Overview

History and evolution of influenza control through vaccination: from the first monovalent vaccine to universal vaccines

I. BARBERIS[1], P. MYLES[2], S.K. AULT[3], N.L. BRAGAZZI[1], M. MARTINI[4]
[1] Department of Health Sciences (DISSAL), University of Genoa, Italy; [2] Division of Epidemiology and Public Health, University of Nottingham, UK.; [3] Pan American Health Organization/World Health Organization (retired), Washington, D.C., United States of America; currently Office of the Dean, School of Public Health, University of Maryland, United States of America; [4] Section of History of Medicine and Ethics, Department of Health Sciences (DISSAL), University of Genoa, Italy

Keywords

Influenza • Vaccination • History of medicine

Summary

Influenza is a highly infectious airborne disease with an important epidemiological and societal burden; annual epidemics and pandemics have occurred since ancient times, causing tens of millions of deaths. A hundred years after this virus was first isolated, influenza vaccines are an important influenza prevention strategy and the preparations used display good safety and tolerability profiles. Innovative tools, such as recombinant technologies and intra-dermal devices, are currently being investigated in order to improve the immunological response. The recurring mutations of influenza strains has prompted the recent introduction of a quadrivalent inactivated vaccine. In the near future, scientific research will strive to produce a long-lasting universal vaccine containing an antigen that will offer protection against all influenza virus strains.

Introduction

Influenza viruses are negative-sense, single-stranded RNA viruses belonging to the *Orthomyxoviridae* family, together with Isavirus, Thogotovirus and Quaranjavirus. Three types of influenza viruses, namely influenza A, B and C, are capable of determining epidemics and pandemics in humans, with influenza A being the most common circulating type and causing significant illness, being most prone to antigenic shifts and the more likely type to lead to a pandemic [1, 2]. Recently, a new genus (termed influenza virus D) has been discovered in pigs and cattle with influenza-like illness syndrome in the United States [3, 4] and in Europe [5].

Influenza is a highly infectious airborne disease that affects a significant percentage of the world's population; local annual epidemics and pandemics have occurred since ancient times, causing tens of millions of deaths [6].

The aim of this mini-review is to provide a brief overview of the history and evolution of influenza and influenza control using vaccines.

A history of influenza: from the classical period to the nineteenth century

In 412 BC, in the "Book of Epidemics", Hippocrates described a putative influenza-like illness syndrome called "fever of Perinthus" or "cough of Perinthus" [7]. While some scholars claim that this is probably the first historical description of influenza (a winter and a spring epidemic of an upper respiratory tract infection occurring regularly every year at Perinthus, a port-town in Marmaraereglisi, a northern part of Greece, now Turkey), others, including the notable 19th-century editor of Hippocrates, Émile Littré (1801-1881), think that a diagnosis of diphtheria would better fit the description of complications (pneumonia, fits of coughing and wheezing, angina and paralysis of soft palate and limbs). On the other hand, symptoms such as disturbed vision and night blindness suggest a combination of diseases, including deficiency syndromes (e.g. vitamin A deficiency) [8]. In the years 1173 and 1500, two other influenza outbreaks were described, though in scant detail [9-11]. The name "influenza" originated in the 15th century in Italy, from an epidemic attributed to the "influence of the stars", which, according to Ginctrac, raged across Europe and perhaps in Asia and Africa [12].

It seems that influenza also reached the Americas. Scholars and historians debate whether influenza was already present in the New World or whether it was carried by contaminated pigs transported on ships. Some Aztec texts speak of a "pestilential catarrh" outbreak in 1450-1456 in an area now corresponding to Mexico, but these manuscripts are difficult to interpret correctly and this hypothesis seems controversial [13].

The first reliable documents regarding influenza-like ill-

ness syndrome date from 1510, when the virus spread from Africa to Europe. The first pandemic, or worldwide epidemic, occurred in 1557, though some scholars deny that it really was an outbreak of influenza. The first pandemic/worldwide epidemic that undoubtedly fits the description of influenza appeared in 1580, beginning in Asia and Russia and spreading to Europe via Asia Minor and North-West Africa. In Rome, it caused the death of over 8,000 people, while in Spain it decimated the populations of entire cities. Subsequently, it also affected the Americas [14].

Over the centuries, other pandemics were described worldwide. From 1404 to the middle of the 19th century, 31 influenza epidemics were recorded, including eight large-scale pandemics. Subsequently, others appeared, including three in the 20th century [14]. Some of the most notable outbreaks occurred in 1729, in 1781-1782 (a pandemic spreading from China to Russia, Europe and North America), in 1830-1833 (a pandemic which again spread from China to India, the Philippines, Indonesia, Russia, Europe and North America), in 1847-1848, and in 1898-1900 (spreading from Europe to India, Australia, and North and South America) [14].

One of the most devastating was the pandemic of "Spanish" influenza in 1918–1919, which caused an estimated 21 million deaths worldwide and was defined by Waring as "the greatest medical holocaust in history" [14, 15].

At the end of the 19th century, the etiology of this disease had yet not been well clarified; it was believed that the disease, termed "winter catarrh", was caused by bacteria (the so-called bacterial hypothesis), such as pneumococcus, streptococcus or *Haemophilus influenzae.* This latter was also named *Bacillus influenzae* or Pfeiffer's bacillus, after Richard Pfeiffer (1858-1945), who described it during the 1889-1892 influenza epidemic. This bacillus had already been discovered by the Polish microbiologist Bujwid Odo Feliks Kazimierz (1857-1942) in biopsy material a year earlier [16].

In the same period, the French microbiologists Charles Nicolle (1866-1936), Charles Lébally and René Dujarric de la Rivière (1885-1969) of the Pasteur Institute showed that the flu pathogen could pass through a fine filter. However, despite their brilliant experiments, the viral hypothesis continued to be neglected until the virus was isolated [16, 17].

In 1889, some Spanish doctors believed that influenza was a variant of dengue fever, whilst others attributed influenza outbreaks to a variety of causes including cannon fire on the western front, the building of the Madrid underground, air pollution, sunspots, or the spread of the habit of smoking poor-quality tobacco [18].

The thirties: virus isolation and the first experimental vaccines

During the 1918-1919 pandemic, some scientists began to suspect that bacteria were not the real agent of influenza disease. One of these was the scholar Richard Edwin Shope (1901-1966), who deeply investigated swine flu in 1920. However, it was only in 1932-1933 that the English scientists Wilson Smith (1897-1965), Sir Christopher Andrewes (1896-1988) and Sir Patrick Laidlaw (1881-1940), working at the Medical Research Council at Mill Hill, first isolated the influenza A virus from nasal secretions of infected patients, thereby demonstrating the intranasal human transmission of this virus [19, 20]. A few years later, the American virologist and epidemiologist Thomas Francis Junior (1900-1969) and Smith, in England, were able to transmit the virus to mice [21]. Subsequently, in 1935, Sir Frank Macfarlane Burnet (1899-1985) and Smith separately discovered that the flu virus could be grown on the chorio-allantoid membrane of embryonated hens' eggs [22], and in 1936 the first neutralized antibodies generated by infection by human influenza virus were isolated [23].

In the next five years, important developments took place: the demonstration that the virus inactivated by formalin was immunogenic in humans, purification of the virus by means of high-speed centrifugation, and the discovery that the influenza virus grew easily in fertilized hen eggs, a procedure that is still used today to manufacture most influenza vaccines [23].

The first clinical trials of influenza vaccines were conducted in the mid-1930s [24, 25].

A study by Smith, Andrewes and Stuart-Harris was conducted among military forces in England in 1937 using a subcutaneous vaccination with an inactivated strain isolated from a mouse lung [25].

In 1938, Francis, together with Jonas Edward Salk (1914-1995), managed to protect USA military forces. Salk would subsequently use this successful experience to develop an effective polio vaccine in 1952 [26, 27].

The forties: inactivated influenza vaccines

Influenza vaccination had two main objectives: (i) to protect against disease, and (ii) to achieve a high vaccination rate in order to ensure protection in unvaccinated people. The first vaccine was an inactivated, monovalent preparation which only contained a subtype of the influenza A virus [26, 27].

In December 1942, large studies were begun to be conducted on the first influenza virus vaccines; these provided the first official proof that inactivated influenza vaccines could yield effective protection against flu epidemics [28].

The efficacy and safety of inactivated vaccines were first studied between 1942 and 1945; in the meantime, a new strain of flu virus was discovered, the influenza virus type B, which is the main cause of seasonal epidemics, as was the phenomenon of so-called "influenza mismatch". Influenza mismatch is caused by major and minor mutations of circulating viruses. As a result, the virus contained in the vaccine does not match the circulating strain, determining a reduction in the effectiveness of subtype A influenza vaccines.

A new route of influenza immunization was tested in December 1942, with the subcutaneous inactivated bivalent vaccine containing viruses of type A and type B.

The following years, the first bivalent vaccine was licensed in the United States and became available for use in the general population [29, 30].

The fifties: influenza mismatch and influenza surveillance

The first system for the surveillance of circulating influenza virus strains in several countries worldwide was created in 1952 by the World Health Organization (WHO) in order to monitor the various virus mismatches reported. This important innovative tool enabled the composition of seasonal influenza vaccines to be determined on the basis of the epidemiology of influenza in the previous season [31]. In 1946, as a result of viral mutation, a new variant of influenza A (H1N1), A/FM/1/47, appeared in Australia. This gave rise to a new influenza subtype, the H2N2 strain, which caused the pandemic known as Asian flu [32].
The following year, the US Commission on Influenza recommended that a representative of this strain be included in subsequent vaccines.
The emergence of an HA subtype different from those circulating in previous seasons determined the need for pandemic influenza vaccines [31].

The sixties: split vaccines

New inactivated compounds were tested for safety and efficacy during seasonal epidemics in the 1960s, in particular two new formulations were created: split and subunit vaccines. The 1968 pandemic led to the development of trivalent inactivated vaccines (TIVs) against influenza viruses; moreover the development of new split or subunit vaccines led to a decrease of adverse reactions in children. These vaccines were split using ether and/or detergent, and haemagglutinin and neuraminidase were, in the case of subunit vaccines, purified and enriched [33].
In the same period, the first flu vaccines were licensed in Europe, while in the US annual influenza vaccination was recommended for individuals at major risk of influenza complications.
In 1968, the new virus strain H3N2 (Hong Kong) appeared, completely replacing the previous type A strain (H2N2, or Asian influenza), and led to another global pandemic with high morbidity and mortality [34]. In the same year, a new type of vaccine, the split vaccine, was authorized in the US after several clinical studies had demonstrated that it was less reactogenic than whole virus vaccines, especially in the early years of life [35].

The seventies: genetic reassortment

Split vaccines were widely used during the pandemic swine influenza in 1976 and in 1977, when the H1N1 subtype re-emerged worldwide. However, they were seen to be less immunogenic than whole virus vaccines in "primed" subjects who had never been vaccinated. Indeed, it was shown that two vaccine doses were needed in order to ensure effective protection [36].
At the beginning of the 1970s, an important innovation was introduced into the production of influenza vaccines: the genetic reassortment of influenza virus strains; this technique enabled the vaccine strains to grow faster in embryonated hen eggs [37].
The first subunit vaccine was created between 1976 and 1977. This contained only the surface antigens, hemagglutinin (HA) and neuraminidase (NA), which were isolated by means of successive purification steps.
This innovative tool proved to be highly immunogenic and well tolerated in humans, especially in children, although two doses were needed to guarantee vaccine effectiveness during epidemics [38].

The eighties: subunit vaccines

In 1980, the first subunit vaccines were licensed in the United Kingdom and are currently available in several countries worldwide.
In 1978, as a result of a major mutation, a new virus strain, H1N1, appeared on the global epidemiological scene. This strain, which was similar to a virus circulating in 1958, emerged in Russia and began to co-circulate, either simultaneously or alternately, with the previous one [39].
Antigenic drift, caused by frequent changes in the composition of the virus, determined the need to update the vaccine composition each year. This necessity prompted both the implementation of the first surveillance systems and the production of the first trivalent vaccine, which included three formulation strains (one strain of influenza A/ H1N1, an influenza virus A /H3N2 and a type B virus), in order to ensure effective protection during the 1978 pandemic.

Live attenuated influenza vaccines

In the period 1935-1941, the first clinical trials involving live attenuated influenza vaccines were conducted. The efficacy of these seasonal vaccines was guaranteed by the correspondence between the circulating strain and the strain contained in the vaccine and by the virus dose grown in hen egg embryos [34].
In 1944, Stanley described in detail the preparation and properties of an influenza virus vaccine produced in embryonated hen eggs; this vaccine was concentrated and purified by means of differential centrifugation and inactivated by means of various procedures [23].
In 1949, an important change in vaccine development involved the introduction of the use of cell cultures for virus growth.
In 1997,the so-called "avian flu" pandemic broke out in Hong Kong. This was caused by influenza virus A/ H5N1, a highly pathogenic strain.
In order to contain this pandemic, the techniques of genetic rearrangement developed in those years enabled a

huge number of vaccine doses to be produced in a short time by applying recombinant DNA technology to the influenza A/H5N1 virus [34].

Recent years

In recent years, scientific research developed new techniques of immunization, which may be more immunogenic and better tolerated during administration, thereby reducing adverse events. In 2003, for instance, the FDA in the United States authorized the use of an intranasally administered live attenuated vaccine, called FluMist®, in adults [40]. In the 2003-2004 influenza season, an outbreak in Asia was caused by an influenza A/H5N1 strain. This was later used to produce a vaccine, which was licensed in the United States by the FDA in 2007.
More recent years saw the development of adjuvanted vaccines, such as those containing alum adjuvants and the oil in water adjuvant MF-59, which significantly enhanced antigenicity [6].
Specifically, MF-59-adjuvanted vaccines were used in the elderly and in young children, and proved to elicit a good response even to pandemic strains with which subjects had not been primed by natural influenza infection. Similar responses were obtained through the use of other emulsions, such as stable emulsion (SE) and AS03, which were included in the 2009 pandemic influenza vaccines [36].
In the most recent pandemic season (2009), the influenza virus H1N1, which was transmitted to humans by pigs, was estimated to have caused more than 200,000 deaths in the first 12 months of its circulation [41].
A massive effort to produce vaccine for the new H1N1 strain began shortly after scientists identified the virus. The virus proved to grow slowly during the manufacturing process, which relies on cultivation of the virus in chicken eggs. Because of manufacturing delay, the vaccine was available in most countries after the second peak of influenza cases at the end of October leaving most people not immunized while influenza H1N1 virus was circulating [42].
In the elderly, the vaccine efficacy normally decreases, because of immunosenescence. For this reason, in 2009 the Advisory Committee on Immunization Practices (ACIP) recommended and authorized the use of high-dose Fluzone®, a new formulation containing a 4-fold higher HA dose than the traditional trivalent vaccine [43].
In 2011, as a result of developments in research into new vaccine delivery techniques, the FDA first authorized the intradermal administration of Fluzone®. This new route of administration involved antigen-presenting cells (APCs) in the dermis; these cells process antigens for subsequent presentation in the lymphoid organs, resulting in the stimulation of both innate and adaptive immunity. The intradermal vaccines elicited a better immunological response than intramuscular vaccines, particularly in the elderly; in healthy adults, it yielded an immune response comparable to that elicited by the traditional vaccines, while saving on the HA dose [44-48].
In 2012, the FDA approved Fluarix®, the first quadrivalent vaccine in the United States. This split vaccine contained two influenza A strains and two influenza B antigens. The presence of an additional influenza B strain reduced the possibility of a mismatch between the circulating viruses and the vaccine composition, while maintaining the same immunogenicity and safety as standard trivalent vaccines [49].
In 2013, the FDA approved FluBlock®, a recombinant trivalent influenza vaccine, for use in people aged between 18 and 49 years. FluBlock® was licensed in a spray formulation and was the first trivalent influenza vaccine made by using recombinant DNA technology. Derived from Baculovirus, it contained a 3-fold higher HA dose than traditional trivalent vaccines [50, 51]. The scale-up potential of the insect cell/baculovirus vector system may offer advantages in terms of rapid antigen change and response to a pandemic situation [31].
Currently, scientists are exploring the fascinating prospect of developing a universal vaccine by exploiting T-cells and by attempting to elicit broadly neutralizing antibodies. Moreover, efforts are being made to design M2e- or stalk-based vaccines, since these proteins (the type-2 matrix protein and the stalk domain of HA, respectively) are quite well conserved from an evolutionary standpoint [52, 53].

Conclusions

In the hundred years since the influenza virus was isolated, influenza vaccine preparations have evolved to ensure effective protection, while maintaining a good safety and tolerability profile.
The recurring mutations of influenza strains prompted the introduction of a quadrivalent inactivated vaccine, the composition of which is determined on the basis of the most frequent strains isolated in the previous season during continuous surveillance by the WHO.
Current research priorities include the development of a universal influenza vaccine that could offer protection against all influenza virus strains, thereby overcoming the challenges faced due to antigenic drift and shift or of co-circulation of different viral strains. Another important priority is to identify sustainable vaccine production platforms capable of rapidly meeting the large global demands for influenza vaccine in the face of an influenza pandemic.

Acknowledgments

No funding declared for this overview. The authors thank Dr. Bernard Patrick for revising the manuscript.

Authors' contribution

MM conceived and designed the overview. IB and PM performed a search of the literature and contributed to the draft of the article. SA and NLB revised critically the manuscript. MM supervised the manuscript. All authors read and approved the final version of the manuscript.

References

[1] Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D. *Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part I: Influenza life-cycle and currently available drugs*. J Prev Med Hyg 2014;55:69-85.

[2] Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D. *Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part II: Future compounds against influenza virus*. J Prev Med Hyg 2014;55:109-29.

[3] Hause BM, Collin EA, Liu R, Huanq B, Shenq Z, Lu W, Wanq D, Nelson EA, Li F. *Characterization of a novel influenza virus in cattle and Swine: proposal for a new genus in the Orthomyxoviridae family*. MBio 2014;5:e00031-14.

[4] Collin EA, Sheng Z, Lang Y, Ma W, Hause BM, Li F. *Cocirculation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle*. J Virol 2015;89:1036-42.

[5] Ducatez MF, Pelletier C, Meyer G. *Influenza D virus in cattle, France, 2011-2014*. Emerg Infect Dis 2015;21:368-71.

[6] Soema PC, Kompier R, Amorij JP, Kersten GF. *Current and next generation influenza vaccines: Formulation and production strategies*. Eur J Pharm Biopharm 2015;94:251-63.

[7] Pappas G, Kiriaze IJ, Falagas ME. *Insights into infectious disease in the era of Hippocrates*. Int J Infect Dis 2008;12:347-50.

[8] Kohn GC *Encyclopedia of Plague and Pestilence: From Ancient Times to the Present*. Infobase Publishing 2007.

[9] Beveridge WIB. *Influenza: The Last Great Plague*. London: Heineman Educational Books 1977.

[10] Wood JM, *Influenza*. In: Crovari P, Principi N, eds. *Le vaccinazioni*. Pisa: Pacini Editore 2000.

[11] Kuszewski K, Brydak. L. *The epidemiology and history of influenza*. Biomed Pharmacoter 2000;54:188-95.

[12] Gintrac H. *Grippe*. In: *Nouveau dictionnaire de médicine et de chirurgie pratiques, directeur de la redaction: le docteur Jaccoud.* 1872, Tome 16, page. 728-753.

[13] de Souza CM. *The Spanish flu epidemic: a challenge to Bahian medicine*. Hist Cienc Saude Manguinhos 2008;15:945-72.

[14] Potter CW. *A history of influenza*. J Appl Microbiol 2001;91:572-9.

[15] Waring J.I. *A History of Medicine in South Carolina 1900-70*. South Carolina Medical Association 1971.

[16] Bazin H. *Vaccination: a history*. France: Editions John Libbey Eurotext Montrouge 2011.

[17] Renaud F, Hansen W, Freney J. *Dictionnaire des precurseurs en bacteriologie*, SFM Éditions Eska, 2005, 249 pages.

[18] Craddock S, Giles-Vernick T, Gunn JL. *Influenza and Public Health: Learning from Past Pandemics*. Earthscan, 2010 - 293 pages.

[19] Smith W, Andrewes CH, Laidlaw PP. *A virus obtained from influenza patients*. Lancet 1933;2:66-8.

[20] Smith W, Andrewes CH, Stuart Harris CH. *The immunization of human volunteers*. Special Rep Ser Med Res Council 1938;228:137-44.

[21] Francis T Jr. *Vaccination against influenza*. Bull World Health Organ 1953;8:725-41.

[22] Burnett FM. *Influenza virus infection of the chick embryo lung*. Br J Exp Pathol 1940;21:147-53.

[23] Stanley WM. *The preparation and properties of influenza virus vaccines concentrated and purified by differential centrifugation*. J Exp Med 1945;81:193-218.

[24] Davenport FM. *The search for the ideal influenza vaccine*. Postgraduate Medical Journal 1979;55:78-86.

[25] Francis T. *Vaccination against influenza.* Bull World Health Org 1953;8:725-41.

[26] Parodi V, de Florentiis D, Martini M, Ansaldi F. *Inactivated influenza vaccines: recent progress and implications for the elderly*. Drugs Aging 2011;28:93-106.

[27] Barberis I, Martini M, Iavarone F, Orsi A. *Available influenza vaccines: immunization strategies, history and new tools for fighting the disease*. J Prev Med Hyg 2016;57:E41-6.

[28] Francis T jr, Salk JE, Pearson HE, Brown PN. *Protective effect of vaccination against induced influenza A*. J Clin Invest 1945;24:536-46.

[29] Weir JP, Gruber MF. *An overview of the regulation of influenza vaccines in the United States*. Influenza and Other Respiratory Viruses 2016;10:354-60.

[30] Keitel WA, Neuzil KM, Treanor J. *Immunogenicity, efficacy of inactivated/live virus seasonal and pandemic vaccines. Textbook of Influenza.* Wiley-Blackwell 2013, pp. 311-326.

[31] WHO Global Influenza Surveillance Network (GISN) http://www.who.int/influenza/gisrs_laboratory/en/ [Accessed 20/9/2016].

[32] Oxford J, Gilbert A, Lambkin-Williams R. *Influenza vaccines have a short but illustrious history of dedicated science enabling the rapid global production of A/swine (H1N1) vaccine in the current pandemic*. In: Rappuoli R, Del Giudice G, eds. *Influenza vaccines for the future.* Springer Verlag, 2011.

[33] Krammer F, Palese P. *Advances in the development of influenza virus vaccines*. Nat Rev Drug Discov 2015;14:167-82.

[34] Zaman M, Ashraf S, Dreyer NA, Toovey S. *Human infection with avian influenza virus, Pakistan, 2007*. Emerg Infect Dis 2011;17:1056-9.

[35] Parkman PD, Hopps HE, Rastogi SC, Meyer HM Jr. *Summary of clinical trials of influenza virus vaccines for adults*. J Infect Dis 1977;136:722-30

[36] Hampson AW. *Vaccines for pandemic influenza. The history of our current vaccines, their limitations and the requirements to deal with a pandemic threat*. Ann Acad Med Singapore 2008;37:510-7.

[37] Crovari P, Alberti M, Alicino C. *History and evolution of influenza vaccines*. J Prev Med Hyg 2011;52:91-4.

[38] Gianchecchi E, Trombetta C, Piccirella S, Montomoli E. *Evaluating influenza vaccines: progress and perspectives*. Future Virology 2016;11:379-93.

[39] Kendal AP, Maassab HF, Alexandrova GI, Ghendon YZ. *Development of cold-adapted recombinant live attenuated influenza vaccines in the U.S.A. and U.S.S.R*. Antiviral Res 1982;1:339-65.

[40] Food and Drug Administration (FDA). FluMist. Prior Approval Supplemental BLA. April 2007. Available at: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4292B1-02.pdf.[Accessed 20/9/2016].

[41] Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, Cheng PY, Bandaranayake D, Breiman RF, Brooks WA, Buchy P, Feikin DR, Fowler KB, Gordon A, Hien NT, Horby P, Huang QS, Katz MA, Krishnan A, Lal R, Montgomery JM, Mølbak K, R Pebody, Presanis AM, Razuri H, Steens A, Tinoco YO, Wallinga J, Yu H, S Vong, Bresee J, Widdowson MA. *Estimated global mortality associated with the* first *12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study*. Lancet Infect Dis 2012;12:687-95.

[42] Sullivan SJ, Jacobson RM, Dowdle WR, Poland GA. *2009 H1N1 influenza*. Mayo Clin Proc 2010;85: 64-76.

[43] Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. *Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older*. J Infect Dis 2009;200:172-80.

[44] Durando P, Iudici R, Alicino C, Alberti M, de Florentiis, Ansaldi F, Icardi G. *Adjuvants and alternative routes of administration towards the development of the ideal influenza vaccine*. Hum Vaccin 2011;7(Suppl):29-40.

[45] Holland D, Booy R, De Looze F, Eizenberg P, Mc Donald J, Karrasch J, Mc Keirnan M, Salem H, Mills G, Reid J, Weber F, Saville M. *Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial*. J Infect Dis 2008;198:650-8.

[46] Arnou R, Icardi G, De Decker M, Ambrozaitis A, Kazek MP, Weber F, Van Damme P. *Intradermal influenza vaccine for older adults: a randomized controlled multicenter phase III study*. Vaccine 2009;27:7304-12.

[47] Belshe RB, Newman FK, Cannon J, Duane C, Treanor J, Van Hoecke C, Howe BJ, Dubin G. *Serum antibody responses after intradermal vaccination against influenza*. N Engl J Med 2004;351:2286-94.

[48] Bragazzi NL, Orsi A, Ansaldi F, Gasparini R, Icardi G. *Fluzone*® Intra-dermal (*Intanza*® / *Istivac*® Intra-dermal): *an updated overview*. Hum Vaccin Immunother 2016, in press.

[49] Tisa V, Barberis I, Faccio V, Paganino C, Trucchi C, Martini M, Ansaldi F. *Quadrivalent influenza vaccine: a new opportunity to reduce the influenza burden*. J Prev Med Hyg 2016;57:E28-33.

[50] Treanor JJ, El Sahly H, King J, Graham I, Izikson R, Kohberger R, Patriarca P, Cox M. *Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok) against influenza in healthy adults: a randomized, placebo-controlled trial*. Vaccine 2011;29:7733-9.

[51] Baxter R, Patriarca PA, Ensor K, Izikson R, Goldenthal KL, Cox M. *Evaluation of the safety, reactogenicity and immunogenicity of FluBlok trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy adults 50-64 years of age*. Vaccine 2011;29:2272-8.

[52] Sridhar S. *Heterosubtypic T-cell immunity to influenza in humans: challenges for universal T-cell influenza vaccines*. Front Immunol 2016;7:195.

[53] Wiersma LC, Rimmelzwaan GF, de Vries RD. *Developing universal influenza vaccines: hitting the nail, not just on the head*. Vaccines (Basel) 2015;3:239-62.

■ Received on July 8, 2016. Accepted on August 25, 2016.

■ Correspondence: N.L. Bragazzi, Department of Health Sciences (DISSAL), University of Genoa, via Antonio Pastore 1, 16132 Genoa, Italy - E-mail: robertobragazzi@gmail.com