Focus

HPV vaccination in Italy: perspectives after 1-year experience

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HPV • Vaccines • Cervical dysplasia

Since January 2008, the Italian Regions, in accordance with their organizational programme, have launched the offer of free human anti-papillomavirus vaccine (HPV) to 12-year-olds, with the possibility to extend this offer to other age groups. With the beginning of this vaccination campaign, a widespread scientific debate developed concerning certain aspects which are still disputed. In our opinion, therefore, it was decided worthwhile making a *review* of articles appearing in the literature over the last year, in particular reports published after the onset of commercialisation, in the attempt to gather together useful elements that would contribute to improve the vaccination strategies.

Development, therefore, of a vaccine to reduce the impact of the infection, in terms of morbidity and mortality, has now become of prime importance as far as concerns public health worldwide.

The European Medicines Evaluation Agency (EMEA) has authorized the use of 2 anti-HPV vaccines: since September 2006, a quadrivalent vaccine (Gardasil®, Sanofi Pasteur MSD) containing Virus-like Particles (VLPs) of the HPV-16, HPV-18, HPV-6 and HPV-11 genotypes, produced in *Saccharomyces cerevisiae* and with an amorphous alluminium hydroxyphosphate sulphate (AAHS) adjuvant and, since September 2007, a bivalent vaccine (Cervarix®, GlaxoSmithKline) containing VLPs of the HPV-16 and HPV-18 genotypes, produced in insect cell lines and with the AS04 (aluminium and monophosphoryl-lipid A hydroxy adjuvant). Since 2006, Gardasil®, has also been granted approval by the Food and Drug Administration (FDA) in the United States.

Treatment indications* for both these products are aimed at prevention of cervical precancerous lesions and cervical cancer caused by HPV 16 and 18, which are held responsible for approximately 70% of all cases of cervical carcinoma [1]; Gardasil® is used, furthermore, in the prevention of lesions correlated with HPV 6 and 11, responsible for 70-90% of genital condylomas and 10-20% of low grade of Cervical Intraepithelial Neoplasia (CIN 1). Evidence that HPV 6 and 11 are associated with CIN 1 lesions confirms the significant frequency of these types in women with *borderline* cytological evidence, such as, abnormal Pap-test, atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGUS) [2-5]. Gardasil® indications also include prevention of precancerous genital lesions at vulva (VIN 2/3) and vagina (VAIN 2/3) level. In this respect, one of the aims in the pharmaceutical development of vaccines was, indeed, that of extending the number of viral types of HPV in order to prevent the related pathological conditions, taking into consideration primarily cervical cancer, but not overlooking other tumours of the female genital tract and the early disorders, such as condylomas and low grade lesions correlated with the various viral types. The treatment used in the management of these lesions involves high costs which are not necessary and could, therefore, be limited with the use of a primary prevention programme [6, 7].

As far as concerns the host immune response, anti-HPV vaccines have been produced with an adjuvant in order to increase antibody response: it is well known, however, that the protection induced by these, even if mediated by the humoral response, cannot be correctly evaluated only by means of the immunological data, but need to be related to prevention of clinical lesions. As mentioned in the technical notes of these two products, no minimal antibody level has been identified as associated with protection against CIN, grades 2 and 3, or against persistent infection associated with the types of virus contained in the vaccine. Also for this reason, World Health Organisation (WHO) recommended undertaking clinical developments with clinical-histological endpoints, aimed at the definition of "precursors" as the surrogate of carcinoma, based upon precise observations focused on the biological behaviour of the intra-epithelial lesions (CIN 1, 2 and 3) [8].

In clinical studies involving young women, both vaccines were found to be efficacious as far as concerns primary and secondary endpoints associated with the types of HPV contained [9]; moreover, Gardasil® was demonstrated to be highly efficacious in the prevention of vaginal/vulvar and genital condyloma lesions [10]. On the other hand, these two preparations do not appear to have any significant effect upon clearance levels and progression of the cervical infections caused by HPV [9], thus confirming their preventive rather than their therapeutic role.

Furthermore, there is evidence of cross protection towards viral strains not directly contained in the quadrivalent preparation thus confirming the difficulty encountered in interpreting the mechanisms of the immune response and

* The therapeutic indications are reported referring to the clinical information appearing on the technical notes of the two products, even if these are preventive vaccines.

the necessity in support of the assumption related to the need to refer to histological endpoints in order to evaluate efficacy. As reported in the technical note, the efficacy of Gardasil® against CIN and CIN 2/3 or adenocarcinoma *in situ* (AIS) caused by HPV not contained in the vaccine, but phylogenetically correlated to HPV 16 or HPV 18, has been evaluated in Phase III studies. After a mean follow-up period of 3.6 years, the results referring to the combined incidence of CIN 2/3 or AIS demonstrate a statistically significant efficacy (55.6% - 95% CI: 26.2-74.1) as far as concerns pathological conditions structurally correlated to HPV 16 (particularly HPV 31). Again, in the technical note, it is stated that the efficacy is not statistically significant for HPV types phylogenetically correlated to HPV 18 (including HPV 45). With regard to Cervarix®, it would appear from published data referring to the GSK 001/007 trial, that the efficacy, calculated in the analysis (modified) intention-to-treat (MITT), was 94% (95% CI: 63-100) for HPV 45 and 55% (95% CI: 12-78) for HPV 31 [11], but only for the incident infection variable (only positivity to HPV). This study made no mention of efficacy findings referring to the above-mentioned and more important histological endpoints. Again as far as concerns Cervarix®, in the MITT analysis performed in the PATRICIA trial, the efficacy against persistent infection since 6 months was found to be 60% (CI 97.9%: 3-85) for HPV 45, 36% (CI 97.9%: 1-60) for HPV 31 and 32% (CI 97.9%: 4-52) for HPV 52, while for persistent infections since 12 month 62% (CI 97.9%: -93; 95), 11% (CI 97.9: -115; 63), 47% (CI 97.9%:-12; 76), respectively [12]. Albeit, these results have not yet been included in the technical notes accompanying the product.

The fact that anti-HPV vaccination offers greater protection compared to specific types of the vaccines, represents, however, an incentive to further develop these preparations. In studies on the immunogenicity, not only the bivalent vaccine but also the quadrivalent type, were found to be highly immunogenic with serum conversion rates of approximately 100% and, generally, after the third dose, peaks of geometric means of the circulating antibody titres (GMT) were observed from 10 to 100 times greater than those observed following a natural infection [11, 13]. Thereafter, a plateau has been shown, that remains stable throughout the period of follow-up (approximately 4 years) [11, 12, 14].

It is worthwhile pointing out that the immunogenicity tests, referring to the clinical trials, were performed by the manufacturers using two different systems to evaluate the post-vaccination antibody response, and, furthermore, it is important to note that the data related to long-term follow-up would appear to be, partially, influenced, in the interpretation, by the technique used to measure the antibodies.

In the clinical trials carried out with Gardasil®, antibodies were measured by means of type-specific immunological samples, in particular the competitive sample based upon Luminex (cLia) technology with type-specific standards, as shown in the technical notes. This sample measures the antibodies against a single neutralizing epitope for each VLP-HPV [15], therefore, this result causes a higher specificity regarding display of the humoral immune response produced following vaccination.. With regard to Cervarix®, the IgGs were measured using a direct type-specific enzyme-linked immunosorbent assay (ELISA) [16], the intrinsic characteristic of which is the capacity to reveal total antibodies employing, during the solid phase, the anti VLPs L1; thus the ELISA test is able to evaluate the complex of the humoral response following vaccination, albeit without discriminating between neutralizing and non-neutralizing antibodies. Thus, considering the different methodological approach, direct comparisons between antibody titres with the cLia test and those obtained with ELISA are impossible and, consequently, also between the antibody "kinetics "of the curves. In addition, the various monoclonal antibodies, selected in the cLia method, display different affinities and perform in a different way according to the different HPV vaccine types, thus at the present state of our knowledge it is absolutely impossible, not only to compare the data concerning the quadrivalent vaccine with those of the bivalent vaccine, but there is not even a common reading of the antibody "kinetics" for the various types of VLP-HPV of the quadrivalent.

For example, the curves of the antibodies of the quadrivalent vaccine reveal that during the long-term follow-up phase, in women vaccinated with Gardasil®, a reduction is observed in the percentage of serum conversion of almost one third for HPV 18 whilst this is not seen to occur for HPV 16, mentioning only the types at high risk. This finding, as previously pointed out, is technique-dependent, therefore it is tempting to hypothesise that this is influenced by the characteristics of the response to the epitope selected for HPV 18. Albeit, this does not appear to be of clinical relevance inasmuch it was not found to be correlated, over time, with the loss of protection as far as concerns the clinical endpoint (CIN 3 or AIS) [13, 14].

Reports, so far concerning laboratory tests, clearly reveal the almost total lack of samples with which to evaluate antibody titres thus, from a practical viewpoint, no standard blood test exists with which these can be evaluated [8], despite the fact that the protection mechanism of HPV vaccines is based upon the production of neutralizing antibodies, as stated in the technical notes.

In conclusion, the above-mentioned data suggest that great caution should be taken when advancing hypotheses and expressing considerations concerning the antibody response, both from a qualitative and quantitative viewpoint.

Again, as far as concerns the immunological response, further reflection, on this point, is triggered by the observation that the antibody response to the VLPs HPV 16 is high following the administration of the quadrivalent vaccine as well as following vaccination with a similar preparation, namely, monovalent HPV 16 [17], thus demonstrating that there is no indication that the VLPs themselves exercise immunological disorders, when contained in a multivalent preparation [9].

Another aspect to be taken into consideration concerns the immunological memory, a mandatory basis for long-term protection.

Suggestions on how this immune memory for HPV vaccines should be investigated have been advanced by international organizations' authorities [8]. For the quadrivalent vaccine, evidence of an anamnestic response has been observed in two different groups, the first one being

vaccinated subjects serum positive to the relative types of HPV prior to vaccination, the second group refferd to vaccinated subjects who had received a challenge dose of Gardasil®, 5 years after the beginning of the vaccination cycle [14]. These results suggest a long-lasting type of vaccine efficacy and mathematical models estimated detectable antibody levels ranging from at least 12 years to throughout lifetime [18]. As far as concerns the bivalent vaccine, no studies *ad hoc* have been reported in the literature evaluating the immune memory detected clinically.

VLPs comprise the capside protein which is highly immunogenic but is not infective since it has no DNA. Thus one would expect to find a safety profile similar to that in other structurally similar vaccines, such as, for example, that of tetanus and hepatitis B [9].

Safety data have been obtained in three clinical trials of phase III in which, in general, the vaccines were found to be well tolerated. Local reactions, such as pain and erythema at the site of administration [11-13, 17] were significantly more frequent than in the control group: local pain was reported in those subjects who received the vaccine and in the controls, 90.5% and 78.0%, respectively, in the PATRICIA trial and 85.3% *vs* 75.4% in the FUTURE I trial [12, 17].

Systemic adverse events, potentially correlated with the vaccine, such as fever, for 7 days following vaccination in 12.4% of those vaccinated and in 10.9% of controls in the PATRICIA trial, or fever for the first 5 days reported by 14.8% of those vaccinated and by 11.5% of controls, in the FUTURE I trial. Neither local nor systemic symptoms were more severe in the successive administrations or in women with previous exposure to one of the types contained in the vaccine [17].

Data regarding current post-marketing monitoring concern primarily Gardasil®: the largest amount of surveillance data have been produced by the USA drug vigilance system where, following FDA authorization, more than 20 million doses have been used. Records were collected in the Vaccine Adverse Event Reporting System (VAERS), in the Vaccine Safety Datalink Project and in the Clinical Immunization Safety Assessment Network. Based upon reports recorded from 2006 to 31/8/2008, 94% of the cases were not severe adverse events, such as weakness, pain in the administration site, headache, nausea and fever. All severe adverse events reported (neuropathy, peripheral paralysis, Guillain-Barré Syndrome, thrombo-embolic events etc), were scrupulously analysed and were not correlated with the vaccine administration [19]. On the basis of this information, Centers for Disease Control and Prevention (CDC) and FDA defined Gardasil® safe and efficacious in the prevention of infections of the 4 types present in HPV.

As far as concerns Italy, the *Agenzia Italiana del Farmaco* (AIFA) (Italian drug agency) authorized the commercialisation of the two HPV vaccines in 2007 (Determination of 28 February for Gardasil® and 21 November for Cervarix®) and defined that the preparations, in the H-RR group, should be made available not only free-of-charge to all 12-year-old females, but also be made available, on sale, at the Chemists. Choice of this strategy is in keeping with the recommendations of the World Health Organization (WHO) [20] and with the scientific evidence currently available, according to which the efficacy of the preparation is based upon immune protection before an eventual HPV infection. The most rational and efficient way of using this new vaccine is, therefore, to actively offer it immediately prior to the beginning of sexual activity and age 12 years was found to be best indicated, because, in this age period, the immune response is higher than that in the years immediately following [21]. Furthermore, coinciding with the compulsory school period, there would be a greater opportunity for families to communicate with health and social workers, thus the offer of vaccination, in this age group, would imply that it be performed through the same services as those already actively involved in infant vaccination, thus maintaining this service in the environment of the professional patrimony and of the programme of the National Health Service, also bearing in mind other important professional figures (specialists in Gynaecology & Obstetrics, Paediatricians in Private Practice, General Medicine Doctors Practitioners, Health Assistants…).

The strategy used in the active offer of the HPV vaccine in Italy was defined in *Conferenza Stato-Regioni (*Conference of State-Regions) (20/12/2007) therefore, from January 2008, each Region, according to its organizational procedure, was able to proceed with the vaccination campaign, with the possibility to extend it to other age groups [22]. In order, not only to guarantee fairness access and parity of offer, but also to monitor coverage of vaccination, at Regional level, the ministerial regulations invites the health services to record, the vaccinations performed in the respective database. This system would also make communication easier as far as concerns the organization of screening programmes: there is, in fact, the not negligible risk that the increase in vaccination coverage would be accompanied by a decrease in compliance by the women who had received the vaccine. Above all, considering the fact that approximately 30% of cervical lesions are not caused by types 16 and 18, it is possible that a considerable increase might occur in incidence and mortality rates in those women not taking part in the screening programme, with consequent reduction of vaccine effectiveness [23]. This is why it is mandatory for the offer of vaccination to be strategically integrated in the programmes, already under way, of secondary prevention, which, moreover, represent an instrument revealing the trend of pre-cancerous lesions and of cervical carcinoma and, thus, the evaluation of the efficacy of the extended immunisation programme.

With regard to coverage, the aims are to reach at least 95% of the target population, with three doses of vaccine, within the five years since the beginning of the vaccination programme, that is, for girls born in 2001, who will be actively invited for vaccination in 2012 and whose coverage for the third dose will be evaluated on 31st December 2013.

Also the possibility to have two products available, which have been demonstrated to be efficacious and well tolerated, have led to good results being obtained, as far as concerns vaccination coverage at territorial level**:** for example, based upon preliminary data recorded in the Basilicata Region, referring only to the 12-year-olds, coverage reached 77%, for the 15-18-year-olds, 67% and 64%, respectively, whilst for 25-year-olds only 37% was reached.

Nevertheless, according to those responsible for decision-making at Public Health level, a better understanding of some important aspects is necessary, since they still remain to be fully elucidated: the efficacy, immunogenicity and the long-term safety profile, the possibility to simultaneously administer other vaccinations, the eventual need to proceed with booster doses. Likewise, further information on the performance of vaccination in mature women, in immunodeficient subjects, in young women with previous exposure and in men, could become available in the near future, when the results of the clinical trials performed in these groups of subjects are published.

In the light of these considerations, it would be interesting to take a closer look at those aspects, which still remain to be fully elucidated, possibly, in a forum focusing on contributions not only from experts in the field but also on queries submitted by readers to the Editorial Board of the Journal of Preventive Medicine and Hygiene.

Declaration of conflict of interest

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