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Autoimmunity in patients reporting long-term complications after exposure to human papilloma virus vaccination

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1. Introduction

Genital Human Papilloma Virus (HPV) infections are commonly acquired soon after onset of sexual activity and persistent HPV infections can result in cervical cancer [1]. Globally, the incidence of cervical cancer is around 570,000 cases and more than 300,000 die each year because of the disease [2]. To counteract the morbidity and mortality, vaccines against HPV were developed and subsequently marketed in 2006, and in 2009, the HPV vaccine was implemented in the Danish childhood vaccination program. The three marketed vaccines - a bivalent (HPV2), a quadrivalent (HPV4), and a nona-valent (HPV9) vaccine - are based on virus-like particles containing aluminum adjuvant [3–5].

Since the inception of vaccination, it has been recognized that adverse events following immunization (AEFI) will occur [6], though the majority of these tends to be minor and linked to the inflammatory response at the injection site [6]. In addition to this, specific AEFI can occur in relation to other nonantigen components and of these, allergic reactions are the most frequent [7]. One example of AEFI leading to serious disease include the increased risk of developing narcolepsy after vaccination with the adjuvanted H1N1-vaccine (Pandemrix [8]) and other examples include the development of Guillain-Barré syndrome following inoculation with a similar swine flu vaccine [9] or SARS-Cov-2 vaccination [10].

The HPV-vaccines are non-live, adjuvanted, and highly immunogenic vaccines and the virus-like particles (VLP) in the HPV-vaccines generate a diversity of responses [11]. The antigen dose in VLP is much higher than in natural infections and the capsids are directly exposed to systemic immune responses as opposed to the natural infection that stays protected by the epithelial barrier [12].

The proteome of parts of the vaccine (HPV-16) has been shown to have several overlaps with the human proteome. These aspects of the HPV-vaccine signal a potential, associated risk of non-specific and autoimmune responses [13].

Almost all pre-licensure studies of the HPV-vaccines used either an aluminum containing adjuvant or another vaccine as control substance [3–5] and thus have several limitations. Two large cohort studies of AEFI with the HPV4 vaccine have been carried out using the hospital-based health care registries of Denmark and Sweden. Both studies only

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included AEFI with a prespecified ICD-10 code. The first study found a significant association between HPV4 vaccine exposure and Bechet's syndrome, Raynaud's disease, and Type-1- diabetes. However, this association was rendered nonsignificant since a clear temporal pattern related to the exposure could not be found [14]. The second study found significantly increased relative risk of Hashimoto's thyroiditis, coeliac disease, localized lupus erythematosus, pemphigus vulgaris, Addison's disease, Raynaud's disease and a mixed group of encephalitis, myelitis, and encephalomyelitis. The statistical significance of these findings disappeared following self-controlled case series analysis, requirement of consistency among the two participating countries, Bonferroni's correction, and a non-descript clustering analysis [15]. A large French register-based study found a higher risk of Guillain Barré syndrome in the HPV-vaccinated population [16]. The epidemiological studies have – in general - several limitations as they only rely on analyzing the occurrence of disease entities that are contained in the databases. Symptoms or syndromes that do not have a specific diagnostic code or in which these codes are not used, will not be included in such analyses. These limitations have been addressed in more recent register-based publications from Denmark by investigating school absence in relation to vaccination [17], or focusing on pain, fatigue, or cardiovascular symptoms [18]. The publication on school absence includes approximately 14,000 girls aged 12–16 years at vaccination and they served as their own controls comparing an unvaccinated period with the period after vaccination. The study did not find any difference between the periods [18]. With the number of girls included and the frequency of side effects reported [19] the study could be expected to have included only 20 to 30 girls with side effect and as such have had a high risk overlooking this signal. The second study [18] did not find any difference in their main analyses of approximately 147,000 girls and could be assumed to have 200 to 300 girls with side effect but would likely overlook this given that their results have broad confidence limits [18].

Two publications based on reported adverse events in the Vaccine Adverse Event Reporting System (VAERS; [20, 21]) found that events with a probable autoimmune background were significantly more frequent after HPV-vaccination compared to other vaccinations. Passive surveillance systems – such as VAERS - are subject to multiple limitations, including underreporting, unconfirmed diagnoses, and lack of denominator data and unbiased comparison groups.

During the past years, case series on suspected side effects to the HPV vaccines have described a collection of signs that resemble symptoms in three main diagnostic entities - postural orthostatic tachycardia syndrome (POTS, 22,23,24), myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS, 24,25), and complex regional pain syndrome (CRPS, [26]).

In 2015, it was decided to establish centers for patients with possible side effects to HPV-vaccination in each of the five Danish Regions, and the Danish State allocated funding for research into possible biological background for the alleged side-effects. The present study is based on clinical observations and diagnostic testing in a large group of patients referred to the "Center for Patients with Possible Side-effects to HPV-vaccination" in the Capital Region of Denmark. The aim was to analyze a possible connection between biologic and/or pathophysiologic changes and symptoms experienced by girls and young women referred to the center. We specifically aimed to relate symptoms to autoimmunity, as the primary sequence of the HPV major capsid L1s antigen is similar to that of several human autonomic nerve receptors and their related proteins, including adrenergic G-protein coupled receptors (GPCRs) [13, 27], and as previous publications have classified the probable side effects as disease entities known to be associated with neuroendocrine GPCR antibodies (POTS – [28]; CRPS – [29]; ME/CFS – 30) as well as in case stories on patients with probable side effects to HPV-vaccination [31–33]. Interestingly, similar symptoms [34] and neuroendocrine GPCR antibodies are reported in patients suffering from long-term complications after SARS-CoV-2 infection (long-COVID, 35).

2. Participants & methods

The participants had all received the quadrivalent HPV-vaccine (Gardasil) and they attended the Syncope Center at Bispebjerg Frederiksberg Hospital, which is a part of the Copenhagen University Hospitals. The Syncope Center became the Center for Possible Side-effects to HPV vaccination in the Capital Region 2015. From 2011 to 2018, the Syncope Center had 845 patients (839 females) with suspected side effects referred and a systematic data collection was started in 2015, when most patients were referred. The total regional number of HPV-vaccinated females in the Capital Region in that age group was 108,231 and thus the study group constituted 0,78% of the target population. The control group was recruited from local educational institutions. The data collection included both patients and age- and sex-matched, healthy vaccinees – unless otherwise indicated - and the protocols on data collections were approved by the Ethics Committee of the Capital Region (reg.no 2012-58-0004).

Data analysis included standard laboratory testing, structured interviews, standardized questionnaires regarding mental and cognitive fatigue and symptoms related to autonomic dysfunction as well as analysis of antinuclear antibodies and antibodies against G-protein coupled receptors. Interviews were conducted at the day of blood sample collection. Venous blood samples were centrifuged immediately after collection at 3000 rpm for 10 min, and serum for autoimmunity analysis were stored at minus 80° Celsius until use.

2.1. Routine biochemistry testing

Blood samples from cases and age- and sex-matched controls were analyzed using routine methods at the Department of Biochemistry at Bispebjerg Frederiksberg Hospitals.

2.2. Symptoms

Patients were interviewed regarding their symptoms and the subjective reports on symptoms were quantified through two questionnaires – the Fatigue Scale for Motor and Cognitive Functions (FSMC, 36) and the abbreviated Composite Autonomic Symptom Score (COMPASS31, [37, 38]). FSMC was developed for the assessment of cognitive and motor fatigue related to multiple sclerosis and has subsequently been used in other condition, where these symptoms are prevalent such as stroke [39]. The scale has a maximum score of 100 and has two principal components – physical and mental fatigue. The score has been subdivided based on standard deviation from control subjects where deviations of more than one, two, or three standard deviations were classified as mild, moderate, or severe fatigue, respectively [36]. COMPASS31 is an abbreviated questionnaire addressing symptoms related to dysfunction of the autonomic nervous system. It was developed by the Mayo Clinic [37] and gives an overall score and contains subdivision relating to orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor dysfunction.

2.3. Antinuclear antibodies

Antinuclear antibodies (ANA) were tested in 83 consecutive patients with possible side-effects to HPV vaccination. Unfortunately, we were unable to have ANA tested in all patients and their controls and had to rely on comparison with an un-matched control group of 289 healthy blood donors constituting the standard control cohort in the laboratory. ANA are a group of diverse autoantibodies often used as key biomarkers in rheumatic diseases. Compared to the background population, ANAs have been found more frequently in POTS [40], CRPS [41], and ME/CFS [42]. The presence of antinuclear antibodies was tested in serum by the classical immunofluorescence assay using HEp-2-cells and a dilution of 1:160 [43]. ANAs were classified in subtypes according to international consensus [44].

2.4. G-protein coupled receptor antibodies

Serum from 108 consecutive patients and 98 age and sex matched vaccinated controls was frozen, stored at minus 80° Celsius and subsequently examined for the presence of antibodies directed towards G-protein coupled receptors in the autonomic nervous system using a bioassay involving spontaneously beating neonatal rat cardiomyocytes as described by Wallukat et al. [43,44]. This bioassay measures the functional activity of the antibodies directed against the GPCRs. In brief, immunoglobulins (IgGs) were prepared from patient and control sera by ammonium sulfate precipitation as described by Wallukat et al. [45]. After registration of the basal beating rate of the cardiomyocytes, the autoantibody (AAB) containing IgG preparation was added and the cells were incubated at 37 °C for 1 h. The beating rate relative to basal levels was then estimated. To characterize the antibody activity and identify the receptors, the cells were measured with and without addition of known receptor antagonists: ICI 118.551 blocking β-2 adrenergic receptors and atropine blocking M-2-receptors. Samples with a change in number of beats/min greater than seven (in the positive direction for β-2 adrenergic receptors and in the negative direction for M-2-receptors) were labelled as "AAB-positive" [46].

2.5. Data analysis

Patients were included on a consecutive basis and for each subgroup analysis, comparisons based on anthropometrical data were made to test if they deviated from the total group referred.

Categorical or ordinal data were compared using non-parametric methods and data are given as median values with range. Continuous data were tested for normality and if the criteria were fulfilled, they were analyzed using parametrical methods and data given as mean values with standard deviations.

When appropriate Bonferroni's correction was made to avoid the possible fallacy of multiple comparisons. The correction is indicated in the text.

3. Results

A total of 845 patients were referred to the center for possible adverse reaction to HPV-vaccination. Six of the patients were males and were excluded from the data analysis. Data on height, weight, and BMI were available for all controls and for 655 vaccinated patients. The mean age at examination for the patients was 23.5 ± 8.0 years (age distribution shown in Fig. 1) mean height was 168 ± 6.5 cm, mean body weight was 62.5 ± 12.6 kg resulting in a mean body mass index of 22.2 ± 4.4 (kg/m^2). The control group included 98 age-matched, HPV-vaccinated females free of symptoms and with a mean age of 23.9 ± 4.9 years, mean height was 169 ± 6.1 cm, mean body weight was 65.4 ± 11.6 kg resulting in a mean body mass index of 23.2 ± 3.2 kg/m^2. The control group was recruited from local educational institutions.

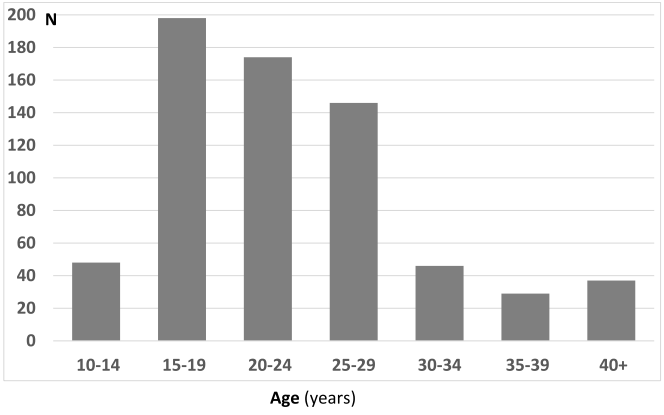

Fig. 1. Age distribution of patients with suspected side-effects to HPV-vaccination. The figure shows the distribution of age in the cohort of patients (n = 839) referred for possible side-effects to HPV-vaccination.

Information on the time between vaccination and clinical evaluation at the specialized center was available in 617 patients and was less than 1 year in 7.9%, 1–3 years in 41.5%, 3–5 years in 18.3% percent and more than 5 years in 32.3%. Information on number of vaccine doses received before persistent symptoms was available in 323 patients with 30.1% having persistent symptoms after the first, 26.6% after the second, and 43.3% after the third vaccination. The time between vaccination and development of symptoms could be reliably accounted for in 206 patients and was less than one month in 59.7%, between 1 and 6 months in 32.0%, between 6 and 12 months in 4.9%, and more than 12 months in 3.4%.

Standard biochemical parameters are given in the Appendix. Using Bonferroni's correction, we found significantly lower values ($p < 0.001$) for HbA1c, Albumin, Creatinine and higher values ($p < 0.001$) for Calcium, D-vitamin, and Monocyte Count in patients compared to controls. None of these differences were considered of clinical significance.

3.1. Symptoms and questionnaires

Systematic reports on selected symptoms were obtained in 612 patients and the results are given in Table 1. The four most prevalent symptoms were fatigue, dizziness, cognitive dysfunction, and headache.

Scores from the FSMC-questionnaire are given in Table 2 and were obtained from a total of 311 patients and 82 age- and sex-matched vaccinees without symptoms resulting in median scores of 72 (range 20–100) and 24 (range 20–49), respectively ($p < 0.001$). 68.5%, 14.8%, 8.7%, and 8% of the patients reported severe, moderate, mild, and no fatigue, respectively. None of the controls reported severe or moderate fatigue and 1.2% and 98.8% reported mild or no fatigue, respectively.

Scores of COMPASS31 were obtained in 649 patients and the distribution in subdivisions is given in Table 3. The median total score in patients was 41.8 compared to 3.0 in 98 vaccinated controls ($p < 0.001$). The scores in sub-items were not statistically analyzed .

3.2. Measures of autoimmunity

A significantly higher proportion of the patients (59% vs 25%) were positive with respect to ANA – especially regarding those with a cytoplasmatic pattern and in mitotic cells (Table 4). Antibodies against G-protein coupled receptors (GPCR) were analyzed in 108 patients and 98 age- and sex-matched, vaccinated control subjects. We found antibodies directed against the adrenergic β-2-receptor in 75% of patients and 17% of controls ($p < 0.001$), against the muscarinic M-2-receptors in 82% and 16% ($p < 0.001$) and against either β-2- or M-2-receptors in 92% and

Table 1
Most frequent symptoms reported by 612 patients.

Symptom	N	%
Fatigue	562	92
Dizziness	560	91
Cognitive dysfunction	532	87
Headache	528	86
Sleep problems	499	81
Nausea	489	80
Abdominal pain	454	74
Heart palpitation	435	71
Muscle weakness	427	70
Tics and cramps	383	63
Urinary problems	346	57
Hypermobility	298	49
Pain	294	48

Symptoms reported in a structured interview by the HPV-vaccinated cohort with possible side-effects.

Table 2
Scores obtained from the FSMC questionnaire.

	N	Total score	No fatigue (%)	Mild fatigue (%)	Moderate Fatigue (%)	Severe Fatigue (%)
Patients	311	72 (20–100)	8.0	8.7	14.8	68.5
Controls	82	24 (20–49)[a]	98.8	1.2	0	0

Scores on fatigue obtained by the Fatigue Scale of Motor and Cognitive function (FSMC, 24). The patients and vaccinated controls were sex- and age-matched.
[a] $p < 0.001$.

19%, respectively ($p < 0.001$).

4. Discussion

This study reports on the to date largest cohort of cases with suspected side-effects to HPV-vaccination in the form of a high proportion of several incapacitating symptoms. Using standardized questionnaires, we have shown that the cohort differs significantly from age-matched HPV-vaccinated controls with respect to symptoms of autonomic dysfunction and physical and mental fatigue. The patients differ from the background population with respect to the presence of ANA, and from age-matched controls with respect to autoantibodies directed at receptors in the autonomic nervous system.

Moderate to severe fatigue was recorded by the FSMC-questionnaire [36] in 83.3% of our patients and in none of the controls. Comparison of results from the FSMC to those in fatigue-associated conditions such as post-stroke [39] shows that patients in our cohort are more severely incapacitated by this symptom (83.3% versus 38.7%, respectively).

We found a high prevalence of symptoms, such as dizziness, palpitations, nausea, and hyperactive bladder suggestive of autonomic dysfunction and quantified these by the COMPASS31 in our cohort. The patients scored overall higher than diabetic patients with known neuropathy [47] and showed a distribution of autonomic dysfunction like that seen in small fiber disease [48]. Dysregulation of cardiovascular control in the form of postural orthostatic tachycardia syndrome (POTS) has been demonstrated in patients with possible adverse events after HPV-vaccination in case reports [22,23] and in a previous analysis in our cohort [24].

We found a high prevalence of autoantibodies to cellular antigens (antinuclear antibodies, ANA) in our patients as compared to the background population which points to possible autoimmunity. Significant differences between the controls and the patients were also observed when the functional autoantibodies against the β-2-adrenoceptor and the muscarinic M − 2 receptor antibodies were analyzed, with 92% and 19% of patients and controls, respectively, showing functional antibodies against either β-2 or M-2-receptors. Antibodies towards GPCRs are a feature of the adaptive immune system and are found in healthy people but the antibodies may become dysregulated and increased in susceptible individuals and cause autoimmune disease [49,50]. A Japanese study of 55 vaccinated adolescent girls reporting possible adverse effects after HPV vaccination and 57 age-matched non-vaccinated healthy girls found significantly higher autoantibody levels against α-1-, α-2, β-1-, β-2-adrenergic receptors, muscarinic M − 1, -2, -3, -4, -5 receptors, and the endothelin receptor using enzyme-linked immunosorbent assay (ELISA, 33). ELISA measures the binding of the immunoglobulins to plated proteins and not the activity of the antibodies and the study could not demonstrate any significant association between dysautonomic symptoms and the serum levels of autoantibodies against the autonomic nervous system.

Autoantibodies against neurotransmitter receptors have been described in patients with POTS [22–24] and CRPS [25], and in subgroups of patients with ME/CFS [30,51–53], and with an apparently higher prevalence in those with post-infectious ME/CFS [54]. Also, recent studies measuring the occurrence of functionally active autoantibodies against the GPCR neurotransmitter receptors in patients suffering from similar and persistent symptoms after recovering from COVID-19 (long-COVID), found active autoantibodies in all investigated patients [35,55]. The higher prevalence of autoantibodies in the long-COVID patients and in our HPV patient cohort as compared to previous findings in ME/CFS could be due to different triggers, pathogenesis/disease cofactors, and duration of disease, or the use of different methodologies that varies in their ability to capture functional or conformational differences among the autoantibodies.

Additional studies are needed to validate if these diseases, HPV vaccination symptoms, CRPS, POTS, ME/CFS and long-COVID, which all share an infectious or infectious-like trigger, belong to the same clinical spectrum and may be caused by some common autoimmune mechanisms. In fact, many of the symptoms, including immune activation and autonomic dysregulation, could be mediated or aggravated by dysregulated autoantibodies against adrenergic receptors and impaired peripheral adrenergic function. The autoantibodies have been shown to persistently stimulate their corresponding receptors and inhibit the normal, physiological and cell-protective desensitization of the receptors [56]. A recent study found that IgG isolated from serum of ME/CFS patients impaired β-2-adrenergic control of the inflammatory response of monocytes, and T cell activation during infection [57]. Moreover, β-2-adrenergic control play an important role in vasodilation and control of vascular microcirculation and blood flow to the brain and muscles [55]. Dysregulated β-2-adrenergic control could result in less blood flow and substrates for mitochondrial energy synthesis with increased lactate excretion [58] and symptoms burden upon exertion [59–61].

In general, the symptoms reported by our patients bear a close resemblance to those found in patients with ME/CFS confirming our initial report on the cohort [23]. A large epidemiological study from

Table 4
Antinuclear antibodies.

	ANA positive (%)	Nuclear (%)	Cytoplasmic (%)	Mitotic (%)
Patients (n = 83)	59	29	10	28
Controls (n = 289)	25	20	2	2
P-value	<0.0001	ns	0.0073	<0.0001

Results of analysis for antinuclear antibodies (ANA, 41) in patients and the control group from the laboratory.

Table 3
Scores obtained from the COMPASS31 questionnaire.

	Total score	Ortho-static	Vaso-motor	Secreto-motor	Gastro-intestinal	Bladder	Pupillo-motor
Patients N = 656	41.8[a] (0.0–86.8)	24.0 (0–40.0)	0.0 (0.0–4.2)	4.3 (0.0–15.0)	8.0 (0.0–25.0)	1.1 (0.0–10.0)	3.0 (0.0–5.0)
Controls N = 98	3.0 (0.0–35.8)	0.0 (0.0–24.0)	0.0 (0.0–3.3)	0.0 (0.0–6.4)	1.8 (0.0–9.8)	0.0 (0.0–3.3)	0.0 (0.0–2.3)
Maximum value	100	40	5	15	25	10	5

Scores on autonomic dysfunction obtained by the Abbreviated Composite Autonomic Symptoms Score (COMPASS31; 35, 36). The patients and vaccinated controls were sex- and age-matched.
[a] $p < 0.001$.

Norway found a general increase in the prevalence of ME/CFS in the years 2009–14 among girls eligible for HPV-vaccination but reported the same increase in boys not vaccinated in that period [62]. A similar finding of increased hospital records of pain, fatigue, or circulatory symptoms was reported from a large Danish cohort of young girls and boys in the years 2000–2014, but without an unusually higher increase after the introduction of the HPV vaccine to girls in 2009 [63]. In girls, however, the hazard ratio of developing ME/CFS was increased two to eight times with increasing number of prior hospital contacts in the Norwegian study [62], which is very much in line with studies of the cohort attending the Danish centers for possible side-effects to HPV-vaccination. Molbak et al. [64] and subsequently two other Danish studies showed that females with suspected adverse events had more frequent contacts to the health care system [65] and were more vulnerable [66] prior to HPV-vaccination. A recent Danish study concluded that patients with hospital treated infections in close proximity to their HPV-vaccination had an increased risk for later referral with suspected adverse vaccine effects [67]. These observations raise the possibility of risk factors for the development of adverse events in vaccinees and should be studied further.

Mitochondria coordinate an evolutionarily conserved, multi-system response used to manage and heal from stress or injury by epigenetic reprogramming of immune-inflammatory, neuroendocrine, and metabolic adaptations to stress [68,69]. Mitochondrial stress adaptive function can be exhausted by physical as well as mental chronic stress, which will decrease the capacity to manage future events of stress, infections or injury [70,71]. The increased health care utilization [64,65], vulnerability [66] and possibly activated immune system [67] among females prior to HPV-vaccination, in those who developed adverse effects, support the concept that they have been biologically vulnerable at the time of vaccination. This may have shaped their mitochondria and immune-metabolic system for autoimmune manifestations when exposed to the HPV-vaccine.

Studies are in progress to characterize mitochondrial and immune-metabolic changes in the symptomatic HPV-vaccinated girls. Even though such studies and the present study will not allow us to establish if HPV vaccination is causal for the observed symptoms and molecular changes, they may provide new insights to risk assessment and treatments directed at dampen autoimmunity and symptom burden in the affected patients. Moreover, future epidemiological studies of possible vaccine side-effects should adjust for the effect of accumulated stress factors, if possible, given the relatively low number of cases.

4.1. Strength and limitations

The present study has a strength in the inclusion of a large cohort and by using age- and sex-matched individuals vaccinated with the same type of HPV-vaccine around the same time. The study is neither placebo controlled, nor prospective or double blinded, which would have been ideal. Due to the circumstances at the time of study, we were unable to measure ANA in an adequate control group, which is a limitation in the interpretation of these findings. The same circumstances caused a lack of clinical and serological follow-up, which would have been ideal. Given that approximately 600,000 girls and young women were HPV-vaccinated at the time in Denmark [19] and 1023 reported possible severe side-effects to the Danish Medicine Agency [72], it would require a group of 50–60,000 participants with a mean age of 24 years to reach an adequate statistical strength and such a study is for many reasons inconceivable.

5. Conclusions

This study has shown that girls and young women with probable side effects to HPV-vaccination have symptoms and biological markers compatible with an autoimmune disease closely resembling that seen in ME/CFS and subsets of long-COVID. Together with previous studies, demonstrating increase morbidity in this group of patients preceding vaccination, this raises the probability that prior disease may precondition some individuals for vaccine related adverse events. The HPV-vaccine possesses a strong immunogenicity, and it is suggested that possible vulnerability should be further investigated and considered when counselling for such vaccines.

Credit author statement

Jesper Mehlsen: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Louise Brinth: Conceptualization, Methodology, Investigation, Writing - Reviewing and Editing, Kirsten Pors: Conceptualization, Methodology, Investigation, Kim Varming: Methodology, Investigation, Gerd Wallukat: Methodology, Investigation, Writing – review & editing, Rikke Katrine Jentoft Olsen: Supervision, Writing – review & editing.

Declaration of competing interest

Jesper Mehlsen: Received study grant from Merck Denmark for participating in studies on Gardasil-9. Owns a private ME/CFS clinic in Denmark. **Louise Brinth:** None. **Kirsten Pors:** None. **Kim Varming:** None. **Gerd Walukat:** Employed by Berlin Cures GmbH, shareholders of Berlin Cures Holding AG, the holding company of Berlin Cures **Rikke Katrine Jentoft Olsen**: Has received financial support for meeting participation from the Danish ME Association.

Data availability

The authors do not have permission to share data.

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Appendix A

	Patients			Controls			
Lab-data	N	Median	Range	N	Median	Range	p-value
Amylase	605	26	5–86	93	26	6–55	0.480
ALAT	601	17	7–368	93	18	10–75	0.660
Alkaline phosphatase	603	59	20–272	93	61	29–279	0.866
Bilirubin	589	6	0–30	93	7	0–28	0.142
Total Cholesterol	595	4.3	2.3–7.9	93	4.3	2.8–6.8	0.779
HDL Cholesterol	598	1.6	0.6–3.1	93	1.7	1.0–3.3	0.019

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(*continued*)

	Patients			Controls			
LDL Cholesterol	587	2,3	0.4–5.4	93	2.1	1.4–4.2	0.161
Triglyceride	592	0.90	0.30–5.1	93	0.78	0.36–2.78	0.167
TSH	569	1.63	0.04–6.34	93	1.65	0.16–4.46	0.948
HbA1c (%)	552	5.0	4.0–6.5	93	5.2	4.6–5.7	<0.001
Albumin	598	45	26–53	93	39	33–45	<0.001
Creatinine	598	66	40–105	93	70	49–94	<0.001
Sodium	593	140	127–145	93	140	131–143	0.658
Potassium	587	4.1	2.0–5.4	93	4.1	3.2–5.0	0.576
Calcium (ionized)	509	1.24	1.13–2.51	93	1.23	1.16–1.30	<0.001
Phosphate	545	1.0	0.44–1.80	93	1.1	0.64–1.50	0.127
D-vitamin	570	81	13–175	93	56	16–145	<0.001
Parathyroid hormone	516	4.1	1.5–12.6	93	4.2	2.2–9.7	0.172
Hemoglobin	582	8.3	5.0–10.1	93	8.2	7.2–9.5	0.158
Cobalamin	570	311	98–1480	93	310	86–1020	0.738
Ferritin	592	48	5–340	93	46	11–195	0.348
Lymphocyte count	571	2.1	0.5–4.5	93	2.0	1.1–3.7	0.503
Monocyte count	576	0.5	0.2–1.2	93	0.4	0.2–0.9	0.001
Neutrophile count	574	3.7	1.2–12.3	93	3.2	1.1–7.1	0.004
C reactive protein	589	1.00	0–74	93	0	0–14	0.010

Result of standard biochemical testing in patients and vaccinated sex- and age-match controls.

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