Influenza in HIV positive patients

Introduction

Influenza illness is a worldwide, public health problem of major concern. It is a highly contagious, ubiquitous disease that can lead to severe complications, especially in the elderly, debilitated or chronically ill patients, children and in immunosuppressed subjects, including those infected with the human immunodeficiency virus (HIV) and transplant recipients [1, 2]. It is difficult to estimate hospitalizations and deaths due to influenza and its complications because infections are not often confirmed virologically or specified on hospital discharge forms or death certificates [3]. For influenza disease a significant burden lies on all individuals, but hospitalization and treatment occur more frequently in high-risk patients. In addition to the clinical burden, influenza also poses a considerable economic burden. There are extensive economic analyses of the influenza burden in the literature. One study estimated a total economic burden of seasonal influenza in the United States (using 2003 population and dollars) to be $87.1 billion, including $10.4 billion in direct medical costs [4]. In addition to the direct costs of medical care, the indirect costs of influenza are substantial due to the increase morbidity. The burden of influenza varies with the age and underlying health of the patient. Estimates of the cost of influenza in the USA, France and Germany have shown that indirect costs can be five– to 10-fold higher than direct costs [5]. In particular, influenza remains a common cause of respiratory illness in adults with HIV, despite the use of novel high retroviral therapies and increase of flu vaccine coverage rate, that lead the mortality rate from 2.2 million (in 2005) to 1.8 million (in 2010) [6, 7]. The studies conducted by Neuizl et al. and Lin et al. confirmed high numbers of hospital admissions and high mortality from influenza in patients with HIV/AIDS not on Highly Active Anti Retroviral Therapy (HAART) [8, 9]. Neuizl et al., in another study reported that cardiopulmonary hospitalisations in influenza infection of patients with Acquired Immunodeficiency Syndrome (AIDS)/HIV were very high in the pre-HAART era, with a rate of 48 per 1000 persons; in the post-HAART era hospitalisation rates decreased by 53% but remained more higher than that of the U.S. population and similar to that of the population at risk [10].

Immunization against influenza can effectively reduce the annual clinical and economic burden of influenza. Nevertheless, despite efforts to vaccinate those at highest risk of severe influenza-related complications, many still go unvaccinated [11, 12]. A study in eleven European countries showed that the highest immunization coverage for the population considered at high risk, was reached in the Netherlands (about 80% of vaccinated subjects), while the lowest rate belongs to Greece with only 25%. This data may be explained by several considerations: first, factors such as education can guide people towards a particular choice in favor of vaccination or not; second, in some countries the reimbursement for the cost of vaccination is partial; third, a smaller number of doses distributed could obviously result in a smaller number of subjects vaccinated [12]. The Advisory Committee on Immunization Practices considers HIV-infected persons to be at increased risk for influenza and recommends annual vaccination [13]. In view of mortality, morbidity and complication risk, already in 1986 there was a rec-
ommendation for influenza vaccination of patients with human T-lymphotrophic virus type III AIDS-related, and since 1988 the CDC recommended vaccination in children with acquired immunodeficiency syndrome. Current guidelines recommend that individuals infected by HIV receive yearly influenza vaccination. Since most previous studies on influenza vaccines in HIV infected individuals focused on their immunogenicity rather than on their clinical effectiveness, it is crucial to set up close post vaccination influenza monitoring to check the persistence of immune response and effective immune protection against viral strains circulating during seasonal epidemics [14-16].

The Strategic Advisory Group of Experts (SAGE) on immunization reviewed evidence on influenza disease burden, vaccine performance (efficacy and safety), and the feasibility and cost-effectiveness of implementing vaccination programmes for population groups at higher risk. In 2012, WHO published updated recommendations for seasonal influenza vaccination based on a systematic literature review performed by SAGE [17-21]. SAGE confirmed the recommendations to consider as priority groups for influenza vaccination individuals > 6 months with chronic heart or lung diseases, metabolic or renal disease, chronic liver disease, chronic neurological conditions and immunodeficiencies.

Prospective studies have looked at the long-term effect of influenza vaccination, confirming the safety and tolerability of currently used influenza vaccines, but questions remain about the immunogenicity and effectiveness of these vaccines in HIV infected patients. New roads could be opened through the use of new vaccines, such as MF59® adjuvant and those administered intradermally, that could overcome problems such as poor immune response and suboptimal effectiveness [22, 23]. The aim of this review is to evaluate tolerability, safety, immunogenicity and efficacy of vaccines actually approved for human use and to consider latest evidence and future perspective in HIV- positive subjects.

**Tolerability and safety**

Several studies were performed to evaluate tolerability and safety of influenza vaccination in HIV positive patients showing a good tolerability profile. In different studies reported symptoms resolved without sequelae in few days. There is lack of data regarding adverse events at long-term [23, 24].

Main evidence emerged by safety and tolerability evaluation of available vaccines in the last decade; serious adverse events, systemic and local reactions are reported below.

**Serious adverse events**

Serious adverse reactions were defined as any untoward medical event that at any dose result in death or is life-threatening, requires hospitalisation, results in disability or congenital anomaly [25]. From global analysis of more than 20 studies that evaluated safety and tolerability profile, no serious adverse events (SAEs) are tight associated with influenza vaccination in HIV- infected patients. Reported adverse events during follow-up of the studies were not associated with influenza vaccination but usually related to syndromes or treatment associated with HIV.

Trivalent Influenza Vaccines (TIV) were the first and the most used vaccine for immunization of HIV positive subjects. As emerged from literature data of the last two decade, TIV showed a good safety profile and no SAEs were reported [26, 27]. Durando et al., compare two influenza virus subunit vaccines with or without MF59® adjuvant showing absence of serious adverse event, except for a case of transitory ischemic attack in a men, episode that was demonstrated to be not correlate to vaccination [28]. Cooper et al., in his study, using a trivalent killed split not adjuvanted influenza vaccine in HIV infected adults, showed that vaccination is well tolerated without increase reactogenicity as consequence of higher antigen dose or boosting dose. In his study the population is divided into three groups, the first of which received one dose of influenza vaccine (0.5 mL or 15 µg hemagglutinin [HA]) in October and November 2008 followed by a booster dose 28 days later, while the second gets a double dose (30 µg HA) at the same time interval, followed by a double dose of booster 28 days later and finally, the third group received a single dose of vaccine [29].

Several studies conducted in HIV infected individuals immunized with A(H1N1) 2009 pandemic inactivated, unadjuvanted vaccine showed that vaccines were safe and well tolerated in this population and no SAEs were reported. Crum-Cianflone et al. evaluated a monovalent non adjuvanted 2009 influenza A/H1N1 vaccine comparing SAE in HIV positive and uninfected subjects showing that only 1 partecipant (HIV- uninfected patients) developed a serious adverse event that consist in angioedema on day 1 post vaccination and resolved after 17 days with antihistamine therapy [30].

It also occurred eight hospitalization but no one related to vaccination (seven in HIV-positive patients and one in healthy subjects) [31-41]. It has been demonstrated in studies involving a total of 892 children and 111 adults that administration of live attenuated influenza vaccine (LAIV) vaccine is safe and well tolerated and not serious adverse events related to vaccination were recorded. Furthermore, the safety profile is very similar to that previously reported in studies including other populations.

Current influenza vaccination guidelines do not recommend LAIV immunization in persons with known or suspected immunodeficiency diseases, but the cited studies demonstrated that this vaccine should be evaluated for a role in immunizing HIV infected people [42-47].

Data from existing studies showed that virosomal influenza vaccination is well tolerated and no complaints of severe adverse reactions are usually reported in both children and adult HIV positive individuals [26].

Gabutti et al., and Durando et al., in their study do not show adverse serious events associated with the vaccination during the follow up period in HIV populations vaccinated both with MF59® influenza vaccine and with
subunit vaccine containing 15 µg of HA per strain and
administered intramuscularly [26, 28].
In the study by Launay et al., three adverse reactions are
reported after 42 days post vaccination, with monovalent 2009 Influenza A/H1N1v vaccine adjuvanted with
AS03®, consisting of hospitalization following the second
dose of adjuvanted vaccine, one recurrent episode of
depression and one increase in serum alanine transami-
nase value associated with other treatment [48]. Palma et al., evaluating safety and immunogenicity of mono-
valent adjuvanted A/H1N1 pandemic influenza vaccina-
tion reported no deaths or serious adverse event during the follow-up [49]. No correlation between SAE and in-
fluenza vaccination was observed. Many studies report
that the pandemic vaccine with or without adjuvant is
safe and well tolerated [32-35, 38, 39, 48, 50-52].
Gelinck et al. also reported no adverse events in the
study which compares the influenza vaccine contain-
ing 0.1 ml of the 2005/2006 trivalent influenza vaccine
administered intradermally and 0.5 ml administered intramuscularly in immunocompromised patients. The
frequency of events is more higher in subjects who re-
ceived the vaccine intramuscularly [22]. The same ob-
servation were reported by Ansaldi et al., in a study in
which intradermal vaccination low-antigen – 9 µg HA
per strain- and vaccine intramuscular containing a stan-
dard dose of antigen -15 µg- were administered to HIV
positive patients and compared. The study, although the
low population size, shows good safety for both vac-
cines and no SAE [53].

Systemic reactions
Systemic reactions occur after a few hours of vaccina-
tion and may last for 24-36 hours. They may be linked
to various causes such as the action of endotoxins, repli-
cation “in vivo” of microorganisms , the toxicity of the
adjuvant and the reactivity of the immune system. They
are usually mild, but in some cases can be so severe as to
require supportive therapy.
Durando et al., comparing two subunit influenza vac-
cines with or without MF59® adjuvant, in patients with
immunodeficiency acquired, reports such as systemic
event most widespread headaches and fever in both
groups with percentages of 25.9% and 28.4% [28].
In different studies, adverse systemic effect in patient
vaccinated with virosomal influenza vaccine are com-
parable to subunit influenza vaccine group. In particular
Evson et al., reported in a population of immunosup-
pressed adults vaccinated with a subunit or a virosomal
influenza vaccine that the fatigue was the most frequent-
ly reaction, followed by headache and malaise in both
vaccine group [54].
Systemic reactions typically occur after a few days post
vaccination and are more frequent in subjects receiving
an adjuvanted vaccine. They are mild or moderate and
of short duration.
Indeed, Gabutti et al., comparing two influenza vaccine
containing 15 µg of superficial haemagglutinin antigen
for strain, MF59®-adjuvanted influenza vaccine (group A) versus a conventional subunit vaccine (group B), re-
ports fever in 5% of patients of group A and 10% of
subjects of groups B [26].
Palma et al., reports in his study as most frequent sys-
temic reactions headache, fatigue and chills. 8% of sub-
jects reports fever , 11% fatigue after the first dose. Fe-
ver is not reported after the second dose, while fatigue
reported by 6% of subjects [49].
Gelinck et al., comparing 156 immunocompromised pa-
tients and 41 healthy controls that were randomized to
receive either 0.5mL of the 2005/2006 trivalent influen-
za vaccine intramuscular or 0.1mL intradermal, estimat-
ed the frequency of adverse reactions after intramuscular
vaccination with symptoms going from muscle pain to
fever with values ranging from 11-48% [22].

Local reactions
Local reactions are defined as reactions that occur at the
site of inoculation of the vaccine and determine redness,
pain, erythema, pruritus. In general the local reactions
are more frequent in subjects vaccinated with adjuvan-
ted or intradermally administered influenza vaccines.
LAIV were well tolerated and have a similar safety
profile compared with TIV; nasopharyngeal symptoms
were minor and of short duration.
Durando et al., comparing two subunit vaccines with or
without MF59® adjuvant, in patients with immuno-
deficiency acquired, reports such as local event most
widestand pain an induration, respectively 54.3% and
22% [28].
Among the adverse local effect in patient vaccinated
with virosomal influenza vaccine, the pain is the most
frequent local reaction, followed by swelling and red-
ness. Reported adverse events were comparable with
those recorded in the groups immunized with conven-
tional vaccines.
Some studies that use an adjuvanted vaccine (MF59®)
and where the population was immunodepressed (HIV
positive or those with chronic illnesses) reported lo-
cal reactions of mild or moderate entity [24, 41, 42]. In
the study by Launay et al., use of adjuvanted vaccine
with aluminum salts determine a higher rate of reac-
tions (71%) in HIV positive subjects compared to the
control group (21%) [48]. Gabuttì et al., comparing the
safety and immunogenicity of a subunit vaccine versus
MF59®-adjuvanted vaccine in HIV-positive patients,
reported pain and redness in 28% of patients vaccinated
with adjuvanted vaccine and 10% of subjects vacci-
nated with the subunit vaccine [26].
The most frequent local effect were pain -18%- , swell-
ing and redness -2%- at injection site. Were classified
as mild and resolved in few days post vaccination, as
reported by Palma et al. [49].
Ansaldi et al. in his study reported pruritus in 29% ID vs
4% IM, redness 46% vs 8%, swelling 43% vs 12%. All
local events were mild or moderate and resolved with-
sout sequelae [53].
The same conclusions are reached by Gelinck et al. in
their study in which they evaluated the administration
of a trivalent vaccine by intradermal route (0.1 ml) or by
intramuscular (0.5 ml), they reported erythema and pain
at the injection site 48 hours after vaccination showing these reactions more frequently in subjects vaccinated intradermally and in the control group compared to immunocompromised patients. In general, all subjects in the control group and 69% of immunocompromised individuals who responded to all three antigens showed a local skin reaction [22].

**HIV-RNA and CD4+ Cell Count Post-Vaccination**

Several studies published between 1995 and 2012 reported controversial data both in favour and against the evidence of increase of viral load and a decrease of CD4+ cell count secondary to the administration of influenza vaccination in HIV infected persons with or without correlation to the immune response. Differences in used vaccines, in sample sizes and features of HIV infected populations (i.e. demographic characteristics, risk factors, stage of HIV disease, patients under anti-retroviral treatment or naive) enrolled in the various studies as well as timing of sampling assays and different sensitivity of laboratory methods for quantification of viral load, could explain at least part of these conflicting reports [32, 41, 43]. Many authors reported an increase in viral load following influenza immunization, this data appears to be related to detectable viral load at baseline and no treatment of HAART therapy, although it is not always observed [24, 27, 55-59, 61-63]. The rebound of viral load is attributed to activation of quiescent HIV infected CD4+ cells and the up regulation of HIV viral replication [52, 58-62]. Günthard et al. and Staprans et al. showed that effects of trivalent influenza vaccine on viral replication in HIV positive adults determined transient increase of plasma HIV-RNA levels [58, 64]. Therefore, the simultaneous decrease in pro-viral DNA and memory phenotype CD4+ cells in association with increased plasma HIV RNA after vaccination in patients with < 400 RNA copies/ml at baseline suggested that in vivo mobilization of the latently infected cell reservoir may occur during potent antiretroviral therapy [58]. The same conclusion has been reported by Fuller et al. and Madhi et al. [56, 65]. Study conducted in HIV infected children reported controversial data about increase of viral load after immunization with trivalent influenza vaccine.

Kosalaraska et al. showed an increase in HIV-RNA levels in children aged ≥ 6 months to < 18 years: authors stated that increase in plasma HIV-RNA was a transient phenomenon and did not have any clinical significance [66]. Machado et al. showed no increase neither in viral load nor in CD4+ cell count in a population of children with a mean age of 12 years [67]. In the study by Tasker et al. CD4+ cell count and viral load are evaluated in HIV positive subjects vaccinated with LAIV influenza vaccine. They showed an increased HIV replication and a decrease in CD4%: according to the authors, this effect is probably due to the absence of treatment with antiretroviral therapy [63]. Levin et al., showed, instead, no significant increase from baseline in plasma HIV viral load and no change in CD4% in HIV infected children aged ≥ 5 months to < 18 years old [44]. Studies in HIV-positive adults immunized with a monovalent unadjuvanted influenza vaccine (H1N1) administered with a single dose, reported no significant change in plasma HIV RNA levels or CD4+ cell count [30, 32]. Tanzi et al. evaluating effects of a virosomal influenza vaccine on viral replication and T-cell activation in HIV–infected children, receiving HAART, observed no increase in plasma HIV-1 RNA or HIV-1 pro-viral DNA and the immunophenotype analyses demonstrated that CD4+ cell counts and percentages and activated lymphocytes remain stable for about a month after immunization [68]. Same results have been obtained by Zuccotti et al., Zanetti et al. and Esposito et al. [24, 69, 70]. In the study by Amendola et al., the mean viral RNA levels did not change in HIV infected and uninfected adults as well as no difference have been showed in HIV proviral DNA levels [25]. However, more recent and better designed studies have not documented a substantial increase in the replication of HIV, immune stimulation resulting from influenza vaccination did not significantly change the levels of plasma virus, CD4+ cell counts, or activation-induced apoptosis in HIV-infected individuals. An increase in the T-cell response to influenza and spontaneous apoptosis, and increase in plasma HIV-1 RNA level following influenza vaccination are rare and transient phenomenon [43-45, 52, 56, 60, 61].

Different studies evaluated the effects of adjuvanted seasonal and pandemic influenza vaccine on HIV viral load and CD4+ cell count in HIV positive subjects. Vaccines used in these studies were adjuvanted with oil-in-water emulsion, in particular with MF59® and AS03®, the only adjuvants approved for human use. Gabutti et al. by comparing groups mean lymphocyte count for time points after immunisation didn’t reveal any statically significant changes in HIV positive adults immunized with MF59 adjuvant or conventional influenza vaccine, with the exception of subjects with lymphocyte counts < 200 cells/mm³, vaccinated with conventional subunit vaccine, whose counts showed a progressive increase after immunization [26]. Durando et al. in HIV positive adults showed a decrease of HIV RNA and an increase of CD4 cells in subjects immunized with adjuvant vaccine compared to patients immunized with conventional not adjuvanted influenza vaccine [28]. Iorio et al. reported, no substantial change in viral load and transitory increase of HIV replication in subjects immunized with a conventional vaccine although the study population were immunized with the same formulation of the above-mentioned study [71]. Palma et al., in HIV positive children and adults immunized with a monovalent pandemic adjuvanted influenza vaccine, showed no altered CD4 count and HIV viral load levels [49]. As regard as influenza vaccines adjuvanted with AS03®, Calmy et al., Tremblay et al. and Launay et al. observed a transient increase of HIV-1 RNA replication within days of the vaccination, but neither increased risk of disease progression nor long-term effects on CD4+ cell
counts, viral load, or progression to AIDS or death have been reported [48, 51, 52]. Lack of data exists in the literature regarding the effect of influenza vaccination with intradermal delivered; therefore, further studies of this preparation are needed.

**IMMUNOGENICITY**

Typically, to evaluate the immunogenicity of influenza vaccines are considered the following parameters: Geometric mean titres (GMTs), mean-fold increase in titres (MFI; ratio of post to pre-vaccination titre), seroprotection rate, defined as the percentage of subjects achieving a significant increase in titre from a non-negative pre-vaccination titre (≥ 1:10) or a rise from < 1:10 to ≥ 1:40 in those who were seronegative), and seroconversion rate defined as either a negative pre-vaccination titer (≤ 10) to a post-vaccination titer ≥ 40, or at least a fourfold increase between pre-and post-vaccination titers where the pre-vaccination titer is ≥ 10. The Committee for Medicinal Products for Human Use (CHMP; formerly CPMP) criteria for approval of influenza vaccine in adults younger than 60 years includes that at least one of the following criteria are met: seroprotection rate > 40%, MFI > 2.5 or seroconversion rate > 70% [72]. As recommended by the WHO the test used to assess the immunogenicity of influenza vaccine are the inhibition of hemagglutination (HI) and microneutralization (MN) assay [17]. The HI assay has been used as the primary means to measure serum antibody responses to influenza vaccines due to the ease of conducting the assay and a generally accepted correlation with seroprotection. However, the information imparted by a demonstration of neutralization capacity is different from that of HI, which measures disruption of binding to red cells without assessing prevention of infection. Although definitive human studies are lacking, animal models suggest that neutralization capacity predicts prevention of infection, while HI correlates with prevention of disease [45, 46]. In clinical trials of vaccines, MN assays may be more sensitive in detecting antibody and demonstrating seroconversion [54, 73], including in immunocompromised subjects. HIV infection is associated with deficiencies in both humoral and cell-mediated immunity, which can alter the course of common infections and influence vaccine immunogenicity [74]. Generally in HIV-infected patients immunogenicity correlates directly with CD4+ cell count and inversely with HIV viral load [57, 58]. Most studies emphasized the importance of a high CD4+ cell count, treatment HAART therapy and use of adjuvant influenza vaccine to increase vaccine response rate; The immune response is closely correlated to the degree of immunosuppression [28, 35, 71, 75]. Hatayema et al.,

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**Fig. 1. Seroprotection rate in studies involving HIV+ population.**
instead, in his study observed no correlation between plasma HIV RNA levels and HI antibody response [31]. Figure 1 shows the seroprotection rates of some studies reported in this review.

**Conventional and LAIV influenza vaccines**

As regard as non-adjuvanted A(H1N1)2009 pandemic vaccine, several studies evaluated antibody responses elicited by this vaccine in different populations of HIV infected individuals. Most of study participants followed an antiretroviral therapy with an high median CD4+ T-cells counts and fully suppressed HIV-RNA levels. Despite a good safety and tolerability profile, antibody responses elicited by conventional pandemic vaccine were insufficient to confer adequate protection in this immunosuppressed, hyporesponsive, at high risk subjects [30, 41].

The collected data regarding the administration of influenza unadjuvanted vaccine are controversial: Lagler et al. and Hatakeyama et al., reported in HIV positive adults a low immune response after two doses of pandemic influenza vaccine, while Bickel et al. and Cooper et al., demonstrate the effect of a booster dose of pandemic influenza vaccine in HIV-positive adults plays a fundamental role to increase the immunogenicity in immunodepressed population [31, 39, 41, 76]. Some authors evaluated the effect of a single dose of non-adjuvanted pandemic vaccine obtaining controversial results: some authors reported an adequate immunogenicity while others suggested that new strategies would be evaluated [30 32, 34, 36, 37, 40, 77]. As regards the evaluation of the immunogenicity of non-adjuvanted pandemic vaccine in children, Phongsamart et al., Flynn et al. and Hakim et al. reported an adequate immune response after two doses. Phongsamart et al., in his study showed a low level of cross reacting antibody to seasonal H1N1 [33, 35, 38].

**Immunogenicity of MF59 adjuvanted influenza vaccine**

In the study by Durando et al. an assessment of humoral and cell-mediated immune responses elicited by the two influenza vaccines was made. Immunocompromised individuals vaccinated with MF59 adjuvanted vaccine showed a better antibody response and also a statistically significant increase in the frequency of proliferating T cells at 30 days after immunization, compared with patients vaccinated with non adjuvanted vaccine. HIV-negative subjects mounted a stronger antibody response than HIV-positive subjects [28]. The same results were obtained by Iorio et al. by comparing the immune response elicited by the same vaccines in HIV patients under antiretroviral therapy [14]: MF59 adjuvanted influenza vaccine induced a better immune responses as compared with conventional vaccine in HIV positive HAART treated subjects [71].

**Oil-in-water emulsion adjuvanted influenza vaccines**

Immunogenicity of MF59 adjuvanted influenza vaccine has been evaluated in different HIV positive populations. The different studies were designed and carried out in different manners: in literature there are comparative and not comparative, prospective trials, and populations involved varied from infected/uninfected children to infected/uninfected young adults and adults. Several studies showed that MF59 adjuvanted influenza vaccine had good safety and immunogenicity profiles similar or better than conventional influenza vaccine. All studies underlined that CD4+ cell count and HAART treatment play a key role in determining antibody response in influenza vaccination. Three studies reported a comparison between MF59 adjuvanted and conventional non adjuvanted seasonal influenza vaccine in HIV positive adults.

In the study by Tanzi et al., showed that use of the virosomal influenza vaccine is moderately immunogenic in HIV infected children. Because there are no EMEA defined criteria for children, immunogenicity was evaluated using criteria for adult. Humoral immune response were adequate for all three influenza strains in primed children and adequate for the two A strains in groups of the unprimed [68].
stimulated by a single or two doses of pandemic MF59 adjuvanted influenza vaccine was comparable in HIV infected patients and in healthy controls. In the study by Kajaste-Rudnitski et al. the seroconversion rate observed after a single dose of MF59®-adjuvanted influenza vaccine was lower in the HIV positive individuals as compared with HIV negative controls; however, the seroprotection rates were similar in both study groups [80]. Soonawala et al. reported the induction of cross reactive antibodies to pH1N1 after vaccination with seasonal TIV in HIV infected adults: authors hypothesized that HIV infected patients could produce larger quantities of cross reactive antibodies after vaccination because of a less well regulated B-cell immune response [75]. Palma et al. and Viganò et al. evaluated the immunogenicity of MF59®-adjuvanted pandemic influenza vaccine in HIV positive children and young adults, showing adequate levels of seroprotection already after a single dose; however, authors suggested a two-dose vaccination schedule in this population, in order to optimize and obtain a long-term antibody response [49, 81].

Thus, MF59®-adjuvanted influenza vaccine should be considered for use in HIV positive adults and children, because it could improve the effectiveness of influenza vaccination programmes in this population, who are at high-risk of influenza-related complications. Another oil-in-water emulsion used as adjuvant in influenza vaccine is the AS03 adjuvant system. The AS03® adjuvant consisted of a 10% oil-in-water emulsion based adjuvant system, containing 5% dl-α-tocopherol (11.86 mg) and squalene (10.69 mg) in the oil phase and 2% of the non-ionic detergent Tween®80 (4.86 mg) in the aqueous phase. Recently, the AS03® adjuvant has been adopted in the licensed formulation of a H1N1 2009 pandemic vaccine. Some authors [51, 82] reported seroprotection rates lower after one dose of AS03 adjuvanted vaccine (61% to 75%) in HIV infected adults [51, 82, 83], while a second dose determines an increase up to 97.7% [76, 84]. According to these authors this difference could be attributed to the different timing of immunization during the pandemic outbreak, in relation to higher baseline or follow-up seroprotection rates. Concerning the seroconversion rates, other studies reported values of 88% to 95.3% after a single dose in HIV positive patients [48, 84, 85]. The collected data regarding the administration of a single dose of influenza AS03 adjuvanted vaccine are controversial: Manuel et al., reported a similar immune response in HIV patients and healthy control, while Kelly et al., showed that immune response correlate with a pre-existing antibodies and a single dose may be insufficient to induce protective immunity [86, 87]. Okike et al., reported that the use of AS03 adjuvant in HIV positive children have likely played an important role in eliciting the high sero-response rates [50]. In general, the use of AS03 adjuvanted pandemic vaccine in HIV positive immunocompromised patients may lead to the expansion of coverage and increase the immune response [55, 83].

Intradermal influenza vaccines

Between strategies implemented to increase the immunogenicity of influenza vaccination in HIV patients, intradermal approach is also to be mentioned. In persons with impaired immunity intradermal vaccination is of particular interest because of different immunologic advantages. The dermis harbours a network of antigen presenting cells, constituting up to 2% of all dermal cells, which forms an optimal environment to deliver a vaccine. In the study by Gelinck et al., 39 HIV positives adults received the 2005/2006 trivalent influenza vaccine delivered intradermally according to the CDC guidelines, using an intradermal injection syringe (BD Micro-Fine 0.5 ml U-100 insulin syringe) and needle (29G), also used for intradermal tuberculin injections. The intradermal vaccine dose contained 3 μg of haemagglutinin of the three strains. The intradermal vaccination with a low dose resulted in similar postvaccination titers as compared to standard intramuscular vaccination in the study groups: in particular only the HIV group had higher titers after intradermal vaccination. Results suggested that the intradermal vaccination is more efficient in inducing an immune response compared to intramuscular vaccination in HIV infected patients [22]. The superiority of the immune response elicited by the intradermal vaccine is shown by the values of the rates of seroprotection, seroconversion, and MFI/GMRT all higher than the vaccine administered intramuscularly to the controls. Ansaldo et al., in his study compared immunogenicity of licensed intradermal trivalent inactivated split vaccine delivered by licensed microsystem device with a lower antigen content (9 μg HA per strain) against the conventional intramuscular (15μg) influenza vaccine in a population of 54 HIV infected adults. Both vaccines meet the criteria for MFI and seroprotection rate but not for seroconversion against the B virus, there are no differences in terms of post-vaccination GMT /MFI, seroprotection and seroconversion rates comparing the two vaccines. The results of this trial showed that the licensed low antigen content ID vaccine was safe and immunogenic in HIV positive adults, although the small sample size of the study [53].

Conclusions

Influenza is a major cause of death, hospitalization and disability in immunocompromised population, resulting in an health and economic burden that may be reduced implementing annual vaccination as recommended by WHO guidelines. The considerable experience accumulated about influenza vaccination refers a good safety and tolerability profile, in fact the adverse events reported are usually mild and resolve in a few days after their appearance. About the vexed question of viral load increase, conflicting data are reported in literature. However, when this increase occurs, it is of transient nature and typically did not associate in CD4+ T cell counts changes.
Some studies reported immunogenicity data in HIV+ population, in which are compared different influenza vaccines, adjuvanted and not, and different administration routes.

The data are encouraging because an adequate immune response is shown, although split/subunit vaccine do not elicited an efficient immune response in these subjects. New strategies were evaluated to increase the immune response in immunocompromised patients, as increase dosage, multiple dose vaccination, use of vaccine adjuvant, immunostimulant patches (the skin, especially its epidermal layer, is an accessible and competent immune environment and an attractive target for vaccine delivery, through transcutaneous delivery or immunostimulant patches) and different routes of vaccine delivery transcutaneous immunization, a topical vaccine application, combines the advantages of needle-free delivery while targeting the immunologically rich milieu of the skin [64, 70, 88, 89]. The administration of an MF59®-adjuvanted influenza subunit vaccine has been shown to improve immunogenicity and to elicited good immune responses and protective antibody [28, 91, 92].

Several studies estimated safety, tolerability and immunogenicity of intradermal influenza vaccine, the majority of which were conducted in elderly population; only one study was conducted on a population of immunocompromised patients. A dose-response relationship together with more efficient antibody response for intradermal vaccine was demonstrated using only a fifth of the normal dose evaluating the humoral response of intradermal influenza vaccination versus intramuscular vaccination. Local skin reactions can be used as predictors for the success of vaccination by identifying patients who may need a booster [54].

In general, the immunogenicity studies have shown encouraging results for the HIV positive subjects with antibody titres protecting, data on safety and tolerability are encouraging reporting only a few cases of serious effects on the population considered (about 10% of patients).

Definitely the most promising data concern the use of vaccine adjuvants, in particular with MF59®, and the use of new vaccine intradermal administration should be more extensively examined.

In the light of data reported in this review, there still is much to do about vaccination in immunocompromised patients. More effective effectiveness studies are needed, to assess the impact of influenza vaccination and to prevent complications associated with it.

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