visit hospitals directly without being referred by clinics in Japan, our prevalence results among unvaccinated girls remained underestimated. Second, we could not analyze the frequency of diverse symptoms by HPV vaccine types among vaccinated girls because we did not obtain such information during the survey. Third, we could not estimate confidence interval (CI) of prevalence. The protocol for a nationwide epidemiological survey on intractable diseases provided a formula to calculate 95\% CIs for prevalence.\textsuperscript{16-18} However, the formula relied on data from the first-stage survey. Since our prevalence was calculated with many parameters from the second-stage survey, estimation of CIs was technically challenging. Even if possible, epidemiological studies are required to consider the influence of chance, bias, and confounding in a balanced manner. Since our survey was subject to substantial bias in comparing prevalence of diverse symptoms between unvaccinated and vaccinated girls, the highest priority was appropriate interpretation of prevalence of diverse symptoms among
unvaccinated girls. This priority led us to not attempt estimation of CIs, as well as statistical testing, because evaluating the extent of chance would not make sense under the considerable influence of bias.

In conclusion, our nationwide epidemiological survey in Japan revealed that adolescent girls without history of HPV vaccination also visited hospitals with diverse symptoms similar to those following HPV vaccination. We hope our results help explain the complexity of HPV vaccine safety issues in Japan, and will provide useful insight into future strategies of signal assessment immediately after detection of AEFI.

Author contributions
TS was the principal investigator of the study group. WF, MH, YK, MS, YU, KH, AO, SM, SuK, SaK, and TS conceptualized and designed the survey. SH supervised the survey planning. WF, MH, YK, MS, YU, KH, AO, SM, SuK, and SaK managed the data. WF and MH analyzed the data. WF, MH, and TS wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest

Masahiko Shibata received honoraria from GlaxoSmithKline K.K. between May 2018 and August 2020. All other authors declare no competing interests.
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Figure legends

Figure 1. Calculation procedure of estimated period prevalence of diverse symptoms by gender and age. Corrected coefficient was calculated by the number of non-eligible cases (e.g., duration of symptoms was <3 months, symptoms did not affect their schooling/working) and duplicated
cases (who were identical for gender, date of birth, name initials, prefectures of residence, and where other information was almost the same) as the numerator and the number of reported patients to the second-stage survey as the denominator. HPV = human papillomavirus.

**Figure 2.** Estimated period prevalence (per 100,000 population) during the period of July to December 2015 among girls with diverse symptoms, according to the number of symptoms.
| Departments                   | Eligible | Sampled (%) | Responded | By reported no. of patients | Reported no. of patients |
|-------------------------------|----------|-------------|-----------|----------------------------|--------------------------|
|                               |          |             | No. (%) |         | 1-9 | 10-29 | ≥30 | Boys | Girls |
| Pediatrics                    | 2,596    | 1,895 (73.0%) | 1,294 (68.3%) | 131 | 110 | 16 | 5 | 243 | 446 |
| Neurology                     | 1,989    | 1,529 (76.9%) | 896 (58.6%) | 64 | 59 | 4 | 1 | 59 | 164 |
| Anesthesiology (pain clinic)  | 319      | 291 (91.2%) | 230 (79.0%) | 38 | 36 | 2 | 0 | 18 | 73 |
| Obstetrics and gynecology     | 1,861    | 1,486 (79.8%) | 1,037 (69.8%) | 12 | 12 | 0 | 0 | 0 | 16 |
| Orthopedic surgery            | 4,780    | 3,186 (66.7%) | 1,949 (61.2%) | 39 | 34 | 5 | 0 | 77 | 87 |
| Gastroenterology              | 7,463    | 4,891 (65.5%) | 2,716 (55.5%) | 29 | 26 | 3 | 0 | 40 | 60 |
| Rheumatology                  | 1,081    | 774 (71.6%) | 469 (60.6%) | 18 | 17 | 1 | 0 | 11 | 33 |
| General practice              | 326      | 293 (89.9%) | 168 (57.3%) | 11 | 11 | 0 | 0 | 5 | 12 |
| Neurosurgery                  | 2,353    | 1,803 (76.6%) | 1,072 (59.5%) | 36 | 35 | 1 | 0 | 53 | 48 |
| Psychiatry/psychosomatic medicine | 2,557 | 2,066 (80.8%) | 1,121 (54.3%) | 97 | 73 | 17 | 7 | 364 | 552 |
| Special departments in the survey | 88    | 88 (100.0%) | 85 (96.6%) | 33 | 28 | 3 | 2 | 33 | 161 |
| **Total**                     | 25,413   | 18,302 (72.0%) | 11,037 (60.3%) | 508 | 441 | 52 | 15 | 903 | 1,652 |

*a* No. of sampled/no. of eligible.

*b* No. of responded/no. of sampled.

*c* The maximum number was 136 patients.
Table 2. Decision process about whether reported symptoms correspond to “diverse symptoms after HPV vaccination”

| Can the doctor adequately explain the patient’s symptoms by the diagnoses? | Decision by the study group whether the reported symptoms correspond to “diverse symptoms” | Frequently reported diagnosis \(^b\) | n (%) \(^c\) |
|---|---|---|---|
| Not explicable | | | |
| A diagnosis which is designated as “the most explicable” by the doctor in charge | Correspond | Orthostatic dysregulation, headache, peripheral neuropathic pain | 82 (14) |
| Explicable | | | |
| The diagnosis includes terms such as “due to HPV vaccine” or “after HPV vaccination” \(^a\) | Correspond | Autoimmune encephalopathy (due to HPV vaccine), HPV vaccine-associated neuroimmunopathic syndrome (HANS), HPV vaccine-related neuropathy | 31 (5) |
| The diagnosis does not include terms such as “due to HPV vaccine” or “after HPV vaccination” \(^a\) | Don’t | Systemic lupus erythematosus, epilepsy | 21 (4) |
| Other \(^a\) | Correspond | Orthostatic dysregulation, adjustment disorder, somatoform disorder | 435 (73) |
| Unknown | Unknown | Somatoform disorder, truancy, depression | 25 (4) |

\(^a\) Don’t correspond.

HPV = human papillomavirus.
a Eight clinicians in the study group independently reviewed the list of diagnoses that were designated as “the most explicable” by the doctor in charge for reported patients in the second-stage survey (a total of 201 diagnoses). During the reviews, no other information from the patients was provided. A diagnosis was judged as being clearly distinguished from “diverse symptoms after HPV vaccination” if all eight clinicians judged so.
b Complete lists of the reported diagnosis are shown in Supplementary Tables 1 to 5.
c Denominator of the proportion was 594 patients who were reported to the second-stage survey and whose age at onset was ≥12 years (see Supplementary Figure 1 and Supplementary Tables 1 to 5).
Table 3. Estimated gender- and age-specific number of patients with diverse symptoms during the period of July to December 2015

| Age a          | Reported number of patients in first-stage survey | Estimated number of patients (crude) b | Correction coefficient c | Estimated number of patients (after correction) | Patients whose age at onset was ≥12 years e |
|----------------|--------------------------------------------------|---------------------------------------|--------------------------|-----------------------------------------------|---------------------------------------------|
|                |                                                  |                                       |                          | All d                                         | Do not correspond to diverse symptoms | Correspond to diverse symptoms | Unknown | Total |
|                |                                                  |                                       |                          |                                               |患者 whose年龄起始≥12年 | | | |
|                |                                                  |                                       |                          |                                               | 非对应 | 对应 | 未知 | 总计 |
| Boys           |                                                  |                                       |                          |                                               | 否 | 是 | 未知 | 数 |
| 12 y           | 99                                               | 228                                   | 0.69                     | 157                                           | 0 | 54 | 4 | 58 |
| 13 y           | 150                                              | 345                                   | 0.63                     | 217                                           | 17 | 152 | 0 | 169 |
| 14 y           | 163                                              | 375                                   | 0.62                     | 233                                           | 8 | 174 | 8 | 191 |
| 15 y           | 150                                              | 345                                   | 0.58                     | 200                                           | 0 | 171 | 9 | 180 |
| 16 y           | 126                                              | 290                                   | 0.56                     | 162                                           | 6 | 128 | 6 | 139 |
| 17 y           | 115                                              | 265                                   | 0.40                     | 106                                           | 9 | 80  | 0 | 89 |
| 18 y           | 100                                              | 230                                   | 0.46                     | 106                                           | 0 | 84  | 5 | 89 |
| 12-18 y        | 903                                              | 2,079                                 | 0.57                     | 1,185                                         | 45 | 829 | 36 | 901 |

Girls

| Age a          | Reported number of patients in first-stage survey | Estimated number of patients (crude) b | Correction coefficient c | Estimated number of patients (after correction) | Patients whose age at onset was ≥12 years e |
|----------------|--------------------------------------------------|---------------------------------------|--------------------------|-----------------------------------------------|---------------------------------------------|
|                |                                                  |                                       |                          |                                               | 非对应 | 对应 | 未知 | 数 |
| 12 y           | 128                                              | 295                                   | 0.59                     | 174                                           | 0 | 45  | 0 | 45  |
| 13 y           | 170                                              | 391                                   | 0.60                     | 235                                           | 0 | 141 | 0 | 141 |
| 14 y           | 231                                              | 532                                   | 0.68                     | 362                                           | 3 | 268 | 8 | 279 |
| 15 y           | 264                                              | 608                                   | 0.56                     | 340                                           | 15 | 260 | 18 | 292 |
| 16 y           | 311                                              | 716                                   | 0.59                     | 422                                           | 11 | 350 | 15 | 376 |
| 17 y           | 315                                              | 725                                   | 0.58                     | 421                                           | 15 | 334 | 26 | 375 |
| 18 y           | 233                                              | 536                                   | 0.51                     | 273                                           | 14 | 207 | 14 | 235 |
| 12-18 y        | 1,652                                            | 3,804                                 | 0.59                     | 2,244                                         | 52 | 1,590 | 86 | 1,728 |

Note: Estimated number of patients aged 12-18 years do not necessarily correspond to age-specific estimated number of patients due to rounding.

a As of October 1, 2015.
Using the information from the first-stage survey, number of reported patients/(sampling fraction × response rate) with stratification by departments and number of beds were calculated within gender- and age-specific strata and summed. See “Step 1” in Figure 1.

Based on information from the second-stage survey. See “Step 2” in Figure 1.

See “Step 3” in Figure 1.

See “Step 4” and “Step 5” in Figure 1.
Table 4. Estimated period prevalence (per 100,000 population) of diverse symptoms during the period of July to December 2015, according to gender

| Age a | Boys | Girls |
|-------|------|-------|
|       | Estimated no. of patients b | Denominator c | Estimated period prevalence (per 100,000 population) | Estimated no. of patients b | Denominator c | Estimated period prevalence (per 100,000 population) |
| 12 y  | 54   | 567,602 | 9.5 | 45   | 539,923 | 8.3 |
| 13 y  | 152  | 584,656 | 26.0 | 141  | 556,169 | 25.4 |
| 14 y  | 174  | 593,163 | 29.3 | 268  | 563,639 | 47.5 |
| 15 y  | 171  | 607,238 | 28.2 | 260  | 572,575 | 45.4 |
| 16 y  | 128  | 607,002 | 21.1 | 350  | 574,169 | 61.0 |
| 17 y  | 80   | 615,670 | 13.0 | 334  | 581,725 | 57.4 |
| 18 y  | 84   | 605,293 | 13.9 | 207  | 576,615 | 35.9 |
| 12-18 y | 829 | 4,180,624 | 19.8 | 1,590 | 3,964,815 | 40.1 |

a As of October 1, 2015.
b Limited to patients whose age at onset was ≥12 years (after correction, reproduced from Table 3).
c The national population in Japan by gender, as of October 1, 2015.
Table 5. Estimated period prevalence (per 100,000 population) during the period of July to December 2015 among girls with diverse symptoms, according to HPV vaccination status

| Age  | All Group (A) | Group (B) | Group (C) | Group (D) | Group (E) |
|------|---------------|-----------|-----------|-----------|-----------|
|      | Estimated no. of patients b | Estimated no. of patients b | Estimated period prevalence (per 100,000 population) | Estimated no. of patients b | Estimated period prevalence (per 100,000 population) | Estimated no. of patients b |
| 12 y | 45            | 30        | 539,567   | 5.6       | 0         | 0         | 0         | 356       | 0.0       | 0.0       | 0.0       | 15       |
| 13 y | 141           | 94        | 552,476   | 17.0      | 0         | 0         | 0         | 3,693     | 0.0       | 0.0       | 0.0       | 47       |
| 14 y | 268           | 126       | 529,150   | 23.8      | 0         | 21        | 3         | 34,489    | 0.0       | 60.9      | 8.7       | 118      |
| 15 y | 260           | 109       | 326,648   | 33.4      | 5         | 31        | 8         | 245,927   | 2.0       | 12.6      | 3.3       | 107      |
| 16 y | 350           | 32        | 148,695   | 21.5      | 4         | 168       | 25        | 425,474   | 0.9       | 39.5      | 5.9       | 123      |
| 17 y | 334           | 30        | 147,400   | 20.4      | 0         | 150       | 23        | 434,325   | 0.0       | 34.5      | 5.3       | 130      |
| 18 y | 207           | 35        | 119,083   | 29.4      | 0         | 114       | 0         | 457,532   | 0.0       | 24.9      | 0.0       | 60       |
| 12-18 y | 1,590     | 477       | 2,363,019 | 20.2      | 16        | 445       | 64        | 1,601,796 | 1.0       | 27.8      | 4.0       | 604      |

HPV = human papillomavirus.

Group (A): Never vaccinated.

Group (B): Vaccinated, symptom occurred before vaccination.

Group (C): Vaccinated, symptom occurred after vaccination.

Group (D): Vaccinated, the temporality between vaccination and symptom onset was unknown.

Group (E): Unknown vaccination status.

a As of October 1, 2015.

b Limited to patients whose age at onset was ≥12 years (after correction, reproduced from Tables 3 and 4).

c Estimated cumulative number of national population by gender and HPV vaccination history, as of October 1, 2015.
Table 6. Potential biases in comparing prevalence or distributions of symptoms by HPV vaccination status

| Situation No. | Process/ point | Persons          | Potential biases                                                                                                                                                                                                                                                                                                                                 | Prevalence among never vaccinated | Prevalence among vaccinated |
|---------------|----------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|---------------------------|
| 1             | Vaccination    | Patients         | If patients already have diverse symptoms, they will not receive HPV vaccine.                                                                                                                                                                                                                                                                  | ↑ a                               | ↓ a                       |
| 2             | Medical visits | Patients         | If patients developed diverse symptoms after HPV vaccination, they are likely to visit the medical institution.                                                                                                                                                                                                                                 |                                   | ↑                         |
| 3             | First-stage survey | Investigators   | Since we targeted the patients who visited hospitals, patients who visited clinics were never identified.                                                                                                                                                                                                                                         | ↓                                 | ↓                         |
| 4             | First-stage survey | Doctors        | Doctors are less likely to respond to the survey if they only have experiences in providing medical care for patients with diverse symptoms who have never been vaccinated.                                                                                                                                                                      | ↓                                 | →                         |
| 5             | First-stage survey | Doctors        | Doctors are less likely to report patients who have been never vaccinated if the doctors wrongly understand that this survey targets only those patients who have been vaccinated.                                                                                                                                                                             | ↓                                 | →                         |
| 6             | First-stage survey | Doctors        | Doctors are less likely to report patients who have been vaccinated if the doctors do not agree with the purpose of the survey.                                                                                                                                                                                                                       | →                                 | ↓ b                       |
| 7             | Second-stage survey | Doctors      | Doctors are less likely to respond to the survey if they only have experiences in providing medical care for patients with diverse symptoms who have never been vaccinated.                                                                                                                                                                         | ↓                                 | →                         |
| 8             | Second-stage survey | Doctors/ Investigators | Patients are categorized as “vaccinated” irrespective of duration from vaccination to symptom onset.                                                                                                                                                                                                                                                  |                                   | ↑                         |
| 9             | Second-stage survey | Doctors        | Doctors are likely to make decisions as “the symptoms cannot be explained medically” or “the symptoms can be explained by HPV vaccine-related diagnosis”, if                                                                                                                                                                                                                     |                                   | ↑                         |
the patients with vaccination history visit them.

10 Second-stage survey Doctors Doctors are likely to report “HPV vaccination history of the patient is unknown” in the case of no definite information on vaccination history in medical records, even if the patients are thought to be unvaccinated, because this survey asks for information to be extracted from already-existing medical data.

11 Second-stage survey Doctors Doctors are likely to report “the date of HPV vaccination of the patient is unknown” in the case of no definite information on the date in medical records, even if the patient’s symptoms are thought to have occurred after vaccination.

In comparison of distributions of symptoms by HPV vaccination status

| Second-stage survey | Patients | Frequency of symptoms among never vaccinated | Frequency of symptoms among vaccinated |
|---------------------|----------|---------------------------------------------|---------------------------------------|
| 12                  | Patients | Patients who have history of vaccination are likely to pay attention to symptoms that have been reported to be associated with the vaccine. | → | ↑ |
| 13                  | Doctors  | Doctors are likely to extract information related to symptoms that are thought to be associated with vaccine when they examine patients who have a history of vaccination. | ↓ | ↑ |

↓: Underestimated, ↑: Overestimated, →: Not affected, HPV = human papillomavirus.

a The bias is negligible if HPV vaccination rate is very low.

b The patients may be reported by another doctor if they visit several medical institutions at the same time.
(Step1) Estimated number of patients aged 12-18 years (crude)
- Solely using information from the first-stage survey
- Number of reported patients / (sampling fraction × response rate), stratified by gender, age, departments, and number of beds or hospital characteristics

(Step 3) Estimated number of patients aged 12-18 years (corrected)

(Step 5) Estimated number of patients aged 12-18 years with diverse symptoms by gender and HPV vaccination history (age at onset: ≥12 years)

(Step 7) Estimated period prevalence (from July 2015 to December 2015) of patients aged 12-18 years with diverse symptoms by gender and HPV vaccination history (age at onset was ≥12 years)

Information from the second-stage survey

(Step 2) Calculation of corrected coefficient (proportion of those who were not eligible or who were duplicated)

(Step 4) Proportion of cases with diverse symptoms by gender and HPV vaccination history (age at onset: ≥12 years)

(Step 6) Estimated cumulative national population aged 12-18 years by gender and HPV vaccination history, as of October 1, 2015
Figure 2

Period prevalence (per 100,000)

- **Group (A) Never vaccinated**
- **Group (C) Vaccinated, symptoms occurred after vaccination**