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FRONTIERS IN AUTOIMMUNITY

A sudden onset of a pseudo-neurological syndrome after HPV-16/18 AS04-adjuvated vaccine: might it be an autoimmune/inflammatory syndrome induced by adjuvants (ASIA) presenting as a somatoform disorder?

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Abstract In last centuries, vaccines reduced the incidence of several infectious diseases. In last decades, some vaccines aimed at preventing also some cancers, where viruses play a causative role. However, several adverse events have been described after vaccines, but a causal relationship has been established only in a minority of cases. Here, we describe a pseudo-neurological syndrome occurred shortly after the administration of the bivalent HPV vaccine. Some autoimmune disorders, including neurological demyelinating diseases, have been reported after HPV vaccines, but the patient showed no organic lesions. The patient was diagnosed as having a functional somatoform syndrome, which was supposed to be autoimmune/inflammatory syndrome induced by adjuvants (ASIA), seen the temporal link with vaccination and the presence of anti-phospholipid autoantibodies. Immunological mechanisms of vaccines—and of adjuvants—have not been completely elucidated yet, and although there is no evidence of statistical association with many post-vaccination events, a causal link with vaccine cannot be excluded in some individuals.

Keywords Vaccine · Human papillomavirus · Adverse event · Functional somatoform syndrome · Autoimmune/inflammatory syndrome induced by adjuvants (ASIA)

Introduction

General overview on vaccines composition and mechanisms of action

Vaccines are the most important tools by which medicine aims to prevent many infectious diseases. Whereas passive immunization provides a time-limited protection through antibody preparations, the active immunization, namely the vaccination, induces a primary immune response, leading to the development of an "immunological memory." The persistent production of specific antibodies by the host is thought to be the principal mechanism of vaccine-related immune protection. Some antigen-specific plasma cells acquire the capacity to migrate in the long-term survival niches in the bone marrow, but such an immunological memory is likely to be supported by T cell- and cytokine-mediated mechanisms too. A protective memory immune response is usually effective some weeks after the vaccination, and people get protected against a specific pathogen for long time or, hopefully, for all the life. In this way, an infectious illness can be prevented and its spreading can be progressively reduced, as the induction of protective immunity in a sufficient proportion of population impairs the circulation of pathogens and eliminates the human reservoirs. By instance, thanks to appropriate vaccine campaigns, poliomyelitis has been reduced by 99 % and smallpox has been completely eradicated [1].

Of course, a vaccine creates an immunological protection for the host without causing a clinical disease. Indeed, vaccines carry into the host only parts or modified versions of the wild pathogens, which must be recognized by the

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immune system. Such an antigenic part of the vaccine can be constituted by: (i) inactivated whole agents; (ii) live attenuated agents; (iii) parts of an agent (e.g., toxoid, proteic subunit, carbohydrate conjugates) [2]. However, the generation of the immunological memory needs a complete activation of the immune system in the host, as it would happen during the natural infection. That is why, in addition to the antigenic component, a vaccine contains an adjuvant, which has to make the antigen to be immunogenic. Many compounds have adjuvant properties, ranging from inorganic molecules (e.g., alum, mineral oil, squalene) to microbiological elements (e.g., bacterial cell wall components and toxins, living viral vectors, cytokines) [3, 4].

Thus, an adjuvant is added to elicit or augment the immune response to the antigens contained in the vaccines. In general, the adjuvant allows the immune system to recognize properly the antigen as a "dangerous" non-self molecule. Historically, diverse mechanisms have been postulated to explain the action of adjuvants: (i) stabilization of epitope conformation; (ii) generation of antigen slow-releasing depot; (iii) formation of multi-molecular aggregates stimulating antigen-presenting cells; (iv) direct antigen presentation to MHC molecules; (v) modulation of cytokine network in the local microenvironment of the inoculation site. Actually, in recent years, adjuvant is seen basically as an activator of the innate immunity, which recognizes some conserved and/or common pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), through some pattern recognition receptors (PPRs). The activation of these receptors induces an inflammatory response, which is a rapid first-line defense against pathogens, at one side, and is a trigger of the specific and complete adaptive immune response, leading to the development of the immunological memory, at the other side [4, 5].

General overview on HPV vaccine

Nowadays, it is known that some microorganisms, especially viruses, can have a role in the process of cellular transformation leading to the development of some cancers. Thus, the prevention of these infections is supposed to reduce the risk of specific tumors. In this perspective, some vaccines against oncogenic viruses have been developed in order to reduce the incidence of related cancers. The cornerstone of this approach for cancer prevention is the vaccine against hepatitis B virus (HBV), causing hepatocellular carcinoma; the vaccine against human papillomavirus (HPV) was introduced eventually with a similar purpose. Indeed, HPV infection sustained by some oncogenic serotypes is considered a fundamental step in the causative process of cervical cancer, representing the third most common malignant tumor in women worldwide [6].

HPV is a sexually transmitted herpes virus producing epithelial lesions of non-genital and genital skin and mucous membranes, and despite the benign and self-limited course in most cases, the persistence of the genome of a subset of HPV genotypes (like HPV-16, HPV-18, HPV-11, HPV-6) has been associated with several epithelial malignancies. Schematically, HPV genome encodes structural L-proteins: (i) major capsid protein (L1), assembling spontaneously to form pentamers, which constitutes the frame of the virion; (ii) and minor capsid protein (L2), which has several functions promoting host cell infection. Moreover, HPV encodes several non-structural E-proteins, some of which interfere with replication mechanisms of host cells and are responsible of cancer promotion by high-risk serotypes. Therefore, the presence of oncogenes in HPV genome precluded the possibility of using live attenuated and inactivated vaccines, shifting toward a subunit approach. In fact, current commercial HPV vaccines contain self-assembled pentamers of L1 proteins, which are produced by the technology of recombinant DNA [7, 8].

So far, two vaccines against HPV are licensed: (i) a quadrivalent vaccine, covering serotypes 6, 11, 16 and 18 (Gardasil®); (ii) a bivalent vaccine directed against high-risk serotypes 16 and 18 (Cervarix®), together causing around 70 % of cervical cancers worldwide. The injection schedule is similar, requiring three sessions in 6 months, and both vaccines are delivered by intramuscular route. Neither vaccine contains any preservative, and further differences are related to the cell system where antigenic viral proteins are produced: Gardasil® is produced in cells of Saccharomyces cerevisiae, whereas Cervarix® is obtained through L1-recombinant baculovirus-infected insect (Spodoptera frugiperda SF9, Trichoplusia ni Hi 5) cells. Moreover, the former contains aluminum hydroxyphosphate sulfate (225 μg) as an adjuvant and the latter uses AS04 adjuvant system, which comprises an aluminum salt (aluminum hydroxide, 500 μg) and 50 μg of the immunostimulatory molecule 3-O-desacyl-4′-monophosphoryl lipid A (MPL). This substance is a detoxified form of lipopolysaccharide (LPS) deriving from Salmonella minnesota (R595), which is able to alert the innate immune system, mainly binding TLR4 [8, 9].

Both HPV vaccines resulted to be able to induce virtually 100 % seroconversion to HPV types contained inside. Antibody titers increase after each dose, peaking 1 month after the third dose and eventually decrease during following 2 years, until reaching a stable plateau, which is characterized by specific titers being greater than those seen after natural infection. The efficacy against the onset of high-grade cervical lesions was evaluated by several randomized, double-blind, placebo-controlled Phase 3 trials, demonstrating a prevention rate of precancerous

lesions greater than 90 % over around 3- to 4-year follow-up [10].

Both vaccines resulted to be well tolerated. The most common side effects are mild and/or localized at the site of injection. Severe reactions are rare, but growing concerns on HPV vaccine are related to the potential connection with some of autoimmune diseases and neurological demyelinating disorders after the vaccination [11, 12].

Case report

In January 2013, a 14-year-old girl was evaluated at the Pediatric Emergency Department of the Hospital, because of the sudden onset of general malaise and other symptoms, after the administration of the second dose of HPV-16/18 vaccine (Cervarix®).

The patient received the first injection around 45 days before and that was well tolerated. At the time of the vaccine boost, she was in a good clinical condition and did not complain any disturbances during the previous days. However, around 60 min after the intramuscular injection, the girl started feeling bad, complaining dizziness, numbness, lower limbs disesthesias and paresis, until she fainted. When she arrived to the hospital, she was awake, showing normal vigilance and reactivity, although above symptoms were still complained. However, general clinical parameters were in the normal range (HR 104 bpm, arterial BP 130/70 mmHg, RR 20/min, SatO2 = 98 %, axillar $T = 36.9$ °C, PGCS = 15). She had headache too, but no vomiting or neck stiffness was recorded. The immediate neurological evaluation evidenced the following findings: significant gait impairment and incapacity to maintain the orthostatic posture, but no cerebellum signs; lower limbs hypo-reflexia, but no impairment of arms and cranial motricity; conserved ocular movements and normally reactive pupils. Urgent blood examinations were in the normal range (blood cell count, biochemistry, C-reactive protein).

Of course, the patient was admitted to our Department of Pediatrics, in order to observe the clinical evolution and to perform some diagnostic examinations. A lumbar puncture was performed: appearance, cells, glucose and proteins in spinal fluid were in the normal range; moreover, no microorganism was microscopically detected or cultured; finally, the immuno-fixation showed no oligo-clonal bands. Eventually, the patient underwent to head and spinal MR imaging, but there was no evidence of demyelinating lesions or other macroscopic pathological processes in the central nervous system.

However, the global clinical picture did not improve significantly in the following days. Actually, gait and posture impairment persisted; moreover, the patient continued to complain headache, in addition to transient and diffuse erythematous skin rashes and worsening myalgias/arthralgias (without arthritis). Therefore, the diagnostic pathway was completed, by performing peripheral nerves and muscle electrophysiologic studies, but no significant disturbance in nerve conduction and/or muscle response was showed. Finally, given the persistence of the clinical manifestations, muscle biopsy was proposed, but the patient and the family refused.

Thus, our diagnostic conclusion was a neuropsychiatric syndrome, as no organic or electrophysiological lesions could be demonstrated. Actually, the immunological workup was not completely negative. Serum globulins, IgG subclasses and complement factors were in the normal range, lymphocyte immuno-phenotype resulted to be balanced, and autoantibodies, such as ANA, ENA and rheumatoid factor, were negative. Interestingly, the patient resulted to be positive for anti-cardiolipin (IgM: 24,7 MPL, IgG: <9,4 GPL) and anti-β2-glycoprotein I (IgM: 21,3 U/ml; IgG: <9,4 U/ml) antibodies.

So far, the patient counts almost 2-year follow-up. Lower limbs motor and sensorial disturbances and gait impairment had persisted at least 6–8 weeks and then those improved slowly until a complete resolution. However, dizziness and headache recurred periodically, but muscular symptoms and myalgias persisted and evolved in a clinical condition recalling a chronic fatigue syndrome (CFS). Moreover, the patient had several admissions to the hospital because of the occurrence of anxiety/panic crisis displaying motor impairment and contact loss as well. These clinical manifestations became so frequent and impressive that her daily life and global functioning were invalidated. Therefore, the patient was addressed to further psychiatric evaluations and neuropsychological support and a course of therapy with antidepressant drugs was needed. Thanks to such a therapeutic approach, the clinical situation ameliorated, but some somatoform symptoms still persisted and anxiety episodes recurred.

Discussion

…on vaccine safety

Here, we describe the onset of a somatoform neuropsychiatric syndrome in a tight temporal linkage with the administration of bivalent HPV vaccine, which made this case to be particularly impressive among many other reports concerning adverse events following this vaccination. Moreover, the patient never suffered physical or psychiatric conditions previously.

Vaccines have been enumerated among the most powerful tools determining the increase in human life expectancy. The success of vaccinations is witnessed by the

control of several infections in the world areas where vaccines are available. Overall, vaccine practice allowed at least 14 infectious illnesses to be controlled: smallpox, diphtheria, tetanus, pertussis, poliomyelitis, measles, mumps, rubella, rabies, hepatitis B, yellow fever, typhoid, rotavirus and Haemophilus influenzae type b disease [13].

However, sometimes vaccines themselves have been recognized as causing some illnesses. In the past time, most famous vaccine-related adverse effects were caused by malpractice in their preparation (e.g., infected materials, incomplete virus inactivation). By instance, in the late nineteenth century, severe rabies-like neurological consequences occurred in more than 1 of every 250 people after the corresponding vaccine. Or, more recently, during the Second World War, more than 300,000 soldiers were infected by HBV, because of a vaccine prepared with contaminated serum; or again, in 1955, one company producing Salk's polio vaccine failed to completely inactivate poliovirus, causing an epidemics of the disease, and 200 children were permanently paralyzed [14].

Apart from these episodes, vaccine practice has some intrinsic risks (being not associated with production errors), and these are not always predictable. Of course, preexisting conditions, being able to increase the risk of post-vaccination adverse effects, must be screened. The knowledge of allergy to one of vaccine constituents or an allergic reaction to a previous administration of vaccine should be carefully evaluated. Moreover, patients affected with severe primary or secondary immunodeficiency must not receive vaccinations containing attenuated, but alive microorganisms and, similarly, pregnant women are dispensed from these vaccinations in order to avoid potential risks of transmission to the fetus [15–17].

Unfortunately, there are some post-vaccination events described as being unpreventable or unexpected. Actually, most should be defined as vaccine events rather than effects, given that there is not sufficient evidence to conclude a certain causal relationship beyond the temporal sequence. In fact, a significant association between vaccines and specific diseases has been established in a few cases: acute encephalopathy after whole-cell pertussis vaccine, acute arthropathy after rubella vaccine, immune-mediated thrombocytopenia after measles-containing vaccines, paralytic polio after live attenuated oral polio vaccine, Guillain–Barrè syndrome after swine flu vaccine. Beyond these associations, there is a magmatic list of post-vaccination diseases, syndromes or clinical conditions, where no epidemiologic/statistical and/or mechanistic evidences have been demonstrated [14, 17, 18].

Among post-vaccination events, neurological/neuropsychiatric syndromes and autoimmune diseases are the main concern for most people. Our clinical case encompasses all these pathologies.

…on vaccines and autoimmunity

Autoimmunity can be defined as the emergence of autoreactive lymphocytes. It can be accompanied by the generation of immunoglobulins reacting against self-antigens, namely autoantibodies. However, most autoantibodies have uncertain pathogenetic activity, and their clinical relevance is often represented by the fact of being markers of specific diseases. Autoimmune disorders happen whenever autoimmunity produces tissue injuries and/or disturbances of physiologic functions leading to clinical manifestations [19].

Indeed, serum autoantibodies can be found also in healthy individuals. They have been reputed to be natural autoantibodies, originating from B1-cells, and these cross-react with bacteria and tumor-associated antigens and are thought to fulfill some immunological roles, such as providing an innate immune protection and/or promoting the maintenance of peripheral tolerance [20]. The emergence of autoantibodies after different vaccines is not surprising, and the generation of autoantibodies has been also described in animal models undergoing to active immunization, as well as in the course of several natural infections [21].

Thus, vaccines are considered as potential causal factors of immune-mediated diseases. Autoimmune diseases are viewed as multifactorial pathologies resulting from the complex interplay between the host genetic background (not only HLA-related) and environmental factors (often undefined) acting as triggers of a dysregulatory process involving both innate and adaptive immune system. Among trigger factors of autoimmune diseases, infectious agents are the most cited, but vaccines could be too. Very schematically, infections are thought to be able to induce autoimmunity through two groups of mechanisms. The antigen-specific way consists in so-called molecular mimicry, which means that some antigenic determinants of microorganisms can display similarities to self-antigens. Non-antigen-specific mechanisms might be more heterogeneous, as infectious process can elicit autoimmunity through the de-sequestration of hidden cell antigens or by activating an immune response against host antigens that immune system is usually anergic to, thus disrupting the mechanisms of peripheral immune tolerance. These principles could be theoretically extended to vaccines as well [22, 23].

There are many experiences that autoimmune diseases can be preceded by a vaccination, but a causal relationship has been established in a very few cases. For instance, an increased relative risk of developing Guillain–Barrè syndrome had been demonstrated in the months following 1976–1977 vaccination campaign against swine influenza, as well as immune-mediated thrombocytopenia has been significantly associated with MMR vaccine [14]. However,

on a large-scale basis statistics, the incidence of autoimmune diseases after vaccines does not result to be greater than in general population, and whenever a causal relationship between an immune-mediated disease and a vaccine is supported, actually it often emerges that the natural infection is more likely to cause the corresponding autoimmune disorder [24].

As for HPV vaccination, which has been approved for the use in females since 2006 in the USA (and, since then, more than 50 millions dosed have been administered), the Center for Disease Control and the post-marketing monitoring have not recorded a greater incidence of autoimmune diseases [25].

Recently, the systematic review by Goncalves et al. [26] showed that most vaccine-related side effects were local reactions and severe adverse reactions were rare. Current evidences supported the acceptance of a causal relationship only for anaphylaxis, even though a higher rate than that expected has been described for venous thrombosis in a few studies [11, 12]. As concerns the specific issue of autoimmunity, the review by Pellegrino et al. [27] found no significant increase in the incidence of several immune-mediated disorders after the regular vaccine schedule, despite the abundance of concerning reports in the medical literature. However, there are still some concerns on the induction some autoimmune diseases and, especially, several inflammatory neurological disorders, reported as arising after HPV vaccines and others. The spectrum of post-vaccination CNS demyelinating diseases included acute disseminated encephalomyelitis (ADEM), multiple sclerosis (MS), myelitis, isolated optic neuritis and neuromyelitis optica (Devic's disease) [28]. Descriptions of the onset of all these neurological diseases after HPV vaccination are present in the medical literature, but there are no studies evaluating the specific risk. Thus, at present, the evidence is inadequate to accept or reject a causal relationship of HPV vaccine with any of above inflammatory demyelinating diseases [12].

At first, our patient developed clinical manifestations, which raised the suspect of a neurological disorder. However, the clinical evolution and all the diagnostic procedures (EMG, EEG, RMN and electromyography) found no abnormalities consistent with any aforementioned neurological diseases. Anyway, because of the persistence of symptoms (including skin rashes, muscles and joint pain), the possibility of a systemic rheumatic disease with a prevalent neuropsychiatric expression was considered.

It is known that some immune-mediated disorders can be characterized by neurological or neuropsychiatric symptoms, even before the emergence of a suggestive rheumatic picture. For instance, nervous system involvement in systemic lupus erythematosus (SLE) can manifest as a range of neurological and psychiatric features. Moreover, several systemic immune-mediated diseases show a high rate of psychiatric comorbidity compared to general population, even though that is more evident during the course of the rheumatic disease rather than at the onset [29].

But we did not found any specific autoantibody panel addressing toward some specific autoimmune disorder and, above all, no diagnostic organic manifestations emerged to support a specific disease. However, the patient resulted to be positive for anti-cardiolipin and anti-β2-glycoprotein I antibodies. These can be found in the setting of several rheumatic diseases and, whenever they are associated with arterial and venous thromboembolic phenomena with multi-organ involvement, they support the diagnosis of anti-phospholipid autoantibodies syndrome (or Hughes syndrome) [30, 31]. Actually, the diagnostic investigations performed in our patient found no hyper-coagulative status and/or thromboembolic lesions in the central nervous system or elsewhere. Therefore, a diagnosis of any definite organic or immune-mediated disease could not be made.

…on vaccines and functional somatic syndromes (FSS)

The inability to record any significant pathological finding in the diagnostic investigations labeled our patient as being affected with a neuropsychiatric syndrome. The cardinal symptom was a medically unexplained localized (headache) and diffuse (musculoskeletal) pain, in addition to not specific manifestations (malaise, fatigue, transient skin rashes). Once any organic lesion was excluded, actually the psychiatric evaluation was not consistent with any major psychiatric disorder, and the conclusion was a functional somatic syndrome (FSS) or somatoform disorder (SD), according to DSM-IV terminology [32].

The term "functional somatic syndromes" was coined in 1990s to refer to several and symptomatically heterogeneous disorders, characterized by general symptoms and by multi-system and not specific clinical manifestations, where no organic lesions or pathological findings could be demonstrated. Since then, the category of FSS has been applied to a growing number of syndromes, characterized by medically unexplained symptoms and by different denominations, depending on the type of symptoms and the main localization of pain (Fig. 1) [33]. That unification under the umbrella of FSS is supported also by the analysis of symptoms prevalence by syndrome, which revealed a considerable clinical overlap. In fact, patients fulfilling criteria for one syndrome often do it for others. At present, diagnostic criteria of FSS require a history of unexplained physical complaints, including several pain or pain-related symptoms (at least four), usually beginning before 30 years of age, persisting years (but, at least 6 months) and leading to significant impairment of individual global functioning, which usually carries to treatment seeking [33–35].

Therefore, the phenomenological denominations are being overcome, and it is supposed that all FSS might recognize similar pathological mechanisms. The observation that, despite the clinical differences among FSS, symptoms seem to be enhanced if the patient is exposed to stressful situations pointed the attention toward social and psychological basis. Moreover, patients with FSS have been shown repeatedly to have greater rates of depression and anxiety, but it does not seem appropriate to reduce FSS as being a nuance of a depressive disorder. On the contrary, the association might derive from a reactive increase of depression and anxiety in patients suffering from chronic physical symptoms, which cannot be medically explained, then properly treated and, finally, solved [36].

Abnormalities of nervous autonomic system and/or hypothalamic–pituitary–adrenal axis and alterations of pain perception mechanisms have been recently hypothesized. In this regard, also serotonergic and noradrenergic pathways have been suggested to play a role: indeed, they are involved in the transmission of emotional inputs, physical (autonomic) functions and pain regulation. Some reports raised a possible relationship between mast cells mediators and hyperalgesia [33, 37]. Recently, Theoharides et al. [38] reviewed a FSS subset as being neuroinflammatory syndromes, suggesting still elusive immunological mechanisms. Indeed, in addition to stressful situations, FSS can be precipitated by physical illnesses, including viral infections.

The viral/immunological hypothesis is claimed especially for chronic fatigue syndrome (CFS), which can be included in FSS in our opinion (Fig. 1). Our clinical case started with pseudo-neurological symptoms, localized pain at the lower limbs and fluctuant skin rashes; however, by time, the clinical picture moved to a condition characterized by chronic fatigue/malaise, recurrent localized or diffuse pain and anxiety, which was consistent with CFS. A history of an infectious illness preceding the onset of symptoms, which is often sudden, has been repeatedly described in CSF. Moreover, disabling fatigue, sleep disturbances, concentration difficulties, memory deficits and psychiatric problems are the principal hallmarks of CFS, and a variably localized and heterogeneous ensemble of subjective complaints can be present. According to Fukuda and colleagues criteria, CFS diagnosis could be made when a patient present with four or more symptoms concurrently for at least 6 months. Moreover, exclusion criteria, such as active medications, past or current major depressive disorder, alcohol or substances abuse and severe obesity, were not present [37, 39].

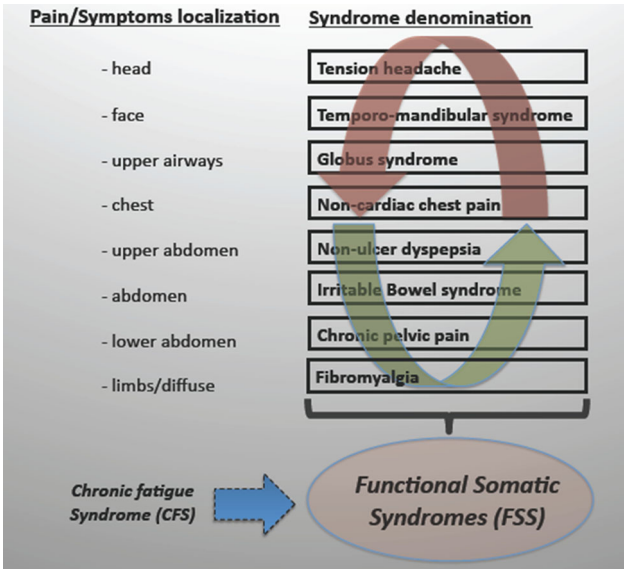

Fig. 1 Variability and overlapping of functional somatic syndromes

CFS was first described in the 1980s, and its etiology has been largely debated, claiming for psychological, infectious, neuroendocrine and immunological causes. Also vaccines have been considered, because of potential hyperactivation on the immune system through the adjuvant component. Recently, some authors proposed to include CFS in the group of autoimmune/inflammatory syndrome induced by adjuvants (ASIA) [40].

…on vaccines and autoimmune/inflammatory syndromes induced by adjuvants (ASIA)

CFS and other FSS developing after a vaccination could be included in the emerging and debated group of autoimmune/inflammatory syndromes induced by adjuvants (ASIA) [40]. Recently, Shoenfeld et al. [41] hypothesized that certain clinical conditions, some of which share several features with FSS, might be determined through still elusive immunological mechanisms elicited by adjuvants contained in vaccines or, more generally, by all environmental substances endowed of adjuvant-like properties on the immune system. ASIA should include both vaccine-related and not vaccine-related disorders as well as both organic and medically unexplained symptoms (Fig. 2).

Indeed, ASIA encompasses a clinical spectrum going from post-vaccination autoimmune phenomena to FSS, characterized mainly by general symptoms (e.g., fever, general malaise, fatigue, sleep disturbances) and manifestations related to several systems and organs (e.g., neuropsychiatric, musculoskeletal, gastrointestinal, mucocutaneous, ophthalmic). Initially, ASIA used to include post-vaccination immune-mediated phenomena (including well-codified autoimmune diseases, such as vasculitis, SLE, rheumatoid arthritis, neurological immune-mediated syndrome, inflammatory myopathies, inflammatory bowel disease) and some heterogeneous clinical entities (siliconosis, macrophagic myophasciitis, Gulf War syndrome and, more recently, CFS) [42].

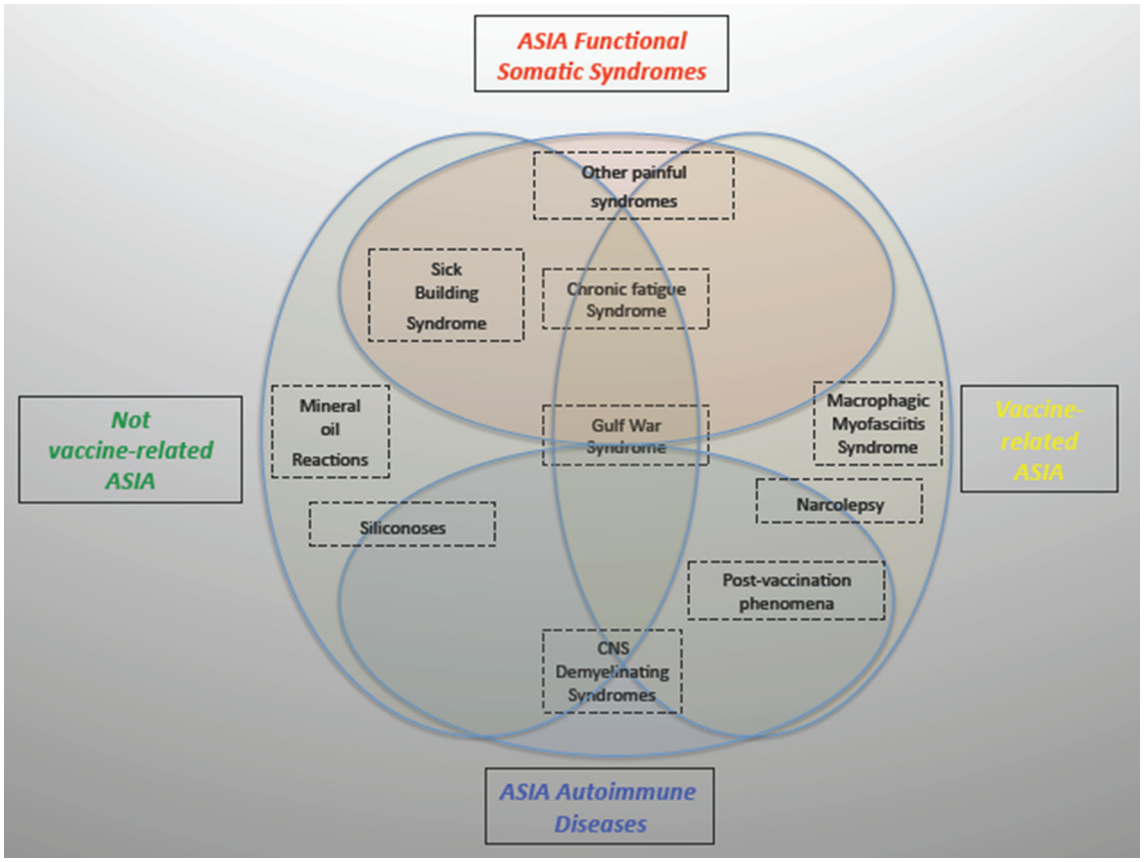

Fig. 2 Proposed perspective of ASIA, including clinical entities which can be related to vaccines, to other adjuvant-like factors or both and which can include organic immune-mediated diseases as well as functional somatic syndromes

Gulf War syndrome (GWS) has been so-called because it was described in soldiers who were sent to battle this conflict. Although multiple vaccinations received over a short time were suggested to be the cause of this syndrome, actually GWS might be linked to many different substances (chemical exposures like oil fire smoke, insect repellents and depleted uranium, etc.) [43]. The largest case series has been reported by Zafrir et al. in patients who received HBV vaccination. Clinical manifestations were general and local symptoms, being consistent with FSS in most cases. Actually, in this study, CNS demyelinating lesions were evidenced in around half patients and, interestingly, almost 80 % demonstrated seropositivity for one or more autoantibodies. In summary, GWS itself seems to be a pathological spectrum including both organic conditions, in the form of CNS demyelinating lesions, and medically unexplained syndromes, namely FSS [44, 45].

As regards siliconoses, those have been correlated with the presence of silicones implants in the body, like breast implants and many other medical devices and products (artificial heart valves, joint implants, ventriculo-peritoneal shunts and more). In addition to several autoimmune diseases following silicone exposure (e.g., systemic sclerosis, SLE, rheumatoid arthritis, vasculitis and polymiositis), variable non-defined immune-mediated phenomena have been described and some meet the criteria of FSS, especially fibromyalgia. In support of immune activation by silicone, many studies describe the occurrence of anti-silicone antibodies and of several autoantibodies (e.g., dsDNA, ssDNA, SSB/La, collagen II) [42, 46].

Macrophagic myofasciitis (MMF) refers to a specific vaccine-related disease characterized by systemic manifestations recalling FSS. However, it is associated with a pathognomonic muscle lesion at the level of injection, which consists in a focal epi-, peri-, endo-mysial infiltration of non-epithelioid PAS-positive macrophages and CD8 T cells in the absence of significant muscle fibers damage. This lesion and the related syndrome have been supposed to be elicited by the alum, which can persist even several years in the muscle. Clinically, the syndrome presents somatoform symptoms resembling FSS, and the frequent complaints of myalgias, muscles weakness and fatigue are very similar to CFS [47, 48].

Very recently, also so-called sick building syndrome has been proposed to be included in ASIA: It refers to a set of FSS-related symptoms, which are reported by a substantial

number of people occupying a building (at least 20 %). The environmental exposure should be represented by materials (asbestos, hydrocarbons, etc.) or by substances present inside (e.g., molds, mycotoxins, phthalates) acting as adjuvants. Finally, emerging evidences support an immunopathological pathogenesis in narcolepsy with cataplexy, which has been associated with influenza vaccine, thus enriching the spectrum of ASIA. In our perspective, ASIA is an emerging and "in fieri" pathological category that is under remodeling and should include organic and medically unexplained conditions, some of which can be induced by vaccines too (Fig. 2) [49–51].

The pseudo-neurological and somatic manifestations complained by our patient defined a FSS, sharing several features with CFS in its evolution, and could be interpreted as a case of ASIA. The sudden onset of the disease, the clinical picture dominated by muscles pain and weakness, the impressive temporal link with HPV vaccine and the finding of anti-phospholipid autoantibodies supported the diagnosis of FSS/CFS and ASIA.

Indeed, the diagnostic criteria for ASIA proposed by Shoenfeld et al. were fulfilled. The patient developed "typical" clinical manifestations after the exposure to an external factor, namely HPV vaccine. Moreover, the appearance of autoantibodies directed against the suspected adjuvants is enumerated among minor criteria supporting the diagnosis of ASIA. In this perspective, the presence of anti-phospholipid autoantibodies was concerning, because in the bivalent HPV vaccine there is the AS04 adjuvant system, which comprises an aluminum salt and the immunostimulatory molecule 3-O-desacyl-4′-monophosphoryl lipid A (MPL) [44, 52]. This substance exploits its adjuvant activity through the interaction with TLR4, but it is interesting to note that it has the biological property to elicit a specific immunity apart from its association with other antigens. Thus, MPL is an immunogenic antigen by itself. This immune system activation can support also the immune response to other antigens administered concomitantly, but can induce a specific adaptive immune response leading to the production of anti-phospholipid antibodies, especially anti-β2-glycoprotein I [9]. Similarly, in animal models being genetically prone to develop anti-phospholipid autoantibodies syndrome, the induction of high levels of anti-phospholipid autoantibodies was demonstrated after adjuvant immunization alone [53, 54].

Moreover, Schwarz et al. [55] observed that >80 % girls had detectable anti-MPL antibodies before receiving bivalent HPV vaccine; however, by month 7 after the vaccination, in previously seropositive girls, they recorded a 3.5-fold increase in anti-MPL antibodies, which appeared in 99 % of previously seronegative girls too.

Finally, the causes of most neuropsychiatric syndromes remain still elusive, but the concept that infections and/or autoimmunity could be related to some neuropsychiatric illnesses has a long history. Some subsets of those showed an increased prevalence of autoimmune and atopic diseases. In some specific post-infectious and immune-mediated disorders, the relationship with autoimmunity resulted particularly impressive, as in Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS) [56, 57]. Further, an altered brain functioning under inflammatory and/or autoimmune conditions promoting the development and the entry of autoantibodies and cytokines in the central nervous system has been speculated in genetically susceptible individuals after the exposure to environmental factors, such as infections and vaccines [58]. Indirect evidence might be the increased levels of pro-inflammatory cytokines in blood and cerebrospinal fluid of patients affected with mood disorders [56]. Interestingly, the concordance rate for some psychiatric disorders (e.g., schizophrenia, autism) in monochorionic monozygotic twins resulted significantly higher than in dichorionic monozygotic twins, having separate placentas, which suggest the influence of factors other than the genotype. Pro-inflammatory cytokines and/or autoantibodies arising from maternal infections and/or autoimmunity might pass through the placenta and fetal blood-barrier brain and affect the neurodevelopmental processes. Similar mechanisms might occur after the birth, leading to minor psychiatric disorder [59, 60].

Conclusion

ASIA is an emerging clinical category needing further studies to support its identity and to better define its diagnostic criteria, as it can include a pathological spectrum ranging from organic (autoimmune and/or demyelinating) diseases to somatoform disorders, which are variously denominated, according to the main symptom, and largely overlap among themselves [51].

Here, we reported an adverse event after bivalent HPV vaccine. It developed around 60 min after the injection in a patient who never suffered with any previous physical and/or psychic disease or complaint. The pseudo-neurological symptoms she developed cannot be ascribed to the well-known occurrence of syncope, which has been largely described by 15 min after the injection, because of the pain experienced during the medical procedure [61]. Neither the development of neuropsychiatric symptoms had a collective occurrence, namely mass psychogenic illness, reported after several vaccines. Apart from the cultural setting, these collective responses are similar and usually include symptoms such as headache, dizziness, weakness and reduced awareness [62]. Mass psychogenic syndrome has been also described in a group of 26 girls, who presented

dizziness, syncope and pseudo-neurological complaints by 2 h after HPV vaccination [63].

Most vaccine-related side effects are local reactions. As concerns the specific issue of autoimmunity, no significant increase in the incidence of several immune-mediated disorders was demonstrated in the medical literature as following HPV vaccine [26, 27]. However, the absence of statistical evidence in the epidemiological studies so far performed should not be automatically translated in the certainty that a causal link between vaccines and autoimmune diseases must be rejected. The exposure to vaccines is common and, in some developed countries, almost universal. Conversely, the development of an adjuvant-related disease is relatively rare, and the clinical diagnosis can be challenging, especially in FSS. Recently, an observational analysis (performed by UK Medicines and Healthcare products Regulatory Agency, MHRA) investigated the association between bivalent HPV vaccine and the development of CFS. However, no change of incidence of spontaneous reports of CFS in girls aged 12–20 years after the introduction of the vaccination was observed [64].

The absence of statistical association does not exclude a causal link, as in post-vaccination ASIA additional risk factors are likely to be required, such as genetic predisposition or the concomitancy of other environmental factors [65]. Thus, although there is no general evidence of increased autoimmune risk in the population, vaccination should be carefully evaluated for the individual patient. Of course, the temporal association is only one point to be considered in the evaluation of a possible causal link between a vaccination and an adverse event, but a small span of time between the vaccine and an eventual disease or disturbance can be even more concerning. However, consistency, strength and specificity of the association between two events must be considered. Unproven causal relationships between adverse events and vaccine can reduce the acceptance of vaccine campaign by some people, compromising the efficacy of the vaccination [22, 66]. Of course, people with risk factors for well-established post-vaccination complications must be dispensed by the vaccine or, at least, the risk/benefit ratio has to be carefully evaluated in the interest of the single individual. However, some concerns can remain for simple adverse events where there is not sufficient evidence to accept the causal link, but neither to reject it.

Then…is it true that, if in the general population well-conducted studies are not able to detect a statistical and significant association between a specific disease and a vaccination, the vaccine is safe for everyone? But are there well-conducted studies? And again, are we looking for any potential adverse event of vaccines, including medically unexplained syndromes, which anyway have a significant impact on the life and functioning of an individual? As well as we must not misinterpret an isolate temporal link as being a causal link, without satisfying all required epidemiological and the biological criteria, we must consider the possibility of an individual (genetic and/or multifactorial?) predisposition to develop an adverse reaction to a vaccine, which could not be apparent through population studies. Indeed, no significant studies evaluating post-vaccination somatoform diseases are currently available.

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